

2003

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Harshman, Lawrence G., "Life Span Extension of *Drosophila melanogaster*: Genetic and Population Studies" (2003). *Lawrence G. Harshman Publications*. 27.

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Published in *Population and Development Review* 29, Supplement: *Life Span: Evolutionary, Ecological, and Demographic Perspectives* (2003), pp. 99–126.

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## **Life Span Extension of *Drosophila melanogaster*: Genetic and Population Studies**

Lawrence G. Harshman

During the past two decades, genetic studies of model organisms have been the most important tool underlying advances in understanding the biological basis of aging and longevity. *Drosophila melanogaster*, the geneticist's "fruit fly," is a model organism because it has been the focus of genetic studies for more than 90 years. This review argues that studies on *D. melanogaster* will make an especially important contribution to the field of aging and longevity at the intersection of research on genetics, complex traits, and fly populations.

Five approaches have been used to study the genetics of longevity of *D. melanogaster*: (1) laboratory selection, (2) quantitative genetics, (3) transgenic overexpression, (4) mutation analysis, and (5) measurement of gene expression. The first two approaches attempt to decompose longevity as a complex character. The third and fourth approaches start by looking for major gene effects on life span. The fifth approach is emerging as part of a major advance in technology in which the expression of almost all genes in the genome can be measured at one time.

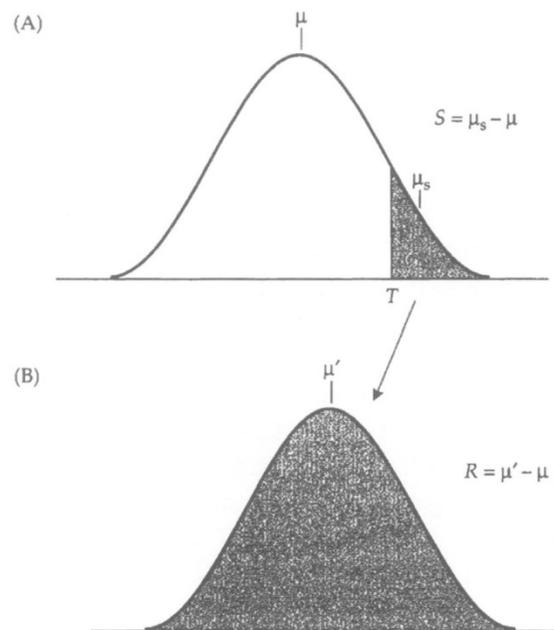
Genetic research on aging and longevity using *D. melanogaster* has been reviewed previously (Arking 1987, 1988; Arking and Dudas 1989; Rose 1991; Curtsinger et al. 1995; Tower 1996; Stearns and Partridge 2001). The present chapter reviews the range of genetic approaches used to study aging and life span (length of life).

### **Selection experiments in the laboratory**

Natural selection has shaped organic evolution whereas artificial selection is a human endeavor, usually with some utilitarian goal. Artificial selection is typically conducted on

complex traits that are controlled by multiple genes. Generation by generation, artificial selection can progressively change the mean value of a complex trait, such as longevity, in a population. The genes that underlie the response to selection, and traits that change as correlated responses to selection, are of interest in the context of understanding aging and longevity.

Figure 1 shows the distribution of a trait in a population of individuals undergoing selection. When individuals from the distribution are nonrepresentatively selected to contribute to the next generation and genetic variation contributes to the trait variation in the population, there can be a genetic-based change in the mean value of the trait in the next generation.



**Figure 1.** The distribution of a trait and population mean ( $\mu$ )

**Notes:** (A) The mean of the subset of individuals used to propagate the next generation is  $\mu_s$ . Selection intensity ( $S$ ) is a function of the difference between  $\mu$  and  $\mu_s$ . (B) The response ( $R$ ) to selection is defined in terms of the mean trait value in the next generation ( $\mu'$ ) and the previous generation ( $\mu$ ). *Source:* Hartl and Clark 1997.

Artificial or natural selection in the laboratory has been used to study a range of traits (Rose 1984; Hoffmann and Parsons 1989; Rose et al. 1990; Huey and Kingsolver 1993; Rose et al. 1996; Gibbs 1999). Selection experiments magnify the difference between selected populations and ancestral or unselected control populations. Large differences are easier to study; selection experiments facilitate investigation by increasing the signal-to-noise ratio in the comparison of selected and control (unselected) populations.

The direct response to selection is the change in the trait targeted for selection. The indirect responses to selection consist of changes in other traits. Indirect responses to selection can be informative because they indicate genetic correlations between traits. Such correlations can reveal traits whose association with the directly selected trait was not anticipated at the beginning of the experiment. Moreover, genetic associations between traits can provide circumstantial evidence about mechanisms underlying the direct response to selection. For example, negative correlations between traits in selection experiments suggest tradeoffs (Rose et al. 1990). Tradeoffs are based on constraints involving energy, space, hormones, or structural biology such that an increase in the expression of one trait results in a decrease in another trait. Tradeoffs are a pivotal consideration in the evolution of longevity and other life-history traits (Williams 1957; Stearns 1989, 1992; Reznick 1985; Zera and Harshman 2001).

A great deal of *D. melanogaster* genetic research on longevity has been based on artificial selection in the laboratory. The following summary of a subset of results is designed to focus on general outcomes and provide perspective on the pluses and minuses associated with the use of selection experiments to study aging and longevity.

#### *Selection experiments on life span*

Although not the first studies of this genre, two artificial selection experiments have had a long-term impact on the field (Rose 1984; Luckinbill et al. 1984). In each case, the mode of selection was to select flies to propagate the next generation using individuals that remained fertile at old ages. For example, an artificial selection experiment by Rose (1984) increased the age of breeding from 4-day-old adults to 28 days (generation 1), then to 35 days (generations 2 and 3), then to 42 days, and ultimately used 70-day-old adults to maintain the selected lines. The important features of the experimental design included a high degree of replication (5 selected and 5 control lines), a relatively large population size in each line to mitigate inbreeding, and a laboratory-adapted ancestral population (Rose 1984; Rose et al. 1996). The control lines were maintained using the same generation time as the ancestral population, meaning that the breeders for the next generation were young adults. Rose (1984) and Luckinbill et al. (1984) substantially increased longevity (by 50 to 100 percent) in their selected lines. Research on their lines highlighted stress resistance as a genetic correlate of longevity.

Selection experiments provided initial evidence for a genetic relationship between stress resistance and longevity. Relative to control lines, the Rose lines, selected for longevity, were resistant to starvation, desiccation, and ethanol fumes, as well as elevated temperature under some conditions (Service et al. 1985). The Luckinbill set of lines provided the first evidence for a genetic correlation between longevity and oxidative stress resistance (Arking et al. 1991). This observation was particularly significant because it provided indirect support for the free radical theory of aging (Harman 1956), a predominant biochemical hypothesis that explains aging in terms of oxidative damage to macromolecules in cells (Wallace 1992; Martin et al. 1996; Johnson et al. 1999; Guarente and Kenyon 2000).

Selection experiments also provided early evidence for a genetic association between early-age reproduction and longevity. Females from control populations exhibited a relatively short life span and high early-age egg production, but the converse was observed

for the lines selected for extended longevity (Rose 1984). Selected-line females were less fecund early in life regardless of whether they had mated or were virgin (Service 1989). Selected-line males also exhibited reduced reproductive function early in life compared to control-line males (Service and Fales 1993; Service and Vossbrink 1996). During the course of many generations of selection, however, early-age egg production evolved to become higher in the selected than in the control lines in some environments (Leroi et al. 1994). This outcome was interesting from an evolutionary standpoint, but it raises questions about the utility of the selection experiment approach because some results can be inconsistent over time.

Ancillary selection experiments have been used to test the direct and correlated responses to selection. For example, direct selection for starvation resistance resulted in flies with extended longevity (Rose et al. 1992). However, Harshman et al. (1999) selected only for female starvation resistance and did not observe an increase in female or male longevity. Both experiments were highly replicated, but they differed in the intensity of selection and in other design features. In general, the relevant literature does not indicate consistent support for an association between starvation resistance and extended longevity in selection experiments (Harshman et al. 1999). The heterogeneity in outcomes suggests the limitations of selection experiments for understanding mechanisms underlying extended longevity when such experiments are conducted in isolation from other genetic approaches.

***Synopsis of results from laboratory selection experiments that extend longevity***

Six selection experiments that extended the life span of *D. melanogaster* are summarized here. The first two have already been described (Rose 1984; Luckinbill et al. 1984). The third and fourth artificial selection experiments were also conducted by selection for advanced age of reproduction (Partridge and Fowler 1992; Partridge et al. 1999). The fifth artificial selection experiment selected from families that exhibited relatively high mean longevity (Zwann et al. 1995). The sixth experiment was based on differential extrinsic mortality controlled by the investigators, not artificial selection for extended longevity (Stearns et al. 2000). Some lines in this experiment experienced high levels of extrinsic survival and others experienced relatively low levels. Flies from lines that experienced low extrinsic mortality became relatively long-lived.

As described previously, genetic correlations can provide insight into the mechanistic underpinnings of a response to selection. The results of the six selection experiments are summarized in table 1 in terms of factors that are genetically correlated (indirect responses to selection) with extended longevity. In some cases the correlated responses to selection were largely consistent. Five of six selection experiments documented that relatively low early-age egg production (EF) was associated with a longer life span. This is an example of a consistent response to selection; consistent indirect responses to similar selection experiments are arguably most suitable for continued study (Harshman and Hoffmann 2000a). A useful approach might be to use the robust genetic correlations to guide the search for underlying longevity factors (Gibbs 1999; Zera and Harshman 2001).

**Table 1.** Life span extension: Correlated responses to selection in six experiments

	SR	Lip	DR	Gly	OR	DT	JV	EF	LF	Met
R	>	>	>	>	>	>	>	<	>	0
L	0	0	0>	0>	>	>	<	<	>	0
P1	na	na	na	na	na	>	<	0	>	na
P2	na	na	na	na	na	0	0	<	0	na
Z	0	0	na	na	na	0	na	<	0	na
S	0	0	>	na	na	>	na	<	0	0

**Notes:** The six selection experiments are identified in the table using the following abbreviations: R = Rose (1984), L = Luckinbill et al. (1984), P1 = Partridge and Fowler (1992), P2 = Partridge et al. (1999), Z = Zwann et al. (1995), S = Stearns et al. (2000). Life-history traits typically have to do with development, growth, size, reproduction, and survival. The life-history traits in table 1 are: DT = development time, JV = juvenile viability (survival rate), EF = early-life female fecundity, and LF = late-life female fecundity. Table 1 also summarizes stress resistance traits: SR = starvation resistance, DR = desiccation resistance, OR = oxidative stress resistance. The metabolic traits in table 1 are: Lip = lipid abundance, Gly = glycogen abundance, Met = metabolic rate (respiration rate). The symbols in the text of the table indicate whether the selected lines exhibit a greater mean trait value (>) compared to the control lines, a relatively reduced mean trait value (<), marginally greater mean trait value than the control line value (0>), no statistically significant difference between selected and control lines (0), or data are not available for a particular experiment (na).

#### *Analysis of selection experiments for extended longevity: Studies of consistent correlated responses*

Stress resistance, primarily resistance to starvation and desiccation, was identified as a correlate of extended longevity in selected lines (Rose 1984; Service et al. 1985; Service 1987). However, selection for extended longevity did not result in significantly increased starvation resistance in three of the four longevity selection experiments in which a starvation assay was conducted (table 1). In one of these experiments, selection on the basis of differential adult mortality did not alter starvation resistance as an indirect response, but the selection response to selection did indicate tradeoffs between early fecundity (egg production), late fecundity, and starvation resistance that was mediated by lipid allocation (Gasser et al. 2000). Desiccation resistance appears to be more consistent as a correlated response to selection for longevity (table 1). Investigation of desiccation resistance in selection lines has revealed that the selected lines have more glycogen, store more water, and have a reduced rate of water loss, but there is no difference in water content at death in the selected and control lines (Bradley et al. 1999; Graves et al. 1992; Gibbs et al. 1997; Nghiem et al. 2000). Oxidative stress resistance in the selected lines might be particularly important for extended longevity. Flies from Luckinbill extended longevity lines (L in table 1) have higher levels of mRNA abundance corresponding to a range of antioxidant defense genes that produce enzymes including superoxide dismutase (SOD), glutathione S-transferase, catalase (CAT), and xanthine dehydrogenase as well as higher levels of CAT and SOD enzyme activity (Dudas and Arking 1995; Force et al. 1995). These results were corroborated by a reverse selection experiment (Arking et al. 2000a), which produced data indicating a time delay in oxidative damage of proteins and lipids in the extended longevity lines. Antioxidant enzymes (CAT and Cu-Zn SOD) are found on the left arm of the 3rd chromo-

some, and chromosome substitution studies showed that the proximal part of this chromosome arm is associated with extended longevity in the Luckinbill selected lines (Buck et al. 1993b). Arking et al. (2000a) used the Luckinbill et al. (1984) lines to reverse selection for longevity by taking young-age breeders for a series of generations. As a correlated response, relatively elevated measurements of antioxidant enzymes decreased in control line levels, but there was no significant decrease in 11 other enzymes that play a general role in metabolism not directly linked to antioxidant defense. Oxidative stress resistance is also present in the Rose lines, suggesting oxidative stress resistance might play a general role underlying increased life span in response to selection for extended longevity (Harshman and Haberer 2000).

Reduced early-age reproduction appears to be a consistent correlated response to selection for extended longevity (table 1). A delay in early-stage egg maturation was found to be responsible for reduced early-age reproduction in the Rose extended longevity lines (Carlson et al. 1998; Carlson and Harshman 1999). As has been described, selection experiments also indicate that increased stress resistance is a consistent correlated response to selection (table 1). Does this imply a negative relationship between reproduction and stress resistance? As an example, selection for cold resistance in *D. melanogaster* and *Drosophila simulans* was correlated with decreased early-age fecundity (Watson and Hoffmann 1996). In general, experiments that have selected for stress resistance have found that decreased early-age reproduction is a correlated response (reviewed in Zera and Harshman 2001). Support for this relationship also comes from phenotypic manipulation experiments. Stimulation of female egg production in the Rose lines resulted in decreased starvation resistance (Chippindale et al. 1996), and stimulation of egg production resulted in decreased oxidative stress resistance (Wang et al. 2001; Salmon et al. 2001). The loss of oxidative stress resistance as a function of reproduction might be particularly relevant because of the role that oxidative damage putatively plays in aging and longevity.

An extension of metabolic life is another consistent outcome of selection experiments for longevity. The rate of gas exchange by animals, the uptake of  $O_2$  and production of  $CO_2$  (respiration rate), is a measure of metabolic activity. Metabolism generates oxygen radicals that are thought to cause aging. Therefore, aging may be an inevitable byproduct of the metabolic functions of life. As a relevant observation, one or more of the mutations that extend the life span of the worm *Caenorhabditis elegans* reduce the metabolic rate (Van Voorhies and Ward 1999).

Is reduced respiration rate associated with extended longevity in *D. melanogaster* selection experiments? A series of respiration rate studies on one set of extended longevity lines is summarized in Rose and Bradley (1998). When fly respiration was measured in chambers smaller than the cages used for selection, Service (1987) found relatively higher rates at young ages in the control (unselected) lines than in the long-lived selected lines, but not at later ages. Djawdan et al. (1996) found no difference between selected and control lines when respiration was measured in cages used for selection. When adults from both lines were provided with supplementary yeast, the control-line females exhibited a slightly higher metabolic rate than selected-line females (Simmons and Bradley 1997). Djawdan et al. (1997) found no difference in respiration rate when the mass of selected and control flies was adjusted by removing the weight of water, lipid, and carbohydrate. Using the Luckin-

bill lines (L in table 1), Arking et al. (1988) demonstrated that selected longevity resulted in appreciably greater lifetime metabolic potential than measured in control lines. The study by Gasser et al. (2000), S in table 1, included a behavioral activity assay and a measurement of respiration rate; neither was reduced in the longer-lived lines. There is little evidence that extended longevity is genetically correlated with reduced metabolic rate. In selected lines of *D. melanogaster*, longevity does not appear to be a simple byproduct of reduced metabolism.

***Analysis of selection experiments for extended longevity: Demography***

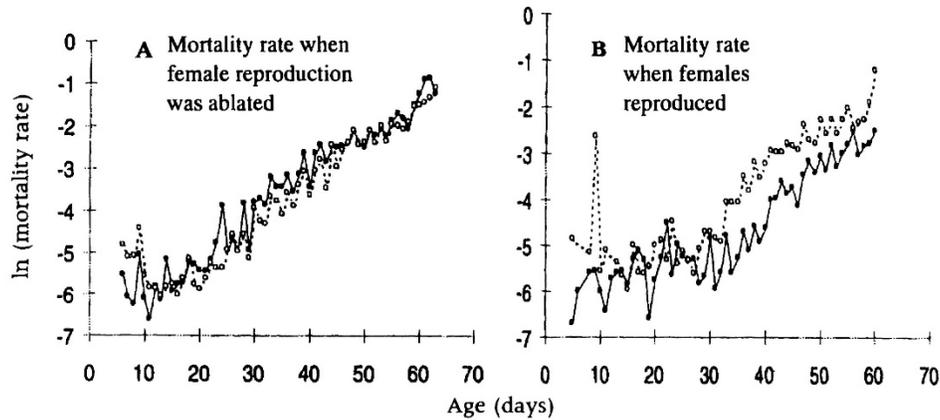
What is the age-specific pattern of mortality in lines selected for extended longevity? At issue is whether selection has reduced the rate of aging or reduced the initial mortality parameter. Demographic analyses have been conducted on the Rose and Luckinbill lines selected for extended longevity (Service et al. 1998; Pletcher et al. 2000). Service et al. (1998) used the Rose selected lines and found that the age-dependent mortality rate parameter was significantly smaller and the frailty parameter had a significantly smaller variance whereas the age-independent mortality parameter did not differ between selected and control lines. Thus, there was evidence that the Rose selected lines evolved a reduced rate of senescence (Nusbaum et al. 1996; Service et al. 1998). Pletcher et al. (2000) investigated the lines selected for extended longevity and found that differences in baseline mortality, compared to the age-dependent mortality parameter, accounted for most of the difference between selected and control lines. In terms of understanding the potential for life span extension, it is important to know whether selected life span extension is based on a delay in the aging process or on reduced age-specific mortality across a range of ages. One of the difficulties in answering this question arises from the demands of adequate sample size because mortality is uncommon among young flies and few flies remain alive at the oldest ages.

***Analysis of selection experiments for extended longevity: The cost of reproduction***

The cost of reproduction is a demographic relationship in which current reproduction is associated with a reduction in future reproduction and decreased longevity (Williams 1966; Bell and Koufopanou 1986; Reznick 1985; Stearns 1989, 1992; Partridge and Sibly 1991; Carey et al. 1998). This relationship has been documented in a wide variety of plants and animals (Roff 1992). The cost of reproduction is so widespread that it would be appropriate to consider it to be a general feature of life.

To investigate the cost of reproduction, experiments were conducted on lines selected for extended longevity (Sgro and Partridge 1999). The selected and control lines used were genetically differentiated in terms of early-age egg reproduction (lower in the selected lines) and longevity (greater in the selected lines). Sgro and Partridge abolished egg maturation in females from selected and control lines to determine whether this manipulation removed the survival-rate differences between selected and control lines. They ablated egg production by using irradiation or introducing a sterile mutation. Thus, the hypothesis was tested in parallel experiments, which helps circumvent potentially idiosyncratic results from any one experimental manipulation. When female reproduction was ablated, age-specific survival differences between selected and control lines disappeared (fig. 2).

With implications that extend beyond *D. melanogaster*, a delayed survival cost of reproduction could play an important role in defining lifetime survival curves (Carey et al. 1998; Sgro and Partridge 1999).



**Figure 2.** Age-specific female mortality of selected populations (closed circles) and control populations (open circles) of *D. melanogaster*

**Note:** When female reproduction was ablated by irradiation (or introduced mutation), the age-specific mortality differences between selected and control lines disappear. *Source:* Sgro and Partridge 1999

The study by Sgro and Partridge (1999) produced results indicating that one phenomenon could underlie both the acceleration of mortality (aging) and mortality deceleration at late ages. The leveling off of mortality at late ages was originally documented in medflies by Carey et al. (1992) and in *D. melanogaster* by Curtsinger et al. (1992). The causes of aging and of the deceleration of mortality at advanced ages are two of the most important problems in biodemography. The results of Sgro and Partridge suggest that reproduction could underlie aging and the deceleration of mortality at late ages. The generality of this study could be tested by repeating it with different lines and with sample sizes that allow finer resolution of age-specific mortality patterns, especially at ages when there were few surviving flies.

#### *Perspectives on selection experiments*

Genetic correlations are the sine qua non of laboratory selection experiments, but they are problematic. First, what appears to be a genetic correlation could be due to inadvertent independent selection on two traits (co-selection). Second, genetic correlations may be based on strong biological connections between traits or very indirect association. Thus, they may or may not provide useful evidence about mechanisms that underlie the response to selection. Third, indirect responses (genetic correlations) to selection for extended longevity are sometimes inconsistent between experiments (table 1; Tower 1996; Harshman and Hoffmann 2000a). This inconsistency could stem from a number of causes including inbreeding, inappropriate assay conditions, genetic differences between base

populations, variable strength of selection in different experiments, container effects, and multiple mechanisms underlying the selection response. Unfortunately, the causes of heterogeneity between experiments are extremely difficult to sort out. In the face of this vagary, it is best to focus on consistent indirect responses (robust genetic correlations among experiments) as the most important targets for further research to elucidate the mechanisms underlying responses to selection for longevity (Harshman and Hoffmann 2000a; Harshman and Haberer 2000).

Flies from the Rose longevity-selected lines have been found to exhibit no greater life span than flies collected from the field and then tested for longevity before numerous generations have passed (Promislow and Tatar 1998). This fact represents a major problem for selection experiments on longevity because selection for extended longevity in the laboratory may merely involve removal of deleterious mutant forms of genes that have accumulated during laboratory culture (Promislow and Tatar 1998). Alternatively, if populations adapt to the laboratory by evolving a reduction in life span, then laboratory selection for extended longevity is an informative reversal of this initial adaptation. Transfer from a natural population to the laboratory environment and subsequent culture of flies in bottles resulted in reduced stress resistance, higher early-age egg production, and a shortened life span (Sgro and Partridge 2000; Hoffmann et al. 2001), indicating that these traits are interrelated (Hoffmann et al. 2001). Overall, the evidence indicates that these changes are due to natural selection in the laboratory.

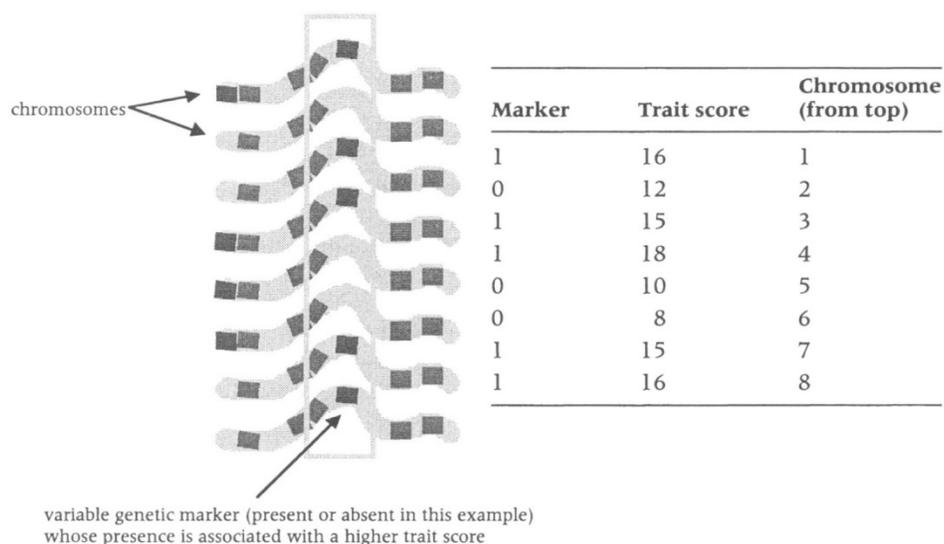
Selection experiments have been useful for identifying properties of organisms whose life span has been genetically extended. And they have proven useful for study of such phenomena as the cost of reproduction (Sgro and Partridge 1999) and oxidative stress resistance (Arking et al. 2000b). They have had limited utility, however, in elucidating specific mechanisms (genes, signaling pathways, cell biology, physiology) that directly cause life span extension. Quantitative genetics provides tools that could identify specific genes that cause extended longevity in selection experiments and other populations.

### **Quantitative genetics**

Quantitative traits are continuously distributed in a population, and multiple genes determine the distribution of these traits. For such genetically complex traits, statistical techniques have been developed to characterize genetic correlations and genetic variances, to estimate the number of major genes controlling the distribution of a trait, and to localize regions of chromosomes that control variation in the trait. The regions are known as quantitative trait loci (QTL). An important goal of QTL analysis is to identify the specific genes responsible for extended longevity in populations.

Using very small sequences and the polymerase chain reaction (PCR) of DNA it is possible to amplify regions of DNA from many locations in the genome of *D. melanogaster*. Curtsinger et al. 1998 used this technique (RAPD) to assess DNA variation at approximately 1,000 positions in the genome in relation to life span. Five genomic regions had a significant effect on the life span of males, females, or both sexes. The largest effect was found for a variant DNA region that does not make a protein product but was associated with reduced mortality at all ages.

A more common approach used to QTL map (localize in the genome) employs recombinant inbred lines (RILs). Briefly, lines from two parental lines are crossed and the hybrid offspring are crossed back to one of the parental lines through a series of generations of matings to siblings. As a consequence, regions of chromosomes are introduced from one parental line into an otherwise homogenous background of the second parental line. These introduced segments can cause variation in the trait of interest among the RILs. The location of the introduced segments can be identified by variable molecular markers distributed across the genome. Association of the trait of interest with specific genetic markers provides a way of mapping regions of chromosomes that contribute to population variation in a complex trait (fig. 3).



**Figure 3.** Quantitative trait loci (QTL) mapping by marker-trait association in recombinant inbred lines (RILs)

A series of QTL analyses of longevity have been conducted. One study, based on a set of RILs derived from laboratory lines, assayed the longevity of virgin males and females among the RIL lines (Nuzhdin et al. 1997; Leips and Mackay 2000; Pasyukova et al. 2000; Viera et al. 2000). Nuzhdin et al. identified 5 QTLs with major effects on longevity, but these QTLs affected life span in only one sex or the other. Viera et al. extended the longevity assays conducted on these recombinant inbred lines by determining mean life span at different temperatures and after heat shock. Seventeen QTLs were identified that increased life span, and they were largely specific to each environment and sex. There was evidence for opposing effects such that a QTL associated with increased life span in one environment tended to decrease life span in other environments.

A few studies have been conducted with outbred RILs. Using recombinant inbred lines crossed to an unrelated stock (outbred), Reiwitch and Nuzhdin (2002) found correspondence between female life span QTLs identified in the recombinant inbred lines and outbred

lines. This study measured longevity with males and females held in the same container, and in this case there were no sex differences in QTLs. In Leips and Mackay (2000), the recombinant inbred lines were crossed to the ancestral lines used to produce them. The investigators studied the effect of another environment, larval (juvenile) density, on adult longevity QTLs. The six significant QTLs identified by Leips and Mackay (2000) typically extended life span only at one density or in one sex. Interactions were common among the life span QTLs, but a combination of interacting QTLs did not necessarily result in exceptional life span. Importantly, the QTLs identified by Nuzhdin et al. (1997) and Veira et al. (2000) tended to correspond to the QTLs identified in the study using relatively outbred lines (Leips and Mackay 2000).

QTL analyses have been conducted on recombinant inbred lines derived from the Luckinbill et al. (1984) selection experiment for extended longevity. In these studies, three life span QTLs have been identified by assaying large numbers of males and females held together from each recombinant inbred line (Curtsinger, personal communication). One of the QTLs, on chromosome 3L, confers resistance to paraquat (oxidative stress), and it maps to a region of the genome that includes a gene that metabolizes oxygen radicals (Curtsinger et al. 1998). Unlike the results of Nuzhdin et al. (1997), these life span QTLs are not sex-specific in their effects. Thus, for the two cases in which males and females were held in the same container during the longevity assays, sex-specific QTLs were not found (Reitwisch and Nuzhdin 2002; Curtsinger, personal communication).

#### *Perspectives on quantitative genetic studies of life span*

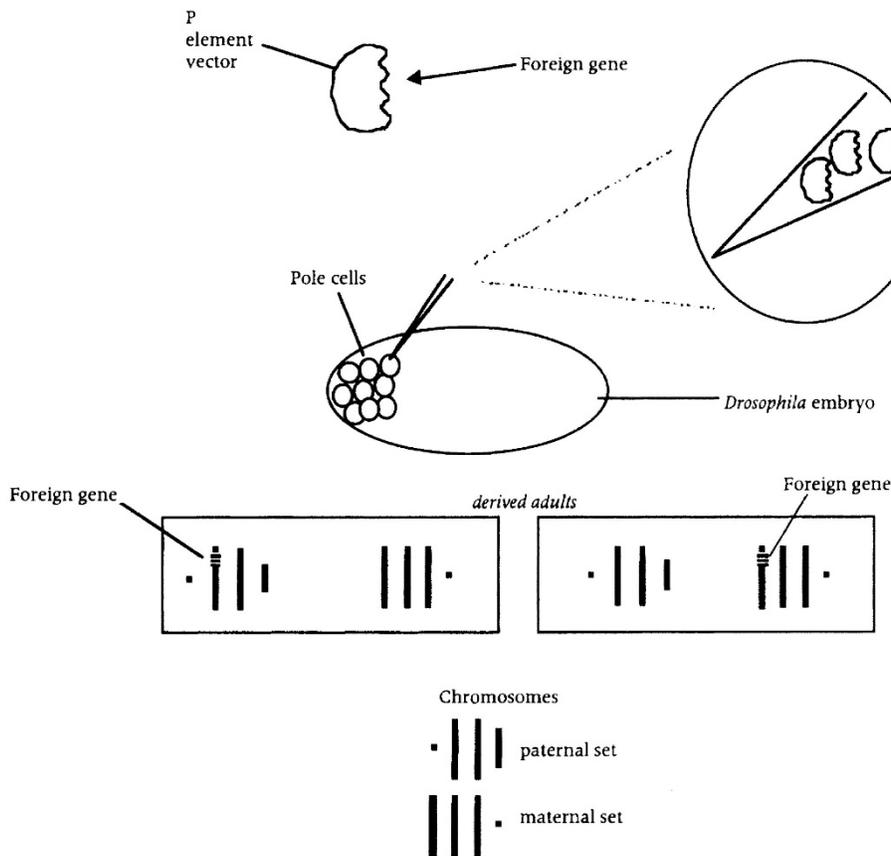
A criticism of recombinant inbred lines for the characterization of QTLs is that inbreeding exposes the effect of deleterious mutations that have accumulated in populations. However, the use of RILs is a legitimate first step in a process that can include outbred lines. Finding the genes that do extend life span in populations is an ultimate goal, and *D. melanogaster* is the best option for such studies among the organisms that are used as models for genetic research. The genetic toolbox available for *D. melanogaster* and genomics/bioinformatics methods will expedite identification of the genes that extend the life span of flies.

QTL analyses of extended life span in *D. melanogaster* selection experiments could be an especially informative avenue of research because metabolic rate apparently has not been reduced as a result of artificial selection for longevity. This implies that there are forms of genes that have the potential to extend life span without sacrificing normal metabolism and activity. In general, quantitative genetics has the potential to vastly improve the power to detect genes that cause extended longevity in *D. melanogaster* populations. Transgenic overexpression and mutation analysis have identified genes that can cause extended longevity in the laboratory.

#### **Transgenic overexpression of candidate genes**

Candidate genes are those that might have an anticipated effect on the trait of interest. Candidate genes in the present context are those that might extend longevity. Foreign genes can be introduced into the genome of *D. melanogaster* as candidate transgenes. The goal is to alter the level of specific gene expression for the purpose of investigating gene

function. Generally, transgenes are introduced into other genomes using modified transposable elements ("jumping genes") that can insert themselves into a recipient genome (fig. 4). For this purpose, a gene of interest can be combined with a modified DNA element (P element) that can integrate into a genome. A population of such recombinant molecules is injected into specific cells in developing embryos. These cells are destined to become the germ line that produces eggs and sperm and eventually adult flies. After recombinant P element vectors are injected into embryonic germ line cells (pole cells), insertion into random chromosome positions can occur because of co-injection with another vector that can make the enzyme (transposase) needed for recombinant vector integration into the genome. In short, it is possible to introduce foreign genes into the recipient genome, and the foreign genes can be transmitted from generation to generation with the rest of the chromosomal genetic material. Candidate genes that have extended life span by overexpression or by mutation are presented in table 2. In general, transgenes that extend life span are associated with stress resistance.



**Figure 4.** P element-mediated introduction of foreign genes

**Note:** A P element is a short segment of DNA that can allow an exogenous carrier molecule with a transgene (foreign gene) to insert at random locations in the genome.

**Table 2.** Genes that have extended the life span of *D. melanogaster* in the laboratory

Genes	Gene product	Possible life span extension effect
<b>Overexpression</b> (high level of gene activity)		
<i>SOD1</i> (CuZn) and <i>SOD2</i> (Mn)	superoxide dismutase (an enzyme)	oxidative stress resistance
<i>hsp70</i>	heat shock protein	high temperature tolerance
<i>DPOSH</i>	signaling protein	stress resistance pathway activation and/or resistance to programmed cell death
<b>Mutation</b> (partial or total loss of gene function)		
<i>mth</i>	G protein-coupled receptor (putative)	multiple stress resistance
<i>Indy</i>	dicarboxylate co-transporter (putative)	reduced intermediary metabolism
<i>InR</i> and <i>chico</i> <sup>1</sup>	insulin receptor and insulin receptor docking protein	altered control of metabolism and some stress resistance

Two enzymes, found in a wide variety of organisms, are known to have a mode of action that nullifies oxygen radicals. Superoxide dismutase (SOD) scavenges superoxide anions and produces hydrogen peroxide as a byproduct. Hydrogen peroxide, also an oxidizing agent, is broken down by catalase (CAT) into molecular water and oxygen. A transgenic extra copy of a *D. melanogaster* CAT gene resulted in increased enzyme activity, but no increase in longevity (Orr and Sohal 1992). Transgenic overexpression of a *SOD1* (CuZn SOD) gene resulted in increased resistance to oxidative stress agents and modestly increased life span in one study but not in another (Reveillaud et al. 1991; Orr and Sohal 1993). Simultaneous introduction of CAT and *SOD1* genes was observed to increase life span by approximately 20 percent (Orr and Sohal 1994). However, this result has been called into question because of inadequate controls for the experiment (Tower 1996).

One of the advantages of *D. melanogaster* transgenic methodology is that it is possible to overexpress introduced genes in specific tissues or at specific times during the life cycle. When human *SOD1* was overexpressed exclusively in adult motor neurons of *D. melanogaster*, life span was extended by 40 percent (Parkes et al. 1998). As an aside, mutations in the human *SOD1* gene are the basis of familial amyotrophic lateral sclerosis (commonly known as Lou Gehrig's disease), which is an inherited life-shortening human disease characterized by deterioration of motor neurons. Sun and Tower (1999) overexpressed *D. melanogaster* *SOD1* in adult *D. melanogaster*, extending life span by 48 percent. In this study, overexpression was not constrained to motor neurons. To the extent that the studies can be compared, the life span-extending effect of *SOD1* overexpression in the whole body (Sun and Tower 1999) was not much greater than that observed by Parkes et al. (1998). This comparison suggests that oxidative damage to motor neurons is a major cause of aging. The *DPOSH* gene, also associated with stress resistance, extends longevity by 14 percent when expressed in the nervous system throughout the life cycle (Seong et al. 2001: table 2). In general, cells that are not replaced by new cells in adults, such as neurons, are especially vulnerable to oxidative damage and may play an important role in aging. Mitochondria are the major source of oxygen radical production that may cause aging and limit life span (Wallace 1992). The gene for the manganese-dependent superoxide dismutase (*SOD2*) is

associated with mitochondria in higher organisms. *SOD2* has been stably introduced into the genome of *D. melanogaster* and overexpressed, thereby extending the life span of *D. melanogaster* adults by approximately 50 percent (Sun et al. 2002).

Oxidative damage, high temperature, and other factors can denature proteins, resulting in deleterious physiological and fitness effects (Feder and Hoffmann 1999). Heat shock proteins (HSP) and molecular chaperones can refold damaged proteins. Thus, investigators tested the hypothesis that a relatively high level of HSP70 production would increase life span (Khazaeli et al. 1997; Tatar et al. 1997). For one test, two P element transgenic strains of *D. melanogaster* were used (Tatar et al. 1997). One of the strains had extra copies of *hsp70* genes. The second strain was very similar in having a remnant P element present at the same location as the extra copy strain, but the extra copies of the *hsp70* genes had been excised. When adults were briefly exposed to high temperature, which stimulates activity of the transgenes, flies from the high copy *hsp70* strain exhibited relatively decreased mortality rates for two weeks and a 4–8 percent increase in life span. If a high level of this heat shock protein is beneficial, why wouldn't heat shock proteins be continuously expressed at high levels? The answer may be that high levels of HSP70 negatively affect other parts of the life cycle. For example, overexpression of *hsp70* can negatively affect larval (juvenile) flies by retarding growth, reducing viability, and reducing egg hatch (Krebs and Feder 1997, 1998; Silberman and Tatar 2000).

#### ***Perspectives on transgenic overexpression***

When transgenes are inserted into genomes, their effects can depend on the position of insertion or on differences between genomes into which they are inserted. Overexpression studies have varied in the extent to which they have controlled for such effects to rule out the possibility of artifacts (Kaiser et al. 1997; Stearns and Partridge 2001). The studies by Sun and Tower (1999) and Sun et al. (2002) are exemplary in their controls for position and genetic background effects and their use of a sufficient number of replicate lines.

#### **Mutation analysis**

Mutation analysis is a preeminent analytical tool in contemporary biology. In general, new mutations are generated and screened to identify genes that affect a biological trait of interest. The goal is to identify the underlying genes controlling trait manifestation and the role of these genes in the process. Mutations in *D. melanogaster* have identified genes that can increase longevity. In all cases, transposable DNA (P elements) was used to induce the mutations (fig. 5). When a P element moves and reinserts itself elsewhere in the genome it can cause a mutation. As opposed to transgenic overexpression, all of the mutations described in this section reduce the expression of, or totally inactivate, specific genes (such as those included in table 2).

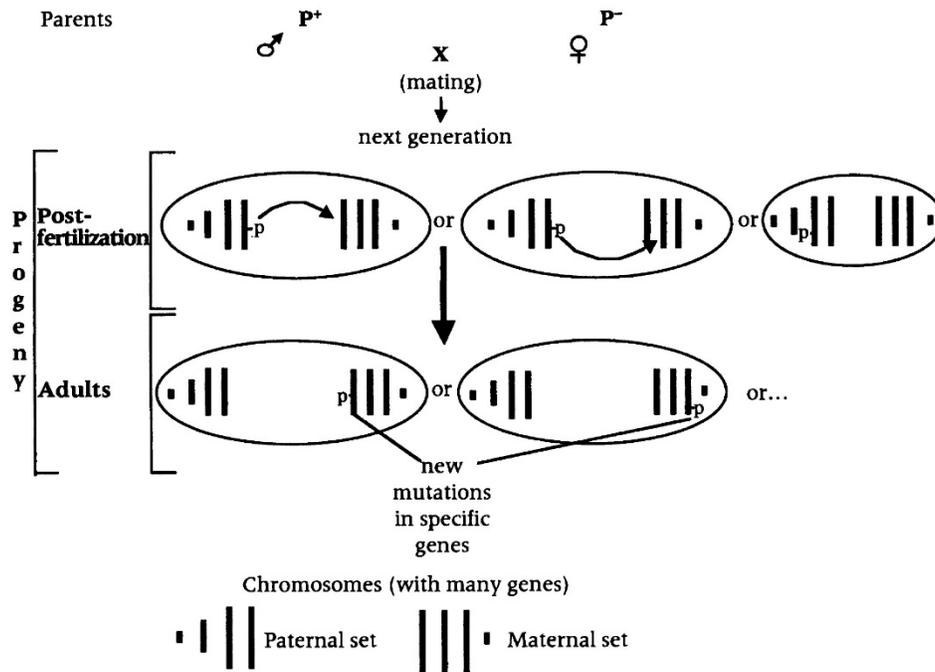


Figure 5. Transposon insertional mutagenesis

**Note:** A P element transposon is a short segment of DNA that can move within the genome and cause mutations. If a male with one or more P elements is mated to a female with none, then P elements can move to new locations in the chromosomes of the progeny, sometimes causing mutation.

The first *D. melanogaster* longevity-extension mutation was in the *Methuselah* (*mth*) gene (Lin et al. 1998). The *mth* mutation results in partial loss of function of the gene and extends longevity by 35 percent. The *mth* gene produces a protein, presumably a G protein-coupled receptor, that belongs to a family of proteins associated with a range of functions in higher organisms including endocrinology, neurology, and response to external stimuli. The putative G protein product of the *mth* gene is not similar to any of the G proteins of known function in other organisms, and thus the specific function of the *mth* gene is not suggested by comparison with other organisms. There is evidence that the *mth* gene plays a role in regulation of neuromuscular neurotransmitter function (Song et al. 2001). Again, neuromuscular function might be especially important for aging and longevity. The *mth* mutation resulted in increased stress resistance (Lin et al. 1998), and the mutant flies were approximately one-third larger than controls. Mutant flies were substantially more resistant to starvation, high temperature, and oxidative stress.

Mutations that markedly extend life span were also found in the *Indy* gene (Rogina et al. 2000). *Indy* mutations can result in a 50 percent increase in maximum longevity and approximately a twofold increase in mean life span. Five independent mutations in the same gene were reported, each of which exhibited a substantial increase in longevity in

combination with a normal gene (as a heterozygote). Moreover, the heterozygote females, derived from crosses between a laboratory stock (Canton S) and mutant stocks, produced substantially more eggs than Canton S females (Rogina et al. 2000), but the heterozygotes derived from crosses to a more relevant control strain had only slightly greater fecundity than the control strain (Helfand, personal communication).

The *Indy* gene product is a protein similar to mammalian dicarboxylate co-transporters. Mammalian co-transporters are membrane proteins that transport intermediates of energy compound metabolism (Krebs cycle intermediates) into cells. The *Indy* gene is expressed at high levels in the gut, fat body, and oenocytes, which are the insect tissues/cells that play a predominant role in intermediary metabolism and storage of metabolic products. A decrease in metabolic product uptake mediated by the *Indy* mutation suggests that the mechanism of life span extension could be caloric restriction (Rogina et al. 2000), which is an environmental intervention that can increase life span in invertebrates and mammals.

For the purposes of experimental rigor, *Indy* mutations were crossed to different stocks to check longevity of the heterozygotes compared to longevity of flies from the homozygote (double mutation) stocks (Rogina et al. 2000). The heterozygotes were substantially longer lived in all but one case. When *Indy* was crossed to flies from Luckinbill selected lines (L in table 1) there was only a 15 percent increase in longevity of the heterozygote compared to the selected line. This result suggests the intriguing possibility that the response to artificial selection for longevity includes the "Indy mechanism" for life span extension (Rogina et al. 2000).

Mutations in the insulin signaling pathway can also extend the life span of *D. melanogaster* (Tatar et al. 2001; Clancy et al. 2001). These results follow the pioneering work using *Caenorhabditis elegans* in which mutations selected for extended life span were found in genes that encode interacting proteins (signaling pathway) that mediate the effects of insulin (Friedman and Johnson 1988; Kenyon et al. 1993; Dorman et al. 1995; Morris et al. 1996; Kimura et al. 1997; Paradis et al. 1998; Gill et al. 1999). The discovery of mutations in *D. melanogaster* that extend life span by reducing activity of the insulin signaling pathway suggests that a general mechanism underlies differential aging and extended longevity in animals.

*D. melanogaster* insulin signaling mutations that extend life span were associated with the *InR* gene that encodes the insulin receptor (Tatar et al. 2001). Increased female longevity resulted only from a specific combination of *InR* mutations. Another longevity mutation was in the *chico* gene that encodes an insulin receptor substrate protein (Clancy et al. 2001). The insulin signaling pathway plays an important role in controlling growth; loss of function associated with the *InR* and *chico* mutations results in dwarf adult flies in addition to extended longevity. *chico* has fewer and smaller cells, resulting in a body size that is 50 percent of normal (Bohni et al. 1999). *chico* mutants are known to have a relatively high proportion of lipid (Bohni et al. 1999), and perhaps correspondingly the *chico* mutant flies were found to be starvation resistant (Clancy et al. 2001). Similarly, Tatar et al. (2001) found that higher levels of energy storage fat (triglyceride lipid) were found than in a comparable fly stock. Small size, stress resistance, and high levels of lipids are to some degree associated with extended longevity of mutant *Caenorhabditis*, *Drosophila*, and mice (Clancy et al. 2001; Tatar et al. 2001).

Tatar et al. (2001) found that *InR* mutations conferred as much as an 85 percent extension of longevity in females and reduced late-age mortality rates in males. There was no decrease in metabolic rate of the mutant flies as measured by mass-specific oxygen consumption in a mixed sample of males and females. Tatar et al. found that the *InR* mutations have approximately 25 percent of normal juvenile hormone biosynthesis and interpreted this deficiency as a basis for increased longevity. Exogenous administration of a juvenile hormone mimic stimulated reproductive activity in *InR* mutation females and reduced life span of the mutant toward that of the wild type control. Tatar et al. argued that the longevity associated with the *InR* mutations was actually due to a deficiency in this class of insect hormone.

Clancy et al. (2001) tested mutations in various genes in the insulin signaling pathway, including the insulin receptor gene, and found that all but one was associated with normal or decreased longevity. Only the *chico*<sup>1</sup> mutation was found to increase male and female longevity, by up to 48 percent when the mutant was present in two copies (homozygote) and 36 percent when the mutant was combined with a normal gene (heterozygote). Resistance to elevated temperature, oxidative stress, and starvation was tested using the *chico* mutation and related normal flies. Starvation resistance was observed for heterozygotes and homozygote mutants, oxidative stress resistance was observed only for heterozygotes, and no resistance to elevated temperature was associated with the mutation. The effect of *chico* on stress resistance was not as consistent as for the long-lived mutations that affect the insulin signaling pathway of *C. elegans*. For *chico*, the homozygotes were half the size, but the moderately long-lived heterozygotes were of normal size (Clancy et al. 2001). Consequently, body size and longevity appear to be at least partially independently determined. Heterozygote *chico* females have reduced fecundity and the homozygote was sterile. Thus, the question arose whether enhanced longevity associated with *chico* was a by-product of reduced reproduction (i.e., a reduced cost of reproduction). Clancy et al. used the *ovo*<sup>D</sup> mutation of normal flies that were comparable to *chico* to test the hypothesis that sterility was the underlying cause of extended longevity of *chico* females. *chico* females were found to live a substantially longer time than sterile females that were otherwise normal. These results indicate that sterility and defective insulin signaling can extend life span by different mechanisms in *D. melanogaster*. However, this result does not exclude the possibility of an interaction between the reproductive system, insulin signaling, and longevity as has been observed in *C. elegans* (Hsin and Kenyon 1999; Lin et al. 2001).

#### ***Perspectives on mutation analysis***

Mutation analysis is a form of site-localized genome perturbation performed to find genes that can extend longevity. Unlike transgenic overexpression, the outcome is not constrained by a priori expectations about which genes could produce the effect. Given this lack of constraint, it is interesting that long-lived mutants have reinforced the outcome of selection experiments in identifying stress resistance as a common component of longevity. In the future, mutation analysis should be able to identify a range of genes that confer longevity in *D. melanogaster*. The potential for synergist studies using long-lived mutants and populations (lines selected for longevity) is indicated by the use of the *Indy* mutation and the Luckinbill selected lines (Rogina et al. 2000).

## Gene expression

A central tenet of biology is that genes produce messenger RNA (mRNA), which in turn is used as a message to make proteins. Gene activity (expression) can be measured by the amount of message (mRNA) produced by specific genes or by reporter genes. Helfand et al. (1995) and Rogina and Helfand (1995) monitored gene expression during aging in *D. melanogaster* using a series of transgenic lines that differed in the chromosomal location of P element reporter gene insertions in the genome. The reporter genes indicated the level of activity of nearby genes. Age-dependent differential gene expression was documented and related to longevity differences among lines.

Another approach has been to monitor the production of mRNA from specific genes as a measurement of age-dependent gene expression. For example, the abundance of mRNA corresponding to heat shock proteins has been quantified as *D. melanogaster* ages. The abundance of heat shock protein 70 (HSP70) mRNA does not increase substantially during aging (Wheeler et al. 1995), but mRNA corresponding to two smaller heat shock proteins (HSP22 and HSP23) increases markedly as a function of age (King and Tower 1999). Moreover, the level of *hsp22* gene mRNA was relatively high in the Rose lines selected for extended longevity (Kurapati et al. 2000). However, overexpression of *hsp22* results in decreased longevity without affecting the level of message production by *hsp70* (Tower, personal communication). Some genes that are expressed at high levels in relatively old individuals may simply represent loss of control of proper expression as a function of age (Tower 1996; Guarente and Kenyon 2000). Alternatively, genes that are highly expressed in aging flies might provide a protective function that could be confirmed by life span extension in transgenic overexpression studies (Tower 1996, 2000).

Technology allows for simultaneous measurement of the relative amount of mRNA produced by almost all of the genes in the genome of *D. melanogaster*. In one study, gene expression was found to be much more strongly affected by sex than by age (Jin et al. 2001). In another study, the investigators measured expression of approximately 8,000 genes as a function of age and oxidative stress (Zou et al. 2000). A total of 127 genes changed expression appreciably during aging, and a third of these genes also responded to oxidative stress. This pattern of co-expression indicates a set of candidate genes that could contribute to longevity. Caloric restriction can extend the life span of *D. melanogaster*. Dietary conditions that extend life span reduce the expression of genes associated with growth, metabolism, reproduction, and stress resistance (Pletcher et al. 2002). In this study (Pletcher et al. 2002), almost a quarter of the genes changed in expression during the process of aging.

### *Perspectives on gene expression studies*

Even at this early stage in application of genome-wide gene expression technology (microarrays), studies have identified many changes in age-related gene expression potentially relevant to extended longevity. Among the questions being explored are whether a few key genes control the expression of many other genes to extend longevity, which genes are suitable candidates for functional tests by transgene overexpression or techniques designed to suppress gene activity, and whether these genes will elucidate the relationship between delayed early-age reproduction, stress resistance, and longevity in populations.

## Future approaches

A theme of this review has been that the diversity of genetic approaches used to study *D. melanogaster* is generating considerable insight into life span extension. Continuing this approach, powerful genetic technologies (mutation analysis, transgenes, genomics) combined with a rich population context (quantitative genetics, laboratory selection experiments, natural population studies such as Mitrovski and Hoffmann 2001) will be the basis for advances in understanding how genes control longevity in populations. Given that overall fitness is requisite for individuals in outbreeding populations, the insight derived from studies on natural populations of flies may be particularly applicable to the design of interventions to extend the span of active and healthy human life.

**Acknowledgments** – Marta Wayne kindly provided figure 3, and Ryan Bailer made major contributions to figures 4 and 5. Discussions with Sergey Nuzhdin, Linda Partridge, Daniel Promislow, and John Tower were valuable for this review. James Curtsinger, Steven Helfand, and John Tower kindly provided information from unpublished studies. Support for this review was provided by the National Institute on Aging (AG08761).

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