ABSTRACT

MAGWIRE, MICHAEL MAHLON. Mutations Increasing *Drosophila melanogaster* Life Span. (Under the direction of Trudy F. C. Mackay.)

Better living conditions and advances in medicine have made it possible for humans to live considerably longer than before. This has uncovered many health problems associated with age. By identifying genes involved in the limitation of life span, we may better understand the processes that lead to aging. In model organisms, we can use mutagenesis to discover mutations that increase life span, and hence infer that the wild-type allele limits life span by some means.

We have assessed longevity in a collection of 1332 co-isogenic gene-trap *P*-element insertion lines and determined changes in life span of each insert line relative to the corresponding control line. Significant lines were determined via two different methods of analysis: A 95% confidence interval was established based on deviations of the mutant life spans from the controls and Dunnett's two tailed t-tests were used to examine differences between mutant and control in each individual block. Based on the 95% confidence interval, 139 inserts displayed an increase in life span and 194 inserts a decrease. Using Dunnett's, 70 lines increased in life span, while 270 decreased in life span. An additional 48 increased longevity lines were close enough to meeting Dunnett's criteria to be considered for additional investigation. Combining these two analyses, we chose 83 inserts associated with increased life span for a secondary screen using an additional twelve replicates. 58 of these lines remained significant. We have determined the *P*-element insertion site for 50 of these lines.

Starvation resistance, chill coma recovery time and climbing activity were measured on the lines remaining significant after the the secondary screen to identify pleiotropic effects. A positive correlation was found for males between life span and starvation resistance as well as between life span and chill coma recovery. Females only displayed a correlation between life span and chill coma recovery, which was negative. None of the lines indicated increased fitness for

all phenotypes, indicating there may be some type of trade-off. There also appears to be a lot of pleiotropic variation depending on background and sex.

Ten of the lines, all with the same parental background, were chosen for a half-diallel cross to identify epistasis between the mutants. There were substantial epistatic interactions between all ten lines. Furthermore, males and females displayed vastly different patterns of epistasis, again indicating major differences in life span regulation between the sexes.

Finally, a subset of seven of the lines used in the epistatic study, in addition to the corresponding parental control, were chosen for microarray analysis to look for possible pathways and novel genes involved in increasing life span. 1,996 probe sets were significant at a false discovery rate q-value threshold of $q \leq 0.0001$. Tukeys tests were carried out for these probe sets to determine how each of the seven mutant backgrounds differed compared to the control and each other. In addition, each mutant background was compared individually with the control to identify any changes in expression. Gene ontologies were determined for each of the lines to identify over-represented biological functions which would indicate possible longevity pathways. Dozens of pathways were suggested, including several novel pathways.

MUTATIONS INCREASING *DROSOPHILA MELANOGASTER* LIFE SPAN

by
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A dissertation to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

GENETICS

Raleigh, North Carolina

2007

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DEDICATION

For those that I love. I hope you know who you are.

BIOGRAPHY

The 5th of May, 1973 was a day that I have difficulties remembering, however, my parents apparently know it quite well. Oddly, my first day out in the world began early in a suburb of an incredibly large, windy city. We moved to subsequently smaller cities until I ended up a city boy in the small rural community of Syracuse, Nebraska. While I spent most my days in Nebraska, I did my growing up on a lake in northern Minnesota. After spending most of my college life studying mathematics and space physics I had an epiphany and realized genetics was my true calling. I was lucky enough to end up in the lab of Dr. Lawrence Harshman working on stressing fruit flies. After two years of being cruel to the flies in the interest of humanity, I decided to make a career of it and headed east to North Carolina State University. Fortunately, I was able to work in the lab of Dr. Trudy Mackay where instead of being cruel to the flies, I let them live long, healthy lives (never minding the forced abstinence). After seven years, many new friends and enjoyable experiences, a new family member and a whole lot of work, work, work, I managed to get to where I am now. My next step will be a temporary move to the lovely city of Edinburgh, Scotland where I hope to make some more friends and enjoyable memories while doing some more satisfying work.

ACKNOWLEDGEMENTS

The long process of completing a PhD is in no way an individual effort. Many, many people have assisted and supported me along the way. Without their help and guidance I would never have made it.

Firstly, I would like to thank Trudy Mackay. Without her assistance, expertise and understanding I would have never been close to finishing a PhD. It has been an honor to work with someone not only prestigious, but a genuinely good person as well.

I am grateful for my committee, both past and present members (Robert Anholt, Greg Gibson, Michael Purugganan and Bruce Weir) for their words of wisdom and guidance over the past seven years. An extra thanks goes out to Robert, who far exceeded the normal role of a committee member.

The path to get here has been heavily influenced by teachers at all levels of education. In particular, I would like to thank:

Larry Harshman who made genetics even more fun as well as giving me the opportunity to work in his lab and to teach. He is one of the primary reasons I continued my education in the pursuit of a PhD.
Kay Miller who introduced me to the beauty of math and the joy of teaching others.
Ed Storey who made science more fun than I ever imagined.
Donald Taylor who was the most entertaining teacher that I have ever encountered.
Jim Mahaffey for much appreciated molecular advice and assistance.

The administrative staff have the difficult task of keeping the department running smoothly. I would like to thank all of them for their help over the years. I would have been lost without Julie and Stephanie. Sometimes I think Jeff Hawley single-handedly keeps Gardner standing.

The faculty, post-docs and technicians at State and especially in the Genetics department are top notch and I am thankful for the privilege of working with them.

It was a great pleasure interacting with the Genetics graduate students during my time at NC State. The early years with Arnar, Brad & Amy, Buck, Jason, Chris, Gretchen and the rest of the crew were a blast, especially after they re-introduced me to alcohol. The ticket campouts and chili parties were some of the most memorable evening I have had. The semi-ex-Chapel Hill crowd (Muk and Shuford) always made sports events more entertaining. Lisa and Ben are two of the kindest, most thoughtful people you could ever meet. I will miss my time at East Village and Sammy's with Eric, Ed, Jason, Ian and the rest. I will especially miss those involved in flag football, soccer and softball.

The members of the Mackay and Anholt labs will be in my heart always. Mere words cannot begin to describe my appreciation for many of them. From the undergraduates who did all the essential behind-the-scenes work, the graduate students who shared the joys and pains of grad-school, the post-docs who were always there with advice, leadership and knowledge and the technical staff and research associates that accomplished more than I thought possible. I would like to thank:

The life span group who were responsible for providing me with the occasional weekend rests and vacations, especially those that helped during my many injuries. Paul, who I swear was involved in half the experiments in both the Mackay and Anholt labs. Laura who not only is an incredibly brilliant and kind individual, but one of the few people with a sense of humor as strange as mine. Gretchen, with whom I once attended a movie that I cannot mention. And Rhonda, who was a shoulder for the entire lab, like a mother to some, a teacher, a listener, a genius, an entertainer and most of all a friend.

The post-docs and research associates. Jeff who got me involved in soccer and guided me the first few months. Juan Jose, my southern friend, for all your help. Aki, the BG keeper, especially for Christmas. Stefanie and Christy, who helped bring out my sense of humor. Richard and Ted for all the computer and statistical assistance. Mark, Harland and Charlene for all the helpful advice. Faye, who making sure I always had enough food (which was no easy task). Gunjan, I'm pretty sure I'll be out of town next time you need to move, seriously, for all the smiles. My favorite new American, Donghui, for all the conversations that brought a smile to my face. Tatiana, for keeping an eye on Dixie and for always willing to help. Mare, one of the kindest people on the planet, who was always there for me. And Stephanie Rollmann, the molecular goddess, for the dog walks, stories, advice and the countless other things you have done.

All the undergraduates and pre-graduates that did all the little things that so often get taken for granted. Especially Stacy for all the lunches and your friendship. And Grace, for being like a sister…you will be missed.

The rest of the Mackay and Anholt graduate students, my comrades in science: Susan, Amanda, Deepa, Ping, Alexis and Reba. All of whom made the journey easier. And Katie, for the Farm, chopping down trees, hot-wings and alcohol, the dogs and all the memories.

I would like to thank all of my family and friends for supporting me all these years in school. Especially Val, who has had to put up with an older brother like me her entire life. Jessica, who brightens each and every day and make me proud to be an uncle. And Ryan for being like a brother.

Dixie, for being there day-in and day-out no matter what. For bringing order to my life when it seemed out of control and chaos when it was too routine. For reminding me that no matter how intelligent I think I am, even a dog cannot outsmart me. For bringing joy not only to me, but to all those around you as well.

Jerry, for being a second father to me and for making Mom so happy. The world lost a great man when you left us.

My grandparents for being role-models and giving me proof that true-love is possible.

ShanShan for making me truly happy among all the other things you have done.

And finally, my parents, who are responsible for molding me into the man I am today. A person could not ask for a better Mother and Father. Thank you!

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CHAPTER 1

Review on the Mechanisms of Aging

Introduction

Longevity has historically been a major interest of humanity. Better living conditions, advanced medical practices and improved technology have resulted in a large increase in the mean expected life span of humans over the last few centuries and this trend is expected to continue in the future. This increase in life span has uncovered many new problems in terms of ailments associated with an aging individual such as Alzheimer's disease, cancer, cardiovascular disease and Huntington's disease. To solve these problems, it is vital to know the genes and mechanisms regulating aging. Furthermore, it is necessary to understand aging from an evolutionary standpoint. Why do we age? Why is there so much variation in aging between different species as well as within the same species? Fortunately, science offers a multitude of methods useful in attacking these problems and significant progress is being made to answer these questions.

The initial difficulty in the quest to understand aging is defining what is meant by the term "aging". Typically when we refer to aging we really mean senescence, which is essentially the summation of all of the intrinsic, detrimental changes that lead to an increase in mortality. Typical changes associated with senescence include increased sensitivity to stress, shortening of the telomeres, accumulation of somatic mutations and an inability of cells to divide.

Perhaps the greatest reason that the answer to the aging dilemma has remained so elusive is the fact that there is not just one mechanism underlying aging, but many. Stresses such as oxidative damage and starvation lead to senescence. Metabolic processes have long been thought to result in aging. Telomere shortening has often been associated with aging. Faulty DNA repair mechanisms, chromatin silencing and growth hormones have all been implicated in the aging process. New mechanisms associated with aging continue to be discovered.

Evolution of Aging

Several studies have shown that life span has a low heritability, indicating that there is indeed some genetic influence on life span. Twin studies have shown that

this is true even among humans (McGue *et al*., 1993). Currently three theories have primarily been tested to explain aging: mutation accumulation, antagonistic pleiotropy and disposable soma. Medawar (1952) first proposed that selection at later ages is much weaker than selection at earlier ages. This means that mutations that occur late in an organism's life will be weakly selected against; hence, late-onset mutations will accumulate leading to aging. Williams (1957) observed that selection favors mutations that have beneficial effects early in life even if they have harmful effects later. This selection for genes with positive early effects and negative later effects is known as the antagonistic pleiotropy theory of evolution. The disposable soma theory is a special case of antagonistic pleiotropy. Kirkwood (1977) suggests that there is a balance of resources between reproduction and somatic maintenance. The fact that few animals survive to an old age in the wild would indicate that resources are more likely to be spent on reproduction than somatic maintenance, which leads to aging. These theories are by no means mutually exclusive, and are in fact all based on the decline in selective pressure with age.

Model Organisms

Model organisms play a very important role in studies of genetic factors affecting life span and mechanisms of aging. Though most studies are done with the benefits to humans in mind, ethical and logistical concerns preclude studies in humans. Hence, scientists must turn to other organisms. *Saccharomyces cerevisiae* is useful as a simple eukaryote. Several conserved genes with a large effect on life span were first found in *S. cerevisiae*, especially those thought to be involved in dietary restriction such as *Sir2* (Kaeberlein, *et al*. 1999), *Sch9* (Fabrizio *et al*., 2001), and *Ras2* (Longo, 2003). Unfortunately the simplicity of yeast is also a drawback of using it as a model system. It becomes difficult to compare many of the possible mechanisms of aging in a single cell to that of an organism composed of many, many different types of cells interacting together.

To better investigate mechanisms underlying aging, we need to work with a more complex system. *Caenorhabditis elegans* and *Drosophila melanogaster* are

the next rungs up the model organism complexity ladder. Though they are not nearly as complex as humans, they do have much more in common with them than yeast. With less than 1000 adult cells, it is fairly easy to explore communication between cells and processes such as apoptosis which may affect life span in *C. elegans.* Nematodes have been major players in the investigation of many possible mechanisms of aging, particularly with respect to the insulin/IGF signaling (IIS) pathway. *age-1* (58), *daf-2* (66) and *daf-16* (Larsen *et al*., 1994) are all major genes in the IIS pathway that were first found in *C. elegans*. One of the most interesting things regarding nematodes is their ability to go into a dormant "dauer" stage (during their second larval stage) that is initiated via external stimuli such as low nutrient availability and larval crowding (Cassada and Russell, 1975; Swanson and Riddle, 1981; Albert *et al*., 1981). Many of the genes involved in dauer formation are also involved in life span extension in adults (Kenyon *et al*., 1993).

D. melanogaster has been a very useful model system over the last century. Although they are quite different in appearance to humans, they have a very large number of genes orthologous to human disease genes (Chien *et al*., 2002). With thousands of mutants available and the genomes of several species of Drosophila sequenced, they are even more useful today. This is especially true in the search for a better understanding of longevity and aging related diseases. Many novel life span genes have been identified using *D. melanogaster*, including *chico* (Clancy *et al*., 2001) and *methuselah* (Lin *et al*., 1998).

Rodents have traditionally been used to model human systems. This is primarily because they are a mammalian system with a short generation time and they are relatively easy to maintain while still being very similar in many respects to humans. The ability to make recombinant inbred strains makes them even more desirable for QTL mapping. In addition, mouse knockouts have proven to be an invaluable genetic tool. Mice have been used extensively to investigate a plethora of processes associated with aging such as dietary restriction (Michan and Sinclair, 2007; Weindruch *et al*., 2001), insulin/IGF signaling (Brown-Borg *et al*., 1996; Bluher *et al*., 2003), stress resistance (Murakami, 2006) and oxidative stress (Migliaccio *et al*., 1999). They have also been widely used in microarray studies to identify

possible genes involved in the mechanisms underlying dietary restriction (Lee *et al*., 1999; Cao *et al*., 2001). Rats were the first rodents to have been used in dietary restriction experiments (McCay *et al*., 1935) and have been examined more recently (Merry and Holehan, 1981; Donati *et al*., 2001), but have recently been surpassed by mice with regard to aging research. Both mice and rats have been used to identify biomarkers of aging that may be used as predictors for possible life span extension in humans (Weindruch and Walford, 1988; Goto *et al*., 2007).

Primates are probably the next best thing to actually studying humans. Though they have fairly long life spans, they are relatively short when compared to humans, which makes them more feasible for longevity studies. They are physiologically very similar to humans, which makes them all the more enticing as a model system. Rhesus monkeys are currently being used to observe the effects of dietary restriction on life span (Ingram *et al*., 1990; Kemnitz *et al*., 1993). The Ingram study was started in 1987 and will be continuing for quite some time (the maximum life span of rhesus monkeys is about 40 years (Ingram *et al*., 2006)). Thus far the study has shown that two common biomarkers of increased longevity via dietary restriction in rodents (reduced body temperature and plasma insulin) also hold for dietary restricted rhesus monkeys (Roth *et al*., 2002). The Kemnitz study is looking at dietary restriction in adult rhesus monkeys. They have similarly found reduced plasma insulin levels in the dietary restricted monkeys (Ramsey *et al*., 2000).

Other organisms are constantly joining current model systems in the study of longevity. Recently the naked mole-rat, the longest known living rodent, was proposed as a possible model system for investigating aging (Buffenstein, 2005). Naked mole-rats weigh only about 35 grams, yet live for more than 28 years (Buffenstein, 2005). They may make a good system for studying human aging because they are one of the few creatures that exhibit a longevity quotient (the ratio of observed maximum species life span to that predicted via the body mass) similar to humans (Buffenstein, 2005). Canines may also turn out to be a good model system for aging research. It has been established that there is a negative correlation between breed size and life span (Greer *et al*., 2007). A recent study

from Sutter *et al*. (2007) has identified an allelic variant of *IGF1* that may be a major determinant of size in dogs. Insulin-like growth factor (IGF) signaling plays a role in the extension of life span in other organisms (Kenyon *et al*., 1993; Tatar *et al*., 2001; Brown-Borg *et al*., 1996). Taken together, these studies help to explain the relationship between dog size and life span. *D. melanogaster* may no longer hold the monopoly on aging studies in flies. Ballard (2005) has suggested that *Drosophila simulans* be used to look at mitochondrial metabolism and aging because of their high diversity of mitochondrial DNA. Even fish are getting in on the act in life span research. Herrera and Jagadeeswaran (2004) have begun using zebrafish and two species of annual fish to understand aging mechanisms. They hope that fish will fill some of the gaps between the invertebrate systems and mice. *Pinus longaeva* (the bristlecone pine), the eukaryote with the longest known life span (Schulman, 1958) has even been used to investigate the possible role of telomere length on life span (Flanary and Kletetschka, 2005). These are just a few of the examples of the many species being used to help gain a better understanding of the mechanisms underlying human longevity.

Techniques

Several techniques are being utilized to discover the mechanisms regulating the aging process as well as the genes responsible for the underlying variation in life span. Mutagenesis exposes genes that affect aging, which in turn leads to the mechanism(s) where those genes play a role. Genome-wide changes in transcription levels with age can point to genes and pathways associated with aging. Finally, QTL mapping can be used to find genomic regions, and ultimately genes, contributing to variation.

Mutagenesis. One of the classic methods of identifying genes affecting any phenotype is the mutagenesis screen. These methods have proven to be quite fruitful for identifying genes and pathways associated with life span. Previously, methods such as ethyl methanesulfonate (EMS) and radiation were commonly employed to generate mutations. EMS techniques have been used to identify

several aging genes in *Caenorhabditis elegans* (Malone and Thomas, 1994; Yang and Wilson, 2000). Unfortunately techniques such as these often create many mutations that are difficult to identify. Recently transposable elements have pushed their way to the forefront of mutagenesis, particularly in *Drosophila* genetics. The use of *P*-elements to both disrupt normal gene function and induce over-expression has become quite common. Longevity studies are no exception (Clark and Guadaloupe, 1995; Seroude *et al*., 2002). Within the last five years several mutations have been discovered using *P*-element mutagenesis techniques including what are some of the most well known *Drosophila melanogaster* aging mutants, *methuselah* (*mth*) (Lin *et al*., 1998) and *I'm not dead yet* (*Indy*) (Rogina *et al*., 2000).

P-elements have been improved over the years. Initially, there was no easy method to check for *P*-elements that inserted near or in a gene, which made screens especially intensive. Fortunately techniques have improved allowing the use of enhancer-trap, gene-trap and gene search *P*-elements. In these methods the *P*-element takes advantage of adjacent genomic sequences to induce expression of some type of marker that allows verification of the presence of the *P*-element and identification of the insertion site, as well as time and location of expression. Enhancer-trap *P*-elements (Bellen *et al*., 1989) use nearby regulatory elements to drive expression of the reporter gene, indicating sites, times and levels of expression in the adjacent gene. Hence, they are typically used for examining patterns of expression and not as useful to mutagenesis screens. Gene-trap *P*-elements (Lukacsovich *et al*., 2001) rely on a gene's promoter for expression and are typically found somewhere within the gene. This allows them to disrupt normal gene function, effectively creating a mutant. The gene search system uses the addition of an upstream activating sequence (UAS) enhancer next to a promoter on each end of the *P*-element, which provides controlled mis-expression of adjacent genes (Toba *et al*., 1999).

While *P*-element mutagenesis can be beneficial, it can also be quite problematic. The location of the insertion can affect the level of expression of the *P*-element, especially in heterochromatin rich regions of the genome. Insertion between two genes can confound analysis. Precise revertants can be difficult to

obtain, making causal analysis wearisome. Finally, the *P*-element itself may affect life span.

RNAi and gene knockouts are two other methods of disrupting gene expression in model organisms. RNAi has commonly been used to examine potential life span genes in *C. elegans* (Lee *et al*., 2003; Arantes-Oliveira *et al*., 2003; Nehrke, 2003; Murphy *et al*., 2003; McElwee *et al*., 2003; Hansen *et al*., 2005; Curran and Ruvkun, 2007). Unfortunately the procedure is new and may cause unknown side effects that affect life span. Gene knockouts in mice have been used to analyze life span genes (Migliaccio *et al*., 1999; Bluher *et al*., 2003; Holzenberger *et al*., 2003). Gene knockouts utilize transgenic recombination techniques to create null mutants of specific genes.

Transgenes are commonly used to rescue gene expression in mutant organisms as well as to over-express select genes. There are several methods to create transgenics, several organism-dependent. Typically the recombinant DNA is inserted into a vector and then the vector is inserted into an embryo. The DNA can exist either as a separate artificial chromosome or be integrated into the genomic DNA. Typically integrated DNA is more stable, but more difficult to accomplish. The common methods of integration include homologous recombination and transposable elements. Unfortunately transposable elements may lead to extra mutations that may affect life span and result in misinterpretation of results. Transposable elements are also subject to positional effects. Recombination is more reliable when successful, but integration via recombination is inefficient.

Microarraying Longevity. DNA microarrays have taken the world by storm. Although genomic sequencing has recently enjoyed the spotlight, microarrays have quickly joined their ranks. Microarrays have primarily been used to make comparisons between two (or more) lines or tissue types to identify differences in expression on a genomic scale. They have additionally filled several other niches. Microarrays have been used in single nucleotide polymorphism (SNP) identification and genotyping (Wang *et al*., 1998), in combination with QTL mapping (Wayne and McIntyre, 2002; Lai *et al*., 2007) and in expression quantitative trait loci (eQTL) mapping (Schadt *et*

al., 2003). In addition, protein microarrays are making progress toward the identification of functional interactions between gene products. In terms of aging, DNA microarrays can serve several purposes including those listed above.

Many labs have taken advantage of microarray technology to observe changes in expression due to aging. 7-9% of genes are estimated to directly affect aging (Pletcher *et al*., 2002; Landis *et al*., 2004). While the studies have lead to many different genes with differential expression between young and old organisms, some similar patterns of expression across studies have been observed, such as a general increase in the expression of inflammatory genes and a decrease in those involved in protein turnover, DNA damage repair, reproduction and metabolism. This has been observed across species including nematodes (Lund *et al*., 2002), fruit flies (Pletcher *et al*., 2002; Kim *et al*., 2005; Zou *et al*., 2000), rodents (Prolla, 2002; Tollet-Egnell *et al*., 2001; Weindruch *et al*., 2001) and humans (Yoon *et al*., 2004). McCarroll *et al*. (2004) found fairly conserved expression profiles between *D. melanogaster* and *C. elegans*. Unfortunately, there does seem to be some variation in changes depending on the tissue and organism studied.

Perhaps the most useful use of microarray technology is in the identification of genes downstream of a known or putative aging gene. Several studies have already proven the usefulness of this aspect of DNA microarrays (Murphy *et al*., 2003; McElwee *et al*., 2003; Lin *et al*., 2001; Dozmorov *et al*., 2001). Lin *et al*. (2001) examined differences in gene expression in yeast due to mutations in Sip2p and Snf4p kinase. A mutation in Snf4p is known to increase life span, while a mutation in Sip2p decreases it (Ashrafi *et al*., 2000). Snf4p activates Snf1p kinase (Ashrafi *et al*., 2000), which is necessary for response to glucose starvation (Hardie *et al*., 1998), and Sip2p is thought to repress Snf1p kinase activation (Ashrafi *et al*., 2000). Lin *et al*. (2001) concluded that a shift from glycolysis to gluconeogenesis as the primary energy source occurs as yeast ages and that a decrease in either Snf1p kinase activity or caloric restriction delays this shift. Only 96 genes differed in expression between young and old yeast cells, while 162 differed in *sip2*Δ cells and 182 genes differed in *snf4*Δ cells out of about 7000. The changes were primarily in energy storage and metabolism in *sip2*Δ cells and due to DNA repair, regulation of

the cell cycle and protein synthesis and modification in *snf4*Δ cells (Lin *et al*., 2001). Dozmorov *et al*. (2001) found that the *Prop1*^{df} mutant primarily affects expression of genes involved in insulin signaling in mice. Out of the 588 genes on their array, 4 differed between young wild-type and mutant mice (5 months old), 7 differed among middle-aged mice (13 months) and 3 differed among old-aged mice (22 months). Almost half of these genes were involved in insulin signaling (Dozmorov *et al*., 2001). Two studies have looked at genome-wide changes in expression due to *daf-16* mutations in *C. elegans* comparing *daf-2* mutants with *daf-2/daf-16* double-mutants (Murphy *et al*., 2003; McElwee *et al*., 2003). Murphy *et al*. (2003) found 263 genes that were induced in *daf-2* mutants while repressed in *daf-2/daf-16* double-mutants and 251 that were repressed in *daf-2* mutants while induced in *daf-2/daf-16* double-mutants. Meanwhile, McElwee *et al*. (2003) found 602 genes up-regulated in daf-2 mutants versus daf-2/daf-16 double-mutants and 1044 down-regulated out of 17,661 genes. A large number of these genes were involved in stress response (McElwee *et al*., 2003).

Insulin signaling is known to affect the life span of several organisms, so examining genome-wide changes in expression due to genes involved in insulin signaling is a logical approach. Fat-specific insulin receptor knock-out (FIRKO) mice have been used to observe changes in expression due to both the lack of an insulin receptor and the size of cells in adipose tissue. The lack of an insulin receptor leads to general decreases in metabolism and immune response genes (Bluher *et al*., 2004). A study of *daf-2* mutants in nematodes displayed a corresponding up-regulation of heat shock proteins, UDP-glucuronosyltransferase and glutathione S-transferase and down-regulation of genes involved in nutrient uptake (McElwee *et al*., 2004). Studies in *daf-16* mutants have shown that it affects expression of genes affecting stress response, metabolism, development and antimicrobial defense (McElwee *et al*., 2003; Murphy *et al*., 2003).

The link between oxidative stress and life span has also been considered via microarrays. Young, oxygen-stressed flies show similar gene-expression patterns to old flies (Landis *et al*., 2004). However, there is evidence from a microarray comparison study that most aging genes are not affected by oxidative stress (Zou *et*

al., 2000). This would indicate that oxidative stress may not be as involved in the aging process as previously thought.

Many experiments have been carried out in attempt to identify the processes underlying caloric restriction. It has been observed that organisms shift to the effects associated with long-term caloric restriction very quickly (Cao *et al*., 2001; Dhahbi *et al*., 2004). Likewise, after resuming normal caloric intake, expression rapidly returns to pre-caloric restriction levels (Dhahbi *et al*., 2004). Caloric restriction appears to modify expression of genes involved in stress response, metabolism, apoptosis, inflammation, DNA damage repair and immune response (Cao *et al*., 2001; Dhahbi *et al*., 2004; Fu *et al*., 2006; Han *et al*., 2000; Lee *et al*., 2002; Sreekumar *et al*., 2002; Pletcher *et al*., 2002; Prolla, 2002; Weindruch *et al*., 2001).

Microarrays have been used in an attempt to identify possible caloric restriction mimetics such as metformin (Dhahbi *et al*., 2005). Mimetics are pharmaceuticals that mimic the effects caused by caloric restriction, primarily an increase in life span as well as extension of health-span. Metformin makes sense as a potential mimetic since it increases insulin-sensitivity (Kirpichnikov *et al*., 2002), which is involved in caloric restriction (Dhahbi *et al*., 2001).

While microarrays show promise in the examination of mechanisms underlying life span, they are not without their drawbacks. The main hindrance to the usage of microarrays is their high cost. Fortunately this has decreased with the utilization of new technological advancements and increased popularity. Data analysis is another large problem associated with microarrays. Many methods of analysis are available and typically most investigators have used different techniques, which makes comparisons difficult. This should also improve as global-standards are developed.

Quantitative Genetics and QTL Mapping. Microarrays and mutagenesis assist in the discovery of genes involved in life span; however, other techniques are necessary to examine why we age, especially from an evolutionary standpoint. Selection experiments and quantitative genetic analysis allow us to break down the variance

components to examine the genetic component of variation. Quantitative trait loci (QTL) mapping can then be used to identify candidate genes that may be responsible for part of the variation.

Selection studies associated with aging are numerous. Typically investigators select for either postponed senescence or stress resistance. In selecting for postponed senescence, only the oldest flies are used to generate successive generations. Hence, the flies are selected based on their ability to reproduce at a late age. Selection for postponed senescence has resulted in an increase in life span (Rose *et al*., 1984; Luckinbill *et al*., 1984; Vettraino *et al*., 2001; Partridge and Fowler, 1992; Harshman *et al*., 1999). In most studies, there was a correlated decrease in early fecundity in the lines with increased life span (Rose *et al*., 1984; Luckinbill *et al*., 1984), which supports the theory of antagonistic pleiotropy (a reduction in fertility results in an increase in life span). However, another study showed no such decrease in fecundity (Partridge and Fowler, 1992). Stress resistances have often been associated with life span and are often used as selection criteria to examine aging. Selection for increased resistance to oxidative stress resulted in an increase in life span (Vettraino *et al*., 2001). Rose *et al*. (1992) selected for resistance to starvation and desiccation and found a corresponding increase in life span. Unfortunately it has been shown that this is not always true, as at least one case of selection for starvation resistance has failed to result in an increase in life span (Harshman *et al*., 1999). This may indicate that there are multiple mechanisms involved in starvation resistance, some of which are not common to the mechanisms of aging.

Quantitative genetic analysis is useful in the determination of the genetic component of the variance (which leads to heritability) and interaction effects between genes and their environment (including other gene products). The genetic component of variance is a vital component of the mutation accumulation theory of aging. According to the theory, the genetic variation should increase with age. Just like with antagonistic pleiotropy, experiments have both supported (Hughes and Charlesworth, 1994) and contradicted (Rose and Charlesworth, 1980) the theory. Gene by environment (GxE) interactions as well as gene by gene (epistatic)

interactions contribute significantly to life span, but neither have been studied to a large degree.

QTL mapping has been commonly used over the last decade. QTL mapping is used to identify genes that affect variation in life span in natural populations. Genes in most of the mechanisms thought to be involved in longevity have been implicated as the result of QTL studies. Studies in *C. elegans* (Ayyadervara *et al*., 2003) have turned up QTL regions containing known longevity related genes such as *rad-8*, *sod-2*, *daf-16*, *clk-1*, *clk-2*, *sir-2* and *mev-1*. *D. melanogaster* have proven to be a QTL mapping gold mine revealing many QTL regions harboring hundreds of possible longevity genes (Nuzhdin *et al*., 1997; Leips and Mackay, 2000; Vieira *et al*., 2000; Leips and Mackay, 2002; Pasyukova *et al*., 2000; Geiger-Thornsberry and Mackay, 2004; Pasyukova *et al*., 2004; Wilson *et al*., 2006). Not only have many longevity genes shown up as candidate genes in QTL regions, but sex-specific QTL have been discovered (Nuzhdin *et al*., 1997). Furthermore, different regions have been revealed in mated flies than in virgin flies (Leips and Mackay, 2002), when there are differences in genetic variation in life span contingent on larval density (Leips and Mackay, 2000), as well as other environmental effects (Vieira *et al*., 2000). Two major problems with this method are that it is necessary to have allelic differences at or near the actual genes affecting life span in the two inbred lines and the initial regions discovered tend to be quite large requiring fine-scale mapping. Because of this, it would be beneficial to complement QTL mapping with other techniques. In fact, linkage disequilibrium/association mapping has been used in combination with QTL mapping to identify SNPs in the *Dopa decarboxylase* (*Ddc*) and *Catecholomines up* (*Catsup*) genes that contribute to the genetic variation in life span (De Luca *et al*., 2003; Carbone *et al*., 2006). More recently, Lai *et al*. (2007) have combined QTL mapping techniques with microarrays to identify novel candidate life span genes.

Demography. Another method of investigating aging is by examination and comparison of mortality curves. Age-specific demographic profiles are more informative than basic knowledge about the mean and variance of life span. Initially

it was thought that mortality increases exponentially with age. It has recently been found that in *Drosophila* this is true during the early and middle stages of life, however, the mortality rate levels off during the later stages (Curtsinger *et al*., 1992). This has forced the re-examination of the theories of aging. Looking at the demographics can help explain differences between lines, sexes or even environments. Classically it has been thought that dietary restriction leads to a decline in the rate of accumulation of damage due to aging, hence a decrease in slope of the mortality curve. It has been shown in *Drosophila* that this is not true. The mortality rates for populations of animals fed normally, calorically restricted from birth and calorically restricted after certain ages all had a similar slope (Mair *et al*., 2003). Instead it appears that initiating dietary restriction at any time drops the curve to the same level as populations calorically restricted from birth indicating that mortality depends only on age and available nutrition. Changes in temperature did change the slope of the curve (slope increased with temperature) indicating that temperature does affect the rate of damage accumulation (Mair *et al*., 2003). Demographic profiles have also indicated multiple mechanisms of life span extension. Mortality curves representing populations selected for delayed female fecundity, populations selected for increased paraquat resistance and populations undergoing a non-lethal heat shock all displayed different patterns of mortality (R. Arking *et al*., 2002). If the mechanisms behind aging in each situation were the same, they would exhibit similar mortality curves.

Mechanisms

The journey to understand the mechanisms underlying aging has been long and difficult. While we still have quite a way to go, a vague picture of the processes involved is starting to develop. Thus far three primary pathways seem to be at the forefront of extending life span: dietary restriction, oxidative stress and insulin/IGF signaling. Of course, these are not the only mechanisms that affect life span. Processes such as stress response, reproduction, telomere length, regulation of expression and protein/DNA protection and repair all seem to play some kind of role

in the regulation of life span. We do not yet fully understand the extent to which these processes affect longevity or if and how they may be related.

Dietary Restriction / Metabolism. Dietary restriction has shown to increase life span in organisms ranging from simple eukaryotes such as yeast (Lin *et al*., 2000; Lin *et al*., 2002) and the nematode (Lakowski and Hekimi, 1998) to more complex eukaryotes like spiders (Austad, 1989), fruitflies (Robertson, 1960), mice (Harrison and Archer, 1987) and primates (Roth *et al*., 1999). The path to discovering the genetics behind dietary restriction has been more difficult than anticipated. This is because many different pathways have been implicated and it is often difficult to establish how independent the pathways actually are. To further complicate matters, orthologous genes in model species often work in different pathways.

Several pathways have been linked to increased life span in *Saccharomyces cerevisiae. Silent information regulator 2 (Sir2)* was first identified as a life span gene in *S. cerevisiae* (Kaeberlein *et al*., 1999). It is a histone deacetylase that requires NAD to function during dietary restriction (Lin *et al*., 2000). *Ras2* and *Sch9* mutants both increase yeast life span (Longo, 2003; Fabrizio *et al*., 2001). There is evidence that they both act independently to inhibit expression of *superoxide dismutase* (*SOD2*) whose gene product is an anti-oxidant (Longo, 2003). The target of rapamycin (TOR) pathway, which regulates carbohydrate and amino acid metabolism, also regulates life span in yeast (Kaeberlein *et al*., 2005).

Sirtuins are the entire family of *Sir2* orthologs and seem to be involved in dietary restriction in higher eukaryotes as well (Dilova *et al*., 2007). In addition to yeast, increased expression of sirtuins has shown to increase life span in *C. elegans* and *D. melanogaster* (Tissenbaum and Guarente, 2001; Rogina and Helfand, 2004). While it has not yet been shown that sirtuins affect life span in mice and primates, they have been linked to de-acetylation of p53 (Vaziri *et al*., 2001) and FOXO (Qiao and Shao, 2006; Motta *et al*., 2004; Daitoku *et al*., 2004) which are known to be involved in regulation of life span in rodents. Sirtuins also affect metabolic pathways. They have been linked to UCP2 (Bordone *et al*., 2006) and activate PGC1- (Rodgers *et al*., 2005) which is a co-activator of peroxisome proliferators-

activated receptor g (PPAR-g) which has independently been shown to be involved in dietary restriction (Masternak and Bartke, 2007). They also show correlated responses to dietary restriction among several biomarkers of aging (Michan and Sinclair, 2007).

Just like in yeast, dietary restriction appears to act via the TOR pathway in higher eukaryotes. TOR has been well studied in *Drosophila* and has been associated with dietary restriction (Kapahi *et al*., 2004) as well as insulin signaling (Oldham *et al*., 2000). TOR and *daf-15*, which encodes a protein that interacts with TOR, extend longevity in *C. elegans* which again seems to be connected with insulin signaling (Jia *et al*., 2004). The TOR pathway has also been correlated to dietary restriction and insulin signaling in mice (Martin and Hall, 2005; Sharp and Bartke, 2005)

Several other metabolic genes have been associated with dietary restriction. In yeast, deletion of the MRG19 gene, which is a transcription factor that regulates the metabolism of nitrogen and carbon, leads to an increase in life span (Kharade *et al*., 2005). It has been shown that a mutation in the *clk-1* gene in *Caenorhabditis elegans*, the nematode, results in a decrease in respiration rate and a decrease in mitochondrial activity leading to an increase in life span (Felkai *et al*., 1999). Likewise, a mutation in the *mev-1* gene, which codes for a subunit of succinate dehydrogenase cytochrome b, leads to a decrease in life span (Ishii *et al*., 1998). Over-expression of the gene demonstrated the opposite effect. The *Drosophila melanogaster* gene *I'm not dead yet* (*Indy*) is a sodium dicarboxylate cotransporter responsible for transporting metabolic intermediates involved in the Krebs cycle. Mutants showed a 37% increase in mean life span over control flies (Rogina *et al*., 2000).

Conditions of dietary restriction have been extensively studied using microarrays (Lee *et al*., 1999; Weindruch *et al*., 2001; Pletcher *et al*., 2002; Sreekumar *et al*., 2002). Pletcher *et al*. (2002) found that genes involved in DNA repair, cell cycle processes, developmental processes, protein metabolism and degradation show a decrease in expression in calorically restricted fruitflies. The *methuselah* gene was also found to have a decrease in expression. A few isolated

protein biosynthesis and nucleic acid metabolism genes showed an increase in expression, but no processes showed a uniform increase in gene expression. Interesting results were found in expression profiles based on age. Most transcript levels showed patterns of expression based on relative age instead of chronological age; however, a few genes did display expression patterns that reflected chronological age. They found that stress and antimicrobial response genes increase expression with age, while developmental processes decrease expression with age (as expected). A study in calorically restricted mice has shown increases in protein synthesis and turnover, gluconeogenesis and fatty acid synthesis, but decreases in damage repair genes (Lee *et al*., 1999). The same study showed an age related increase in stress response genes and neuronal injury, with a decrease in glycolysis.

Oxidative Stress. The free radical theory of aging is often reviewed when dealing with dietary restriction and longevity. This theory attributes aging to the accumulation of oxygen free radicals, also known as reactive oxygen species (ROS), which damage DNA, proteins and lipids. Metabolic processes create these harmful by-products, but are kept under control by antioxidants such as superoxide dismutase (SOD) and catalase. An overabundance of ROS or decline in SOD and catalase function can lead to macromolecule damage and eventually cell death. The theory is still very controversial, as most studies have proven to be inconclusive. Over-expression studies using transgenic Cu-Zn SOD and catalase in *D. melanogaster* have shown major increases in life span (about 34%when both are over-expressed, while the increase is minimal when individually over-expressed (Orr and Sohal, 1994). Another study showed increased life span in *Drosophila* when the human SOD1 transgene was over-expressed (Parkes *et al*., 1998). However, a decrease in life span has not always been observed when SOD and catalase are under-expressed. A mutation in a cytosolic catalase gene in the nematode, *ctl-1*, was thought to result in a decrease in life span, however, this was later retracted due to experimental error (Taub *et al*., 1999; Taub *et al*., 2003). Another *C. elegans* gene, *age-1*, has been shown to increase life span (Friedman and Johnson, 1987).

It is associated with an increase in resistance to oxidative stress (Morris *et al*., 1996). Mitochondrial uncoupling proteins, which are responsible for thermogenesis and regulation of body mass, may also be involved in protection against ROS (Sluse and Jarmuszkiewicz, 2002; Borecky *et al*., 2001), but currently there is little evidence of this. More recently several candidate genes in relieving oxidative stress have been proposed (Kharade *et al*., 2005; Fabrizio *et al*., 2001; Migliaccio *et al*., 1999; Ishii *et al*., 1998).

Finally, differences in aging profiles have been examined in *Drosophila* under conditions of oxidative stress (Zou *et al*., 2000). When compared with normal conditions, genes involved in fatty acid metabolism were decreased in oxidative conditions, while antioxidants like glutathione S-transferases displayed increases in expression. Few differences in expression were found between the two environmental conditions, however, there were interesting gene expression changes with age consistent in both environments. These included reductions in genes involved in protein turnover, Acp proteins and mitochondrial metabolism; and increases in genes involved in detoxification. Oxidative damage may play a part in aging, but based on the low number of genes resulting from this study it is by no means the only process involved in aging.

Insulin Signaling. Insulin/insulin-like growth factor (IGF) signaling may help explain part of the fecundity/longevity trade-off often discussed in the antagonistic pleiotropy theory of aging. In the nematode, fruitfly and mouse, insulin-like receptors regulate growth hormones leading to fertility as well as metabolic processes that lead to aging (Tatar *et al*., 2003). The conservation of these results across species indicates that a similar process may occur in humans.

The nematode was one of the first organisms used to study insulin/IGF pathways. Early in development nematodes can enter a state of developmental arrest called the dauer stage in the absence of necessary nutrients. The shift to dauer larva is triggered by pheromone levels based on population density as well as food availability (Cassada and Russell, 1975; Swanson and Riddle, 1981; Albert *et al*., 1981; Golden and Riddle, 1984). They can return to normal development with

environmental cues such as a decrease in temperature and an increase in food sources (Golden and Riddle, 1984). This stage can last for several months (Malone and Thomas, 1994), which significantly increases the life span of the nematode. Obviously this kind of increase in life span caught the world's attention and the race was on to find the genes involved in dauer formation with the hope that they may related to genes affecting life span. Subsequently, many genes have been identified in the dauer pathway, which have successfully been linked to life span. *daf-2* was one of the first genes discovered in this pathway. Decreased *daf-2* expression in *C. elegans* is accompanied by a shift to the dauer stage, a decrease in metabolic processes and an increase in life span (Kimura *et al*., 1997). *C. elegans* hermaphrodites with mutations in *daf-2* have been shown to live twice as long as controls (Kenyon *et al*., 1993). RNAi experiments have shown dauer arrest and increases in life span as well (Arantes-Oliveira *et al*., 2003). The *age-1* gene, the *C. elegans* homolog of phosphatidylinositol 3-OH kinase (PI3K), has a similar effect. It was found that *age-1* mutants that did have maternal *age-1* expression lived 2.6 times as long as controls, while mutants that lacked maternal expression became dauer larva (Morris *et al*., 1996). *daf-18*, which is a homolog of the tumor suppressor gene *PTEN* in humans, was found to be involved in the insulin pathway (Gil *et al*., 1999). Mutants in *akt-1* and *akt-2* were found that worked downstream from *daf-2* in the insulin signaling pathway that seem to inhibit the function of DAF-16 (Paradis and Ruvkun, 1998).

Considerable work has been done in examining expression downstream of DAF-16, a transcription factor involved in insulin signaling and longevity in *C. elegans* (Murphy *et al*., 2003; McElwee *et al*., 2003). Many potential targets of DAF-16 have been identified including those among several processes thought to be involved in aging. McElwee *et al.* (2003) found up-regulation (*daf-16* [+] vs. *daf-16* [-]) of several stress response genes (including *sod-3* and *hsp70*), down-regulation in genes involved in digestion (such as antibacterial proteins and proteases) and down-regulation in metabolism genes when comparing a *daf-2* mutant strain with a *daf-2*/*daf-16* double mutant strain of *C. elegans*. They identified DAF-16 binding sequences in the promoter regions of genes found in the microarray study to identify

putative DAF-16 transcriptional targets. They followed this up with RNA-mediated interference (RNAi) studies on the putative targets to identify any associations to life span. RNAi of *sod-3* (Cu/Zn superoxide dismutase) and *ins-7* (an insulin-like peptide) both resulted in a small increase in life span, while RNAi in a predicted protease of unknown function (ZK384.3) showed a decrease in life span. Murphy *et al*. (2003) conducted a similar experiment comparing several strains of *daf-2* single mutants and *daf-2*/*daf-16* double mutants. They found up-regulation of several signaling molecules (including *scl-1*), stress response genes (including *mtl-1*, *sod-3*, *ctl-1*, *ctl-2*, *gst-4* and heat shock proteins) and antimicrobial genes (*lys-7*, *lys-8*, *spp-1*). They found down-regulation in *gcy-6* and *gcy-18* (receptor guanylate cyclases), vitellogenin (yolk protein) genes (*vit-2*, *vit-5*) and a few proteases and metabolic genes (including protein degradation proteins *skr-8*, *skr-9*, *pes-2*). One other up-regulated gene of note is *gei-7*, which encodes isocitrate lyase/malate synthase, a gene thought to be involved in hibernating mammals (Davis *et al*., 1990). RNAi analysis of most of the up-regulated genes resulted in a decrease in life span, while down-regulated genes showed an increase in life span. Potential regulatory sequences were obtained based on common sequences found in the upstream regulatory regions. They also found that *ins-7* was down-regulated and *ins-7* RNAi resulted in an increase in life span. It was found that INS-7 actually activates DAF-2 in a positive feedback loop (Murphy *et al*., 2003).

In *D. melanogaster* a mutation in an insulin receptor substrate gene called *chico* has produced 48% and 36% increases in mean life span in homozygotes and heterozygotes respectively (Clancy *et al*., 2001). Mutations in the *Drosophila* gene *insulin-like receptor* (*Inr*) have led to an 85% increase in life span in females and a decrease in late-age mortality in males (Tatar *et al*., 2001). The *Drosophila* homolog of TOR (*dTor*) is involved in growth and may be associated with insulin signaling via the PI3 pathway (Oldham *et al*., 2000; Kapahi *et al*., 2004).

In mice, there is an association between life span and growth hormone (GH) expression via the IIS pathway (Brown-Borg *et al*., 1996). Mice lacking the growth hormone receptor show an increase in life span (Zhou *et al*., 1997). The insulin-like growth factor-1 receptor gene (*Ifg1r*) increases life span in females by 33% (Tatar *et*

al., 2003). The *p66Shc* gene is activated by insulin and increases oxidant levels (Purdom and Chen, 2003a).

Reproduction. The antagonistic pleiotropy and disposable soma theories of aging both implicate a trade-off between reproduction and life span. Experimental evidence supports this via dietary restriction in model systems. Fowler and Partridge (1989) found that mating results in a decrease in life span in female *D. melanogaster*. This is thought to be due to toxic effects of male accessory gland proteins that are transferred with sperm (Wolfner, 2002). It appears that there is a negative correlation between dietary restriction and fecundity in *C. elegans* and *D. melanogaster*, while there is a positive correlation between dietary restriction and life span up to the point at which the organism is getting too few nutrients (starvation) where upon life span decreases (Klass, 1977; Chapman and Partridge, 1996). Hence, there is a tradeoff between life span and fecundity due to dietary restriction. Rats and mice similarly show the tradeoff under dietary restriction (Holehan and Merry, 1985; Merry and Holehan, 1981; Weindruch and Walford, 1988).

Most experiments involving the IIS pathway also support a trade-off between life span and reproduction (Fantin *et al*., 2000; Tissenbaum and Ruvkun, 1998; Friedman and Johnson, 1987; Giannakou *et al*., 2004). However, some mutations in the IIS pathway that extend life span do not always reduce fecundity. For example, some *daf-2* mutants in *C. elegans* have increased life span with no change in fecundity (Gems *et al*., 1998). Hwangbo *et al*. (2004) found that over-expression of *dFOXO* in the head fat body of adult *D. melanogaster* also increases life span without a reduction in fecundity. Furthermore, female heterozygous knockout mice for the *Ifg1r* gene have increased longevity with no loss in fecundity (Holzenberger *et al*., 2003). Unfortunately, fecundity has not been measured in many of the mutants that exhibit an increase in life span, so it is difficult to say whether the cases involving the IIS pathway are incredibly rare or quite common.

There is evidence of a trade-off between life span and reproduction in female *Drosophila* with mutations in *methuselah* (*mth*) (Mockett and Sohal, 2006). Mockett

and Sohal (2006) also found that the direction of this trade-off changes with temperature.

Protection and Damage Repair / Hormesis. Hormesis is the term used to describe it when a detrimental stress is actually beneficial when applied at low levels (Furst, 1987). Non-lethal heat shock has been known to increase life span (Khazaeli *et al*., 1997). Heat shock elicits a stress response where a set of genes normally inactive are activated. Responses such as this are induced by other environmental stressors as well and are often called heat-shock proteins or chaperone proteins. These proteins are typically responsible for the repair and/or destruction of damaged proteins (Soti and Csermely, 2003). The *RAS2* gene is an example of this in yeast that also extends life span (Shama *et al*., 1998). In *Drosophila*, the heat shock protein 70 gene (*hsp70*) may play a role in life span extension (Wheeler *et al*., 1995). The *methuselah* (*mth)* gene in *D. melanogaster* is thought to be involved in stress response (Lin *et al*., 1998). DNA repair has also been linked to longevity in mammals by posttranslational modification of proteins (Burkle, 2000). Many genes preventing oxidative stress (above) would also fall under this category.

Telomeres. Telomeres are the protective caps at the ends of linear chromosomes to protect against DNA damage and the ligation of unnecessary DNA (such as other chromosomes). They also prevent the loss of vital DNA during each round of mitosis, where polymerase is unable to replicate a short sequence of DNA at each end of the chromosome. In the absence of telomerase, the telomeres shorten after each mitotic division. The shortening of telomeres has long been thought to be correlated with aging. In fact, there is evidence that the rate of decay in telomere length is correlated to life span in birds, mammals and trees (Haussmann *et al*., 2003; Flanary and Kletetschka, 2005). One of the longer-lived species of bird, Leach's storm-petrel (Huntington *et al*., 1996), showed an increase of telomere length with age, whereas shorter-lived species displayed the typical decrease in telomere length with age (Haussmann *et al*., 2003). Flanary and Kletetschka (2005) provided support amongst trees by showing that longer-lived trees had longer

telomeres and greater telomerase activity than medium- and shorter-lived trees. However, this does not hold for all species as longevity in *C. elegans* does not appear to be correlated with telomere length (Raices *et al*., 2005; Lim *et al*., 2001).

Expression Regulation. Chromatin remodeling and methylation may be involved in longevity. Several histone deacetylase genes involved in gene silencing have been associated with changes in life span. Mutations in the *RPD3* (Kim *et al*., 1999) and *sir-2* (Tissenbaum and Guarente, 2001) genes in *S. cerevisiae* have been identified that increase life span. DNA methylation may regulate aging in mammals (Dahl and Guldberg, 2003). In mice, p66Shc signaling may be controlled by methylation (Purdom and Chen, 2003b). The *Drosophila* DNA methyltransferase gene *dDnmt2* regulates expression of several heat shock genes (Lin *et al*., 2005). *dDnmt2* mutants show decreased life span while flies with over-expression had increased life span. Several genes discussed above, such as *daf-16*, also regulate expression of other genes affecting life span.

Human Studies on Longevity

Although studying longevity in humans is quite limited due to many ethical and logistic factors, there has been a great deal accomplished. Family and twin studies have established a moderate genetic component to variation in life span ranging from 0.10 to 0.33 (Philippe, 1978; Mayer, 1991; Cournil *et al*., 2000; McGue *et al*., 1993; Herskind *et al*., 1996; Ljungquist *et al*., 1998). Several techniques have been used to identify human longevity genes such as linkage studies, association studies and genes orthologous to longevity genes in other organisms.

Linkage studies look at related individuals with a similar life span and try to identify genomic regions shared by the individuals more often than chance. A recent example is the *microsomal transfer protein* (*MTP*) gene which was found to be a modifier of human life span in a group of US families (Puca *et al*., 2001; Geesaman *et al*., 2003). With the completion of the sequencing of the human genome and the recent completion of the human haplotype map (HapMap), more linkage studies are likely to be common in the future. Unfortunately this type of study is currently

severely limited by small sample sizes. Furthermore, reproducing results is difficult, likely due to the fact that they are typically biased toward a particular group of people. For example, a follow up study of *MTP* in French families showed no association to life span for the same haplotype as in the US study (Geesaman *et al*., 2003).

Association studies are another of the typical methods used to examine candidate longevity genes in humans. Centenarian studies have indicated that particular alleles of the human genes *apolipoprotein E* (*ApoE*), *angiotensin-converting enzyme* (*ACE*) and *cholesteryl ester transfer protein* (*CETP*), which code for proteins that affect LDL-cholesterol levels and size, may be associated with an increase in human life span (Schachter *et al*., 1994; Barzilai *et al*., 2003). A study examining polymorphisms of sirtuin 3 (*SIRT3*), the human ortholog to *Sir2*, identified a genotype associated with increased longevity in males (Rose *et al*., 2003). The KL-VS allele of the human ortholog to the mouse gene *klotho* has been associated with a decrease in life span (D.E. Arking *et al*., 2002). Like with linkage studies, association studies display low reproducibility for similar reasons.

The use of model systems has already proven beneficial to humans. Many genes identified in model systems have human orthologs with similar function. *LAG1* (*Longevity Assurance Gene 1*) was found in *S. cerevisiae* (D'mello *et al*., 1994). Orthologs were found in humans and nematodes that were able to complement lethal *lag1*/*lac1* double mutants in *S. cerevisiae* (Jiang *et al*., 1998). Furthermore, the human ortholog (*LAG1H*) restored 85% of the wild-type life span (Jiang *et al*., 1998). A human ortholog to the *Drosophila* gene *Indy* was identified that codes for a sodium-coupled citrate transporter (NaCT) likely responsible for transporting citrates into cells for synthesis of fatty acids (Inoue *et al*., 2002). Evidence suggests that the transporter is stimulated by lithium, a common treatment for bipolar affective disorder, which may result in obesity (Inoue *et al*., 2003).

Dietary restriction studies in humans may give an indication on the possible effects on life span. Several biomarkers of aging due to dietary restriction in humans are similar to those in other species that also display an increase in life span. Individuals participating in the Biosphere 2 study were calorically restricted for two

years. After the period of caloric restriction they showed decreases in body mass index, blood pressure, insulin levels, blood sugar levels, cholesterol and several other biomarkers found in rodents and primates associated with an increase in life span (Walford *et al*., 2002). Members of the Caloric Restriction Society (CRS) eat self-imposed calorie restricted diets. Studies of members who have been on the diet for an average of six years show decreases in body mass index, blood pressure, insulin and blood sugar levels and cholesterol similar to those in the Biosphere 2 study (Holloszy and Fontana, 2007).

Conclusions and Future Directions

It has long been thought that there is some kind of trade-off between life span and reproduction, some evidence now suggests that this is not necessarily the case. Likewise, biomarkers are not always correlated (for instance, life span and starvation resistance are not always positively correlated) so they may not be as beneficial as formerly thought. Unfortunately, many pathways that regulate longevity overlap so it is difficult to determine exactly how they function. There is evidence that mechanisms work independently in some cases, while they obviously depend on each other in other situations. There is evidence that dietary restriction acts via relieving oxidative stress as well as insulin signaling. It is becoming more apparent that the networks that regulate aging are quite complex indeed. At the same time it is clear that we are making great strides in the understanding of the mechanisms underlying aging and the future looks quite hopeful.

One of the most powerful tools in the genetics toolbox is to use unbiased, forward genetic screens to identify mutations affecting traits of interest, and by inference, the genes and pathways required for normal manifestation of the trait. For quantitative traits such as life span, it is important that (1) mutations are not homozygous lethal; (2) replicate measurements can be obtained for each homozygous mutation, since life span is highly sensitive to uncontrollable environmental variance; (3) the effects of mutations can be compared to a control genotype that only differs by the absence of the mutations; and (4) the mutation can be mapped and cloned independent of its effect on life span. The short life span of

C. elegans and the ability to conduct whole genome RNAi screens by simply feeding bacteria containing the RNAi constructs have greatly facilitated such screens in this organism (Duhon *et al*., 1996; Lee *et al*., 2003; Hamilton *et al*., 2005; Hansen *et al*., 2005; Curran and Ruvkun, 2007).

In *Drosophila*, *P* transposable element mutagenesis can fulfill the criteria for an unbiased mutant screen, but the highly labor-intensive nature of such a screen and the difficulty in constructing suitable control genotypes has diminished enthusiasm for adopting this approach. Nevertheless, *P*-element insertions in *methuselah* (Lin *et al*., 1998) and *Indy* (Rogina *et al*., 2000) are associated with increased life span, demonstrating the feasibility of this approach. Clark and Guadalupe (1996) generated 48 *P*-element insertions in a co-isogenic background, and identified mutations that both increased and decreased life span, although the locations of the insertions were not reported. Landis *et al*. (2003) conducted a screen using the *PdL P*-element: insertions of this *P*-element near the 5′end of a gene causes over-expression of the gene when flies are fed doxycycline. Six novel genes associated with modest (5-17%) increases in life span were identified in this screen (*VhaSFD*, *Sugar baby*, *filamin*, *four wheel drive*, *CTP:cytidylyltransferase-1*, and *Red herring*); these genes are presumably required for normal life span (Landis *et al*., 2003). Thus, a large-scale screen to identify mutations that increase *Drosophila* life span would be invaluable for furthering our understanding of the genetic mechanisms affecting *Drosophila* longevity.

Here, we report the results of such a screen for mutations increasing *Drosophila* life span, utilizing a collection of 1,332 *P{GT1}* single, homozygous *P*-element insertion lines that have been constructed in isogenic backgrounds (Bellen *et al*., 2004). We identify 58 mutations with increased longevity, the largest collection of such mutations reported to date. Only one mutation is in a gene that had previously been associated with the regulation of life span; the rest are in novel candidate genes. The effects of the mutations are highly sex-specific, but modest, with life span extensions ranging from 5% - 33%. The mutations have pleiotropic effects on resistance to starvation stress, chill coma recovery time, and locomotor behavior, but the pleiotropic effects are highly variable. All of the mutations

associated with increased life span have at least one deleterious pleiotropic effect on stress resistance or general health, indicating the complicated mutational basis of trade-offs between putative fitness components. A half-diallel cross analysis of 10 of these mutations reveals pervasive epistatic effects that are sex-specific. Whole genome transcript profiles of seven mutant lines and the co-isogenic control indicates complex effects of the mutations on transcript abundance. These results set the stage for a more comprehensive understanding of the genetics of aging in a complex eukaryote.

CHAPTER 2

Materials and Methods

Drosophila Stocks

The *P{GT1}* insertion lines (Lukacsovich *et al*., 2001) used in this study were constructed in six co-isogenic w^{1118} Canton-S backgrounds (A, B, C, D, E and F) as part of the Berkeley *Drosophila* Gene Disruption Project (Bellen *et al*., 2004), and were obtained from Dr. Hugo Bellen (Baylor College of Medicine, Houston, TX). All lines were maintained at 60-80% humidity and 25°C under a 12:12 hour light:dark cycle.

Screen for Mutations Affecting Life Span

We screened 1332 homozygous viable *P{GT1}* insertion lines for changes in life span relative to their control line. The initial screen was conducted in blocks consisting of approximately 50-100 insert lines and the appropriate Canton S control (backgrounds A-F). Each block was initiated with virgin males and females from the insert lines and the control line, all of which had eclosed within 48 hours of each other. Five flies of a single sex were placed in vials containing standard cornmeal/molasses medium, with two replicate vials per sex per insert line, and ten replicate vials per sex of the control line. Vials were scored for deaths every two days until all flies were dead. The media was changed every 1-2 days. Lines with more than three individuals lost due to causes other than natural death were retested in a later block.

Mutational variation for life span was evaluated using analyses of variance (ANOVAs) of the mean life span of replicate vials, expressed as deviations from the appropriate contemporaneous control means for each sex. The full two-factor mixed effects model for pooled sexes was $Y = \mu + L + S + L\times S + \varepsilon$, and the reduced model for the analysis of sexes separately was $Y = \mu + L + \varepsilon$, where μ is the overall mean, L is the line effect (random), S is the sex effect (fixed) and ε is the environmental variance between replicate vials. The mutational broad sense heritability (H_M^2) was computed from the full model as $H_M^2 = (\sigma_L^2 + \sigma_{SL}^2)/(\sigma_L^2 + \sigma_{SL}^2 + \sigma_\varepsilon^2)$, where σ_L^2, σ_{SL}^2 and σ_ε^2 are, respectively, line, sex by line, and environmental variance components. The cross-sex genetic correlation (r_{MF}) was computed as $r_{MF} = cov_{FM}/\sigma_{LF}\sigma_{LM}$, where cov_{FM} is the covariance between the mean life span of males

and females, and σ_{LF} and σ_{LM} are the square roots of the variance components from the separate sex analyses of females and males, respectively.

Two methods were used to identify insert lines with mean life spans that were significantly deviant from the control. First, the 95%, 99% and 99.9 % confidence intervals of the deviation from the control mean were computed, assuming normality, as $\pm z_\alpha\sigma/(n)^{½}$. z_α is the critical value for the normal distribution (1.96, 2.575 and 3.3 respectively for the 95%, 99% and 99.9% confidence intervals). σ is the phenotypic standard deviation, estimated as $(\sigma_L^2 + \sigma_{SL}^2 + \sigma_\varepsilon^2)^{½}$ for the full model and $(\sigma_L^2 + \sigma_\varepsilon^2)^{½}$ for the reduced models. n is the number of replicate vials for each insert line ($n = 4$ in the full model and $n = 2$ in the reduced models). Second, Dunnett's *t*-test, which corrects for multiple tests of different insert lines relative to a common control, was used to identify insert lines that were significantly different from the control within each block.

The mean life spans of 83 lines either exceeded the 95% upper confidence limit and/or were suggestive as significant via Dunnett's test. The life span of these lines was re-tested using the same conditions as the previous assay, but with larger sample sizes of at least 12 replicate vials per sex per line. The significance of the difference in life span between each insert line and the control was evaluated by ANOVAs pooled across sexes and for each sex separately. The models were $Y = \mu + L + S + L\times S + R(L\times S) + \varepsilon$ (full model) and $Y = \mu + L + R(L) + \varepsilon$ (reduced model); where μ and S are defined above; L, the fixed effect of line, is the difference between the *P*-element insertion line and the co-isogenic control; R is the random effect of replicate vial; and ε is the environmental variance between individuals within each replicate vial. We computed the standardized effect of each mutation as a/σ_P, where a is one-half the difference in mean life span between the homozygous mutant and the corresponding control line, and σ_P is the phenotypic variation of the control line (Falconer and Mackay, 1996).

SAS for Windows 8.2 and 9.0 were used for all statistical computations.

P-element Insertion Sites

Bellen *et al*. (2004) identified flanking sequences for the majority of lines using inverse PCR techniques (Liao *et al*., 2000). Several more were identified using the same technique at Model System Genomics (Duke University) and in our laboratory. DNA was isolated from roughly 25 individuals using the Puregene® protocol. The isolated DNA was digested with *Hin*p1I and then ligated to obtain circular fragments containing both genomic and *P*-element DNA from both ends of the insert. The 5' end was PCR amplified with oligonucleotide primers pGT1.5 (CCGCACGTAAGGGTTAATG) and pGT1.d (GAAGTTAAGCGTCTCCAGG) with a 55^{o}C annealing temperature. The 3' end was PCR amplified using oligonucleotide primers Pry1 (CCTTAGCATGTCCGTG-GGGTTTGAAT) and Pry4 (CAATCATATCGCTGTCTCACTCA) again with a 55^{o}C annealing temperature. Resulting products were sequenced using (5') Sp1 (ACACAAC-CTTTCCTCTCAACAA) and (3') Spep1 (GACACTCAGAATACTATTC). Flanking sequences were then aligned to the fly genome using BLAST (Crosby *et al*., 2007). *in situ* squashes were done by Dr. Tatiana Morozova for three of the lines where inverse PCR did not work in order to determine the cytological location of the inserts. A modified Ashburner protocol was used for the preparation of the squashes. Biotin labeled probes were generated using the BioNick Labeling System (Invitrogen) protocol. We used Vectastain ABC kit (Vector Laboratories) for signal detection. Dr. Elena Pasyukova verified the location of the BG00121 (77E-F) and BG01700 (49F) inserts, while Drs. Tatiana Morozova and Jim Mahaffey verified the location of the BG00817 (88A) insert.

Revertants

Revertant stocks were created for two chromosome *2* insert lines (BG00346, BG01042) and one chromosome *3* insert line (BG00043), using crossing schemes designed to preserve the co-isogenic background of the revertant lines (Figure 2.1). The life span of the revertant lines was assessed simultaneously with the corresponding insert and control lines, with 12 replicates for each line and sex. The analysis of the BG00043 revertants was done in Raleigh under the same conditions

described for the previous tests. The analysis of the BG00346 and BG01042 revertants was done by Dr. Elena Pasyukova in Moscow, Russia. The same full ($Y = \mu + L + S + L\times S + R(L\times S) + \varepsilon$) and reduced ($Y = \mu + L + R(L) + \varepsilon$) ANOVA models described above were used to assess differences in life span among the lines. Tukey tests were used to identify significant differences between mutant, revertant and control lines.

Epistasis

We evaluated epistatic interactions among 10 mutations, generated in different genes in the F background, that had increased life span relative to the Canton S F strain (BG00004, BG00010, BG00028, BG00043, BG00297, BG00346, BG00495, BG00761, BG00817, BG01042). The lines were crossed using a half-diallel crossing scheme (excluding homozygous insert lines and reciprocal crosses) to create all 45 possible double heterozygote F1 genotypes following Griffing's (1956) Method 4 and Model 1. The life span of each F1 genotype was determined as described above, with eight replicate vials per genotype per sex. The general combining ability (*GCA*) for each mutation is the difference between the mean life span of all genotypes containing that mutation and the overall mean (Sprague and Tatum, 1942). *GCA* values were estimated as

$$GCA_i = [T_i/(n-2)] - \Sigma T/n(n-2)$$

where T_i is the sum of mean life spans for all genotypes with the ith mutation, ΣT is twice the sum of mean life spans of all double-heterozygote genotypes, and n is the total number of mutant lines (Falconer and Mackay, 1996). The specific combining ability (*SCA*) for any particular genotype is the difference between the mean life span of the genotype and the life span expected from the sum of the *GCA*s of the mutants involved in the cross (Sprague and Tatum, 1942). *SCA* values were estimated as

$$SCA_{ij} = X_{ij} - (T_i + T_j)/(n-2) + \Sigma T/(n-1)(n-2)$$

where X_{ij} is the mean life span of the offspring resulting from the cross of the ith and jth mutant lines. *GCA*s and *SCA*s were also estimated for each sex separately. The SAS program Diallel-SAS05 (Zhang *et al*., 2005) was used to estimate individual

GCA and *SCA* effects and their standard errors. This program was also used to perform ANOVAs to assess the significance of variation among the double heterozygous genotypes (*G*) for the full model pooled across sexes ($Y = \mu + G + S + G\times S + R(G\times S) + \varepsilon$) and for the analyses of each sex separately ($Y = \mu + G + R(G) + \varepsilon$); as well as to partition the *G* term into its *GCA* and *SCA* components, both pooled across sexes ($Y = GCA + SCA + S + GCA\times S + SCA\times S + R(S) + \varepsilon$) and separately by sex ($Y = GCA + SCA + R(S) + \varepsilon$).

Pleiotropy

We assessed pleiotropic effects of mutations with significantly increased life span on stress resistance (chill coma recovery and starvation resistance) as well as a general measure of health (climbing activity) at one week and six weeks of age. Virgin flies were used for all assays. The F lines were separated into three blocks and the A lines into two blocks; all B lines were tested simultaneously. Each block included the appropriate control line. Individuals within each block and assay were tested within 48 hours of each other (with the exception of the starvation assay where all individuals within a block were scored at the same time).

Chill coma recovery. 30 males and 30 females of each line were placed in empty vials. Beginning between 9 am and 10 am, two randomly selected vials were placed in ice every thirty minutes (twenty minutes for the F background) until all vials were in ice. After three hours, vials were emptied onto two plates and the flies were placed on their backs. Flies were scored for flipping themselves upright every minute up to a maximum of 30 minutes for one week old flies and 45 minutes for six week old flies. The significance of the difference in chill coma recovery time between each insert line and the control was evaluated by ANOVAs pooled across sexes ($Y = \mu + L + S + L\times S + \varepsilon$) and for each sex separately($Y = \mu + L + \varepsilon$).

Starvation resistance. We assessed survival time of flies in vials containing starvation medium (1.5% agar in 5 ml water). There were three replicate vials per sex per line, with 10 flies of a single sex in each vial. Flies were scored for survival every eight hours until all were dead. All assays were started at 4:00 pm. The significance of the difference in survival between each insert line and the control was

evaluated by ANOVAs pooled across sexes ($Y = \mu + L + S + L\times S + R(L\times S) + \varepsilon$) and for each sex separately ($Y = \mu + L + R(L) + \varepsilon$).

Climbing activity assay. We assessed the climbing activity of 30 flies per sex per line. Single individuals were placed in empty glass vials (15cm high x 2.5cm diameter) and starved for approximately one hour. Vials were "banged" on a pad to stimulate a negative geotactic climbing response, and scored the height of the fly in the vial after 10 seconds (1 height unit = 5 mm). All flies were scored between 11:00 am and 12:30 pm. The significance of the difference in climbing activity between each insert line and the control was evaluated by ANOVAs pooled across sexes ($Y = \mu + L + S + L\times S + \varepsilon$) and for each sex separately($Y = \mu + L + \varepsilon$).

We also assessed variation for these three traits among all the long-lived mutant lines, expressed as deviations from controls, by similar two-factor mixed effects ANOVA pooled across sexes and for sexes separately, but treating the *L* term as a random effect. Cross-sex genetic correlations were computed as described above. We estimated Pearson's product-moment correlations among lines means, expressed as deviations from the control, to quantify directional pleiotropy among the traits.

Transcriptional Co-regulation

Preparation. We chose seven *P*-element insertion lines for whole genome expression analysis with inserts in the same Canton S genetic background (F) that were associated with a large effect on increased life span in at least one sex, and for which we knew the exact *P*-element insertion sites (with the exception of BG00817). These seven lines were a subset of the lines used for the epistasis analysis: BG00028 (*pyd*), BG00043 (*mub*), BG00346 (*crol*), BG00495 (*CG10990*), BG00761 (*CG9238*), BG00817 and BG01042 (*esg*). We collected approximately 150 flies of each sex after eclosion over a 4-day interval from each of the seven *P*-element insert lines and the co-isogenic Canton S F background control (with the following exceptions: *pyd* – 100 males and 126 females, *crol* – 103 males). Flies were maintained in the same manner as the previous life span assays. After 42 days,

surviving flies of a single line and sex were randomly grouped into replicates of 25 flies and prepared for RNA extraction. Two replicates were randomly chosen for each line and sex. Dr. Mary Anna Carbone prepared RNA and cRNA through fragmentation. Expression Analysis® hybridized, scanned and normalized the GeneChips®. RNA was collected following a TRIZOL (Gibco BRL)/chloroform extraction protocol using whole flies. cRNA was prepared using 5 g of RNA following the protocol in Affymetrix® Genechip® Expression Analysis Technical Manual (2004) for eukaryotic one-cycle target labeling. Fragmented cRNA was hybridized to Affymetrix® Drosophila Genome 2.0 GeneChip® arrays. The arrays were washed and scanned using the same protocol.

Analysis. For each of the 18,800 probe sets on the array there are 14 perfect match (PM) and 14 single nucleotide mis-match (MM) pairs. The oligonucleotides are 25 bases long and the mis-match is located at the 13th base. We used the weighted log(PM-MM) intensity of each probe set as the quantitative measure of expression. The expression scores were then scaled to a median intensity of 500. Each probe set is identified as being present, marginal or absent. Probe sets that did not have a present signal for both replicates for at least one sex and line were excluded from analysis. A total of 12,635 probe sets passed this criterion and were used in the analysis. ANOVAs were run on each probe set using the following two factor model: $Y = \mu + L + S + L\times S + \varepsilon$. Probe sets for which the p-value for the L term exceeded a q-value threshold of $q < 0.0001$ (Storey and Tibshirani, 2003) were considered to be significant after correction for multiple tests and were further examined. Tukey's tests were run for each probe set for which the L or $L\times S$ terms were significant to identify in which mutant lines gene expression was different from the control line. Reduced ANOVAs were run for males and females separately for the probe sets with a significant $L\times S$ term. ANOVAs were also run for each individual line with the control using the full model. Gene ontology (GO) information was collected for the probes with a significant L effect for all mutant lines together ($q < 0.0001$) as well as individual mutant lines ($q < 0.05$) and individual sexes ($q < 0.001$). NetAffx™ Analysis Center was used to obtain the information and a χ^2 test was used to test for over- and under-representation of GO categories (expected

values were based on the ratio of the biological process probe sets in the significant list to the total number of biological process probe sets on the array).

Statistical tests were run using SAS® 8.2, 9.1 and Microsoft® Office Excel, while *q*-values were computed with QVALUE software (Storey and Tibshirani, 2003).

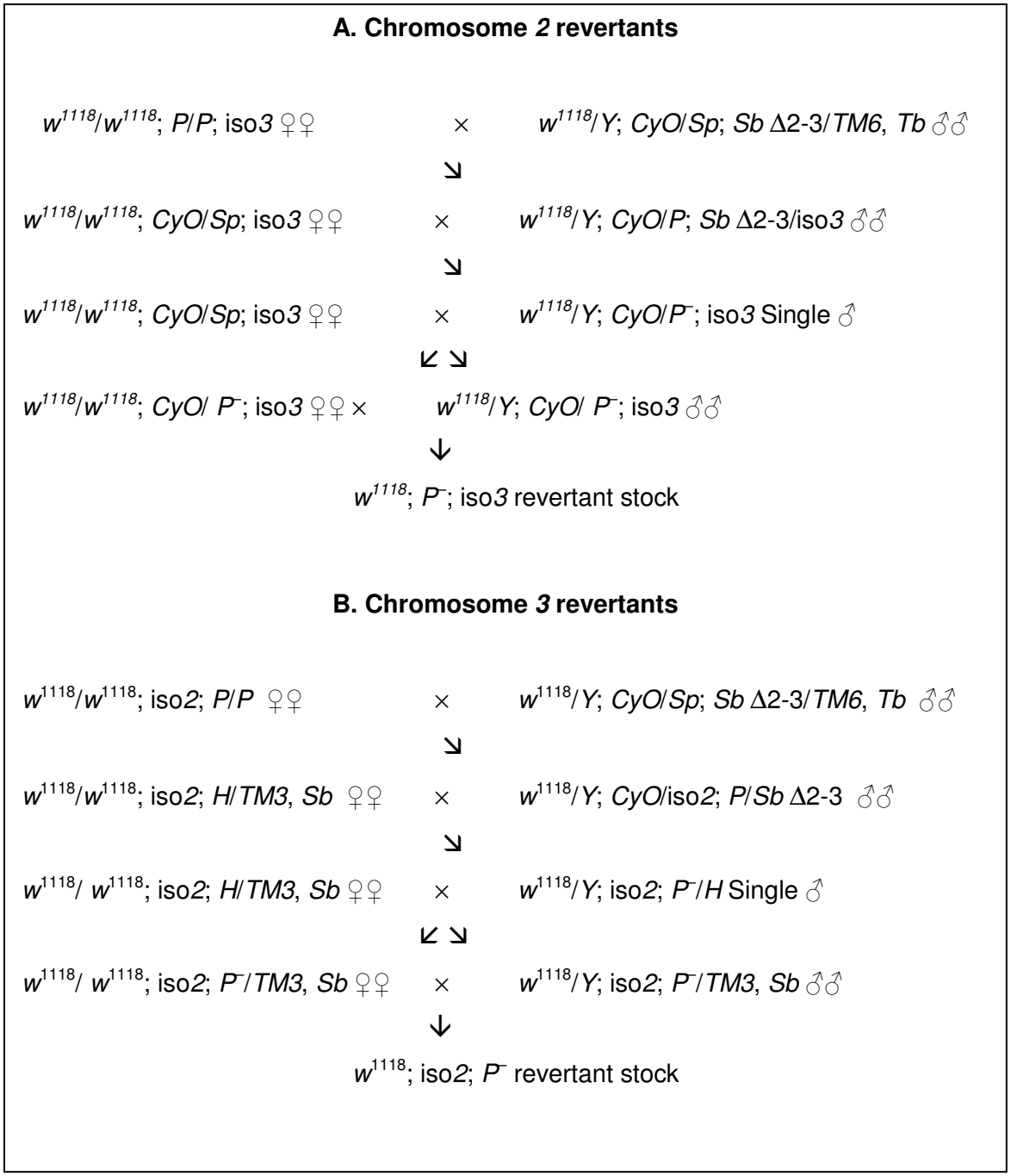


Figure 2.1 - Crosses to generate *P*-element revertant lines. P^{-} denotes a chromosome in which the *P*-element has excised, as inferred by the loss of the *mini-white* selectable marker; iso denotes an isogenic chromosome of the appropriate Canton S genotype. The visible mutations are *white* (*w*), *Curly* (*Cy*), *Sternopleural*

(*Sp*), *Stubble* (*Sb*), *Tubby* (*Tb*) and *Hairless* (*H*). Δ2-3 is a chromosomally stable source of *P*-element transposase.

CHAPTER 3

Results

Identification of Co-isogenic *P{GT1}* Insertion Lines with Increased Life Span

We performed a forward genetic screen for mutations affecting life span, utilizing a panel of 1,332 homozygous *P{GT1}* insertion lines that had been constructed in one of six Canton S strain isogenic backgrounds (labeled A-F) (Bellen *et al*., 2004; Harbison *et al*., 2004; Sambandan *et al*., 2006). We quantified the life span of males and females of the *P*-element insertion lines simultaneously with their co-isogenic control line. Two-way analysis of variance (ANOVA) of the deviations of life span from the control, pooled over sexes, revealed highly significant variation between males and females, among the *P*-element insert lines, and for the line by sex interaction term (Table 3.1). The broad-sense mutational heritability (H_M^2) of life span was high: $H_M^2 = 0.557$. The significant line by sex interaction term indicates that mutational effects on life span can be sex-specific. Indeed, the estimate of the cross-sex genetic correlation (± SE) is $r_{FM} = 0.555 \pm 0.025$, which is significantly different from both a correlation of zero (which would indicate no correlation of mutational effects between the sexes) and a perfect correlation of unity.

We used two statistical analyses to identify the lines that contributed to the significant mutational variance. In the first analysis, we considered the entire data set simultaneously, and determined 95%, 99% and 99.9% confidence intervals (CI) of deviations of line means from their corresponding controls, both averaged over sexes and for individual sexes (Figure 3.1). At the 95%, 99% and 99.9% CIs, respectively, 139 (194), 55 (95) and 12 (49) lines had significantly increased (decreased) life span in at least one of the sexes or averaged over males and females. Second, we analyzed the deviations of insert lines from the control line within each block separately, using Dunnett's *t*-tests to correct for multiple comparisons with a common control. These tests indicated 270 lines had significantly decreased life span, and 70 lines had significantly increased life span, relative to the control ($P < 0.05$, after correction for multiple tests). In addition, 48 lines approached formal significance for an increase in life span. Both analyses indicate an asymmetrical distribution of mutational effects, with more mutations decreasing than increasing longevity, as expected for components of fitness.

We chose 54 lines that both exceeded the upper 95% CI and were significant based on Dunnett's test, as well as 29 lines that met the upper CI criterion, but only approached formal significance based on the Dunnett's test, for re-testing using larger sample sizes. Analyses of variance of these data, both pooled over sexes and for each sex separately, showed that 58 of the 83 lines (70%) remained formally significant for at least one sex, and for 43 lines the *P*-values survive Bonferroni's correction for multiple testing ($P \leq 0.0006$) (Table 3.2). Thus, 4.3% of the mutations screened are associated with increased longevity.

Mutational Effects on Life Span

Consistent with the imperfect correlation between the life span of males and females from the initial screen, we observed substantial variation in sexual dimorphism for the effects of *P{GT1}*-element insertions on longevity, as indicated by significant line by sex interaction terms in the ANOVAs pooled across sexes (Table 3.2). Indeed, the cross-sex genetic correlation for longevity among the 58 long-lived mutant lines was negative and significantly different from zero ($r_{FM} = -0.295 \pm 0.128$, $t_{56} = 2.308$, $P < 0.05$). Therefore, mutations associated with an increase in longevity have highly sex-specific effects, with a trend towards opposite effects in males and females. A total of 17 lines were considered to affect both sexes based on a significant line term but no significant line by sex interaction. The remaining 41 mutations (70.7%) affected males and females differently. We categorized the mutational effects as "sex-specific" if the line by sex interaction from the analysis pooled across sexes was significant, and the line term from the separate sex analysis was significant only in one sex; "sex-biased" if the line and line by sex interaction terms from the analysis pooled across sexes were both significant, and the line term from the separate sex analysis was significant in both sexes; and "sex-antagonistic" if the line term from the analysis pooled across sexes was not significant, but the line by sex interaction was significant, and the line term from the separate sex analysis was significant in both sexes. We found 22 male-specific, two male-biased, nine female-specific, two female-biased, and six sex-antagonistic mutations (Table 3.2).

We quantified the standardized mutational effects of the *P*-element mutations as a/σ_P, where a = one half of the difference in life span between the homozygous mutant and control line, and σ_P is the phenotypic standard deviation of the control (Falconer and Mackay, 1996). The average absolute value of the standardized mutational effect of the significant *P*-element insertions was 0.27 pooled across sexes (with a range of 0.12 – 0.55), 0.43 in males (with a range of 0.13 – 0.73), and 0.39 in females (with a range of 0.13 – 0.69) (Table 2). These effects correspond to an average change in longevity relative to the control of 12% pooled across sexes (with a range of 4% - 27%), 17% in males (with a range of 5% - 33%) and 15% in females (with a range of 5% - 25%) (Table 3.2). Thus the effects of *P*-element insertions on longevity, although significant, are rather subtle.

Candidate genes

Flanking sequences of 49 of the *P*-element inserts associated with increased life span were identified by inverse PCR and sequencing (Table 3.3). Two of the rescued fragments were too short to give unique insertion sites. Eight of the rescued fragments mapped to unique genomic locations, but were ≥ 2 kb from the nearest annotated gene. These inserts possibly have long-range effects on the nearest neighboring gene(s); or, more likely, affect an un-annotated gene in the more immediate vicinity. The remaining 39 *P*-element inserts were < 2 kb from the nearest gene. Of these, 27 were adjacent to or within the predicted transcript of the only gene in the region, and most parsimoniously affect these genes. A total of 13 inserts were located in an intergenic region, < 2 kb from each flanking gene, and could affect either or both adjacent genes.

Several of the *P*-elements inserted into identical or nearly identical positions: five inserts in the first intron of *mushroom-body expressed* (*mub*), two inserts 500 base pairs upstream of *polychaetoid* (*pyd*), two inserts adjacent to *CG9238*, two inserts in the *Tre1/Gr5a* intergenic region, and two inserts between *CG8418* and *Gef64C*. Since this screen is far from saturation, these sites likely represent hotspots for *P{GT1}* element insertion.

The effects of multiple inserts in the same genomic region are often, but not always, heterogeneous. Two of the inserts in *mub* affected both sexes, two were male-specific, and one was female-specific. One insert near *CG9238* was female-specific, while the other affected both sexes. One of the inserts in the *CG18418/Gef64C* intergenic region affected both sexes, while the other was strongly female-biased. On the other hand, both inserts in the *Tre1/Gr5a* intergenic region affected both sexes, and both inserts near *polychaetoid* were male-specific.

To add to the complexity, not all inserts in the same gene affect longevity in the same direction. The mutations in *esg*, *pyd* and *mub* associated with increased life span were all in the Canton S F genetic background. In addition, seven mutations in *esg*, two mutations in *pyd*, and two mutations in *mub* were associated with reduced life span in the initial screen. All of these mutations were in the Canton S A or B genetic backgrounds, and with one exception ($mub^{BG02497}$) were in different locations from the mutations in these genes associated with increased life span.

Only one of the *P*-element tagged candidate genes, *forkhead box, sub-group O* (*foxo*) has been previously implicated to regulate adult life span (Giannakou *et al.*, 2004; Hwangbo *et al.*, 2004). All others are novel candidate genes affecting longevity, and fall into a wide range of gene ontology categories, including early development, metabolism, chemosensation, immune response and transcription factors (Table 3.3).

Revertants

We re-mobilized the *P*-element insertions in three of the lines associated with increased life span ($mub^{BG00043}$, $crol^{BG00346}$ and $esg^{BG01042}$) to create revertant alleles in which the *P*-element was precisely (or nearly precisely) excised, while maintaining the co-isogenic background (Figure 2.1). We measured the life span of the revertant alleles, the parental strains, and the *P*-element insert line simultaneously. The *mub* experiments were done in Raleigh, USA, and the other two experiments were done by Dr. E. G. Pasyukova in Moscow, Russia. If the disruption of the adjacent gene by the *P*-element insertion causes the increase in life span, we expect that the life span of the revertant alleles will not be significantly different from the control. This

expectation was realized for at least one sex for each of the revertant alleles. There was at least a partial reversion to the control phenotype for almost all revertants. The cases where precise revertants did not fully revert may have been due to DNA damage from imprecise excisions outside of the sequenced region when the transposase was reintroduced or some other side effect of the excision process.

The *mub*^{BG00043} allele was associated with increased life span in both sexes. We obtained one precise revertant (*mub*^{rev1}) and one imprecise revertant (*mub*^{rev3}). Both revertant alleles had mean female life spans that fully reverted to the control, whereas the mean male life spans were intermediate between the control and mutant line (Figure 3.2A). When measured in Raleigh, the *crol*^{BG00346} allele was associated with increased life span in males, but the life span of females was not significantly different from the control (Table 3.2). When measured in Moscow, *crol*^{BG00346} males were also longer-lived than the controls, but females had significantly reduced life span (Figure 3.2B). The life span of the precise revertant allele of *crol* (*crol*^{rev4}) was not significantly different from the control in males, but was actually significantly greater than that of both the control and *crol*^{BG00346} lines in females (Figure 3.2B). Similarly, the *esg*^{BG01042} allele was associated with increased male life span in both Raleigh and Moscow, while females had decreased life span in Raleigh but were not significantly different from the control in Moscow (Table 3.2, Figure 3.2C). The life span of the precise revertant allele of *esg* (*esg*^{rev3}) was not different from the control in either sex (Fig 3.2C). Life span is sensitive to variation in environmental conditions (Vieira *et al*., 2000; Leips and Mackay, 2000); therefore, the difference in life span of female *crol*^{BG00346} and *esg*^{BG01042} flies between Raleigh and Moscow could easily be explained by differences in the two environments. It is encouraging that the increased longevity of *crol*^{BG00346} and *esg*^{BG01042} males was independently replicated in a different location.

Epistasis

We selected 10 *P*-element insertion lines in the Canton S F genetic background to assess epistatic interactions affecting life span, using a half-diallel crossing scheme in which all possible double heterozygotes were constructed

(without reciprocals) (Fedorowicz *et al*., 1998; Sambandan *et al*., 2006). The mean life span of all double heterozygote genotypes is given in Table 3.4. Analysis of variance indicated highly significant variation in life span among the double heterozygote genotypes, between males and females, and the genotype by sex interaction (Table 3.5). The effect of double heterozygous genotype was also highly significant in the individual analyses of males and females (Table 3.5). However, the estimate of the cross-sex genetic correlation was $r_{FM} = -0.276 \pm 0.146$, which is not significantly different from zero ($t_{43} = 1.88$, $P > 0.05$). Thus, the effects of the double heterozygous genotypes on life span are independent in the two sexes.

Variation among the double heterozygote genotypes can arise from two sources: variation in mean heterozygous effects of the different mutations, and variation from epistatic interactions. Since all mutations are in the same co-isogenic background, all genetic variation among the genotypes must be attributable to one of these sources. Classical diallel cross analysis enables us to partition the variation among the double heterozygous genotypes into their general (*GCA*) and specific (*SCA*) combining abilities. The *GCA* of each mutation estimates its average dominance in combination with all other mutations. The *SCA* of each double heterozygous genotype is the difference in the observed life span of the genotype from that expected given the *GCA*s of the two parental lines. Since alleles at all other loci are fixed and homozygous, any statistically significant *SCA* values must be due to (dominance × dominance) epistasis. Epistatic effects can either suppress or enhance the mutant phenotype: the former occurs when the life span of a double heterozygote genotype is less than expected (closer to the wild-type), and the latter when the life span of the double heterozygote genotype is greater than expected.

Analysis of variance revealed significant variation in *GCA* and *SCA* values when pooled over both sexes (Table 3.6). In addition to variation due to the difference in mean life span between males and females, both the *GCA* × Sex and *SCA* × Sex interaction terms were highly significant (Table 3.6). Variation attributable to both *GCA* and *SCA* was also highly significant when analyses were performed for each sex separately (Table 3.6). Estimates of individual *GCA* and *SCA* values are given in Table 3.4 and Tables 3.22 and 3.23. We identified seven

statistically significant epistatic interactions in the analysis pooled across sexes; 10 significant interactions for females and 14 significant interactions for males (Figure 3.3, Table 3.23). The observed epistatic effects are quite large. The absolute values of significant *SCA* effects are 2.95, 5.34 and 3.96 for the analyses pooled across sexes, females, and males, respectively (and are, respectively, 1.74×, 2.10× and 2.14× the average absolute *GCA* values for these analyses).

Of the seven epistatic interactions observed in the analysis pooled across sexes, four showed enhancing effects (*CG9238–mub*, *esg*–BG00297*, CG31531–*BG000297, *pyd–crol*) while three displayed suppressing effects (*CG10990–*BG00817, *CG10990–*BG00297, *CG9238–CG31531*). However, the cross-sex correlation of *SCA* values was $r_{FM} = -0.137 \pm 0.151$, which is not significantly different from zero ($t_{43} = 0.907$, $P > 0.05$). Thus, when examined separately the two sexes displayed vastly different epistatic interactions (Figure 3.3, Table 3.23). For females, we found enhancing effects between *CG10990–CG9238,* BG00817–*esg*, BG00004–*CG9238*, BG00004–*CG31531*, *pyd–crol* and BG00297–*crol* and suppressing effects between BG00817–BG00004, BG00004–BG00297, *CG9238–CG31531 and esg–mub*. In males, we found enhancing effects between *CG10990–BG00004*, *CG10990–crol*, BG00817–BG00004, BG00817–BG00297, *CG9238–pyd, CG9238–mub, esg*–BG00297, and *CG31531–*BG00297 and suppressing effects between *CG10990–*BG00297, BG00817–*esg*, BG00817–*CG31531*, BG00004–*CG9238*, BG00004–BG00297 and *mub–crol*. Thus, of the 20 significant epistatic interactions in males and/or females, only one was common to both sexes, 16 were unique to each sex, and three epistatic interactions were sexually antagonistic with enhancing effects in one sex and suppressing effects in the other (BG00817–BG00004, BG00817–*esg*, BG00004–*CG9238*).

Pleiotropy

We assessed whether mutations with significantly increased life span had pleiotropic effects on stress resistance (chill coma recovery and starvation resistance) as well as a general measure of health (climbing activity) at one week and six weeks of age. We observed substantial pleiotropy. Of the 50 lines tested for

starvation resistance, 44 were significantly different from the control at one week (16 with increased starvation resistance and 28 with decreased starvation resistance in one or both sexes), and 46 were significantly different from the control at six weeks (five with increased starvation resistance and 42 with decreased starvation resistance – one line had sexually antagonistic effects) (Figure 3.4, Tables 3.24 and 3.25). Of the 50 lines tested for chill coma recovery, 32 were significantly different from the control at one week (15 with decreased chill coma recovery times and 17 with increased chill coma recovery times), and 42 were significantly different from the control at six weeks (29 with decreased chill coma recovery times and 13 with increased chill coma recovery times) (Figure 3.4, Tables 3.26 and 3.27). We only assessed 40 of the lines for climbing activity. Of these, 23 were significantly different from the control at one week (14 with increased climbing activity and 9 with decreased climbing activity), and 30 were significantly different from the control at six weeks (28 with increased climbing activity and 2 with decreased climbing activity) (Figure 3.4, Tables 3.28 and 3.29). Thus, on average, by six weeks of age the lines with increased longevity have overall decreased resistance to starvation stress and increased resistance to chill coma stress and increased general activity relative to the controls.

There was significant variation among the lines and significant sex by line interactions for all three traits (Tables 3.7 – 3.9), indicating that the mutations do indeed have heterogeneous pleiotropic effects, and that the effects are sex-specific. Broad sense mutational heritabilities ranged from $H^2 = 0.43 – 0.60$ for starvation resistance and chill coma recovery, but were lower for climbing activity ($H^2 = 0.21$ averaged over week 1 and week 6 measurements) (Table 3.10). Although all cross-sex genetic correlations were significantly different from unity, the estimates of r_{MF} were high for all traits except for climbing activity at six weeks (Table 3.10).

If the mutations affecting increased life span are generally more robust, we would expect positive correlations between life span and stress resistance and general health, expressed as deviations from the control. Similarly, if the mutations affecting increased life span have delayed senescence, the correlations between longevity and the other traits should be positive at six weeks of age, when the

control line flies are beginning to die, but the long-lived mutant individuals are still alive. However, this was not the pattern observed. We consider the overall pleiotropic effects of the mutations separately for males and females, since the effects of the mutations on life span were not correlated between the sexes. In females, the correlation (± s.e.) between longevity and chill coma recovery time was positive and significant at both one week ($r = 0.328 \pm 0.136$, $t_{48} = 2.41$, $P = 0.0198$) and six weeks ($r = 0.418 \pm 0.131$, $t_{48} = 3.19$, $P = 0.0025$) (Table 3.11). Thus, there is a tendency for mutations affecting female life span to be inversely associated with resistance to chill coma stress, at either age. The correlation between starvation resistance and climbing activity was significant and negative at one week ($r = -0.329 \pm 0.153$, $t_{38} = 2.15$, $P = 0.0380$). None of the other correlations were significantly different from zero (Table 3.11). In males, however, the correlation (± s.e.) between longevity and starvation resistance was positive and significant at both one week ($r = 0.303 \pm 0.138$, $t_{48} = 2.20$, $P = 0.0327$) and six weeks ($r = 0.554 \pm 0.120$, $t_{48} = 4.61$, $P < 0.0001$) (Table 3.11). Further, the correlation between male life span and chill coma recovery time was negative and significant at six weeks ($r = -0.577 \pm 0.118$, $t_{48} = 4.90$, $P < 0.0001$) (Table 3.11). Thus, mutations affecting male life span do show the expected positive associations with stress resistance and delayed senescence for stress resistance. However, the correlation between male starvation stress resistance and climbing activity was significant and negative at one week ($r = -0.619 \pm 0.127$, $t_{38} = 4.86$, $P < 0.0001$); *i.e.*, mutations associated with increased stress resistance were less active (Table 3.11).

The combination of significant pleiotropy but little directional correlation between longevity and other traits indicates that the pleiotropic effects are highly variable, as illustrated in Figs. 3.5 – 3.8. The complex pattern of variation in pleiotropic effects among the *P*-insert lines associated with increased life span in at least one sex is depicted in Figure 3.4. Notably, all of the mutations are associated with at least one deleterious pleiotropic effect on stress resistance or general health, indicating the complicated mutational basis of trade-offs between putative fitness components.

Transcriptional Co-regulation

We assessed the extent to which seven of the mutations associated with increased life span (*pyd*^{BG00028}, *mub*^{BG00043}, *crol*^{BG00346}, *CG10990*^{BG00495}, *CG9238*^{BG00761}, BG00817 and *esg*^{BG01042}) affected whole genome transcriptional regulation. Co-regulated transcripts are in turn candidate genes affecting longevity, and the clustering of co-regulated transcripts can yield insight as to the function of predicted genes. We assessed whole genome transcript abundance at six weeks of age for all mutant lines and the Canton S F co-isogenic control – the age at which the control lines are beginning to die, but at which most of the *P*-element insert lines remain alive. This is the same age at which we assessed differences among these lines in senescence for resistance to starvation and chill coma stress, and climbing activity. The survival curves for these mutants are given in Figure 3.9.

Not all transcripts on the array are expressed in six week old adults. We eliminated all transcripts that were not considered present in both replicates of at least one line and sex, leaving 12,636 transcripts for analysis. First, we performed two-way ANOVA on each of these transcripts, to assess how many transcripts varied significantly among all lines for the main effects of sex and genotype, and the sex by genotype interaction, using a false discovery rate criterion to account for multiple tests (Storey and Tibshirani, 2003). At a q-value of 0.001 (0.0001), we found 11,111 (10,603) sexually dimorphic transcripts. Clearly, the sexes represent different gene expression environments. Remarkably, genotype was significant for 4,488 transcripts (35.5%) at $q \leq 0.001$, and 1,996 transcripts (15.8%) at $q \leq 0.0001$. The genotype by sex interaction was significant for 1,621 transcripts (12.8%) at $q \leq 0.001$, and 434 transcripts (3.4%) at $q \leq 0.0001$. We also ran reduced ANOVAs separately for each sex, and for each of the mutant lines compared to the control. A total of 619 and 561 transcripts were significant at $q \leq 0.001$ for females and males, respectively. The magnitude of transcriptional co-regulation varied among the mutant lines. At a significance level of $q \leq 0.05$, we observed 276 significant transcripts for *CG10990*^{BG00495}; 313 for *pyd*^{BG00028}, 777 for *CG9238*^{BG00761}, 2,141 for *crol*^{BG00346}, 2,193 for *esg*^{BG01042}, and 4,750 for *mub*^{BG00043}.

We analyzed the Biological Process Gene Ontology (GO) categories represented by the significant transcripts to determine if particular categories are over-represented. In the separate sex analyses of all genotypes, there was over-representation of significant transcripts in the DNA integration, metabolism (particularly carbohydrate metabolism) and proteolysis categories in both sexes (Tables 3.12 and 3.13). Genes affecting detection of external stimuli, particularly light and abiotic stimuli, were enriched in females (Table 3.12), while genes affecting mating and reproductive behavior and muscle development were enriched in males (Table 3.13).

Although all of the mutations assessed are long-lived, they have variable and sex-specific pleiotropic effects on longevity, resistance to starvation and chill coma stress, and climbing activity (Figs. 3.4, 3.10). Thus, we expected to find both common and variable patterns of transcriptional co-regulation among the mutations. This is indeed what we observed.

pyd affects the biological processes of the JNK cascade, dorsal closure, electron transport and establishment and/or maintenance of cell polarity (Table 3.3). Perhaps unexpectedly, over-represented co-regulated transcripts in the $pyd^{BG00028}$ mutant background fell into the categories of DNA integration; prosthetic group, pyruvate, nucleoside, lipid, chitin and glucosamine metabolism; proteolysis; and mating and reproductive behavior (Table 3.14).

mub is involved in multiple biological processes: histone mRNA 3'-end processing, induction of apoptosis, mRNA polyadenylylation, protein targeting, regulation of alternative nuclear mRNA splicing via the spliceosome, regulation of progression through the cell cycle, regulation of translation, signal transduction, and transcription from the RNA polymerase II promoter (Table 3.3). Categories that are over-represented among co-regulated $mub^{BG00043}$ probe sets are consistent with this annotation, and include DNA replication and repair, RNA processing, the cell cycle, and chromatin modification and silencing. However, the largest over-represented categories in this mutation were in DNA, RNA, cellular and macromolecular metabolism (Table 3.15).

crol is an RNA polymerase II transcription factor that has pleiotropic effects on cell adhesion and proliferation, homoiothermy, mesoderm development, regulation of transcription, response to freezing, skeletal development and wing morphogenesis. Over-represented transcripts in the *crol*^{BG00346} mutation primarily affect ribosome biogenesis, histone mRNA 3′ end processing and metabolism, transcription, protein metabolism and proteolysis, sleep, and reproductive and mating behavior (Table 3.16).

CG10990 is a predicted gene inferred to affect the induction of apoptosis and protein biosynthesis and metabolism (Table 3.3). The top over-represented GO categories in *CG10990*^{BG00495} mutants are DNA integration, peptidyl-proline modification and amino acid derivative metabolism; but insulin signaling, proteolysis, and mating, reproductive and locomotor behavior are also over-represented (Table 3.17).

CG9238 is a predicted gene that is annotated to affect glycogen metabolism. Although aspects of metabolism are indeed over-represented among transcripts that are co-regulated in the *CG9238*^{BG00761} mutant background, genes affecting embryonic and larval development, as well as visual, locomotor, mating, reproductive and rhythmic (circadian) behaviors are also over-represented (Table 3.18).

We do not know the exact insertion site of the *P*-element in line BG00817. However, many GO categories associated with muscle development are highly over-represented among significant co-regulated transcripts in this line. Lipid catabolism, proteolysis, and lipid, carbohydrate and protein metabolism are also over-represented (Table 3.19).

esg is an RNA polymerase II transcription factor with pleiotropic effects on multiple biological processes: electron transport, fusion cell fate specification, maintenance of imaginal histoblast diploidy, neuroblast division, peripheral nervous system development, regulation of transcription from the RNA polymerase II promoter, tracheal branch fusion and tracheal system development. The large number of over-represented GO categories among the co-regulated transcripts in the *esg*^{BG01042} mutation is consistent with highly pleiotropic functions of *esg*. Genes

involved in RNA processing and localization, ribosome biogenesis, RNA and DNA metabolism, primary metabolism and fertilization are over-represented. However, the most significant over-representation of co-regulated transcripts in *esg*^{BG01042} is related to vision (response to light, visual perception, phototransduction) (Table 3.20).

We assessed the extent to which the same genes were co-regulated in the background of the seven mutations by performing Tukey's tests for each of the 1996 probe sets that were significant for the main effect of genotype at $q \leq 0.0001$ in the analyses pooled across sexes. Via Tukey's criteria, 1970 probe sets had at least one mutant line significantly different from the control (pooled across sexes). Most probe sets that were significant were shared in more than one line. In females, there were 14 probe sets unique to *pyd*^{BG00028}, 51 unique to *mub*^{BG00043}, 11 unique to *crol*^{BG00346}, 6 unique to *CG10990*^{BG00495}, 13 unique to *CG9238*^{BG00761}, 21 unique to BG00817 and 10 unique to *esg*^{BG01042}. In contrast, the number of significant probe sets shared by the focal line and at least one other line were 222 for *pyd*^{BG00028}, 380 for *mub*^{BG00043}, 221 for *crol*^{BG00346}, 133 for *CG10990*^{BG00495}, 149 for *CG9238*^{BG00761}, 214 for BG00817 and 275 for *esg*^{BG01042}. A total of 16 probe sets were in common to all of the mutant lines. Similarly, in males, there were 47 probe sets unique to *pyd*^{BG00028}, 4 unique to *mub*^{BG00043}, 44 unique to *crol*^{BG00346}, 4 unique to *CG10990*^{BG00495}, 7 unique to *CG9238*^{BG00761}, 48 unique to BG00817 and 2 unique to *esg*^{BG01042}. The number of significant probe sets shared by the focal line and at least one other line were 264 for *pyd*^{BG00028}, 161 for *mub*^{BG00043}, 222 for *crol*^{BG00346}, 218 for *CG10990*^{BG00495}, 190 for *CG9238*^{BG00761}, 217 for BG00817 and 129 for *esg*^{BG01042}. A total of 25 probe sets were in common to all of the mutant lines. Although the numbers of probe sets shared by 6 or all 7 mutations are small, they are enriched for genes affecting lipid catabolism and metabolism, DNA integration, sex determination, mating and reproductive behavior, and neurotransmission (Figure 3.11, data not shown).

Since genes that are co-regulated in the mutant backgrounds are themselves candidate genes affecting life span, we tabulated variation in gene expression of known genes affecting life span in the mutant lines associated with increased life

span (Table 3.21). First, five of the six focal genes for which we know the genes tagged by the *P*-element (*pyd*, *mub*, *CG10990*, *CG9238* and *esg*) are themselves significantly differentially expressed in the analysis considering all genotypes. Three of these genes (*mub*, *CG10990* and *CG9238*) are also differentially expressed relative to the control in their own mutant backgrounds; further, *mub* is differentially expressed in the $pyd^{BG00028}$ $esg^{BG01042}$ mutant lines, and *esg* is differentially expressed in the $pyd^{BG00028}$, $crol^{BG00346}$ and $CG9238^{BG00761}$ mutant lines. Six additional genes in which *P*-element mutations were associated with increased life span in our screen were differentially regulated among the seven mutations profiled in the array analysis (*CG31531*, *Trapped in endoderm-1*, *CG18418*, *meiotic from via Salaria 332*, *kayak* and *Dek*). A further 13 genes in which *P*-element insertions were associated with decreased life span had differentially regulated transcripts in the mutant backgrounds (*CG14478*, *CG31176*, *CG4004*, *CG6854*, *couch potato*, *inaF*, *ken and barbie*, *Laminin A*, *Lipid storage droplet-2*, *Malic enzyme*, *Protein kinase 61C*, *Rab23* and *singed*). Finally, eight genes in which mutations have been described to negatively regulate life span were also differentially co-regulated in the mutant backgrounds (*I'm not dead yet*, *chico*, *Insulin-like receptor*, *Superoxide dismutase*, *Alcohol dehydrogenase*, *Sirt2*, *Vacuolar H^+-ATPase SFD subunit* and *CTP:phosphocholine cytidylyltransferase 1*).

TABLE 3.1

Analyses of variance of life span of 1,332 *P{GT1}* insertion lines

Analysis	Source	d.f.	MS	*F*	*P*	σ^{2a}
Sexes pooled	Sex	1	274.89	6.11	0.0135	—
	Line	1331	241.89	5.37	< 0.0001	31.39
	Line×Sex	1312	101.56	2.26	< 0.0001	25.24
	Error	3301	45.02	—	—	45.02
Males	Line	1321	164.50	4.75	< 0.0001	57.93
	Error	1643	34.60	—	—	34.60
Females	Line	1322	180.11	3.25	< 0.0001	55.37
	Error	1658	55.36	—	—	55.36

[a]Variance component

TABLE 3.2

Increased life span of *P{GT1}* insertion lines

Line	Mutational Effects						*P*-values from ANOVA					Category
	Life Span (% [a])			a/σ_p [b]			Sexes Pooled [c]			Sexes Separate		
	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀	
BG00004 (F)	60.1 (9.0)	61.7 (16.6)	58.4 (2.3)	0.18	0.36	0.04	ns	*	ns	**	ns	both sexes
BG00008 (F)	60.6 (9.8)	64.1 (21.1)	57.1 (0.1)	0.20	0.46	0.00	ns	***	**	****	ns	male-specific
BG00010 (F)	63.1 (14.4)	64.6 (22.1)	61.6 (8.0)	0.29	0.48	0.15	ns	****	ns	****	ns	both sexes
BG00028 (F)	61.8 (12.1)	67.7 (27.8)	56.4 (–1.2)	0.24	0.61	–0.02	*	****	****	****	ns	male-specific
BG00037 (F)	60.6 (10.0)	54.6 (3.3)	66.5 (16.5)	0.20	0.07	0.32	****	***	*	ns	****	female-specific
BG00039 (F)	59.5 (8.0)	52.6 (–0.5)	66.3 (16.2)	0.16	–0.01	0.31	****	*	**	ns	***	female-specific
BG00041 (F)	57.2 (3.7)	60.7 (14.7)	53.7 (–5.8)	0.07	0.32	–0.11	ns	ns	**	***	ns	male-specific
BG00042 (F)	64.1 (16.3)	70.6 (33.4)	57.8 (1.2)	0.33	0.73	0.02	*	****	***	****	ns	male-specific
BG00043 (F)	66.9 (21.4)	67.3 (27.2)	66.6 (16.7)	0.43	0.60	0.32	ns	****	ns	****	****	both sexes
BG00080 (B)	63.1 (8.3)	58.3 (7.7)	66.7 (6.9)	0.24	0.18	0.33	****	*	ns	ns	**	both sexes
BG00106 (F)	65.3 (19.8)	65.2 (25.6)	65.4 (14.6)	0.40	0.68	0.26	ns	****	ns	****	*	both sexes
BG00121 (F)	68.3 (3.7)	68.0 (9.3)	68.6 (–1.2)	0.12	0.27	–0.05	**	*	**	**	ns	male-specific
BG00218 (F)	61.7 (12.6)	61.8 (22.8)	61.6 (4.6)	0.32	0.65	0.13	****	****	****	****	*	male-biased
BG00297 (F)	62.9 (14.0)	65.5 (23.8)	60.3 (5.6)	0.28	0.52	0.11	ns	****	**	****	ns	male-specific
BG00336 (B)	66.7 (14.6)	64.2 (18.6)	69.3 (11.0)	0.42	0.44	0.54	****	****	ns	****	***	both sexes
BG00346 (F)	66.7 (12.8)	67.4 (26.8)	65.8 (1.5)	0.27	0.57	0.04	***	****	****	****	ns	male-specific
BG00472 (F)	60.5 (9.7)	62.5 (18.0)	58.5 (2.4)	0.19	0.40	0.05	ns	**	*	****	ns	male-specific
BG00495 (F)	70.2 (27.3)	70.2 (32.7)	70.1 (22.9)	0.55	0.72	0.44	ns	****	ns	****	****	both sexes
BG00528 (B)	67.4 (11.1)	68.0 (0.6)	66.9 (22.8)	0.24	0.01	0.63	****	****	***	ns	****	female-specific

Table 3.2 (continued)

Line	Mutational Effects						*P*-values from ANOVA					Category
	Life Span (% [a])			a/σ_p [b]			Sexes Pooled [c]			Sexes Separate		
	♂,♀	♂	♀	♂,♀	♂	♀	***S***	***L***	***L*×*S***	***L*♂**	***L*♀**	
BG00719 (F)	65.4 (–0.7)	65.6 (5.5)	65.1 (–6.2)	–0.02	0.16	–0.25	**	ns	**	*	*	sex-antagonistic
BG00757 (F)	60.7 (10.2)	67.1 (26.8)	54.5 (–4.5)	0.20	0.59	–0.09	*	**	****	****	ns	male-specific
BG00761 (F)	63.1 (14.5)	60.3 (14.0)	65.8 (15.3)	0.29	0.31	0.29	**	****	ns	**	****	both sexes
BG00767 (B)	69.2 (13.9)	71.5 (5.8)	66.7 (22.6)	0.30	0.13	0.62	****	****	**	*	****	female-biased
BG00817 (F)	69.2 (5.1)	70.1 (12.7)	68.3 (–1.7)	0.16	0.37	–0.07	*	*	**	***	ns	male-specific
BG00864 (B)	59.9 (–1.3)	57.5 (–14.9)	62.3 (14.4)	–0.03	–0.35	0.40	*	ns	****	****	**	sex-antagonistic
BG00890 (F)	60.9 (10.4)	63.0 (19.1)	58.8 (3.0)	0.21	0.42	0.06	ns	**	*	***	ns	male-specific
BG00907 (F)	61.4 (12.5)	65.8 (26.7)	57.4 (0.6)	0.25	0.71	0.01	ns	***	***	****	ns	male-specific
BG00915 (F)	64.0 (16.1)	66.7 (26.0)	61.3 (7.4)	0.32	0.57	0.14	ns	****	*	****	ns	male-specific
BG01004 (F)	59.6 (9.3)	61.3 (18.0)	58.0 (1.5)	0.19	0.48	0.03	ns	**	*	****	ns	male-specific
BG01030 (A)	62.7 (3.0)	64.8 (5.3)	74.8 (27.7)	0.08	0.15	0.64	*	****	****	ns	****	female-specific
BG01031 (A)	69.8 (16.3)	69.1 (13.3)	55.6 (–8.4)	0.35	0.34	–0.16	**	ns	**	**	ns	male-specific
BG01042 (F)	57.1 (3.9)	61.0 (21.1)	53.4 (–9.8)	0.10	0.55	–0.29	ns	ns	****	****	**	sex-antagonistic
BG01085 (F)	60.9 (11.7)	62.3 (20.1)	59.4 (4.1)	0.24	0.53	0.07	ns	**	*	****	ns	male-specific
BG01121 (F)	63.5 (0.6)	66.3 (6.6)	60.6 (–5.0)	0.02	0.23	–0.13	ns	ns	*	**	ns	male-specific
BG01283 (F)	61.7 (12.3)	60.5 (20.2)	62.8 (6.1)	0.32	0.53	0.18	**	****	*	****	ns	male-specific
BG01345 (A)	72.9 (21.3)	74.8 (21.5)	70.8 (21.0)	0.45	0.55	0.38	*	****	ns	****	****	both sexes
BG01403 (A)	68.2 (13.6)	65.9 (7.1)	70.5 (20.4)	0.29	0.18	0.37	ns	****	*	*	****	female-biased
BG01540 (B)	67.3 (4.6)	60.5 (–5.3)	73.5 (13.4)	0.14	–0.16	0.46	****	ns	****	ns	****	female-specific
BG01550 (F)	61.2 (–3.0)	66.1 (6.2)	56.4 (–11.6)	–0.09	0.21	–0.30	ns	ns	**	*	ns†	sex-antagonistic
BG01551 (F)	66.4 (5.2)	68.4 (10.0)	64.2 (0.6)	0.15	0.35	0.01	ns	*	ns	**	ns	both sexes
BG01553 (F)	59.9 (8.9)	58.8 (16.7)	61.0 (3.0)	0.23	0.44	0.09	****	***	*	****	ns	male-specific
BG01615 (A)	70.3 (17.0)	75.2 (22.0)	65.5 (11.8)	0.36	0.57	0.22	**	****	ns	****	*	both sexes

Table 3.2 (continued)

Line	Mutational Effects						*P*-values from ANOVA					Category
	Life Span (% [a])			a/σ_p [b]			Sexes Pooled [c]			Sexes Separate		
	♂,♀	♂	♀	♂,♀	♂	♀	***S***	***L***	***L*×*S***	***L*♂**	***L*♀**	
BG01677 (A)	71.7 (19.4)	72.0 (16.9)	71.4 (21.9)	0.41	0.44	0.40	ns	****	ns	****	****	both sexes
BG01700 (F)	70.6 (11.9)	73.2 (17.6)	68.2 (6.9)	0.35	0.61	0.18	ns	****	*	****	*	male-biased
BG01701 (F)	70.0 (11.0)	65.8 (5.8)	74.0 (15.8)	0.32	0.20	0.41	ns	*	ns	*	ns†	both sexes
BG01702 (A)	61.4 (1.0)	66.0 (8.2)	56.5 (–7.0)	0.03	0.24	–0.17	**	ns	**	*	ns	male-specific
BG01710 (A)	64.1 (5.3)	62.9 (3.2)	65.1 (7.2)	0.14	0.09	0.17	ns	*	ns	ns	*	both sexes
BG01878 (B)	65.7 (8.3)	63.5 (–6.1)	68.0 (24.9)	0.18	–0.14	0.69	**	***	****	ns	****	female-specific
BG01918 (A)	63.8 (4.8)	66.5 (9.0)	61.3 (1.0)	0.13	0.26	0.02	ns	ns†	ns	*	ns	male-specific
BG01950 (B)	52.7 (–13.1)	47.5 (–29.8)	63.1 (15.9)	–0.29	–0.69	0.44	ns	***	****	****	****	sex-antagonistic
BG01976 (B)	64.5 (6.2)	61.4 (–9.2)	67.5 (24.0)	0.13	–0.21	0.66	*	*	****	*	****	sex-antagonistic
BG02019 (B)	62.5 (3.0)	63.1 (–6.7)	62.0 (14.0)	0.07	–0.16	0.39	****	ns	****	ns	****	female-specific
BG02039 (A)	66.3 (9.0)	67.5 (10.7)	65.2 (7.3)	0.23	0.31	0.18	ns	***	ns	**	ns	both sexes
BG02049 (B)	68.1 (12.2)	71.3 (5.5)	65.1 (19.6)	0.27	0.13	0.54	****	****	*	ns	****	female-specific
BG02128 (B)	63.8 (5.0)	67.2 (–0.7)	60.2 (10.7)	0.11	–0.02	0.30	****	ns	*	ns	***	female-specific
BG02257 (B)	63.5 (8.9)	63.7 (10.3)	63.3 (7.4)	0.25	0.30	0.20	ns	****	ns	****	**	both sexes
BG02395 (B)	62.8 (8.0)	59.7 (10.4)	65.3 (4.6)	0.23	0.24	0.23	****	**	ns	*	ns	both sexes
BG02644 (B)	61.3 (5.2)	61.1 (12.9)	61.4 (–1.5)	0.15	0.30	–0.07	***	*	***	***	ns	male-specific

Candidate genes used for analysis of epistasis are shown in bold font. Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$. Red font denotes *P*-values that are significant after Bonferroni correction for multiple tests.

TABLE 3.3

P{GT1} insertion lines with increased life span

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	Biological Process
BG00004 (F)	32F3	No gene in region			
BG00008 (F)	85B7	*polychaetoid*	630 bp upstream	guanylate kinase activity; oxidoreductase activity; protein binding; structural molecule activity	JNK cascade; dorsal closure; electron transport; establishment and/or maintenance of cell polarity
BG00010 (F)	82D1	*CG31531*	170 bp upstream	Unknown	Unknown
BG00028 (F)	85B7	*polychaetoid*	630 bp upstream	See BG00008	See BG00008
BG00037 (F)	70E2	*CG9238*	230 bp upstream	protein phosphatase 1 binding; protein phosphatase type 1 regulator activity	glycogen metabolism
BG00039 (F)	79A2	*mushroom body expressed*	1.1 kb into gene (1st intron)	poly(rC) binding	histone mRNA 3'-end processing; induction of apoptosis; mRNA polyadenylylation; protein targeting; regulation of alternative nuclear mRNA splicing, via spliceosome; regulation of progression through cell cycle; regulation of translation; signal transduction; transcription from RNA polymerase II promoter
BG00041 (F)	79A2	*mushroom body expressed*	1 kb into gene (1st intron)	See BG00039	See BG00039
BG00042 (F)	79A2	*mushroom body expressed*	1 kb into gene (1st intron)	See BG00039	See BG00039
BG00043 (F)	79A2	*mushroom body expressed*	1 kb into gene (1st intron)	See BG00039	See BG00039
BG00080 (B)	82E6	No gene in region			
BG00106 (F)	79A2	*mushroom body*	1 kb into gene (1st	See BG00039	See BG00039

Table 3.3 (continued)

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	Biological Process
		expressed	intron)		
BG00121 (F)	77E-F	No sequence			
BG00218 (F)	5A12	*Trapped in endoderm 1* / *Gustatory receptor 5a*	Adjacent *Trapped in endoderm 1* / 750 bp upstream *Gustatory receptor 5a*	G-protein coupled receptor activity, unknown ligand/ G-protein coupled receptor activity; taste receptor activity	G-protein coupled receptor protein signaling pathway; germ cell development; germ cell migration; germ cell programmed cell death; pole cell migration / G-protein coupled receptor protein signaling pathway; detection of chemical stimulus during sensory perception of taste
BG00297 (F)	75B7	No gene in region			
BG00336 (B)	64B13	*CG18418* / *Guanine nucleotide exchange factor 64C*	1.6 kb upstream *CG18418* / 1.1 kb upstream *Guanine nucleotide exchange factor 64C*	binding; carrier activity; oxoglutarate:malate antiporter activity / Rho guanyl-nucleotide exchange factor activity	alpha-ketoglutarate transport; cation transport; malate transport; mitochondrial transport / axon guidance; regulation of Rho protein signal transduction
BG00346 (F)	33A2	*crooked legs* / *CG14939*	60 bp into gene (1st exon) *crooked legs* / 700 bp downstream from *CG14939*	RNA polymerase II transcription factor activity; ice binding; nucleic acid binding; zinc ion binding / Unknown	cell adhesion; cell proliferation; homoiothermy; mesoderm development; regulation of transcription from RNA polymerase II promoter; response to freezing; skeletal development; wing morphogenesis / Unknown
BG00472 (F)	6D8	No gene in region			
BG00495 (F)	12B4	*CG10990*	8 kb into gene (3rd	nucleic acid binding; translation	induction of apoptosis;

Table 3.3 (continued)

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	**Biological Process**
			intron)	elongation factor activity	protein biosynthesis; protein metabolism
BG00528 (B)	83E2	*Osiris 9*	150 bp upstream	Unknown	Unknown
BG00719 (F)	Unknown	No sequence			
BG00757 (F)	Unknown	No sequence			
BG00761 (F)	70E2	*CG9238*	200 bp upstream	**See** BG00037	**See** BG00037
BG00767 (B)	64B13	*CG18418* / *Guanine nucleotide exchange factor 64C*	1.6 kb upstream *CG18418* / 1.1 kb upstream *Guanine nucleotide exchange factor 64C*	See BG00336	See BG00336
BG00817 (F)	88A	No sequence			
BG00864 (B)	42E5	*Tetraspanin 42Ef*	12 bp into gene (1st exon)	Unknown	Unknown
BG00890 (F)	Unknown	No sequence			
BG00907 (F)	Unknown	No sequence			
BG00915 (F)	50B1	*CG13334* / *CG13333*	350 bp upstream *CG13334* / 850 bp downstream *CG13333*	L-lactate dehydrogenase activity / Unknown	glycolysis; tricarboxylic acid cycle intermediate metabolism / Unknown
BG01004 (F)	32E1	No gene in region			
BG01030 (A)	58D2	*CG13503* / *mei-S332*	400 bp upstream *CG13503* / 850 bp into gene (2nd exon) *mei-S332*	Unknown / Unknown	actin filament organization; regulation of cell shape / female meiosis; male meiosis; meiotic chromosome segregation; meiotic metaphase I; sister chromatid cohesion
BG01031 (A)	47A13	*pipsqueak*	7.3 kb into gene (3rd intron)	DNA binding; protein binding; transcription factor activity	gene silencing; pole plasm assembly; regulation of transcription, DNA-dependent
BG01042 (F)	35D2	*escargot*	500 bp downstream	DNA binding; RNA polymerase II transcription factor activity; electron carrier activity; specific RNA polymerase II transcription	electron transport; fusion cell fate specification; maintenance of imaginal histoblast diploidy;

Table 3.3 (continued)

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	**Biological Process**
				factor activity; zinc ion binding	neuroblast division; peripheral nervous system development; regulation of transcription from RNA polymerase II promoter; tracheal branch fusion; tracheal system development (sensu Insecta)
BG01085 (F)	Unknown	No sequence			
BG01121 (F)	86C7	*CG14696* / *CG4674*	200 bp upstream *CG14696*/ 500 bp upstream *CG4674*	Unknown / Unknown	Unknown / Unknown
BG01283 (F)	9B11-12	*l(1)G0289* / *CG32679*	60 bp into gene (1st exon) *l(1)G0289* / 250 bp downstream *CG32679*	Unknown / Unknown	Unknown / defense response
BG01345 (A)	75B4	*Ecdysone-induced protein 75B*	30 kb into gene (1st intron)	ligand-dependent nuclear receptor activity; sequence-specific DNA binding; specific RNA polymerase II transcription factor activity; steroid hormone receptor activity; transcription factor activity; zinc ion binding	antimicrobial humoral response; cell proliferation; cell-cell signaling; ecdysis (sensu Insecta); ecdysone-mediated induction of salivary gland cell autophagic cell death; intracellular signaling cascade; molting cycle (sensu Insecta); oogenesis (sensu Insecta); regulation of ecdysteroid metabolism; regulation of transcription from RNA polymerase II promoter; regulation of transcription, DNA-dependent
BG01403 (A)	50B2	No gene in region			

Table 3.3 (continued)

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	Biological Process
BG01540 (B)	13F1	*scalloped*	350 bp upstream	specific RNA polymerase II transcription factor activity; transcription factor activity	nervous system development; regulation of transcription from RNA polymerase II promoter; sensory organ development; wing morphogenesis
BG01550 (F)	99B11	*kayak*	5.3 kb into gene (1st intron)	DNA binding; RNA polymerase II transcription factor activity; protein binding; protein heterodimerization activity; sequence-specific DNA binding; specific RNA polymerase II transcription factor activity; transcription factor activity; transcription factor binding	JNK cascade; R3/R4 cell fate commitment; antimicrobial humoral response; central nervous system development; dorsal closure; establishment of planar polarity; follicle cell migration (sensu Insecta); head involution; imaginal disc fusion, thorax closure; midgut development; regulation of transcription, DNA-dependent; response to wounding; wound healing
BG01551 (F)	30F5	*CG13130* / *big brain*	35 bp in 1st exon of *CG13130* / 25 bp upstream *big brain*	Unknown / binding; carrier activity; nonselective cation channel activity; water channel activity	Unknown / Notch signaling pathway; cell homeostasis; ectoderm development; mesoderm development; nervous system development; ovarian follicle cell development (sensu Insecta); transport
BG01553 (F)	88A5	*forkhead box, sub-group O*	100 bp into gene (1st exon)	sequence-specific DNA binding; transcription factor activity	determination of adult life span; induction of apoptosis; insulin receptor signaling pathway; negative

Table 3.3 (continued)

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	**Biological Process**
BG01615 (A)	39D2	*nervana 3*	110 bp into gene (1st exon)	sodium:potassium-exchanging ATPase activity	regulation of body size; negative regulation of cell proliferation; negative regulation of cell size; negative regulation of insulin receptor signaling pathway; regulation of cell growth; regulation of insulin receptor signaling pathway; regulation of progression through cell cycle; regulation of transcription, DNA-dependent; response to stress cation transport; potassium ion transport; sodium ion transport
BG01677 (A)	9B12	*CG17841*	Adjacent	Unknown	Unknown
BG01700 (F)	49F10	*CG4630* / *CG4646*	200 bp upstream /40 bp downstream	carbohydrate transporter activity; carnitine transporter activity; cation transporter activity / unknown	anion transport; carbohydrate metabolism; carbohydrate transport; cation transport; extracellular transport / unknown
BG01701 (F)	18C1	No gene in region			
BG01702 (A)	53D14	*Dek*	1 kb into gene (2nd intron)	DNA binding	mRNA processing
BG01710 (A)	61E1	*Glucose transporter 1*	45 kb into gene (3rd intron)	GTP binding; glucose transporter activity; protein binding; sugar porter activity	carbohydrate metabolism; carbohydrate transport; glucose transport; regulation of cell proliferation; regulation of progression through cell cycle

Table 3.3 (continued)

Line	Cytological Location	Nearest Gene(s)	*P{GT1}* Insertion Site Relative to Gene(s)	Gene Ontology	
				Molecular Function	Biological Process
BG01878 (B)	Unknown	No sequence			
BG01918 (A)	23A3	*Phosphoglycerate kinase*	1.8 kb upstream *P*	carbohydrate kinase activity; phosphoglycerate kinase activity	glycolysis; phosphorylation
BG01950 (B)	Unknown	No sequence			
BG01976 (B)	Unknown	No sequence			
BG02019 (B)	25F5	*CG9171* / *CG14005*	15 bp in gene (1st exon) *CG9171* / 300 bp downstream *CG14005*	N-acetyllactosaminide beta-1,6-N-acetylglucosaminyltransferase activity / unknown	unknown / unknown
BG02039 (A)	58E9	*Defense repressor 1*	22 kb into gene (1st intron)	protein binding; structural constituent of cytoskeleton; zinc ion binding	immune response; transmission of nerve impulse
BG02049 (B)	Unknown	No sequence			
BG02128 (B)	12E5	*CG32604*	17.4 kb into gene (2nd intron)	ATP binding; ATP-dependent RNA helicase activity; ATP-dependent helicase activity; RNA splicing factor activity, transesterification mechanism	nuclear mRNA splicing, via spliceosome
BG02257 (B)	5A12	*Trapped in endoderm 1* / *Gustatory receptor 5a*	Adjacent *Trapped in endoderm 1* / 750 bp upstream *Gustatory receptor 5a*	See BG00218	See BG00218
BG02395 (B)	2B17	No gene in region			
BG02644 (B)	57E6	*Fkbp13*	400 bp into gene (1st intron)	FK506 binding; calcium ion binding; peptidyl-prolyl cis-trans isomerase activity	protein folding

Candidate genes used for analysis of epistasis are shown in bold font. Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion.

TABLE 3.4

Diallel cross between ten *P{GT1}* insertion lines with increased life span

A. Pooled sexes

	Male parent										
Female parent	**BG00817**	**BG00004**	***CG9238***	***esg***	***CG31531***	***pyd***	***mub***	**BG00297**	***crol***	T_i	***GCA***
CG10990	69.39	72.48	72.45	71.31	73.48	70.36	69.01	65.44	70.05	633.97	–0.6638
BG00817		73.81	72.39	72.49	72.32	73.12	73.73	72.30	72.57	652.11	**1.5670**
BG00004			73.46	71.35	74.85	72.51	69.95	64.72	70.40	643.52	0.4812
CG9238				69.59	68.87	72.33	73.76	68.61	70.15	641.62	0.2563
esg					72.81	68.74	68.39	70.89	68.09	633.66	–0.7269
CG31531						70.87	71.16	73.15	74.35	651.87	**1.5870**
pyd							70.28	67.72	74.06	640.00	0.0561
mub								70.27	68.54	635.10	–0.5655
BG00297									70.99	624.09	**–1.9396**
crol										639.22	–0.0518

Table 3.4 (continued)

B. Females

	Male parent										
Female parent	**BG00817**	**BG00004**	***CG9238***	***esg***	***CG31531***	***pyd***	***mub***	**BG00297**	***crol***	T_i	***GCA***
CG10990	68.45	71.90	79.18	69.98	75.00	70.69	68.80	67.38	65.25	636.62	0.5955
BG00817		70.28	75.58	73.33	73.50	72.00	72.92	68.85	67.95	642.85	1.2635
BG00004			82.48	70.77	78.28	73.20	71.76	63.80	70.30	652.76	**2.4923**
CG9238				69.31	67.71	71.50	74.02	71.02	68.18	658.98	**3.2779**
esg					70.18	63.54	61.31	65.38	62.68	606.47	**–3.2864**
CG31531						68.31	69.50	70.03	71.75	644.26	**1.5548**
pyd							70.84	65.08	73.68	628.84	–0.4742
mub								70.56	66.44	626.16	–0.8261
BG00297									70.22	612.31	**–2.5550**
crol										616.44	**–2.0422**

Table 3.4 (continued)

C. Males

	Male parent										
Female parent	**BG00817**	**BG00004**	***CG9238***	***esg***	***CG31531***	***pyd***	***mub***	**BG00297**	***crol***	T_i	***GCA***
CG10990	70.35	73.05	66.05	72.65	72.74	70.03	69.23	63.50	74.97	632.58	**–1.8068**
BG00817		77.62	69.21	71.64	71.10	74.24	74.54	75.84	77.08	661.62	**1.8424**
BG00004			64.21	71.92	71.41	71.82	68.22	65.68	70.51	634.46	**–1.5666**
CG9238				69.91	69.98	73.18	73.49	66.20	72.18	624.40	**–2.8056**
esg					75.51	74.23	76.06	76.54	73.37	661.82	**1.9000**
CG31531						73.50	72.87	76.20	76.82	660.13	**1.6623**
pyd							69.75	70.38	74.44	651.54	0.5674
mub								69.97	70.60	644.73	–0.2794
BG00297									71.77	636.08	**–1.3586**
crol										661.73	**1.8449**

The table lists mean life span of double heterozygous genotypes and estimated *GCA* values for (A) sexes pooled; (B) females and (C) males. Parental homozygous *P{GT1}* insertion lines are indicated on the top row and first column of each panel. T_i and *GCA* are defined in the text. Significant *GCA* values are indicated in bold font (see Table 3.22).

TABLE 3.5

Analyses of variance of life span of double heterozygote genotypes

Analysis	Source	d.f.	SS	*F*	*P*
Pooled Sexes	Genotype	44	18338.5	5.70	< 0.0001
	Sex	1	2335.2	31.94	< 0.0001
	Genotype × Sex	44	32427.4	10.08	< 0.0001
	Rep (Genotype × Sex)	78	259583.8	45.51	< 0.0001
	Error	3316	242467.4	—	—
Females	Genotype	44	30021.3	7.06	< 0.0001
	Rep (Sex)	39	152482.6	40.48	< 0.0001
	Error	1655	15986.5	—	—
Males	Genotype	44	20712.2	9.47	< 0.0001
	Rep (Sex)	39	107101.2	55.22	< 0.0001
	Error	1661	82606.9	—	—

TABLE 3.6

Analyses of variance of general and specific combining abilities

Analysis	Source	d.f.	SS	*F*	*P*
Pooled Sexes	*GCA*	9	6427.9	9.76	< 0.0001
	SCA	35	11770.5	4.60	< 0.0001
	Sex	1	2335.2	31.94	< 0.0001
	GCA × Sex	9	16099.2	24.46	< 0.0001
	SCA × Sex	35	15465.5	6.04	< 0.0001
	Rep(Sex)	78	259583.8	45.51	< 0.0001
	Error	3316	242467.4	—	—
Females	*GCA*	9	13511.7	15.54	< 0.0001
	SCA	35	16556.0	4.90	< 0.0001
	Rep	39	152482.6	40.48	< 0.0001
	Error	1655	15986.5	—	—
Males	*GCA*	9	9015.4	20.14	< 0.0001
	SCA	35	10680.1	6.14	< 0.0001
	Rep	39	107101.2	55.22	< 0.0001
	Error	1661	82606.9	—	—

TABLE 3.7

Analyses of variance of starvation resistance in mutations with increased life span

Time	Analysis	Source	d.f.	SS	*F*	*P*	σ^2
Week 1	**Pooled Sexes**	Line	49	357621	75.23	<0.00001	89.297
		Sex	1	28939.9	298.29	<0.00001	—
		Line × Sex	49	95333.5	20.05	<0.00001	61.380
		Rep (Line × Sex)	200	21042	1.08	0.21	0.819
		Error	2699	261853	—	—	97.019
	Females	Line	49	344694	47.17	<0.00001	230.254
		Rep (Sex)	100	13536.9	0.91	0.73	0.000
		Error	1349	201162	—	—	149.119
	Males	Line	49	108275	49.15	<0.00001	71.155
		Rep (Sex)	100	7505.07	1.67	0.00007	3.009
		Error	1350	60691.2	—	—	44.956
Week 6	**Pooled Sexes**	Line	49	130708	35.44	<0.00001	32.251
		Sex	1	26367.5	350.28	<0.00001	—
		Line × Sex	49	39396.5	10.68	<0.00001	25.506
		Rep (Line × Sex)	195	13734.5	0.94	0.72	0.000
		Error	2627	197749	—	—	75.276
	Females	Line	49	135660	22.67	<0.00001	89.697
		Rep (Sex)	99	9959	0.82	0.89	0.000
		Error	1339	163549	—	—	122.142
	Males	Line	49	34917.1	26.84	<0.00001	24.632
		Rep (Sex)	96	3775.51	1.48	0.0024	1.306
		Error	1288	34200	—	—	26.553

TABLE 3.8

Analyses of variance of chill coma recovery in mutations with increased life span

Time	Analysis	Source	d.f.	SS	*F*	*P*	σ^2
Week 1	**Pooled Sexes**	Line	49	17257	46.81	<0.00001	5.152
		Sex	1	460.106	61.16	<0.00001	—
		Line × Sex	49	2341.26	6.35	<0.00001	1.349
		Error	2886	21712.4	—	—	7.523
	Females	Line	49	12544.7	33.91	<0.00001	8.316
		Error	1444	10900.9	—	—	7.549
	Males	Line	49	7215.4	19.64	<0.00001	4.684
		Error	1442	10811.4	—	—	7.498
Week 6	**Pooled Sexes**	Line	49	168114	66.96	<0.00001	54.676
		Sex	1	5243.63	102.34	<0.00001	—
		Line × Sex	49	11939.8	4.76	<0.00001	6.603
		Error	2820	144493	—	—	51.238
	Females	Line	49	79774.7	40.08	<0.00001	54.070
		Error	1418	57599.4	—	—	40.620
	Males	Line	49	100592	33.12	<0.00001	68.564
		Error	1402	86893.5	—	—	61.978

TABLE 3.9

Analyses of variance of climbing activity in mutations with increased life span

Time	Analysis	Source	d.f.	SS	*F*	*P*	σ^2
Week 1	**Pooled Sexes**	Line	39	56660.1	20.49	<0.00001	21.216
		Sex	1	1728.9	24.38	<0.00001	—
		Line × Sex	39	7015.11	2.54	<0.00001	3.632
		Error	2320	164527	—	—	70.917
	Females	Line	39	26184.2	9.29	<0.00001	19.970
		Error	1160	83867.7	—	—	72.300
	Males	Line	39	37491	13.82	<0.00001	29.726
		Error	1160	80659.3	—	—	69.534
Week 6	**Pooled Sexes**	Line	39	13554.3	9.06	<0.00001	2.953
		Sex	1	1560.81	40.69	<0.00001	—
		Line × Sex	39	6734.11	4.50	<0.00001	4.522
		Error	2300	88229.1	—	—	38.360
	Females	Line	39	11327.1	7.31	<0.00001	8.356
		Error	1160	46104.3	—	—	39.745
	Males	Line	39	9009.81	6.25	<0.00001	6.580
		Error	1140	42124.9	—	—	36.952

TABLE 3.10

Effects of mutations increasing life span on starvation resistance, chill coma recovery and climbing activity

Trait	Week	Mean[a]	H^{2b}	r_{MF}[c]
Starvation resistance	1	39.24	0.608	0.698
	6	28.70	0.434	0.686
Chill coma recovery	1	10.95	0.464	0.826
	6	19.30	0.545	0.898
Climbing Activity	1	14.15	0.259	0.871
	6	6.23	0.163	0.398

[a] Not corrected as deviation from control

[b] Broad sense heritability, $H^2 = (\sigma_L^2 + \sigma_{SL}^2)/(\sigma_L^2 + \sigma_{SL}^2 + \sigma_E^2)$

[c] Cross-sex genetic correlation, $r_{MF} = \sigma_L^2/(\sigma_{LM}\,\sigma_{LF})$

TABLE 3.11

Mutational correlations between life span, starvation resistance, chill coma recovery and climbing activity

A. Week 1

		Males			
		LS	SR	CC	CA
Females	LS		**0.303**	–0.153	0.159
	SR	–0.080		0.236	**–0.619**
	CC	**0.328**	–0.266		–0.282
	CA	–0.083	**–0.329**	–0.238	

B. Week 6

		Males			
		LS	SR	CC	CA
Females	LS		**0.554**	**–0.577**	–0.149
	SR	–0.093		–0.162	–0.145
	CC	**0.418**	0.096		–0.024
	CA	0.151	0.048	–0.060	

Correlations in bold font are significantly different from zero. LS= life span, SR = starvation resistance, CC = chill coma recovery, CA = climbing activity.

Table 3.12

Over-represented GO categories – Females

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
15074	DNA integration	17	5	0.72	25.24	5.05E-07
9581	detection of external stimulus	67	10	2.85	17.89	2.34E-05
8015	circulation	15	4	0.64	17.68	2.62E-05
8016	regulation of heart contraction	15	4	0.64	17.68	2.62E-05
51606	detection of stimulus	74	10	3.15	14.87	0.000115
51663	oocyte nucleus localization during oocyte axis determination	11	3	0.47	13.67	0.000217
6875	metal ion homeostasis	36	6	1.53	13.01	0.00031
51647	nucleus localization	27	5	1.15	12.89	0.000331
9583	detection of light stimulus	57	8	2.43	12.78	0.000349
5975	carbohydrate metabolism	592	43	25.22	12.54	0.000399
7602	phototransduction	47	7	2.00	12.47	0.000413
6873	cell ion homeostasis	38	6	1.62	11.86	0.000574
30003	cation homeostasis	38	6	1.62	11.86	0.000574
9416	response to light stimulus	83	10	3.54	11.82	0.000587
9628	response to abiotic stimulus	179	17	7.63	11.52	0.000687
8643	carbohydrate transport	85	10	3.62	11.24	0.000802
9582	detection of abiotic stimulus	62	8	2.64	10.87	0.000976
6508	proteolysis	851	56	36.25	10.76	0.00104
6865	amino acid transport	52	7	2.22	10.33	0.001306
15837	amine transport	52	7	2.22	10.33	0.001306
51189	prosthetic group metabolism	65	8	2.77	9.88	0.001669
7440	foregut morphogenesis	14	3	0.60	9.69	0.001856
50801	ion homeostasis	43	6	1.83	9.48	0.002073
9314	response to radiation	92	10	3.92	9.43	0.00213
7097	nuclear migration	23	4	0.98	9.31	0.00228
16059	deactivation of rhodopsin mediated signaling	15	3	0.64	8.72	0.003142
30005	di-, tri-valent inorganic cation homeostasis	34	5	1.45	8.71	0.003167
6874	calcium ion homeostasis	25	4	1.07	8.09	0.004456
48123	oocyte dorsal/ventral axis determination (sensu Insecta)	16	3	0.68	7.89	0.004983

Table 3.12 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
40023	establishment of nucleus localization	26	4	1.11	7.55	0.005991
6858	extracellular transport	130	12	5.54	7.54	0.006036
6631	fatty acid metabolism	116	11	4.94	7.43	0.006425
15849	organic acid transport	62	7	2.64	7.19	0.007319
46942	carboxylic acid transport	62	7	2.64	7.19	0.007319
44255	cellular lipid metabolism	416	29	17.72	7.18	0.007384
6986	response to unfolded protein	28	4	1.19	6.61	0.010162
51789	response to protein stimulus	28	4	1.19	6.61	0.010162
8152	metabolism	6114	301	260.46	6.31	0.012014
44262	cellular carbohydrate metabolism	375	26	15.98	6.29	0.012139
51239	regulation of organismal physiological process	41	5	1.75	6.06	0.013829
7026	negative regulation of microtubule depolymerization	10	2	0.43	5.82	0.015886
30722	establishment of oocyte nucleus localization during oocyte axis determination	10	2	0.43	5.82	0.015886
31111	negative regulation of microtubule polymerization or depolymerization	10	2	0.43	5.82	0.015886
48126	establishment of oocyte nucleus localization during oocyte axis determination (sensu Insecta)	10	2	0.43	5.82	0.015886
51662	oocyte nucleus localization during oocyte axis determination (sensu Insecta)	10	2	0.43	5.82	0.015886
6936	muscle contraction	113	10	4.81	5.59	0.018094
7051	spindle organization and biogenesis	43	5	1.83	5.48	0.019243
9408	response to heat	56	6	2.39	5.48	0.019281
7601	visual perception	133	11	5.67	5.02	0.025033
50953	sensory perception of light stimulus	133	11	5.67	5.02	0.025033
6665	sphingolipid metabolism	21	3	0.89	4.95	0.02602
6082	organic acid metabolism	499	31	21.26	4.46	0.034605
19752	carboxylic acid metabolism	499	31	21.26	4.46	0.034605
82	G1/S transition of mitotic cell cycle	12	2	0.51	4.34	0.03732
6541	glutamine metabolism	12	2	0.51	4.34	0.03732
6821	chloride transport	12	2	0.51	4.34	0.03732
7019	microtubule depolymerization	12	2	0.51	4.34	0.03732
9253	peptidoglycan catabolism	12	2	0.51	4.34	0.03732
31110	regulation of microtubule polymerization or depolymerization	12	2	0.51	4.34	0.03732

Table 3.12 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
31114	regulation of microtubule depolymerization	12	2	0.51	4.34	0.03732
51640	organelle localization	48	5	2.04	4.27	0.038776
9605	response to external stimulus	157	12	6.69	4.22	0.039991
6030	chitin metabolism	108	9	4.60	4.21	0.040279
6629	lipid metabolism	582	35	24.79	4.20	0.040394
7498	mesoderm development	262	18	11.16	4.19	0.040666

Table 3.13

Over-represented GO categories – Males

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
45297	post-mating behavior	22	6	0.84	31.79	1.72E-08
15074	DNA integration	17	5	0.65	29.25	6.36E-08
5975	carbohydrate metabolism	592	48	22.55	28.71	8.39E-08
30239	myofibril assembly	15	4	0.57	20.57	5.74E-06
51146	striated muscle cell differentiation	15	4	0.57	20.57	5.74E-06
55001	muscle cell development	15	4	0.57	20.57	5.74E-06
55002	striated muscle cell development	15	4	0.57	20.57	5.74E-06
44262	cellular carbohydrate metabolism	375	31	14.29	19.56	9.77E-06
18208	peptidyl-proline modification	16	4	0.61	18.86	1.41E-05
18401	peptidyl-proline hydroxylation to 4-hydroxy-L-proline	16	4	0.61	18.86	1.41E-05
19471	4-hydroxyproline metabolism	16	4	0.61	18.86	1.41E-05
19511	peptidyl-proline hydroxylation	16	4	0.61	18.86	1.41E-05
6508	proteolysis	851	57	32.42	18.64	1.58E-05
51704	interaction between organisms	87	11	3.31	17.82	2.42E-05
7617	mating behavior	81	10	3.09	15.49	8.28E-05
51705	behavioral interaction between organisms	81	10	3.09	15.49	8.28E-05
9116	nucleoside metabolism	19	4	0.72	14.83	0.000118
19098	reproductive behavior	85	10	3.24	14.12	0.000171
5976	polysaccharide metabolism	214	18	8.15	11.90	0.000563
6030	chitin metabolism	108	11	4.11	11.52	0.000687
6807	nitrogen compound metabolism	506	34	0.53	11.41	0.000731
16052	carbohydrate catabolism	99	10	19.28	11.25	0.000798
44275	cellular carbohydrate catabolism	99	10	3.77	10.29	0.00134
44264	cellular polysaccharide metabolism	129	12	3.77	10.29	0.00134
6041	glucosamine metabolism	116	11	4.91	10.22	0.001392
6044	N-acetylglucosamine metabolism	116	11	4.42	9.80	0.001745
6040	amino sugar metabolism	117	11	4.42	9.80	0.001745
44248	cellular catabolism	394	27	4.46	9.60	0.001941
9308	amine metabolism	491	32	15.01	9.58	0.001968

Table 3.13 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
6066	alcohol metabolism	238	18	18.70	9.45	0.002111
6633	fatty acid biosynthesis	39	5	9.07	8.80	0.003009
6090	pyruvate metabolism	18	3	1.49	8.31	0.003937
7062	sister chromatid cohesion	18	3	0.69	7.81	0.005194
9056	catabolism	439	28	0.69	7.81	0.005194
272	polysaccharide catabolism	19	3	16.72	7.60	0.005827
6032	chitin catabolism	19	3	0.72	7.16	0.007463
6043	glucosamine catabolism	19	3	0.72	7.16	0.007463
6046	N-acetylglucosamine catabolism	19	3	0.72	7.16	0.007463
44247	cellular polysaccharide catabolism	19	3	0.72	7.16	0.007463
46348	amino sugar catabolism	19	3	0.72	7.16	0.007463
45861	negative regulation of proteolysis	10	2	0.72	7.16	0.007463
6510	ATP-dependent proteolysis	20	3	0.38	6.88	0.008712
5996	monosaccharide metabolism	136	11	0.76	6.57	0.010346
6629	lipid metabolism	582	34	5.18	6.54	0.010573
18193	peptidyl-amino acid modification	45	5	22.17	6.31	0.012002
8355	olfactory learning	59	6	1.71	6.30	0.01209
19722	calcium-mediated signaling	75	7	2.25	6.26	0.012318
6638	neutral lipid metabolism	11	2	2.86	6.01	0.014248
6639	acylglycerol metabolism	11	2	0.42	5.96	0.014597
46483	heterocycle metabolism	176	13	0.42	5.96	0.014597
16053	organic acid biosynthesis	47	5	6.70	5.91	0.015049
46394	carboxylic acid biosynthesis	47	5	1.79	5.75	0.016458
48627	myoblast development	34	4	1.79	5.75	0.016458
48628	myoblast maturation	34	4	1.30	5.65	0.017474
45445	myoblast differentiation	35	4	1.30	5.65	0.017474
9253	peptidoglycan catabolism	12	2	1.33	5.33	0.020921
16998	cell wall catabolism	12	2	0.46	5.21	0.022494
45924	regulation of female receptivity	12	2	0.46	5.21	0.022494
6575	amino acid derivative metabolism	64	6	0.46	5.21	0.022494
7612	learning	64	6	2.44	5.20	0.022539
6006	glucose metabolism	81	7	2.44	5.20	0.022539
70	mitotic sister chromatid segregation	37	4	3.09	4.97	0.02586

Table 3.13 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
819	sister chromatid segregation	37	4	1.41	4.76	0.029114
6100	tricarboxylic acid cycle intermediate metabolism	24	3	1.41	4.76	0.029114
9166	nucleotide catabolism	13	2	0.91	4.76	0.029162
46486	glycerolipid metabolism	13	2	0.50	4.57	0.032496
51186	cofactor metabolism	308	19	0.50	4.57	0.032496
6961	antibacterial humoral response (sensu Protostomia)	25	3	11.73	4.50	0.033887
21700	developmental maturation	39	4	0.95	4.40	0.035889
48469	cell maturation	39	4	1.49	4.25	0.039136
8610	lipid biosynthesis	121	9	1.49	4.25	0.039136
6092	main pathways of carbohydrate metabolism	122	9	4.61	4.18	0.040859
16082	synaptic vesicle priming	14	2	4.65	4.08	0.043499
50795	regulation of behavior	14	2	0.53	4.03	0.04461
8652	amino acid biosynthesis	106	8	0.53	4.03	0.04461
6732	coenzyme metabolism	278	17	4.04	3.89	0.048657
9408	response to heat	56	5	10.59	3.88	0.048889

Table 3.14

Over-represented GO categories – *pyd*

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
51189	prosthetic group metabolism	65	7	1.43	21.67	3.24E-06
15074	DNA integration	17	3	0.37	18.42	1.77E-05
6090	pyruvate metabolism	18	3	0.40	17.11	3.54E-05
9116	nucleoside metabolism	19	3	0.42	15.93	6.56E-05
16042	lipid catabolism	34	4	0.75	14.12	0.000171
6030	chitin metabolism	108	8	2.38	13.29	0.000266
6721	terpenoid metabolism	11	2	0.24	12.76	0.000354
16096	polyisoprenoid metabolism	11	2	0.24	12.76	0.000354
42214	terpene metabolism	11	2	0.24	12.76	0.000354
43449	alkene metabolism	11	2	0.24	12.76	0.000354
6041	glucosamine metabolism	116	8	2.55	11.61	0.000655
6044	N-acetylglucosamine metabolism	116	8	2.55	11.61	0.000655
6040	amino sugar metabolism	117	8	2.58	11.42	0.000726
6508	proteolysis	851	33	18.74	10.86	0.000984
44242	cellular lipid catabolism	13	2	0.29	10.26	0.001359
44264	cellular polysaccharide metabolism	129	8	2.84	9.37	0.002201
18991	oviposition	14	2	0.31	9.28	0.00231
6858	extracellular transport	130	8	2.86	9.22	0.002391
6820	anion transport	112	7	2.47	8.34	0.003885
6732	coenzyme metabolism	278	13	6.12	7.73	0.005427
7632	visual behavior	16	2	0.35	7.71	0.005501
6398	histone mRNA 3'-end processing	18	2	0.40	6.49	0.010852
8334	histone mRNA metabolism	18	2	0.40	6.49	0.010852
7617	mating behavior	81	5	1.78	5.80	0.016013
51705	behavioral interaction between organisms	81	5	1.78	5.80	0.016013
51186	cofactor metabolism	308	13	6.78	5.70	0.01694
6807	nitrogen compound metabolism	506	19	11.14	5.54	0.018542
9126	purine nucleoside monophosphate metabolism	20	2	0.44	5.52	0.018757
9127	purine nucleoside monophosphate biosynthesis	20	2	0.44	5.52	0.018757

Table 3.14 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
9167	purine ribonucleoside monophosphate metabolism	20	2	0.44	5.52	0.018757
9168	purine ribonucleoside monophosphate biosynthesis	20	2	0.44	5.52	0.018757
6790	sulfur metabolism	85	5	1.87	5.23	0.022202
19098	reproductive behavior	85	5	1.87	5.23	0.022202
2778	antibacterial peptide production	21	2	0.46	5.11	0.02374
2780	antibacterial peptide biosynthesis	21	2	0.46	5.11	0.02374
2808	regulation of antibacterial peptide biosynthesis	21	2	0.46	5.11	0.02374
6665	sphingolipid metabolism	21	2	0.46	5.11	0.02374
6963	positive regulation of antibacterial peptide biosynthesis	21	2	0.46	5.11	0.02374
51704	interaction between organisms	87	5	1.92	4.97	0.025838
6626	protein targeting to mitochondrion	41	3	0.90	4.87	0.027286
6720	isoprenoid metabolism	22	2	0.48	4.74	0.02943
45297	post-mating behavior	22	2	0.48	4.74	0.02943
6839	mitochondrial transport	66	4	1.45	4.46	0.034622
7097	nuclear migration	23	2	0.51	4.41	0.035828
9156	ribonucleoside monophosphate biosynthesis	23	2	0.51	4.41	0.035828
9161	ribonucleoside monophosphate metabolism	23	2	0.51	4.41	0.035828
44272	sulfur compound biosynthesis	44	3	0.97	4.26	0.039044
44260	cellular macromolecule metabolism	2570	72	56.58	4.20	0.040435
9123	nucleoside monophosphate metabolism	24	2	0.53	4.10	0.042929
9124	nucleoside monophosphate biosynthesis	24	2	0.53	4.10	0.042929
6960	antimicrobial humoral response (sensu Protostomia)	45	3	0.99	4.07	0.043535
19731	antibacterial humoral response	45	3	0.99	4.07	0.043535
5976	polysaccharide metabolism	214	9	4.71	3.90	0.048203

Table 3.15

Over-represented GO categories – *mub*

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
6139	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	2234	954	753.36	53.44	2.67E-13
44238	primary metabolism	5502	2165	1855.41	51.66	6.61E-13
6259	DNA metabolism	526	272	177.38	50.47	1.21E-12
44237	cellular metabolism	5627	2199	1897.56	47.88	4.52E-12
43170	macromolecule metabolism	4600	1807	1551.23	42.17	8.37E-11
8152	metabolism	6114	2356	2061.79	41.98	9.21E-11
6260	DNA replication	143	90	48.22	36.19	1.79E-09
6396	RNA processing	374	190	126.12	32.35	1.29E-08
6281	DNA repair	128	80	43.16	31.43	2.06E-08
43283	biopolymer metabolism	2978	1176	1004.26	29.37	5.98E-08
6974	response to DNA damage stimulus	140	84	47.21	28.67	8.6E-08
16070	RNA metabolism	1579	655	532.48	28.19	1.1E-07
50875	cellular physiological process	7572	2812	2553.47	26.18	3.12E-07
6261	DNA-dependent DNA replication	74	50	24.95	25.14	5.34E-07
9719	response to endogenous stimulus	167	92	56.32	22.61	1.98E-06
16071	mRNA metabolism	286	141	96.45	20.58	5.71E-06
6397	mRNA processing	274	135	92.40	19.64	9.35E-06
51276	chromosome organization and biogenesis	294	142	99.14	18.52	1.68E-05
7067	mitosis	288	138	97.12	17.21	3.35E-05
6270	DNA replication initiation	16	15	5.40	17.10	3.55E-05
279	M phase	383	176	129.16	16.99	3.76E-05
87	M phase of mitotic cell cycle	289	138	97.46	16.87	4.01E-05
16072	rRNA metabolism	59	38	19.90	16.47	4.94E-05
7049	cell cycle	605	261	204.02	15.91	6.63E-05
16568	chromatin modification	132	71	44.51	15.76	7.19E-05
7001	chromosome organization and biogenesis (sensu Eukaryota)	273	130	92.06	15.63	7.69E-05
6323	DNA packaging	237	115	79.92	15.40	8.72E-05
6325	establishment and/or maintenance of chromatin architecture	237	115	79.92	15.40	8.72E-05

Table 3.15 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
42254	ribosome biogenesis and assembly	59	37	19.90	14.70	0.000126
7046	ribosome biogenesis	51	33	17.20	14.52	0.000139
19538	protein metabolism	2606	991	878.81	14.32	0.000154
74	regulation of progression through cell cycle	236	113	79.59	14.03	0.00018
51726	regulation of cell cycle	237	113	79.92	13.69	0.000216
44267	cellular protein metabolism	2451	932	826.54	13.46	0.000244
8380	RNA splicing	211	102	71.15	13.37	0.000255
278	mitotic cell cycle	341	154	114.99	13.23	0.000275
44249	cellular biosynthesis	1093	438	368.59	13.07	0.0003
31497	chromatin assembly	64	38	21.58	12.49	0.000409
9058	biosynthesis	1180	467	397.93	11.99	0.000535
6277	DNA amplification	17	14	2753.78	11.93	0.000554
9987	cellular process	8166	2935	5.73	11.92	0.000555
6352	transcription initiation	96	52	32.37	11.90	0.000562
9892	negative regulation of metabolism	218	103	73.52	11.83	0.000584
9059	macromolecule biosynthesis	618	258	208.40	11.80	0.000592
7059	chromosome segregation	162	80	54.63	11.78	0.000598
6367	transcription initiation from RNA polymerase II promoter	94	51	31.70	11.75	0.000608
6342	chromatin silencing	32	22	10.79	11.64	0.000645
31507	heterochromatin formation	32	22	10.79	11.64	0.000645
45814	negative regulation of gene expression, epigenetic	32	22	10.79	11.64	0.000645
6412	protein biosynthesis	577	242	194.58	11.56	0.000675
7582	physiological process	8256	2962	2784.13	11.36	0.000749
6333	chromatin assembly or disassembly	171	83	57.67	11.13	0.000849
375	RNA splicing, via transesterification reactions	198	94	66.77	11.10	0.000861
377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	198	94	66.77	11.10	0.000861
398	nuclear mRNA splicing, via spliceosome	198	94	66.77	11.10	0.000861
7307	chorion gene amplification	16	13	5.40	10.72	0.001061
30261	chromosome condensation	29	20	9.78	10.68	0.001082
45934	negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	158	77	53.28	10.56	0.001157
44260	cellular macromolecule metabolism	2570	962	866.67	10.49	0.001203

Table 3.15 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
51261	protein depolymerization	20	15	6.74	10.11	0.001479
16569	covalent chromatin modification	42	26	14.16	9.89	0.00166
16570	histone modification	42	26	14.16	9.89	0.00166
6364	rRNA processing	40	25	13.49	9.82	0.001723
31123	RNA 3'-end processing	34	22	11.47	9.68	0.001864
31124	mRNA 3'-end processing	34	22	11.47	9.68	0.001864
9566	fertilization	15	12	5.06	9.53	0.002026
6220	pyrimidine nucleotide metabolism	17	13	5.73	9.21	0.002404
6996	organelle organization and biogenesis	1019	399	343.63	8.92	0.002819
70	mitotic sister chromatid segregation	37	23	12.48	8.87	0.002892
819	sister chromatid segregation	37	23	12.48	8.87	0.002892
9299	mRNA transcription	25	17	8.43	8.71	0.003164
6338	chromatin remodeling	59	33	19.90	8.63	0.003306
15931	nucleobase, nucleoside, nucleotide and nucleic acid transport	48	28	16.19	8.62	0.003322
7338	fertilization (sensu Metazoa)	14	11	4.72	8.35	0.003856
31324	negative regulation of cellular metabolism	203	92	68.46	8.10	0.004434
6221	pyrimidine nucleotide biosynthesis	16	12	5.40	8.08	0.004466
6379	mRNA cleavage	16	12	5.40	8.08	0.004466
45132	meiotic chromosome segregation	49	28	16.52	7.97	0.004756
16042	lipid catabolism	34	21	11.47	7.93	0.004867
6398	histone mRNA 3'-end processing	18	13	6.07	7.91	0.004912
8334	histone mRNA metabolism	18	13	6.07	7.91	0.004912
6913	nucleocytoplasmic transport	81	42	27.32	7.89	0.004958
51168	nuclear export	30	19	10.12	7.80	0.005224
6366	transcription from RNA polymerase II promoter	845	332	284.95	7.77	0.005321
6378	mRNA polyadenylation	24	16	8.09	7.72	0.005449
43631	RNA polyadenylation	24	16	8.09	7.72	0.005449
6399	tRNA metabolism	110	54	37.09	7.70	0.005509
16458	gene silencing	54	30	18.21	7.63	0.00573
16481	negative regulation of transcription	142	67	47.89	7.63	0.005742
45892	negative regulation of transcription, DNA-dependent	126	60	42.49	7.22	0.007228
31109	microtubule polymerization or depolymerization	13	10	4.38	7.19	0.007313
44242	cellular lipid catabolism	13	10	4.38	7.19	0.007313

Table 3.15 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
6206	pyrimidine base metabolism	35	21	11.80	7.17	0.007427
50791	regulation of physiological process	1734	648	584.75	6.84	0.008904
9116	nucleoside metabolism	19	13	6.41	6.78	0.0092
245	spliceosome assembly	23	15	7.76	6.77	0.009295
6631	fatty acid metabolism	116	55	39.12	6.45	0.011107
7143	female meiosis	52	28	17.54	6.24	0.012458
7051	spindle organization and biogenesis	43	24	14.50	6.22	0.01261
6310	DNA recombination	50	27	16.86	6.10	0.013545
6275	regulation of DNA replication	12	9	4.05	6.06	0.013804
6383	transcription from RNA polymerase III promoter	12	9	4.05	6.06	0.013804
7019	microtubule depolymerization	12	9	4.05	6.06	0.013804
8062	eclosion rhythm	12	9	4.05	6.06	0.013804
31110	regulation of microtubule polymerization or depolymerization	12	9	4.05	6.06	0.013804
31114	regulation of microtubule depolymerization	12	9	4.05	6.06	0.013804
51322	anaphase	12	9	4.05	6.06	0.013804
51244	regulation of cellular physiological process	1683	626	567.55	6.02	0.014147
6479	protein amino acid methylation	26	16	8.77	5.97	0.014589
8213	protein amino acid alkylation	26	16	8.77	5.97	0.014589
51129	negative regulation of cell organization and biogenesis	14	10	4.72	5.90	0.01512
6405	RNA export from nucleus	24	15	8.09	5.89	0.015194
6950	response to stress	312	130	105.21	5.84	0.015675
6720	isoprenoid metabolism	22	14	7.42	5.84	0.015685
1775	cell activation	18	12	6.07	5.79	0.016089
9451	RNA modification	18	12	6.07	5.79	0.016089
8033	tRNA processing	33	19	11.13	5.57	0.018293
51327	M phase of meiotic cell cycle	134	61	45.19	5.53	0.018664
9314	response to radiation	92	44	31.02	5.43	0.019832
51169	nuclear transport	75	37	25.29	5.42	0.019908
43118	negative regulation of physiological process	323	133	108.92	5.32	0.02106
51243	negative regulation of cellular physiological process	315	130	106.23	5.32	0.021072
51321	meiotic cell cycle	135	61	45.53	5.26	0.021821
7126	meiosis	130	59	43.84	5.24	0.022035
51128	regulation of cell organization and biogenesis	66	33	22.26	5.19	0.022774

Table 3.15 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
9416	response to light stimulus	83	40	27.99	5.15	0.023198
43037	translation	226	96	76.21	5.14	0.023416
44255	cellular lipid metabolism	416	167	140.29	5.09	0.024103
226	microtubule cytoskeleton organization and biogenesis	113	52	38.11	5.07	0.024405
6350	transcription	1212	454	408.72	5.02	0.025097
6721	terpenoid metabolism	11	8	3.71	4.96	0.025901
16096	polyisoprenoid metabolism	11	8	3.71	4.96	0.025901
42214	terpene metabolism	11	8	3.71	4.96	0.025901
43449	alkene metabolism	11	8	3.71	4.96	0.025901
6665	sphingolipid metabolism	21	13	7.08	4.95	0.026151
32774	RNA biosynthesis	1140	428	384.44	4.94	0.026294
6082	organic acid metabolism	499	197	168.28	4.90	0.026804
19752	carboxylic acid metabolism	499	197	168.28	4.90	0.026804
6406	mRNA export from nucleus	19	12	6.41	4.88	0.027142
42168	heme metabolism	13	9	4.38	4.86	0.027478
7076	mitotic chromosome condensation	15	10	5.06	4.83	0.028008
46011	regulation of oskar mRNA translation	15	10	5.06	4.83	0.028008
6351	transcription, DNA-dependent	1137	426	383.42	4.73	0.029683
6633	fatty acid biosynthesis	39	21	13.15	4.68	0.030456
122	negative regulation of transcription from RNA polymerase II promoter	70	34	23.61	4.58	0.032406
9056	catabolism	439	174	148.04	4.55	0.032887
7028	cytoplasm organization and biogenesis	95	44	32.04	4.47	0.034541
9586	rhodopsin mediated phototransduction	24	14	8.09	4.31	0.037874
51236	establishment of RNA localization	33	18	11.13	4.24	0.039411
6414	translational elongation	20	12	6.74	4.10	0.043004
7304	eggshell formation (sensu Insecta)	74	35	24.95	4.04	0.044336
30703	eggshell formation	74	35	24.95	4.04	0.044336
6997	nuclear organization and biogenesis	45	23	15.18	4.03	0.04457
9583	detection of light stimulus	57	28	19.22	4.01	0.045263
43543	protein amino acid acylation	29	16	9.78	3.96	0.046686
16571	histone methylation	16	10	5.40	3.93	0.047454
6013	mannose metabolism	10	7	3.37	3.90	0.048211

Table 3.15 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
7026	negative regulation of microtubule depolymerization	10	7	3.37	3.90	0.048211
7094	mitotic spindle checkpoint	10	7	3.37	3.90	0.048211
31111	negative regulation of microtubule polymerization or depolymerization	10	7	3.37	3.90	0.048211
31577	spindle checkpoint	10	7	3.37	3.90	0.048211
6123	mitochondrial electron transport, cytochrome c to oxygen	14	9	4.72	3.88	0.048923
16043	cell organization and biogenesis	1833	667	618.13	3.86	0.049356
50794	regulation of cellular process	1833	667	618.13	3.86	0.049356
212	meiotic spindle organization and biogenesis	12	8	4.05	3.86	0.04939
6563	L-serine metabolism	12	8	4.05	3.86	0.04939
6783	heme biosynthesis	12	8	4.05	3.86	0.04939

Table 3.16

Over-represented GO categories – *crol*

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
42254	ribosome biogenesis and assembly	59	23	8.40	25.35	4.79E-07
7046	ribosome biogenesis	51	20	7.26	22.32	2.3E-06
16072	rRNA metabolism	59	21	8.40	18.88	1.39E-05
6364	rRNA processing	40	16	5.70	18.63	1.59E-05
7279	pole cell formation	14	7	1.99	12.56	0.000393
6398	histone mRNA 3'-end processing	18	8	2.56	11.52	0.000687
8334	histone mRNA metabolism	18	8	2.56	11.52	0.000687
6383	transcription from RNA polymerase III promoter	12	6	1.71	10.77	0.001032
16042	lipid catabolism	34	12	4.84	10.58	0.001146
6732	coenzyme metabolism	278	60	39.60	10.51	0.001188
7028	cytoplasm organization and biogenesis	95	25	13.53	9.72	0.001825
19538	protein metabolism	2606	431	371.22	9.63	0.001917
44267	cellular protein metabolism	2451	407	349.14	9.59	0.001957
44242	cellular lipid catabolism	13	6	1.85	9.29	0.002301
44260	cellular macromolecule metabolism	2570	423	366.09	8.85	0.002936
15074	DNA integration	17	7	2.42	8.66	0.00326
51186	cofactor metabolism	308	63	43.87	8.34	0.003883
51169	nuclear transport	75	20	10.68	8.12	0.004368
6508	proteolysis	851	151	121.22	7.31	0.00684
30431	sleep	15	6	2.14	6.99	0.008219
7277	pole cell development	19	7	2.71	6.81	0.00906
18193	peptidyl-amino acid modification	45	13	6.41	6.77	0.009246
6913	nucleocytoplasmic transport	81	20	11.54	6.21	0.012735
18208	peptidyl-proline modification	16	6	2.28	6.07	0.013715
18401	peptidyl-proline hydroxylation to 4-hydroxy-L-proline	16	6	2.28	6.07	0.013715
19471	4-hydroxyproline metabolism	16	6	2.28	6.07	0.013715
19511	peptidyl-proline hydroxylation	16	6	2.28	6.07	0.013715
51704	interaction between organisms	87	21	12.39	5.98	0.014488
19236	response to pheromone	13	5	1.85	5.35	0.020698

Table 3.16 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
6606	protein import into nucleus	49	13	6.98	5.19	0.022688
19098	reproductive behavior	85	20	12.11	5.14	0.023328
6479	protein amino acid methylation	26	8	3.70	4.98	0.025583
8213	protein amino acid alkylation	26	8	3.70	4.98	0.025583
9310	amine catabolism	65	16	9.26	4.91	0.026739
44270	nitrogen compound catabolism	65	16	9.26	4.91	0.026739
51189	prosthetic group metabolism	65	16	9.26	4.91	0.026739
6858	extracellular transport	130	28	18.52	4.85	0.027567
51170	nuclear import	50	13	7.12	4.85	0.02764
7617	mating behavior	81	19	11.54	4.83	0.028042
51705	behavioral interaction between organisms	81	19	11.54	4.83	0.028042
45297	post-mating behavior	22	7	3.13	4.77	0.028967
7320	insemination	10	4	1.42	4.66	0.030933
48609	reproductive organismal physiological process	10	4	1.42	4.66	0.030933
50876	reproductive physiological process	10	4	1.42	4.66	0.030933
6418	tRNA aminoacylation for protein translation	66	16	9.40	4.63	0.031397
43039	tRNA aminoacylation	66	16	9.40	4.63	0.031397
18991	oviposition	14	5	1.99	4.53	0.033302
6399	tRNA metabolism	110	24	15.67	4.43	0.03533
43038	amino acid activation	67	16	9.54	4.37	0.036638
43037	translation	226	44	32.19	4.33	0.037442
9063	amino acid catabolism	62	15	8.83	4.31	0.037933
7602	phototransduction	47	12	6.70	4.20	0.04034
9059	macromolecule biosynthesis	618	107	88.03	4.09	0.043222
15931	nucleobase, nucleoside, nucleotide and nucleic acid transport	48	12	6.84	3.90	0.048347

Table 3.17

Over-represented GO categories – *CG10990*

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
18208	peptidyl-proline modification	16	4	0.30	45.66	1.41E-11
18401	peptidyl-proline hydroxylation to 4-hydroxy-L-proline	16	4	0.30	45.66	1.41E-11
19471	4-hydroxyproline metabolism	16	4	0.30	45.66	1.41E-11
19511	peptidyl-proline hydroxylation	16	4	0.30	45.66	1.41E-11
15074	DNA integration	17	4	0.32	42.54	6.92E-11
6575	amino acid derivative metabolism	64	6	1.20	19.21	1.17E-05
6875	metal ion homeostasis	36	4	0.67	16.39	5.15E-05
45297	post-mating behavior	22	3	0.41	16.24	5.58E-05
15012	heparan sulfate proteoglycan biosynthesis	11	2	0.21	15.61	7.78E-05
30201	heparan sulfate proteoglycan metabolism	11	2	0.21	15.61	7.78E-05
40018	positive regulation of body size	11	2	0.21	15.61	7.78E-05
6873	cell ion homeostasis	38	4	0.71	15.18	9.77E-05
30003	cation homeostasis	38	4	0.71	15.18	9.77E-05
7629	flight behavior	24	3	0.45	14.46	0.000143
6023	aminoglycan biosynthesis	12	2	0.22	14.01	0.000182
6024	glycosaminoglycan biosynthesis	12	2	0.22	14.01	0.000182
30307	positive regulation of cell growth	13	2	0.24	12.66	0.000373
44242	cellular lipid catabolism	13	2	0.24	12.66	0.000373
50801	ion homeostasis	43	4	0.81	12.66	0.000373
44272	sulfur compound biosynthesis	44	4	0.82	12.23	0.000471
18193	peptidyl-amino acid modification	45	4	0.84	11.82	0.000587
51704	interaction between organisms	87	6	1.63	11.71	0.000621
6022	aminoglycan metabolism	14	2	0.26	11.51	0.000693
18991	oviposition	14	2	0.26	11.51	0.000693
30203	glycosaminoglycan metabolism	14	2	0.26	11.51	0.000693
7549	dosage compensation	16	2	0.30	9.64	0.001904
45793	positive regulation of cell size	16	2	0.30	9.64	0.001904
7584	response to nutrient	17	2	0.32	8.87	0.002893
8286	insulin receptor signaling pathway	17	2	0.32	8.87	0.002893

Table 3.17 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
30166	proteoglycan biosynthesis	17	2	0.32	8.87	0.002893
30005	di-, tri-valent inorganic cation homeostasis	34	3	0.64	8.76	0.003076
6090	pyruvate metabolism	18	2	0.34	8.20	0.0042
6398	histone mRNA 3'-end processing	18	2	0.34	8.20	0.0042
7016	cytoskeletal anchoring	18	2	0.34	8.20	0.0042
8334	histone mRNA metabolism	18	2	0.34	8.20	0.0042
7617	mating behavior	81	5	1.52	7.99	0.004711
51705	behavioral interaction between organisms	81	5	1.52	7.99	0.004711
6508	proteolysis	851	27	15.95	7.66	0.005649
6029	proteoglycan metabolism	19	2	0.36	7.59	0.00587
7160	cell-matrix adhesion	38	3	0.71	7.35	0.006706
31589	cell-substrate adhesion	38	3	0.71	7.35	0.006706
19098	reproductive behavior	85	5	1.59	7.29	0.006945
6936	muscle contraction	113	6	2.12	7.12	0.007633
21700	developmental maturation	39	3	0.73	7.04	0.007949
48469	cell maturation	39	3	0.73	7.04	0.007949
6807	nitrogen compound metabolism	506	17	9.48	5.96	0.014639
8344	adult locomotory behavior	44	3	0.82	5.74	0.01659
1558	regulation of cell growth	23	2	0.43	5.71	0.016858
7475	apposition of dorsal and ventral wing surfaces	24	2	0.45	5.34	0.020803
45927	positive regulation of growth	24	2	0.45	5.34	0.020803
9308	amine metabolism	491	16	9.20	5.02	0.025013
6874	calcium ion homeostasis	25	2	0.47	5.01	0.025256
9991	response to extracellular stimulus	25	2	0.47	5.01	0.025256
31667	response to nutrient levels	25	2	0.47	5.01	0.025256
7365	periodic partitioning	48	3	0.90	4.90	0.026784
19722	calcium-mediated signaling	75	4	1.41	4.79	0.02864
6812	cation transport	461	15	8.64	4.68	0.030462
1539	ciliary or flagellar motility	27	2	0.51	4.41	0.035702
46483	heterocycle metabolism	176	7	3.30	4.15	0.041526
1501	skeletal development	28	2	0.52	4.15	0.041692
9725	response to hormone stimulus	28	2	0.52	4.15	0.041692
35265	organ growth	28	2	0.52	4.15	0.041692

Table 3.17 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
46620	regulation of organ size	28	2	0.52	4.15	0.041692
6519	amino acid and derivative metabolism	362	12	6.78	4.01	0.045221

Table 3.18

Over-represented GO categories – *CG9238*

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
8586	wing vein morphogenesis	26	6	1.48	13.84	0.000199
578	embryonic axis specification	72	11	4.09	11.66	0.000638
35222	wing disc pattern formation	37	7	2.10	11.40	0.000733
7632	visual behavior	16	4	0.91	10.50	0.001191
48512	circadian behavior	39	7	2.22	10.32	0.001314
7623	circadian rhythm	58	9	3.30	9.87	0.001682
48511	rhythmic process	59	9	3.35	9.51	0.002045
51239	regulation of organismal physiological process	41	7	2.33	9.36	0.00222
45475	locomotor rhythm	33	6	1.88	9.07	0.002599
8362	embryonic cuticle biosynthesis (sensu Insecta)	11	3	0.63	9.02	0.002669
35296	regulation of tube diameter	11	3	0.63	9.02	0.002669
7622	rhythmic behavior	42	7	2.39	8.91	0.00283
7367	segment polarity determination	34	6	1.93	8.56	0.003433
7351	regional subdivision	62	9	3.52	8.51	0.003531
8595	determination of anterior/posterior axis, embryo	62	9	3.52	8.51	0.003531
9063	amino acid catabolism	62	9	3.52	8.51	0.003531
50801	ion homeostasis	43	7	2.44	8.49	0.003564
2168	larval development (sensu Insecta)	53	8	3.01	8.26	0.004056
6807	nitrogen compound metabolism	506	44	28.76	8.08	0.004482
42306	regulation of protein import into nucleus	19	4	1.08	7.90	0.004953
48100	wing disc anterior/posterior pattern formation	19	4	1.08	7.90	0.004953
6541	glutamine metabolism	12	3	0.68	7.88	0.005004
7458	progression of morphogenetic furrow (sensu Endopterygota)	12	3	0.68	7.88	0.005004
8062	eclosion rhythm	12	3	0.68	7.88	0.005004
7610	behavior	319	30	18.13	7.77	0.005311
6875	metal ion homeostasis	36	6	2.05	7.64	0.005707
9310	amine catabolism	65	9	3.69	7.62	0.005773
44270	nitrogen compound catabolism	65	9	3.69	7.62	0.005773
51704	interaction between organisms	87	11	4.94	7.42	0.006467

Table 3.18 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
6112	energy reserve metabolism	20	4	1.14	7.21	0.00724
51223	regulation of protein transport	20	4	1.14	7.21	0.00724
16335	morphogenesis of larval imaginal disc epithelium	13	3	0.74	6.92	0.008525
6873	cell ion homeostasis	38	6	2.16	6.83	0.008972
30003	cation homeostasis	38	6	2.16	6.83	0.008972
7474	wing vein specification	21	4	1.19	6.60	0.010204
46822	regulation of nucleocytoplasmic transport	21	4	1.19	6.60	0.010204
6606	protein import into nucleus	49	7	2.78	6.38	0.011544
7617	mating behavior	81	10	4.60	6.33	0.011902
51705	behavioral interaction between organisms	81	10	4.60	6.33	0.011902
9308	amine metabolism	491	41	27.91	6.14	0.013189
5977	glycogen metabolism	14	3	0.80	6.11	0.013468
6073	glucan metabolism	14	3	0.80	6.11	0.013468
6376	mRNA splice site selection	14	3	0.80	6.11	0.013468
7440	foregut morphogenesis	14	3	0.80	6.11	0.013468
9453	energy taxis	14	3	0.80	6.11	0.013468
16082	synaptic vesicle priming	14	3	0.80	6.11	0.013468
18991	oviposition	14	3	0.80	6.11	0.013468
30237	female sex determination	14	3	0.80	6.11	0.013468
35161	imaginal disc lineage restriction	14	3	0.80	6.11	0.013468
42331	phototaxis	14	3	0.80	6.11	0.013468
48190	wing disc dorsal/ventral pattern formation	14	3	0.80	6.11	0.013468
51170	nuclear import	50	7	2.84	6.08	0.013637
9967	positive regulation of signal transduction	22	4	1.25	6.05	0.013934
45297	post-mating behavior	22	4	1.25	6.05	0.013934
9952	anterior/posterior pattern formation	179	18	10.17	6.02	0.014138
51248	negative regulation of protein metabolism	61	8	3.47	5.93	0.014912
6520	amino acid metabolism	328	29	18.64	5.76	0.016441
2376	immune system process	261	24	14.83	5.66	0.017321
35282	segmentation	157	16	8.92	5.61	0.017833
7448	anterior/posterior pattern formation, imaginal disc	23	4	1.31	5.55	0.018513
19098	reproductive behavior	85	10	4.83	5.53	0.018687
8015	circulation	15	3	0.85	5.41	0.02003

Table 3.18 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
8016	regulation of heart contraction	15	3	0.85	5.41	0.02003
9070	serine family amino acid biosynthesis	15	3	0.85	5.41	0.02003
30713	stalk formation (sensu Insecta)	15	3	0.85	5.41	0.02003
19748	secondary metabolism	86	10	4.89	5.35	0.020762
7450	dorsal/ventral pattern formation, imaginal disc	24	4	1.36	5.09	0.024012
6508	proteolysis	851	64	48.37	5.05	0.024587
7350	blastoderm segmentation	137	14	7.79	4.96	0.025966
42127	regulation of cell proliferation	44	6	2.50	4.90	0.026914
16042	lipid catabolism	34	5	1.93	4.87	0.027334
30005	di-, tri-valent inorganic cation homeostasis	34	5	1.93	4.87	0.027334
8589	regulation of smoothened signaling pathway	16	3	0.91	4.81	0.028355
6874	calcium ion homeostasis	25	4	1.42	4.68	0.03049
50803	regulation of synapse structure and function	25	4	1.42	4.68	0.03049
7447	imaginal disc pattern formation	67	8	3.81	4.61	0.031698
6206	pyrimidine base metabolism	35	5	1.99	4.56	0.032788
16333	morphogenesis of follicular epithelium	35	5	1.99	4.56	0.032788
5975	carbohydrate metabolism	592	46	33.65	4.54	0.033199
7611	learning and/or memory	91	10	5.17	4.51	0.033761
9636	response to toxin	142	14	8.07	4.36	0.036875
6519	amino acid and derivative metabolism	362	30	20.57	4.32	0.037712
271	polysaccharide biosynthesis	17	3	0.97	4.28	0.038541
15074	DNA integration	17	3	0.97	4.28	0.038541
43284	biopolymer biosynthesis	17	3	0.97	4.28	0.038541
9880	embryonic pattern specification	169	16	9.61	4.26	0.03908
8355	olfactory learning	59	7	3.35	3.97	0.046434
1539	ciliary or flagellar motility	27	4	1.53	3.96	0.046566
16334	establishment and/or maintenance of polarity of follicular epithelium	27	4	1.53	3.96	0.046566
9948	anterior/posterior axis specification	159	15	9.04	3.93	0.047295
2217	physiological defense response	120	12	6.82	3.93	0.047325
7365	periodic partitioning	48	6	2.73	3.92	0.0476
5976	polysaccharide metabolism	214	19	12.16	3.84	0.049941

Table 3.19

Over-represented GO categories – BG00817

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
21700	developmental maturation	39	15	4.74	22.24	2.41E-06
48469	cell maturation	39	15	4.74	22.24	2.41E-06
16042	lipid catabolism	34	12	4.13	15.00	0.000107
48627	myoblast development	34	12	4.13	15.00	0.000107
48628	myoblast maturation	34	12	4.13	15.00	0.000107
30239	myofibril assembly	15	7	1.82	14.72	0.000125
51146	striated muscle cell differentiation	15	7	1.82	14.72	0.000125
55001	muscle cell development	15	7	1.82	14.72	0.000125
55002	striated muscle cell development	15	7	1.82	14.72	0.000125
45445	myoblast differentiation	35	12	4.25	14.13	0.000171
51189	prosthetic group metabolism	65	18	7.89	12.94	0.000322
42692	muscle cell differentiation	44	13	5.34	10.97	0.000927
7519	striated muscle development	80	20	9.72	10.88	0.00097
7520	myoblast fusion	22	8	2.67	10.62	0.001116
6508	proteolysis	851	136	103.36	10.31	0.001324
16202	regulation of striated muscle development	13	5	1.58	7.41	0.006477
44242	cellular lipid catabolism	13	5	1.58	7.41	0.006477
44255	cellular lipid metabolism	416	69	50.52	6.76	0.009345
48637	skeletal muscle development	65	15	7.89	6.40	0.011442
48741	skeletal muscle fiber development	65	15	7.89	6.40	0.011442
48747	muscle fiber development	65	15	7.89	6.40	0.011442
44260	cellular macromolecule metabolism	2570	356	312.14	6.16	0.013039
44267	cellular protein metabolism	2451	340	297.68	6.02	0.014183
6732	coenzyme metabolism	278	48	33.76	6.00	0.014289
6629	lipid metabolism	582	91	70.69	5.84	0.015686
19538	protein metabolism	2606	359	316.51	5.70	0.016924
43543	protein amino acid acylation	29	8	3.52	5.69	0.017035
51186	cofactor metabolism	308	52	37.41	5.69	0.017041
5975	carbohydrate metabolism	592	92	71.90	5.62	0.017772

Table 3.19 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
7076	mitotic chromosome condensation	15	5	1.82	5.54	0.01854
8152	metabolism	6114	806	742.57	5.42	0.01993
51247	positive regulation of protein metabolism	11	4	1.34	5.31	0.021178
6473	protein amino acid acetylation	25	7	3.04	5.17	0.022926
6643	membrane lipid metabolism	119	23	14.45	5.05	0.024565
6575	amino acid derivative metabolism	64	14	7.77	4.99	0.025519
7632	visual behavior	16	5	1.94	4.81	0.028325
18208	peptidyl-proline modification	16	5	1.94	4.81	0.028325
18401	peptidyl-proline hydroxylation to 4-hydroxy-L-proline	16	5	1.94	4.81	0.028325
19471	4-hydroxyproline metabolism	16	5	1.94	4.81	0.028325
19511	peptidyl-proline hydroxylation	16	5	1.94	4.81	0.028325
7413	axonal fasciculation	21	6	2.55	4.67	0.03078
6644	phospholipid metabolism	103	20	12.51	4.48	0.034198
8062	eclosion rhythm	12	4	1.46	4.44	0.035198
9253	peptidoglycan catabolism	12	4	1.46	4.44	0.035198
16998	cell wall catabolism	12	4	1.46	4.44	0.035198
51235	maintenance of localization	32	8	3.89	4.35	0.03693
15074	DNA integration	17	5	2.06	4.17	0.041076
45475	locomotor rhythm	33	8	4.01	3.98	0.04615
6936	muscle contraction	113	21	13.72	3.86	0.049537
6457	protein folding	173	30	21.01	3.85	0.049891

Table 3.20

Over-represented GO categories – *esg*

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
9416	response to light stimulus	83	33	13.01	30.70	3.01E-08
9314	response to radiation	92	34	14.42	26.57	2.54E-07
9583	detection of light stimulus	57	24	8.94	25.39	4.68E-07
7602	phototransduction	47	21	7.37	25.22	5.13E-07
7601	visual perception	133	43	20.85	23.52	1.23E-06
50953	sensory perception of light stimulus	133	43	20.85	23.52	1.23E-06
9581	detection of external stimulus	67	26	10.50	22.86	1.74E-06
6396	RNA processing	374	94	58.64	21.33	3.87E-06
9582	detection of abiotic stimulus	62	24	9.72	20.98	4.65E-06
51606	detection of stimulus	74	26	11.60	17.87	2.37E-05
16072	rRNA metabolism	59	22	9.25	17.57	2.76E-05
43283	biopolymer metabolism	2978	555	466.90	16.62	4.56E-05
6403	RNA localization	127	38	19.91	16.43	5.04E-05
7046	ribosome biogenesis	51	19	8.00	15.14	9.96E-05
42254	ribosome biogenesis and assembly	59	21	9.25	14.92	0.000112
9586	rhodopsin mediated phototransduction	24	11	3.76	13.92	0.000191
6139	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	2234	417	350.26	12.72	0.000362
43170	macromolecule metabolism	4600	816	721.21	12.46	0.000416
9605	response to external stimulus	157	42	24.62	12.28	0.000458
6364	rRNA processing	40	15	6.27	12.15	0.000491
44242	cellular lipid catabolism	13	7	2.04	12.08	0.00051
6259	DNA metabolism	526	114	82.47	12.06	0.000516
6270	DNA replication initiation	16	8	2.51	12.02	0.000526
16071	mRNA metabolism	286	68	44.84	11.96	0.000543
44238	primary metabolism	5502	962	862.63	11.45	0.000716
16070	RNA metabolism	1579	299	247.56	10.69	0.001079
7338	fertilization (sensu Metazoa)	14	7	2.19	10.52	0.001182
6397	mRNA processing	274	64	42.96	10.31	0.001326
15931	nucleobase, nucleoside, nucleotide and nucleic acid transport	48	16	7.53	9.54	0.002008

Table 3.20 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
9451	RNA modification	18	8	2.82	9.50	0.002055
6399	tRNA metabolism	110	30	17.25	9.43	0.002133
9566	fertilization	15	7	2.35	9.19	0.002437
16059	deactivation of rhodopsin mediated signaling	15	7	2.35	9.19	0.002437
8152	metabolism	6114	1052	958.58	9.10	0.00255
8062	eclosion rhythm	12	6	1.88	9.02	0.002676
51236	establishment of RNA localization	33	12	5.17	9.01	0.002691
6406	mRNA export from nucleus	19	8	2.98	8.46	0.003624
6281	DNA repair	128	33	20.07	8.33	0.003894
7549	dosage compensation	16	7	2.51	8.04	0.004571
7315	pole plasm assembly	64	19	10.03	8.01	0.004649
9628	response to abiotic stimulus	179	43	28.06	7.95	0.004813
6505	GPI anchor metabolism	13	6	2.04	7.70	0.005519
6506	GPI anchor biosynthesis	13	6	2.04	7.70	0.005519
44237	cellular metabolism	5627	964	882.23	7.58	0.005903
9620	response to fungus	20	8	3.14	7.55	0.006015
6405	RNA export from nucleus	24	9	3.76	7.29	0.006937
51028	mRNA transport	24	9	3.76	7.29	0.006937
19094	pole plasm mRNA localization	57	17	8.94	7.28	0.006991
9893	positive regulation of metabolism	118	30	18.50	7.15	0.007506
31325	positive regulation of cellular metabolism	118	30	18.50	7.15	0.007506
50657	nucleic acid transport	32	11	5.02	7.13	0.007561
50658	RNA transport	32	11	5.02	7.13	0.007561
48113	pole plasm assembly (sensu Insecta)	62	18	9.72	7.05	0.007919
46474	glycerophospholipid biosynthesis	17	7	2.67	7.05	0.007929
7316	pole plasm RNA localization	58	17	9.09	6.87	0.008744
7028	cytoplasm organization and biogenesis	95	25	14.89	6.86	0.008833
6913	nucleocytoplasmic transport	81	22	12.70	6.81	0.009059
8298	intracellular mRNA localization	72	20	11.29	6.72	0.009519
9453	energy taxis	14	6	2.19	6.60	0.010221
30237	female sex determination	14	6	2.19	6.60	0.010221
42331	phototaxis	14	6	2.19	6.60	0.010221
45475	locomotor rhythm	33	11	5.17	6.56	0.010426

Table 3.20 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
278	mitotic cell cycle	341	72	53.46	6.43	0.011241
50875	cellular physiological process	7572	1274	1187.17	6.35	0.011735
45087	innate immune response	55	16	8.62	6.31	0.012001
7613	memory	38	12	5.96	6.13	0.013307
16042	lipid catabolism	34	11	5.33	6.03	0.014069
31123	RNA 3'-end processing	34	11	5.33	6.03	0.014069
31124	mRNA 3'-end processing	34	11	5.33	6.03	0.014069
51168	nuclear export	30	10	4.70	5.96	0.0146
51169	nuclear transport	75	20	11.76	5.78	0.016248
48512	circadian behavior	39	12	6.11	5.66	0.017309
48149	behavioral response to ethanol	15	6	2.35	5.66	0.017362
6418	tRNA aminoacylation for protein translation	66	18	10.35	5.66	0.017367
43039	tRNA aminoacylation	66	18	10.35	5.66	0.017367
45893	positive regulation of transcription, DNA-dependent	85	22	13.33	5.64	0.017507
6974	response to DNA damage stimulus	140	33	21.95	5.56	0.018344
7067	mitosis	288	61	45.15	5.56	0.018366
87	M phase of mitotic cell cycle	289	61	45.31	5.43	0.019764
50832	defense response to fungus	19	7	2.98	5.43	0.019817
43038	amino acid activation	67	18	10.50	5.35	0.020742
48112	oocyte anterior/posterior axis determination (sensu Insecta)	67	18	10.50	5.35	0.020742
7623	circadian rhythm	58	16	9.09	5.25	0.022004
6519	amino acid and derivative metabolism	362	74	56.76	5.24	0.022083
6023	aminoglycan biosynthesis	12	5	1.88	5.17	0.02299
6024	glycosaminoglycan biosynthesis	12	5	1.88	5.17	0.02299
6275	regulation of DNA replication	12	5	1.88	5.17	0.02299
9253	peptidoglycan catabolism	12	5	1.88	5.17	0.02299
45934	negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	158	36	24.77	5.09	0.024075
8654	phospholipid biosynthesis	36	11	5.64	5.08	0.024175
8380	RNA splicing	211	46	33.08	5.04	0.024701
48511	rhythmic process	59	16	9.25	4.93	0.026469
7632	visual behavior	16	6	2.51	4.86	0.027495
9584	detection of visible light	16	6	2.51	4.86	0.027495

Table 3.20 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
19732	antifungal humoral response	16	6	2.51	4.86	0.027495
46489	phosphoinositide biosynthesis	16	6	2.51	4.86	0.027495
9892	negative regulation of metabolism	218	47	34.18	4.81	0.028306
279	M phase	383	77	60.05	4.79	0.028702
6261	DNA-dependent DNA replication	74	19	11.60	4.72	0.029862
70	mitotic sister chromatid segregation	37	11	5.80	4.66	0.030884
819	sister chromatid segregation	37	11	5.80	4.66	0.030884
19538	protein metabolism	2606	452	408.58	4.61	0.031708
45451	pole plasm oskar mRNA localization	51	14	8.00	4.51	0.033732
8033	tRNA processing	33	10	5.17	4.50	0.033861
2217	physiological defense response	120	28	18.81	4.48	0.034195
9719	response to endogenous stimulus	167	37	26.18	4.47	0.034519
43037	translation	226	48	35.43	4.46	0.034761
7622	rhythmic behavior	42	12	6.58	4.45	0.03484
45941	positive regulation of transcription	105	25	16.46	4.43	0.035359
7059	chromosome segregation	162	36	25.40	4.42	0.035425
6807	nitrogen compound metabolism	506	98	79.33	4.39	0.0361
30261	chromosome condensation	29	9	4.55	4.36	0.036756
6873	cell ion homeostasis	38	11	5.96	4.27	0.038853
30003	cation homeostasis	38	11	5.96	4.27	0.038853
6961	antibacterial humoral response (sensu Protostomia)	25	8	3.92	4.25	0.039302
45935	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	106	25	16.62	4.23	0.039801
6865	amino acid transport	52	14	8.15	4.19	0.040576
15837	amine transport	52	14	8.15	4.19	0.040576
6277	DNA amplification	17	6	2.67	4.17	0.041095
15074	DNA integration	17	6	2.67	4.17	0.041095
30717	karyosome formation	17	6	2.67	4.17	0.041095
45471	response to ethanol	17	6	2.67	4.17	0.041095
50830	defense response to Gram-positive bacterium	17	6	2.67	4.17	0.041095
6260	DNA replication	143	32	22.42	4.09	0.043053
45944	positive regulation of transcription from RNA polymerase II promoter	34	10	5.33	4.09	0.043137

Table 3.20 (continued)

GO ID	Biological Process	Total probes on array	Probe sets in list	Expected # probes	χ^2	*p*-value
48111	oocyte axis determination (sensu Insecta)	77	19	12.07	3.98	0.046172
31324	negative regulation of cellular metabolism	203	43	31.83	3.92	0.047654
375	RNA splicing, via transesterification reactions	198	42	31.04	3.87	0.04924
377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	198	42	31.04	3.87	0.04924
398	nuclear mRNA splicing, via spliceosome	198	42	31.04	3.87	0.04924
7049	cell cycle	605	114	94.85	3.86	0.049324
7582	physiological process	8256	1365	1294.41	3.85	0.049766

Table 3.21

Candidate life span genes on the array

Reason	Candidate gene name	Gene symbol	Probe sets	Line *p*-value	Sex *p*-value	Line x Sex *p*-value
Lines on Microarray	*polychaetoid*	*pyd*	1627360_at	0.00371	0.153104	2.15E-10
		pyd	1631905_a_at	0.32705	0.380906	0.118432
		pyd	1630791_a_at	0.39715	0.592731	0.033877
		pyd	1637322_at	0.00059	0.174538	8.53E-08
		pyd	1637428_a_at	0.00021	0.008277	2.13E-10
	mushroom-body expressed	*mub*	1627456_s_at	0.00048	0.06327	0.00026
	crooked legs	*crol*	1626018_a_at	0.08785	0.008821	3.81E-10
	CG10990	*CG10990*	1640020_at	0.00000	0.006239	3.12E-10
	CG9238	*CG9238*	1632222_a_at	0.00002	0.222866	1.51E-05
	BG00817	BG00817	Unknown			
	escargot	*esg*	1641639_at	0.00001	0.003489	1.60E-07
Screen Increased	*CG31531*	*CG31531*	1623254_s_at	0.00016	0.000115	2.39E-14
	Trapped in endoderm-1	*Tre1*	1625691_at	0.00323	0.001547	3.21E-17
	Gustatory receptor 5a	*Gr5a*	No expression			
	CG18418	*CG18418*	1627208_at	0.00075	0.001425	1.71E-14
	Guanine nucleotide exchange factor GEF64C	*Gef64C*	1623087_at	0.66474	0.93185	0.001093
	Osiris 9	*Osi9*	No expression			
	Tetraspanin 42Ef	*Tsp42Ef*	1634845_at	0.38113	0.595013	0.694978
	CG13334	*CG13334*	No expression			
	CG13503	*CG13503*	1634562_s_at	0.17543	0.49916	6.13E-08
	meiotic from via Salaria 332	*mei-S332*	1639029_at	0.00119	0.006952	2.51E-14
	pipsqueak	*psq*	1639136_s_at	0.14164	0.080615	8.37E-14
	CG14696	*CG14696*	1635764_at	0.50798	0.884622	2.92E-07
	CG4674	*CG4674*	1627043_at	0.13420	0.170006	8.27E-12
	CG32679	*CG32679*	No expression			
	Ecdysone-induced protein 75B	*Eip75B*	1635393_s_at	0.35954	0.075864	0.19305
		Eip75B	1640781_a_at	0.04023	0.079918	3.72E-09

Table 3.21 (continued)

Reason	Candidate gene name	Gene symbol	Probe sets	Line *p*-value	Sex *p*-value	Line x Sex *p*-value
	scalloped	*sd*	1625515_a_at	0.03109	0.571102	0.227793
	kayak	*kay*	1640886_a_at	0.00136	0.019123	2.00E-09
	CG13130	*CG13130*	No expression			
	big brain	*bib*	1634044_at	0.10398	0.332684	9.98E-05
	foxhead box, sub-group O	*foxo*	1631597_at	0.24744	0.010422	0.800791
	nervana 3	*nrv3*	1640729_s_at	0.15210	0.658699	1.91E-11
	CG17841	*CG17841*	1623704_at	0.03622	0.07664	5.02E-12
	CG4630	*CG4630*	1633536_at	0.09748	0.037183	5.85E-13
	CG4646	*CG4646*	1638233_at	0.03458	0.024135	2.54E-10
	Dek	*Dek*	1628041_a_at	0.00128	0.006991	7.32E-18
	Glucose transporter 1	*Glut1*	1634033_s_at	0.13956	0.076951	1.36E-10
		Glut1	1628757_at	0.75927	0.800139	0.051221
	Phosphogycerate kinase	*Pgk*	1639766_at	0.15942	0.03507	0.003108
	CG9171	*CG9171*	1633801_s_at	0.08264	0.420647	0.000134
	Defense repressor 1	*Dnr1*	1626336_at	0.11620	0.243392	7.55E-11
	CG32604	*CG32604*	Not on array			
	Fkbp13	*Fkbp13*	1633836_a_at	0.02725	0.383334	1.73E-07
Screen Decreased	*6-phosphofructo-2-kinase*	*Pfrx*	1633454_s_at	0.04065	0.421154	6.91E-10
	Actin 5C	*Act5c*	1626163_s_at	0.01550	0.327268	0.007675
	Adh-related	*Adhr*	Not on array			
	outspread	*osp*	1627054_at	0.44994	0.134978	1.82E-09
	β4GalNAcTA	*β4GalNAcTA*	1638113_at	0.31412	0.10037	2.75E-10
	Basigin	*Bsg*	1628276_s_at	0.04587	0.433909	3.48E-07
		Bsg	1640457_s_at	0.11869	0.504995	0.941386
	CG13334	*CG13334*	No expression			
	CG13333	*CG13333*	No expression			
	CG14478	*CG14478*	1638359_s_at	0.00009	0.084546	7.27E-13
	CG14709	*CG14709*	1637439_at	0.92822	0.553751	3.52E-05
	CG31176	*CG31176*	1637788_at	0.00055	0.044339	1.25E-07
	CG32541	*CG32541*	1640125_s_at	0.19032	0.24999	6.15E-09
		CG32541	1639917_at	0.32928	0.327388	3.90E-12

Table 3.21 (continued)

Reason	Candidate gene name	Gene symbol	Probe sets	Line *p*-value	Sex *p*-value	Line x Sex *p*-value
	CG3777	*CG3777*	1639917_at	0.32928	0.327388	3.90E-12
		CG3777	1637077_s_at	0.00071	8.48E-05	8.25E-09
	CG4004	*CG4004*	1638751_a_at	0.00000	0.001401	5.07E-11
	CG6854	*CG6854*	1639986_at	0.00000	4.33E-10	7.23E-16
		CG6854	1640191_a_at	0.01321	0.056292	2.34E-11
	CG9650	*CG9650*	1625641_s_at	0.09188	0.224863	0.000946
		CG9650	1639550_a_at	0.00006	0.385491	0.890127
	CG9894	*CG9894*	1628814_s_at	0.30507	0.884755	0.001577
	chameau	*chm*	1631863_at	0.03701	0.069558	1.16E-15
	couch potato	*cpo*	1632644_s_at	0.00005	0.001899	5.35E-12
		cpo	1624608_s_at	0.02512	0.104114	5.62E-09
	dacapo	*dap*	1625199_s_at	0.08879	0.121556	2.04E-13
	division abnormally delayed	*dally*	1628489_at	0.05406	0.598904	1.47E-05
	Defense repressor 1	*Dnr1*	1626336_at	0.11620	0.243392	7.55E-11
	escargot	*esg*	1641639_at	0.00001	0.003489	1.60E-07
	failed axon connections	*fax*	1624705_a_at	0.46868	0.174456	4.36E-07
	GDP dissociation inhibitor	*Gdi*	1641129_at	0.70223	0.07848	1.72E-07
	Gliotactin	*Gli*	1624203_s_at	0.18260	0.254794	1.54E-08
	grapes	*grp*	1629880_at	0.48372	0.519405	0.477172
		grp	1634230_s_at	0.03433	0.089501	1.90E-18
		grp	1625416_x_at	0.20469	0.378622	0.029202
	inaF	*inaF*	1633646_at	0.00001	0.01086	2.16E-12
	innexin 7	*inx7*	1638225_a_at	0.01022	0.033513	0.01024
	ken and barbie	*ken*	1628840_at	0.00369	0.003084	1.02E-16
	Laminin A	*LanA*	1641324_at	0.00000	0.211264	0.003946
	Lipid storage droplet-2	*Lsd-2*	1637359_at	0.00007	0.02482	4.60E-13
	longitudinals lacking	*lola*	1623411_at	0.03613	0.064539	8.80E-11
		lola	1634495_s_at	0.03664	0.672669	7.56E-08
		lola	1627324_at	0.04434	0.00672	4.73E-14
		lola	1637581_at	0.04663	0.074247	3.57E-11
		lola	1625768_s_at	0.12377	0.106139	1.58E-16
		lola	1641609_at	0.13149	0.694246	1.45E-05
		lola	1629523_at	0.19323	0.207933	0.703383

Table 3.21 (continued)

Reason	Candidate gene name	Gene symbol	Probe sets	Line *p*-value	Sex *p*-value	Line x Sex *p*-value
		lola	1640945_at	0.20505	0.132804	0.014554
		lola	1633089_a_at	0.22648	0.838396	4.12E-07
		lola	1630936_at	0.55668	0.727902	0.018777
		lola	1624729_at	0.01158	0.251913	1.38E-06
		lola	1633422_a_at	0.01160	0.314343	1.03E-09
		lola	1628421_at	0.01769	0.117611	6.07E-11
		lola	1640280_at	0.02480	0.016341	1.98E-09
	Malic enzyme	*Men*	1629095_a_at	0.00003	0.008492	3.49E-15
	mushroom-body expressed	*mub*	1627456_s_at	0.00048	0.06327	0.00026
	neuralized	*neur*	1638682_a_at	0.79723	0.093373	1.06E-10
	plexus	*px*	1638412_at	0.12987	0.023615	0.008999
	polychaetoid	*pyd*	1627360_at	0.00371	0.153104	2.15E-10
		pyd	1631905_a_at	0.32705	0.380906	0.118432
		pyd	1630791_a_at	0.39715	0.592731	0.033877
		pyd	1637322_at	0.00059	0.174538	8.53E-08
		pyd	1637428_a_at	0.00021	0.008277	2.13E-10
	Protein kinase 61C	*Pk61C*	1629078_s_at	0.00001	0.205837	0.000459
	Protein tyrosine phosphatase 10D	*Ptp10D*	1624660_at	0.58279	0.440217	1.94E-09
		Ptp10D	1634653_a_at	0.04158	0.345119	2.15E-08
	Rab23	*Rab23*	1635643_at	0.00163	0.001611	1.54E-17
	rutabaga	*rut*	1628594_at	0.28583	0.022281	3.28E-05
	Sema-1a	*Sema-1a*	1634588_at	0.43898	0.163681	7.17E-07
	serrano	*sano*	1627971_s_at	0.25419	0.710066	1.47E-11
	singed	*sn*	1622932_s_at	0.00002	0.026886	2.87E-12
	Tob	*Tob*	1628358_at	0.00714	0.026936	0.000435
	Vacuolar H+ ATPase 16kD subunit	*Vha16*	1632117_s_at	0.05983	0.382292	0.002188
Known life span genes	*I'm not dead yet*	*Indy*	1641566_s_at	0.00108	0.343714	8.12E-06
	chico	*chico*	1631374_at	0.00223	0.167928	1.01E-05
	Insulin-like receptor	*InR*	1629141_at	0.00000	0.02448	0.080178
	Superoxide dismutase	*Sod*	1630845_at	0.00047	0.029841	2.99E-08

Table 3.21 (continued)

Reason	Candidate gene name	Gene symbol	Probe sets	Line *p*-value	Sex *p*-value	Line x Sex *p*-value
	Superoxide dismutase 2 (Mn)	*Sod2*	1622984_at	0.06031	0.269416	0.002475
	Alcohol dehydrogenase	*Adh*	1632699_s_at	0.00907	0.367264	1.35E-10
		Adh	1631333_s_at	0.00008	0.015023	0.020979
	shuttle craft	*stc*	1640237_a_at	0.00716	0.072542	7.62E-13
	Dopa decarboxylase	*Ddc*	1639320_a_at	0.58643	0.643968	4.64E-11
	Target of rapamycin	*Tor*	1641510_at	0.08786	0.27943	2.32E-09
	Catecholamines up	*Catsup*	1627458_at	0.12968	0.241096	1.57E-09
	Glutathione S transferase S1	*GstS1*	1623957_s_at	0.00369	0.006176	1.36E-16
	Sirt2	*Sirt2*	1626458_at	0.00013	0.006002	2.11E-10
	methuselah	*mth*	1632989_a_at	0.03618	0.551107	0.155524
	Pten	*Pten*	1628114_s_at	0.13704	0.016861	2.89E-13
		Pten	1630738_s_at	0.90044	0.12625	4.30E-06
	Akt1	*Akt1*	1639064_s_at	0.01945	0.173143	1.34E-09
	Vacuolar H^+-ATPase SFD subunit	*VhaSFD*	1633931_a_at	0.00037	0.227853	1.54E-06
	sugarbabe	*sug*	1624195_at	0.01664	0.350361	9.81E-07
	cheerio	*cher*	1632339_s_at	0.13092	0.114286	0.385455
	four wheel drive	*fwd*	1634509_s_at	0.44338	0.222732	0.008595
	CTP:phosphocholine cytidylyltransferase 1	*Cctl*	1629930_s_at	0.00107	0.05407	2.96E-13
	Red-herring	*Rdh*	Not on array			

TABLE 3.22

Estimates of general combining abilities

		Sexes Pooled		Females		Males	
Line	**Gene**	***GCA***	***P***	***GCA***	***P***	***GCA***	***P***
BG00495	*CG10990*	–0.6638	0.1641	0.5955	0.4325	**–1.8068**	**0.0012**
BG00817	BG00817	**1.5670**	**0.0009**	1.2635	0.084	**1.8424**	**0.0011**
BG00004	BG00004	0.4812	0.3059	**2.4923**	**0.0006**	**–1.5666**	**0.0051**
BG00761	*CG9238*	0.2563	0.5867	**3.2779**	**<0.0001**	**–2.8056**	**<0.0001**
BG01042	*esg*	–0.7269	0.1254	**–3.2864**	**<0.0001**	**1.9000**	**0.0008**
BG00010	*CG31531*	**1.5870**	**0.0009**	**1.5548**	**0.0418**	**1.6623**	**0.0031**
BG00028	*pyd*	0.0561	0.9053	–0.4742	0.5155	0.5674	0.3109
BG00043	*mub*	–0.5655	0.2304	–0.8261	0.2569	–0.2794	0.6177
BG00297	BG00297	**–1.9396**	**<.0001**	**–2.5550**	**0.0004**	**–1.3586**	**0.0151**
BG00346	*crol*	–0.0518	0.9127	**–2.0422**	**0.0053**	**1.8449**	**0.0010**

Significant GCA values are indicated in bold font

TABLE 3.23

Estimates of specific combining abilities

Parents		Sexes pooled		Females		Males	
Female	Male	*SCA*	*P*	*SCA*	*P*	*SCA*	*P*
CG10990	BG00817	**–2.6385**	**0.0362**	–3.8020	0.0505	–1.5620	0.2978
CG10990	BG00004	1.5840	0.1971	–1.4972	0.4317	**4.5304**	**0.0019**
CG10990	*CG9238*	1.7647	0.1548	**4.9736**	**0.0105**	–1.2306	0.3978
CG10990	*esg*	1.6296	0.1851	2.3565	0.2164	0.6638	0.6488
CG10990	*CG31531*	1.9008	0.1730	3.5255	0.1733	0.9725	0.5081
CG10990	*pyd*	–0.0433	0.9722	0.3098	0.8720	–0.5479	0.7092
CG10990	*mub*	–0.8485	0.4918	–1.2787	0.5021	–0.6191	0.6734
CG10990	BG00297	**–3.0326**	**0.0136**	–0.9749	0.6086	**–5.2276**	**0.0003**
CG10990	*crol*	–0.3162	0.7979	–3.6126	0.0582	**3.0207**	**0.0399**
BG00817	BG00004	0.6810	0.5842	**–3.7901**	**0.0455**	**5.4464**	**0.0003**
BG00817	*CG9238*	–0.4150	0.7402	0.7992	0.6791	–1.6181	0.2762
BG00817	*esg*	0.5964	0.6305	**5.0580**	**0.0083**	**–3.9553**	**0.0073**
BG00817	*CG31531*	–1.8877	0.1267	0.3724	0.8453	**–4.2355**	**0.0040**
BG00817	*pyd*	0.4603	0.7130	0.9369	0.6278	–0.0279	0.9850
BG00817	*mub*	1.6460	0.1841	2.1875	0.2530	1.0394	0.4798
BG00817	BG00297	1.5871	0.2022	–0.1482	0.9382	**3.3979**	**0.0222**
BG00817	*crol*	–0.0297	0.981	–1.6136	0.4040	1.5152	0.3030
BG00004	*CG9238*	1.6761	0.1737	**6.3954**	**0.0007**	**–3.2356**	**0.0278**
BG00004	*esg*	0.5017	0.6840	1.2157	0.5249	–0.3014	0.8361
BG00004	*CG31531*	1.7028	0.1699	**3.9120**	**0.0421**	–0.5600	0.7031
BG00004	*pyd*	0.9017	0.4621	0.8725	0.6449	0.9312	0.5223
BG00004	*mub*	–1.0340	0.4035	–0.2022	0.9166	–1.8220	0.2107
BG00004	BG00297	–4.8906	<.0001	**–6.4467**	**0.0007**	**–3.2667**	**0.0278**
BG00004	*crol*	–1.1228	0.3622	–0.4594	0.8084	–1.7222	0.2412
CG9238	*esg*	–0.9705	0.4427	–0.9697	0.6122	–1.0160	0.5078
CG9238	*CG31531*	**–4.0712**	**0.0010**	**–7.4721**	**0.0001**	–0.7747	0.5947
CG9238	*pyd*	0.9181	0.4563	–1.6130	0.3945	**3.4610**	**0.0186**

Table 3.23 (continued)

Parents		Sexes pooled		Females		Males	
Female	Male	*SCA*	*P*	*SCA*	*P*	*SCA*	*P*
CG9238	*mub*	**2.9702**	**0.0160**	1.2639	0.5046	**4.6155**	**0.0017**
CG9238	BG00297	–0.7777	0.5258	–0.0072	0.9969	–1.5288	0.2937
CG9238	*crol*	–1.0947	0.3745	–3.3700	0.0755	1.3273	0.3666
esg	*CG31531*	0.8908	0.4798	1.5912	0.4132	0.1123	0.9404
esg	*pyd*	–1.6157	0.1968	–2.9622	0.1217	–0.0987	0.9476
esg	*mub*	–1.3070	0.2988	**–4.8410**	**0.0115**	2.6844	0.0770
esg	BG00297	**2.5025**	**0.0424**	0.9071	0.6319	**4.1636**	**0.0047**
esg	*crol*	–2.2278	0.0767	–2.3558	0.2278	–2.2526	0.1297
CG31531	*pyd*	–1.8490	0.1383	–3.0835	0.1094	–0.6269	0.6728
CG31531	*mub*	–0.9071	0.4627	–1.5380	0.4200	–0.3445	0.8147
CG31531	BG00297	**2.4624**	**0.0461**	0.7646	0.6910	**4.0033**	**0.0060**
CG31531	*crol*	1.7581	0.1651	1.9280	0.3314	1.4536	0.3275
pyd	*mub*	–0.3082	0.8034	1.7926	0.3534	–2.4309	0.0951
pyd	BG00297	–1.4650	0.2321	–2.2051	0.2442	–0.7268	0.6175
pyd	*crol*	**3.0011**	**0.0159**	**5.9521**	**0.0021**	0.0670	0.9637
mub	BG00297	1.6853	0.1754	3.5976	0.0599	–0.2644	0.8586
mub	*crol*	–1.8966	0.1240	–0.9816	0.6080	**–2.8584**	**0.0497**
BG00297	*crol*	1.9286	0.1176	**4.5129**	**0.0173**	–0.5505	0.7079

TABLE 3.24

Pleiotropic effects of mutations increasing life span on starvation resistance (week 1)

	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
Line	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG00004 (F)	36.7 (-0.4)	30.9 (-6.5)	42.4 (4.6)	-0.01	-0.12	0.09	****	ns	ns	ns	ns
BG00008 (F)	34.0 (-24.1)	31.7 (-24.7)	36.3 (-23.6)	-0.38	-1.02	-0.29	*	****	ns	****	*
BG00010 (F)	41.2 (12.0)	29.3 (-11.3)	53.1 (30.9)	0.21	-0.21	0.60	****	*	****	ns†	****
BG00028 (F)	68.7 (86.6)	62.9 (90.3)	74.4 (83.6)	1.53	1.64	1.61	****	****	ns	****	****
BG00037 (F)	34.9 (-22.0)	29.3 (-30.4)	40.5 (-14.6)	-0.35	-1.25	-0.18	***	****	ns	****	ns
BG00041 (F)	35.5 (-17.9)	29.3 (-24.1)	41.6 (-12.8)	-0.30	-0.70	-0.20	****	****	ns	****	ns†
BG00042 (F)	32.3 (-25.3)	41.1 (6.2)	23.5 (-50.8)	-0.43	0.18	-0.78	ns†	****	****	ns	****
BG00043 (F)	49.9 (35.5)	42.7 (29.0)	57.1 (40.8)	0.63	0.53	0.79	****	****	ns†	****	****
BG00080 (B)	41.1 (-23.0)	38.1 (-8.9)	44.0 (-32.1)	-0.42	-0.32	-0.91	****	****	****	*	****
BG00121 (F)	35.1 (-21.7)	32.3 (-23.4)	37.9 (-20.2)	-0.34	-0.96	-0.25	*	****	ns	****	*
BG00297 (F)	41.3 (12.3)	37.6 (13.7)	45.1 (11.2)	0.22	0.25	0.22	****	*	ns	*	ns
BG00336 (B)	38.0 (-28.8)	36.8 (-12.1)	39.2 (-39.5)	-0.52	-0.44	-1.12	****	****	****	**	****
BG00346 (F)	41.1 (11.6)	41.6 (25.8)	40.5 (0.0)	0.20	0.47	0.00	ns†	*	*	***	ns
BG00472 (F)	28.8 (-35.7)	27.7 (-34.2)	29.9 (-37.1)	-0.56	-1.41	-0.46	ns†	****	ns	****	***
BG00495 (F)	43.1 (17.0)	35.7 (8.1)	50.4 (24.3)	0.30	0.15	0.47	****	**	ns†	ns	**
BG00528 (B)	39.2 (-26.5)	38.1 (-8.9)	40.3 (-37.9)	-0.48	-0.32	-1.08	****	****	****	*	****
BG00757 (F)	25.9 (-42.3)	28.3 (-32.9)	23.5 (-50.6)	-0.67	-1.35	-0.62	ns	****	*	****	****
BG00761 (F)	37.3 (1.4)	32.8 (-0.8)	41.9 (3.3)	0.03	-0.01	0.06	****	ns	ns	ns	ns
BG00767 (B)	36.5 (-31.5)	40.3 (-3.8)	32.8 (-49.4)	-0.57	-0.14	-1.41	****	****	****	ns	****
BG00817 (F)	41.9 (13.8)	42.9 (29.8)	40.8 (0.7)	0.24	0.54	0.01	ns	**	*	****	ns
BG00864 (B)	36.9 (-30.8)	38.1 (-8.9)	35.7 (-44.9)	-0.56	-0.32	-1.28	****	****	****	*	****
BG00890 (F)	30.5 (-29.3)	30.4 (-21.4)	30.7 (-35.8)	-0.50	-0.62	-0.55	**	****	*	****	****
BG00907 (F)	35.2 (-21.4)	32.0 (-24.1)	38.4 (-19.1)	-0.34	-0.99	-0.24	**	****	ns	****	*
BG00915 (F)	30.0 (-30.6)	31.2 (-19.3)	28.8 (-39.7)	-0.52	-0.56	-0.60	ns	****	*	***	****
BG01004 (F)	36.7 (-15.1)	34.4 (-11.0)	38.9 (-18.4)	-0.26	-0.32	-0.28	***	**	ns	*	*
BG01030 (A)	47.7 (33.1)	38.7 (13.3)	56.8 (51.1)	0.55	0.28	0.74	****	****	***	*	****
BG01031 (A)	32.8 (-3.1)	36.0 (3.1)	29.6 (-9.8)	-0.06	0.14	-0.13	**	ns	ns	ns	ns
BG01042 (F)	39.2 (6.5)	39.7 (20.2)	38.7 (-4.6)	0.11	0.37	-0.09	ns†	ns	*	**	ns

Table 3.24 (continued)

Line	Mutational effects: Mean (%[a]) ♂,♀	♂	♀	Mutational effects: a/σ_p[b] ♂,♀	♂	♀	*P*-values from ANOVA: Sexes Pooled[c] *S*	*L*	*L*×*S*	Sexes Separate *L*♂	*L*♀
BG01121 (F)	32.4 (-27.7)	34.1 (-19.0)	30.7 (-35.4)	-0.44	-0.78	-0.44	ns	****	*	****	***
BG01345 (A)	37.1 (3.3)	42.4 (24.2)	31.7 (-15.6)	0.06	0.51	-0.23	ns†	ns	***	***	ns†
BG01403 (A)	38.7 (7.8)	35.7 (4.7)	41.6 (10.6)	0.13	0.10	0.15	*	ns	ns	ns	ns
BG01540 (B)	37.6 (-29.5)	37.3 (-10.8)	37.9 (-41.6)	-0.54	-0.39	-1.18	****	****	****	*	****
BG01550 (F)	33.6 (-25.0)	34.1 (-19.0)	33.1 (-30.3)	-0.39	-0.78	-0.37	ns	****	ns	****	**
BG01553 (F)	33.2 (-23.1)	32.0 (-17.2)	34.4 (-27.9)	-0.39	-0.50	-0.43	**	****	ns†	***	****
BG01615 (A)	39.5 (10.0)	44.3 (29.7)	34.7 (-7.8)	0.17	0.62	-0.11	ns	ns†	**	****	ns
BG01677 (A)	39.9 (11.2)	38.7 (13.3)	41.1 (9.2)	0.18	0.28	0.13	ns	*	ns	*	ns
BG01700 (F)	38.1 (-14.9)	36.0 (-14.6)	40.3 (-15.2)	-0.23	-0.60	-0.19	*	**	ns	****	ns†
BG01701 (F)	35.1 (-21.7)	33.1 (-21.5)	37.1 (-21.9)	-0.34	-0.89	-0.27	*	****	ns	****	**
BG01702 (A)	36.5 (7.9)	35.7 (2.3)	37.3 (13.8)	0.14	0.10	0.18	ns	ns†	ns	ns	ns
BG01710 (A)	54.9 (62.2)	46.1 (32.1)	63.7 (94.3)	1.14	1.43	1.24	****	****	****	****	****
BG01878 (B)	37.1 (-30.5)	37.6 (-10.2)	36.5 (-43.6)	-0.56	-0.37	-1.24	****	****	****	*	****
BG01918 (A)	34.7 (2.4)	37.6 (7.6)	31.7 (-3.3)	0.04	0.34	-0.04	*	ns	ns	*	ns
BG01950 (B)	36.1 (-32.3)	28.3 (-32.5)	44.0 (-32.1)	-0.59	-1.17	-0.91	****	****	*	****	****
BG01976 (B)	39.6 (-25.8)	42.9 (2.5)	36.3 (-44.0)	-0.47	0.09	-1.25	****	****	****	ns	****
BG02019 (B)	41.7 (-27.2)	40.3 (-18.4)	43.2 (-33.9)	-0.64	-0.76	-0.94	****	****	***	****	****
BG02039 (A)	43.6 (28.7)	45.9 (31.3)	41.3 (26.0)	0.53	1.39	0.34	ns†	****	ns	****	*
BG02049 (B)	52.3 (-2.0)	42.7 (1.9)	61.9 (-4.5)	-0.04	0.07	-0.13	****	ns	ns	ns	ns
BG02128 (B)	44.7 (-16.3)	38.9 (-7.0)	50.4 (-22.2)	-0.30	-0.25	-0.63	****	****	**	ns†	****
BG02395 (B)	40.4 (-24.3)	37.6 (-10.2)	43.1 (-33.5)	-0.44	-0.37	-0.95	****	****	****	*	****
BG02644 (B)	63.9 (19.7)	54.4 (29.9)	74.0 (14.2)	0.36	1.08	0.40	****	****	ns	****	*

Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$.

TABLE 3.25

Pleiotropic effects of mutations increasing life span on starvation resistance (week 6)

	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
Line	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG00004 (F)	26.5 (-19.8)	23.5 (-8.3)	29.6 (-27.0)	-0.32	-0.20	-0.65	****	****	**	ns†	****
BG00008 (F)	28.7 (-23.5)	23.6 (-18.2)	31.7 (-31.2)	-0.27	-0.40	-0.38	****	****	*	**	***
BG00010 (F)	28.8 (-12.9)	21.9 (-14.6)	35.7 (-11.8)	-0.21	-0.35	-0.29	****	***	ns	**	*
BG00028 (F)	39.1 (18.1)	31.5 (22.9)	46.7 (15.1)	0.29	0.55	0.37	****	***	ns	***	*
BG00037 (F)	20.8 (-44.4)	10.1 (-65.0)	29.1 (-37.0)	-0.50	-1.44	-0.45	****	****	ns	****	***
BG00041 (F)	29.6 (-9.8)	25.1 (-13.0)	34.1 (-7.2)	-0.14	-0.29	-0.10	****	ns†	ns	*	ns
BG00042 (F)	36.9 (12.6)	41.9 (45.4)	32.0 (-13.0)	0.18	1.00	-0.17	ns	ns†	***	****	ns
BG00043 (F)	29.8 (-9.9)	24.9 (-2.9)	34.4 (-15.1)	-0.16	-0.07	-0.37	****	*	ns†	ns	*
BG00080 (B)	31.7 (-22.6)	26.1 (-13.3)	37.2 (-28.1)	-0.32	-0.40	-0.60	****	****	***	**	****
BG00121 (F)	24.0 (-35.9)	24.0 (-16.7)	24.0 (-48.0)	-0.41	-0.37	-0.59	****	****	****	***	****
BG00297 (F)	29.5 (-10.9)	25.1 (-2.1)	33.9 (-16.4)	-0.18	-0.05	-0.40	****	*	ns†	ns	*
BG00336 (B)	28.9 (-29.3)	22.4 (-25.7)	35.5 (-31.4)	-0.42	-0.77	-0.67	****	****	**	****	****
BG00346 (F)	26.3 (-20.6)	22.1 (-13.5)	30.4 (-25.0)	-0.33	-0.33	-0.60	****	****	*	**	***
BG00472 (F)	23.6 (-37.0)	19.7 (-31.5)	27.5 (-40.5)	-0.42	-0.70	-0.49	****	****	*	****	****
BG00495 (F)	25.6 (-22.6)	23.5 (-8.3)	27.7 (-31.6)	-0.36	-0.20	-0.76	****	****	****	ns†	****
BG00528 (B)	26.5 (-35.2)	20.8 (-31.0)	32.3 (-37.6)	-0.50	-0.93	-0.80	****	****	***	****	****
BG00757 (F)	20.8 (-44.5)	19.2 (-33.3)	22.4 (-51.4)	-0.51	-0.74	-0.63	****	****	***	****	****
BG00761 (F)	25.1 (-24.2)	20.5 (-19.8)	29.6 (-27.0)	-0.39	-0.48	-0.65	****	****	*	***	****
BG00767 (B)	29.2 (-28.6)	24.0 (-20.4)	33.6 (-35.1)	-0.41	-0.61	-0.74	****	****	**	****	****
BG00817 (F)	28.0 (-15.3)	24.3 (-5.2)	31.7 (-21.7)	-0.25	-0.13	-0.52	****	**	*	ns	**
BG00864 (B)	30.2 (-26.2)	23.7 (-21.5)	35.5 (-31.4)	-0.37	-0.65	-0.67	****	****	**	****	****
BG00890 (F)	28.5 (-13.0)	24.3 (-15.7)	32.8 (-10.9)	-0.19	-0.35	-0.14	****	*	ns	*	ns
BG00907 (F)	27.6 (-26.3)	22.1 (-23.1)	33.1 (-28.3)	-0.30	-0.51	-0.35	****	****	ns	****	**
BG00915 (F)	30.8 (-6.2)	35.5 (23.1)	25.9 (-29.5)	-0.09	0.51	-0.39	ns	ns	****	***	**
BG01004 (F)	28.9 (-11.8)	24.5 (-14.8)	33.3 (-9.4)	-0.17	-0.33	-0.12	****	*	ns	**	ns
BG01030 (A)	35.3 (11.8)	26.7 (5.3)	44.0 (16.2)	0.14	0.13	0.20	****	ns†	ns	ns	ns
BG01031 (A)	24.3 (-18.0)	21.3 (-7.0)	27.2 (-25.0)	-0.26	-0.23	-0.42	****	****	**	ns	***
BG01042 (F)	28.4 (-14.1)	24.0 (-6.3)	32.8 (-19.1)	-0.23	-0.15	-0.46	****	***	*	ns	**

Table 3.25 (continued)

Line	Mutational effects: Mean (%[a]) ♂,♀	Mean (%[a]) ♂	Mean (%[a]) ♀	a/σ_p[b] ♂,♀	a/σ_p[b] ♂	a/σ_p[b] ♀	*P*-values from ANOVA: Sexes Pooled[c] *S*	Sexes Pooled[c] *L*	Sexes Pooled[c] *L*×*S*	Sexes Separate *L*♂	Sexes Separate *L*♀
BG01121 (F)	22.3 (-40.6)	21.1 (-26.9)	23.5 (-49.1)	-0.46	-0.59	-0.60	****	****	***	****	****
BG01345 (A)	26.8 (-15.2)	22.7 (-10.5)	30.9 (-18.3)	-0.18	-0.26	-0.22	****	*	ns	*	ns†
BG01403 (A)	26.1 (-17.3)	20.8 (-17.9)	31.5 (-16.9)	-0.21	-0.44	-0.21	****	**	ns	***	ns†
BG01540 (B)	33.1 (-19.2)	26.1 (-13.3)	40.0 (-22.7)	-0.28	-0.40	-0.48	****	****	***	**	****
BG01550 (F)	25.2 (-32.7)	22.9 (-20.4)	27.5 (-40.5)	-0.37	-0.45	-0.49	****	****	**	****	****
BG01553 (F)	26.3 (-19.9)	22.4 (-22.2)	30.1 (-18.1)	-0.28	-0.49	-0.24	****	***	ns	****	*
BG01615 (A)	28.7 (-9.3)	25.9 (2.1)	31.5 (-16.9)	-0.11	0.05	-0.21	****	ns†	ns†	ns	ns†
BG01677 (A)	27.7 (-12.2)	21.9 (-13.7)	33.6 (-11.3)	-0.15	-0.33	-0.14	****	*	ns	**	ns
BG01700 (F)	32.9 (-12.1)	26.9 (-6.5)	38.9 (-15.6)	-0.14	-0.14	-0.19	****	*	ns	ns	ns†
BG01701 (F)	27.7 (-26.0)	22.1 (-23.1)	33.3 (-27.7)	-0.29	-0.51	-0.34	****	****	ns	****	**
BG01702 (A)	29.2 (-1.4)	21.6 (-5.8)	36.8 (1.5)	-0.02	-0.19	0.02	****	ns	ns	ns	ns
BG01710 (A)	39.3 (32.9)	23.7 (3.5)	54.9 (51.5)	0.47	0.12	0.86	****	****	****	ns	****
BG01878 (B)	28.1 (-31.3)	22.7 (-24.8)	33.6 (-35.1)	-0.45	-0.75	-0.74	****	****	***	****	****
BG01918 (A)	22.5 (-23.9)	20.0 (-12.8)	25.1 (-30.9)	-0.34	-0.42	-0.52	****	****	**	**	****
BG01950 (B)	27.0 (-34.0)	13.6 (-54.9)	31.5 (-39.2)	-0.49	-1.65	-0.83	****	****	ns	****	****
BG01976 (B)	25.9 (-36.7)	21.6 (-28.3)	28.8 (-44.3)	-0.53	-0.85	-0.94	****	****	****	****	****
BG02019 (B)	28.7 (-40.1)	20.8 (-31.0)	36.5 (-44.3)	-0.47	-0.93	-1.13	****	****	****	****	****
BG02039 (A)	28.5 (-3.6)	22.9 (0.0)	34.1 (-5.9)	-0.05	0.00	-0.10	****	ns	ns	ns	ns
BG02049 (B)	39.6 (-3.3)	25.1 (-16.8)	54.6 (5.6)	-0.05	-0.51	0.12	****	ns	*	***	ns
BG02128 (B)	26.7 (-34.9)	20.0 (-33.6)	33.3 (-35.6)	-0.50	-1.01	-0.75	****	****	**	****	****
BG02395 (B)	25.1 (-38.8)	18.7 (-38.1)	31.5 (-39.2)	-0.56	-1.14	-0.83	****	****	**	****	****
BG02644 (B)	50.3 (22.8)	34.8 (15.4)	70.4 (36.1)	0.33	0.46	0.76	****	****	***	*	****

Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$.

TABLE 3.26

Pleiotropic effects of mutations increasing life span on chill coma recovery (week 1)

	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
Line	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG00004 (F)	9.4 (-14.3)	10.3 (-11.2)	8.4 (-18.5)	-0.27	-0.26	-0.30	**	**	ns	ns†	*
BG00008 (F)	12.7 (2.6)	13.7 (2.2)	11.8 (3.4)	0.07	0.06	0.09	****	ns	ns	ns	ns
BG00010 (F)	9.9 (-9.9)	12.2 (4.8)	7.6 (-26.7)	-0.18	0.11	-0.43	****	*	***	ns	****
BG00028 (F)	11.9 (8.4)	12.7 (9.4)	11.1 (6.8)	0.16	0.21	0.11	*	ns	ns	ns	ns
BG00037 (F)	11.9 (-4.4)	12.7 (-5.5)	11.0 (-3.0)	-0.11	-0.15	-0.08	****	ns	ns	ns	ns
BG00041 (F)	13.0 (5.2)	12.7 (-5.0)	13.4 (17.4)	0.13	-0.14	0.46	ns	ns	**	ns	**
BG00042 (F)	16.0 (34.9)	15.0 (20.5)	16.9 (50.2)	0.86	0.64	1.08	ns	****	*	**	****
BG00043 (F)	10.7 (-2.3)	10.5 (-9.5)	10.9 (5.5)	-0.04	-0.22	0.09	ns	ns	ns	ns	ns
BG00080 (B)	18.6 (84.6)	20.5 (78.3)	16.7 (93.1)	2.14	2.95	3.30	***	****	ns	****	****
BG00121 (F)	9.6 (-22.8)	10.4 (-22.1)	8.7 (-23.3)	-0.57	-0.61	-0.62	***	****	ns	***	****
BG00297 (F)	10.2 (-7.1)	10.8 (-7.2)	9.6 (-7.4)	-0.13	-0.16	-0.12	*	ns	ns	ns	ns
BG00336 (B)	11.4 (13.3)	12.7 (10.6)	10.1 (17.4)	0.34	0.40	0.62	****	***	ns	*	**
BG00346 (F)	10.1 (-7.9)	10.5 (-10.1)	9.8 (-5.6)	-0.15	-0.23	-0.09	ns	ns	ns	ns	ns
BG00472 (F)	10.1 (-18.4)	11.1 (-16.9)	9.1 (-20.3)	-0.46	-0.47	-0.54	****	****	ns	****	****
BG00495 (F)	10.7 (-2.6)	12.0 (3.1)	9.4 (-9.3)	-0.05	0.07	-0.15	***	ns	ns	ns	ns
BG00528 (B)	11.6 (15.7)	11.6 (1.0)	11.7 (35.1)	0.40	0.04	1.25	****	****	****	ns	****
BG00757 (F)	10.4 (-16.6)	11.1 (-17.4)	9.6 (-15.3)	-0.41	-0.48	-0.41	**	***	ns	*	*
BG00761 (F)	10.5 (-4.7)	11.9 (2.1)	9.1 (-11.9)	-0.09	0.05	-0.19	**	ns	ns	ns	ns
BG00767 (B)	17.5 (73.3)	17.3 (50.7)	17.6 (103.5)	1.85	1.91	3.67	**	****	**	****	****
BG00817 (F)	10.8 (-2.1)	11.0 (-5.8)	10.5 (1.6)	-0.04	-0.13	0.03	ns	ns	ns	ns	ns
BG00864 (B)	11.4 (13.6)	11.8 (2.2)	11.1 (28.8)	0.34	0.08	1.02	***	**	*	ns	***
BG00890 (F)	9.8 (-17.4)	9.1 (-27.1)	10.5 (-7.0)	-0.43	-0.85	-0.15	ns	****	**	****	ns
BG00907 (F)	8.7 (-29.5)	10.1 (-24.9)	7.4 (-34.8)	-0.74	-0.69	-0.93	****	****	ns	****	****
BG00915 (F)	12.4 (4.3)	12.7 (1.9)	12.0 (6.7)	0.11	0.06	0.14	*	ns	ns	ns	ns
BG01004 (F)	9.0 (-23.8)	10.4 (-16.6)	7.8 (-30.8)	-0.59	-0.52	-0.66	****	****	ns†	***	****
BG01030 (A)	11.6 (37.5)	12.5 (32.4)	10.7 (44.8)	0.79	1.09	0.83	****	****	ns	****	****
BG01031 (A)	9.1 (-4.3)	9.7 (-10.0)	8.5 (3.7)	-0.09	-0.27	0.10	****	ns	*	**	ns
BG01042 (F)	10.6 (-3.6)	11.8 (1.6)	9.3 (-10.0)	-0.07	0.04	-0.16	****	ns	ns	ns	ns

Table 3.26 (continued)

	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
Line	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG01121 (F)	10.5 (-15.8)	11.6 (-13.7)	9.3 (-18.0)	-0.39	-0.38	-0.48	****	****	ns	**	**
BG01345 (A)	8.5 (0.8)	8.8 (-6.8)	8.2 (11.2)	0.02	-0.23	0.21	****	ns	*	ns	ns
BG01403 (A)	9.5 (11.8)	10.5 (11.3)	8.3 (11.4)	0.25	0.38	0.21	****	**	ns	**	ns
BG01540 (B)	9.1 (-9.8)	10.3 (-10.5)	7.8 (-9.3)	-0.25	-0.40	-0.33	****	***	ns	**	*
BG01550 (F)	10.5 (-15.6)	11.4 (-14.7)	9.5 (-16.5)	-0.39	-0.40	-0.44	****	****	ns	**	**
BG01553 (F)	11.3 (-5.1)	12.2 (-2.0)	10.3 (-9.0)	-0.13	-0.06	-0.19	**	ns	ns	ns	ns
BG01615 (A)	9.9 (17.0)	11.5 (21.8)	8.1 (8.9)	0.36	0.73	0.16	****	***	ns†	***	ns
BG01677 (A)	9.0 (6.6)	10.4 (9.7)	7.8 (5.1)	0.14	0.33	0.09	****	ns†	ns	ns†	ns
BG01700 (F)	9.7 (-22.2)	9.9 (-26.1)	9.4 (-17.4)	-0.55	-0.72	-0.47	**	****	*	****	***
BG01701 (F)	8.7 (-30.1)	9.3 (-30.8)	8.1 (-29.1)	-0.75	-0.85	-0.78	****	****	ns	****	****
BG01702 (A)	8.8 (-6.9)	9.9 (-7.8)	7.7 (-5.3)	-0.15	-0.21	-0.15	****	ns†	ns	ns	ns
BG01710 (A)	10.6 (11.6)	11.1 (3.0)	10.1 (23.3)	0.26	0.08	0.65	****	**	*	ns	***
BG01878 (B)	10.4 (3.6)	11.5 (0.1)	9.3 (7.4)	0.09	0.01	0.26	****	ns	ns	ns	ns
BG01918 (A)	9.5 (-0.3)	10.0 (-7.5)	9.0 (9.8)	-0.01	-0.21	0.27	****	ns	ns†	ns	ns
BG01950 (B)	16.1 (59.6)	13.4 (16.5)	18.5 (114.1)	1.51	0.62	4.05	*	****	****	***	****
BG01976 (B)	12.2 (21.6)	11.9 (3.5)	12.6 (45.9)	0.55	0.13	1.63	**	****	****	ns	****
BG02019 (B)	11.6 (14.8)	12.6 (9.6)	10.5 (22.2)	0.37	0.36	0.79	****	****	ns	*	***
BG02039 (A)	9.0 (-5.5)	10.3 (-4.1)	7.5 (-8.2)	-0.12	-0.11	-0.23	****	ns†	ns	ns	ns†
BG02049 (B)	9.6 (-4.4)	9.7 (-15.6)	9.5 (10.4)	-0.11	-0.59	0.37	****	ns	****	****	ns†
BG02128 (B)	10.7 (6.1)	11.2 (-2.9)	10.2 (17.8)	0.15	-0.11	0.63	****	*	***	ns	***
BG02395 (B)	11.5 (14.1)	11.3 (-2.0)	11.7 (35.5)	0.36	-0.08	1.26	**	**	***	ns	****
BG02644 (B)	11.6 (15.2)	11.8 (2.3)	11.4 (32.4)	0.39	0.09	1.15	****	****	***	ns	****

Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$.

TABLE 3.27

Pleiotropic effects of mutations increasing life span on chill coma recovery (week 6)

	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
Line	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG00004 (F)	18.7 (-31.2)	20.9 (-27.0)	16.6 (-36.8)	-0.46	-0.46	-0.50	*	****	ns	**	****
BG00008 (F)	31.1 (-8.8)	29.8 (-20.3)	32.7 (3.9)	-0.15	-0.45	0.06	ns	ns	*	**	ns
BG00010 (F)	21.6 (-20.7)	24.4 (-14.9)	18.8 (-28.6)	-0.31	-0.25	-0.39	*	**	ns	ns	**
BG00028 (F)	18.9 (-30.7)	17.2 (-40.0)	20.6 (-21.6)	-0.46	-0.68	-0.30	ns	****	*	****	*
BG00037 (F)	24.7 (-27.8)	28.0 (-24.9)	21.8 (-30.7)	-0.48	-0.55	-0.47	***	****	ns	**	****
BG00041 (F)	19.1 (-44.1)	18.3 (-51.1)	20.2 (-35.8)	-0.76	-1.13	-0.55	ns	****	*	****	****
BG00042 (F)	28.1 (-22.4)	29.2 (-30.1)	27.0 (-11.4)	-0.43	-1.35	-0.18	****	****	***	****	ns†
BG00043 (F)	23.6 (-13.4)	23.5 (-17.9)	23.6 (-10.2)	-0.20	-0.31	-0.14	ns	*	ns	ns†	ns
BG00080 (B)	19.7 (16.6)	20.0 (8.0)	19.3 (25.4)	0.21	0.09	0.39	ns	ns†	ns	ns	*
BG00121 (F)	15.4 (-55.0)	17.9 (-52.2)	12.9 (-59.2)	-0.95	-1.15	-0.91	***	****	ns	****	****
BG00297 (F)	17.6 (-35.1)	16.7 (-41.8)	18.7 (-29.1)	-0.52	-0.71	-0.40	ns	****	ns	****	**
BG00336 (B)	13.9 (-17.7)	13.3 (-28.0)	14.5 (-6.0)	-0.22	-0.32	-0.09	ns	**	*	**	ns
BG00346 (F)	15.0 (-44.7)	14.4 (-49.6)	15.6 (-40.6)	-0.67	-0.84	-0.55	ns	****	ns	****	****
BG00472 (F)	23.8 (-30.3)	22.5 (-39.7)	25.1 (-20.2)	-0.53	-0.87	-0.31	ns	****	**	****	*
BG00495 (F)	14.8 (-45.5)	16.1 (-43.9)	13.6 (-48.3)	-0.68	-0.75	-0.66	ns†	****	ns	****	****
BG00528 (B)	20.4 (20.9)	21.0 (13.6)	19.8 (28.8)	0.26	0.15	0.44	ns†	**	ns	ns	**
BG00757 (F)	20.5 (-40.0)	22.5 (-39.9)	18.4 (-41.4)	-0.69	-0.88	-0.64	**	****	ns	****	****
BG00761 (F)	24.9 (-8.7)	26.8 (-6.5)	22.9 (-12.8)	-0.13	-0.11	-0.18	ns	ns	ns	ns	ns
BG00767 (B)	19.1 (13.4)	19.0 (2.6)	19.3 (25.2)	0.17	0.03	0.39	ns	ns	ns	ns	*
BG00817 (F)	19.8 (-27.1)	24.8 (-13.6)	15.3 (-42.0)	-0.40	-0.23	-0.57	***	****	*	ns	****
BG00864 (B)	25.6 (51.7)	24.9 (34.3)	26.3 (70.9)	0.64	0.39	1.09	ns	****	ns	**	****
BG00890 (F)	20.4 (-43.8)	20.9 (-50.0)	19.9 (-34.8)	-0.85	-2.25	-0.56	****	****	***	****	****
BG00907 (F)	15.8 (-53.6)	17.1 (-54.2)	14.6 (-53.5)	-0.93	-1.19	-0.82	**	****	ns	****	****
BG00915 (F)	19.4 (-46.5)	20.9 (-50.1)	18.4 (-39.8)	-0.90	-2.25	-0.64	****	****	**	****	****
BG01004 (F)	21.8 (-39.8)	23.7 (-43.2)	20.1 (-34.0)	-0.77	-1.94	-0.54	****	****	*	****	****
BG01030 (A)	19.51 (24.2)	19.76 (11.8)	19.27 (41.6)	0.43	0.21	1.26	*	****	ns	ns	****
BG01031 (A)	16.9 (30.2)	16.1 (12.6)	17.7 (50.9)	0.49	0.19	1.21	ns	****	**	ns	****
BG01042 (F)	15.7 (-42.3)	14.8 (-48.3)	16.6 (-37.0)	-0.63	-0.82	-0.51	ns	****	ns	****	****

Table 3.27 (continued)

Line	Mutational effects: Mean (%[a]) ♂,♀	Mean (%[a]) ♂	Mean (%[a]) ♀	a/σ_p[b] ♂,♀	a/σ_p[b] ♂	a/σ_p[b] ♀	*P*-values from ANOVA: Sexes Pooled[c] *S*	Sexes Pooled[c] *L*	Sexes Pooled[c] *L*×*S*	Sexes Separate *L*♂	Sexes Separate *L*♀
BG01121 (F)	21.2 (-38.1)	21.8 (-41.6)	20.6 (-34.7)	-0.66	-0.92	-0.53	*	****	ns	****	****
BG01345 (A)	18.05 (14.9)	17.33 (-1.9)	18.79 (38.1)	0.26	-0.03	1.15	ns	ns	*	ns	**
BG01403 (A)	22.54 (43.5)	20.11 (13.8)	24.74 (81.8)	0.77	0.24	2.47	ns	****	***	ns	****
BG01540 (B)	12.8 (-24.4)	12.8 (-30.9)	12.7 (-17.5)	-0.30	-0.35	-0.27	ns	****	ns	**	*
BG01550 (F)	23.8 (-30.2)	28.7 (-23.1)	19.1 (-39.4)	-0.52	-0.51	-0.61	****	****	ns	**	****
BG01553 (F)	24.2 (-33.3)	24.9 (-40.3)	23.4 (-23.3)	-0.65	-1.81	-0.37	****	****	***	****	**
BG01615 (A)	17.4 (10.8)	17.89 (1.2)	16.93 (24.4)	0.19	0.02	0.74	*	ns	ns	ns	*
BG01677 (A)	13.83 (-12.0)	16.11 (-8.8)	11.77 (-13.5)	-0.21	-0.15	-0.41	****	ns	ns	ns	**
BG01700 (F)	19.0 (-44.3)	20.3 (-45.6)	17.7 (-43.8)	-0.77	-1.00	-0.67	*	****	ns	****	****
BG01701 (F)	21.7 (-36.6)	23.1 (-38.1)	20.1 (-36.1)	-0.63	-0.84	-0.55	**	****	ns	****	****
BG01702 (A)	16.2 (24.5)	15.5 (8.9)	16.8 (43.2)	0.40	0.13	1.03	ns	***	*	ns	***
BG01710 (A)	14.6 (12.4)	15.9 (11.4)	13.2 (12.8)	0.20	0.17	0.31	**	ns†	ns	ns	ns†
BG01878 (B)	15.5 (-8.3)	17.2 (-6.9)	13.7 (-11.0)	-0.10	-0.08	-0.17	**	ns	ns	ns	ns
BG01918 (A)	15.2 (16.9)	14.4 (0.8)	15.9 (35.8)	0.27	0.01	0.85	ns	*	*	ns	***
BG01950 (B)	21.7 (28.4)	24.1 (30.1)	19.2 (24.7)	0.35	0.34	0.38	**	**	ns	*	**
BG01976 (B)	25.8 (53.2)	30.5 (64.7)	21.0 (36.7)	0.66	0.73	0.57	****	****	*	****	***
BG02019 (B)	18.5 (9.7)	20.2 (8.9)	16.9 (9.8)	0.12	0.10	0.15	**	ns	ns	ns	ns
BG02039 (A)	12.8 (-1.7)	14.3 (0.5)	11.2 (-4.6)	-0.03	0.01	-0.11	***	ns	ns	ns	ns
BG02049 (B)	12.4 (-26.6)	12.5 (-32.7)	12.3 (-20.1)	-0.33	-0.37	-0.31	ns†	****	ns	***	**
BG02128 (B)	17.7 (4.9)	21.5 (16.3)	13.9 (-9.9)	0.06	0.18	-0.15	***	ns	ns	ns	ns
BG02395 (B)	16.2 (-3.8)	17.8 (-3.9)	14.6 (-5.2)	-0.05	-0.04	-0.08	*	ns	ns	ns	ns
BG02644 (B)	17.2 (1.8)	17.0 (-8.4)	17.4 (13.2)	0.02	-0.10	0.20	ns	ns	ns	ns	ns

Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$.

TABLE 3.28

Pleiotropic effects of mutations increasing life span on climbing activity (week 1)

	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
Line	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG00004 (F)	14.4 (-4.1)	13.7 (-11.6)	15.0 (3.9)	-0.03	-0.09	0.03	ns	ns	ns	ns	ns
BG00008 (F)	17.2 (91.3)	19.6 (154.1)	14.7 (44.0)	0.55	0.83	0.30	ns	****	**	****	*
BG00010 (F)	15.6 (4.0)	20.2 (30.3)	10.9 (-24.2)	0.03	0.24	-0.17	**	ns	*	*	ns
BG00028 (F)	6.6 (-56.2)	7.6 (-51.3)	5.6 (-61.4)	-0.42	-0.41	-0.44	ns	****	ns	**	***
BG00037 (F)	16.1 (79.2)	14.3 (85.7)	17.8 (74.3)	0.48	0.46	0.50	*	****	ns	**	***
BG00041 (F)	14.5 (62.1)	15.8 (105.6)	13.2 (29.3)	0.38	0.57	0.20	ns	****	ns†	****	ns
BG00043 (F)	8.3 (-44.9)	10.0 (-35.8)	6.5 (-54.7)	-0.34	-0.28	-0.39	ns	****	ns	*	***
BG00080 (B)	17.4 (15.5)	14.5 (7.4)	20.3 (22.0)	0.14	0.06	0.22	**	ns	ns	ns	ns†
BG00121 (F)	16.4 (82.3)	15.7 (103.5)	17.0 (66.4)	0.50	0.56	0.45	ns	****	ns	***	**
BG00297 (F)	14.1 (-6.0)	12.0 (-22.7)	16.2 (12.0)	-0.05	-0.18	0.09	ns	ns	ns	ns	ns
BG00336 (B)	17.9 (18.2)	17.0 (25.6)	18.7 (12.2)	0.17	0.22	0.12	ns	ns†	ns	ns†	ns
BG00346 (F)	19.9 (32.8)	19.0 (22.3)	20.8 (44.1)	0.25	0.18	0.31	ns	**	ns	ns	**
BG00472 (F)	15.2 (69.3)	14.3 (85.3)	16.1 (57.3)	0.42	0.46	0.38	ns	****	ns	**	**
BG00495 (F)	16.5 (9.8)	18.9 (21.5)	14.0 (-2.8)	0.07	0.17	-0.02	ns	ns	ns	ns	ns
BG00528 (B)	16.0 (6.0)	14.4 (6.2)	17.6 (5.8)	0.05	0.05	0.06	*	ns	ns	ns	ns
BG00757 (F)	16.0 (78.8)	16.4 (113.0)	15.7 (53.1)	0.48	0.61	0.36	ns	****	ns	***	*
BG00761 (F)	19.3 (28.7)	18.7 (20.6)	19.8 (37.4)	0.22	0.16	0.27	ns	*	ns	ns	*
BG00767 (B)	16.1 (6.3)	15.7 (16.0)	16.4 (-1.6)	0.06	0.14	-0.02	ns	ns	ns	ns	ns
BG00817 (F)	13.6 (-9.1)	13.3 (-14.4)	13.9 (-3.5)	-0.07	-0.11	-0.02	ns	ns	ns	ns	ns
BG00864 (B)	18.4 (22.0)	19.7 (45.8)	17.1 (2.6)	0.20	0.39	0.03	ns	*	*	**	ns
BG00907 (F)	16.1 (79.9)	16.1 (109.5)	16.1 (57.7)	0.48	0.59	0.39	ns	****	ns	***	*
BG01031 (A)	11.8 (-15.6)	13.9 (10.3)	9.7 (-37.0)	-0.12	0.07	-0.32	ns	ns	*	ns	*
BG01042 (F)	15.6 (4.2)	16.0 (3.0)	15.2 (5.5)	0.03	0.02	0.04	ns	ns	ns	ns	ns
BG01121 (F)	8.0 (-10.8)	8.0 (4.3)	8.0 (-22.1)	-0.07	0.02	-0.15	ns	ns	ns	ns	ns
BG01540 (B)	13.9 (-8.1)	12.6 (-6.9)	15.2 (-9.0)	-0.07	-0.06	-0.09	ns†	ns	ns	ns	ns
BG01550 (F)	16.1 (79.0)	19.2 (149.4)	12.9 (26.1)	0.48	0.81	0.17	ns	****	**	****	ns
BG01700 (F)	16.1 (79.2)	18.2 (136.4)	13.9 (36.2)	0.48	0.74	0.24	ns	****	*	****	ns
BG01701 (F)	20.4 (127.5)	21.4 (177.5)	19.4 (89.9)	0.77	0.96	0.60	ns	****	ns	****	****

Table 3.28 (continued)

Line	Mutational effects: Mean (%[a]) ♂,♀	Mean (%[a]) ♂	Mean (%[a]) ♀	a/σ_p[b] ♂,♀	a/σ_p[b] ♂	a/σ_p[b] ♀	*P*-values from ANOVA: Sexes Pooled[c] *S*	Sexes Pooled[c] *L*	Sexes Pooled[c] *L*×*S*	Sexes Separate *L*♂	Sexes Separate *L*♀
BG01702 (A)	11.9 (-15.3)	11.2 (-11.1)	12.5 (-18.7)	-0.12	-0.08	-0.16	ns	ns	ns	ns	ns
BG01710 (A)	10.3 (-26.3)	8.6 (-31.9)	12.0 (-21.7)	-0.20	-0.22	-0.19	ns†	*	ns	ns†	ns
BG01878 (B)	13.5 (-10.7)	10.0 (-25.9)	16.9 (1.6)	-0.10	-0.22	0.02	***	ns	ns	ns†	ns
BG01918 (A)	7.4 (-47.3)	6.3 (-49.9)	8.4 (-45.2)	-0.36	-0.34	-0.39	ns	****	ns	**	**
BG01950 (B)	15.0 (-1.0)	13.2 (-2.2)	16.7 (0.0)	-0.01	-0.02	0.00	*	ns	ns	ns	ns
BG01976 (B)	9.4 (-37.6)	7.5 (-44.6)	11.3 (-32.0)	-0.35	-0.38	-0.32	*	****	ns	**	*
BG02019 (B)	11.9 (-21.4)	11.8 (-13.1)	12.0 (-28.2)	-0.20	-0.11	-0.28	ns	*	ns	ns	*
BG02039 (A)	7.9 (-43.7)	6.7 (-46.7)	9.0 (-41.3)	-0.34	-0.32	-0.35	ns	****	ns	**	**
BG02049 (B)	7.7 (-49.0)	7.4 (-45.6)	8.0 (-51.8)	-0.45	-0.39	-0.51	ns	****	ns	*	***
BG02128 (B)	14.0 (-7.2)	15.3 (13.3)	12.7 (-23.8)	-0.07	0.11	-0.24	ns	ns	ns†	ns	ns†
BG02395 (B)	13.5 (-10.5)	10.9 (-19.5)	16.1 (-3.2)	-0.10	-0.17	-0.03	**	ns	ns	ns	ns
BG02644 (B)	16.6 (9.8)	17.6 (30.0)	15.6 (-6.6)	0.09	0.26	-0.07	ns	ns	ns†	ns†	ns

Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$.

TABLE 3.29

Pleiotropic effects of mutations increasing life span on climbing activity (week 6)

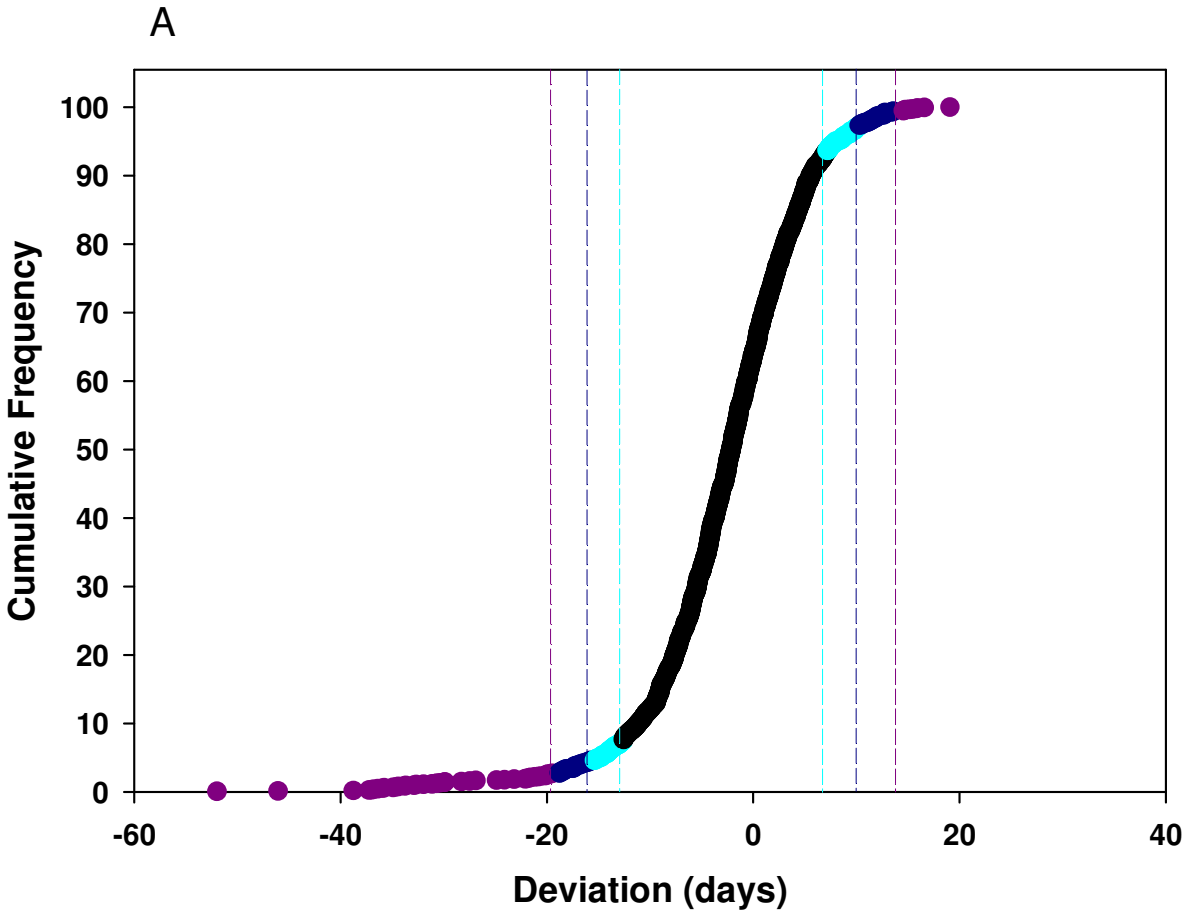
Line	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
	♂,♀	♂	♀	♂,♀	♂	♀	*S*	*L*	*L*×*S*	*L*♂	*L*♀
BG00004 (F)	8.1 (170.6)	6.2 (210.0)	10.0 (150.8)	0.67	0.59	0.77	**	****	ns	**	****
BG00008 (F)	3.6 (8.0)	1.1 (-46.7)	6.1 (31.4)	-0.07	-0.13	0.16	****	ns	ns	ns	ns
BG00010 (F)	5.2 (74.4)	3.4 (71.7)	7.0 (75.8)	0.29	0.20	0.39	***	**	ns	ns	*
BG00028 (F)	3.8 (26.1)	4.5 (123.3)	3.1 (-22.5)	0.10	0.35	-0.11	ns	ns	ns†	ns†	ns
BG00037 (F)	4.8 (42.5)	3.9 (93.3)	5.6 (20.7)	0.07	0.26	0.11	*	ns	ns	ns	ns
BG00041 (F)	4.3 (29.5)	4.3 (113.3)	4.4 (-6.4)	0.02	0.32	-0.03	ns†	ns	ns	*	ns
BG00043 (F)	2.1 (-28.9)	1.4 (-30.0)	2.9 (-28.3)	-0.11	-0.08	-0.14	*	ns	ns	ns	ns
BG00080 (B)	10.6 (183.4)	11.5 (817.3)	9.7 (80.2)	0.60	2.46	0.33	ns	****	*	****	*
BG00121 (F)	8.9 (167.5)	7.5 (273.3)	10.4 (122.1)	0.56	0.77	0.63	**	****	ns	***	***
BG00297 (F)	12.1 (302.2)	9.9 (393.3)	14.3 (256.7)	1.18	1.11	1.30	**	****	ns	****	****
BG00336 (B)	7.9 (111.7)	9.2 (638.7)	6.6 (22.2)	0.37	1.92	0.09	ns	***	**	****	ns
BG00346 (F)	8.1 (168.3)	4.7 (136.7)	11.4 (184.2)	0.66	0.39	0.94	***	****	*	ns†	****
BG00472 (F)	4.1 (23.0)	2.2 (10.0)	6.0 (28.6)	-0.01	0.03	0.15	****	ns	ns	ns	ns
BG00495 (F)	10.5 (248.3)	10.4 (420.0)	10.5 (162.5)	0.97	1.19	0.83	ns	****	ns	****	***
BG00528 (B)	7.8 (107.2)	3.0 (137.3)	12.5 (132.1)	0.35	0.41	0.54	****	****	*	ns†	****
BG00757 (F)	5.2 (57.0)	4.9 (146.7)	5.5 (18.6)	0.12	0.42	0.10	ns†	*	ns	*	ns
BG00761 (F)	7.9 (163.3)	6.7 (233.3)	9.1 (128.3)	0.64	0.66	0.65	*	****	ns	**	***
BG00767 (B)	6.1 (63.1)	3.5 (180.0)	8.7 (61.1)	0.21	0.54	0.25	****	**	ns	*	ns†
BG00817 (F)	4.8 (60.0)	2.2 (10.0)	7.4 (85.0)	0.23	0.03	0.43	****	*	ns†	ns	*
BG00864 (B)	6.4 (72.0)	5.8 (361.3)	7.1 (31.5)	0.24	1.09	0.13	*	*	ns	**	ns
BG00907 (F)	3.9 (17.0)	3.5 (75.0)	4.3 (-7.9)	-0.03	0.21	-0.04	*	ns	ns	ns	ns
BG01031 (A)	2.6 (-33.8)	3.1 (12.2)	2.2 (-58.1)	-0.13	0.04	-0.26	ns	ns	ns†	ns	*
BG01042 (F)	8.7 (190.6)	10.6 (431.7)	6.8 (70.0)	0.74	1.22	0.36	ns	****	**	****	*
BG01121 (F)	1.1 (-67.5)	1.2 (-41.7)	1.0 (-78.6)	-0.37	-0.12	-0.41	*	***	*	ns	***
BG01540 (B)	5.9 (58.6)	4.4 (252.0)	7.5 (38.3)	0.19	0.76	0.16	**	*	ns	*	ns
BG01550 (F)	4.0 (20.5)	3.5 (73.3)	4.6 (-2.1)	-0.02	0.21	-0.01	*	ns	ns	ns	ns
BG01700 (F)	3.3 (0.0)	3.2 (58.3)	3.5 (-25.0)	-0.10	0.17	-0.13	ns†	ns	ns	ns	ns
BG01701 (F)	6.9 (107.5)	5.4 (171.7)	8.4 (80.0)	0.32	0.49	0.41	**	***	ns	*	*

Table 3.29 (continued)

Line	Mutational effects						*P*-values from ANOVA				
	Mean (%[a])			a/σ_p[b]			Sexes Pooled[c]			Sexes Separate	
	♂,♀	♂	♀	♂,♀	♂	♀	***S***	***L***	***L*×*S***	***L*♂**	***L*♀**
BG01702 (A)	8.1 (105.1)	4.6 (69.5)	11.6 (123.9)	0.41	0.24	0.56	****	****	*	ns	***
BG01710 (A)	3.9 (-2.5)	3.7 (34.1)	4.0 (-21.9)	-0.01	0.12	-0.10	ns	ns	ns	ns	ns
BG01878 (B)	8.5 (126.4)	9.4 (654.7)	7.5 (38.9)	0.42	1.97	0.16	ns	***	*	****	ns
BG01918 (A)	6.9 (73.4)	6.4 (134.1)	7.3 (41.3)	0.29	0.47	0.19	ns	**	ns	*	ns
BG01950 (B)	5.7 (52.9)	8.5 (580.0)	3.9 (-28.4)	0.17	1.75	-0.12	ns	*	***	***	ns
BG01976 (B)	6.4 (70.6)	7.1 (464.0)	5.9 (9.9)	0.23	1.40	0.04	ns	**	*	****	ns
BG02019 (B)	3.2 (-14.4)	2.1 (68.0)	4.3 (-20.4)	-0.05	0.20	-0.08	**	ns	ns	ns	ns
BG02039 (A)	7.2 (81.0)	3.4 (24.4)	10.9 (111.0)	0.32	0.09	0.50	****	**	*	ns	**
BG02049 (B)	8.5 (126.4)	9.0 (622.7)	7.9 (46.3)	0.42	1.88	0.19	ns	****	*	****	ns
BG02128 (B)	6.3 (69.3)	6.4 (409.3)	6.3 (16.7)	0.23	1.23	0.07	ns†	*	ns†	**	ns
BG02395 (B)	8.2 (119.3)	5.8 (366.7)	10.6 (95.7)	0.39	1.10	0.39	***	***	ns	**	**
BG02644 (B)	7.3 (94.7)	6.4 (412.0)	8.2 (51.2)	0.31	1.24	0.21	**	***	ns	***	ns†

Letters in parenthesis after the Line name denote different co-isogenic *Canton S* host strains for *P{GT1}*-element insertion. [a] Percent deviation from the mean life span of the control line. [b] standardized mutational effect (see text for explanation), [c] *S* and *L* denote the main cross-classified effects of Sex and Line, respectively in the ANOVA of life span. ns $P > 0.1$, † $0.05 < P < 0.1$, * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, **** $P < 0.0001$.

Figure 3.1 – Cumulative frequency distributions of effects of mutations on life span, expressed as deviations from the mean of co-isogenic controls. Lines with mean life spans exceeding the 95%, 99% and 99.9% confidence intervals are depicted in cyan, dark blue and dark pink, respectively. (A) Sexes pooled. (B) Males. (C) Females.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Cumulative Frequency
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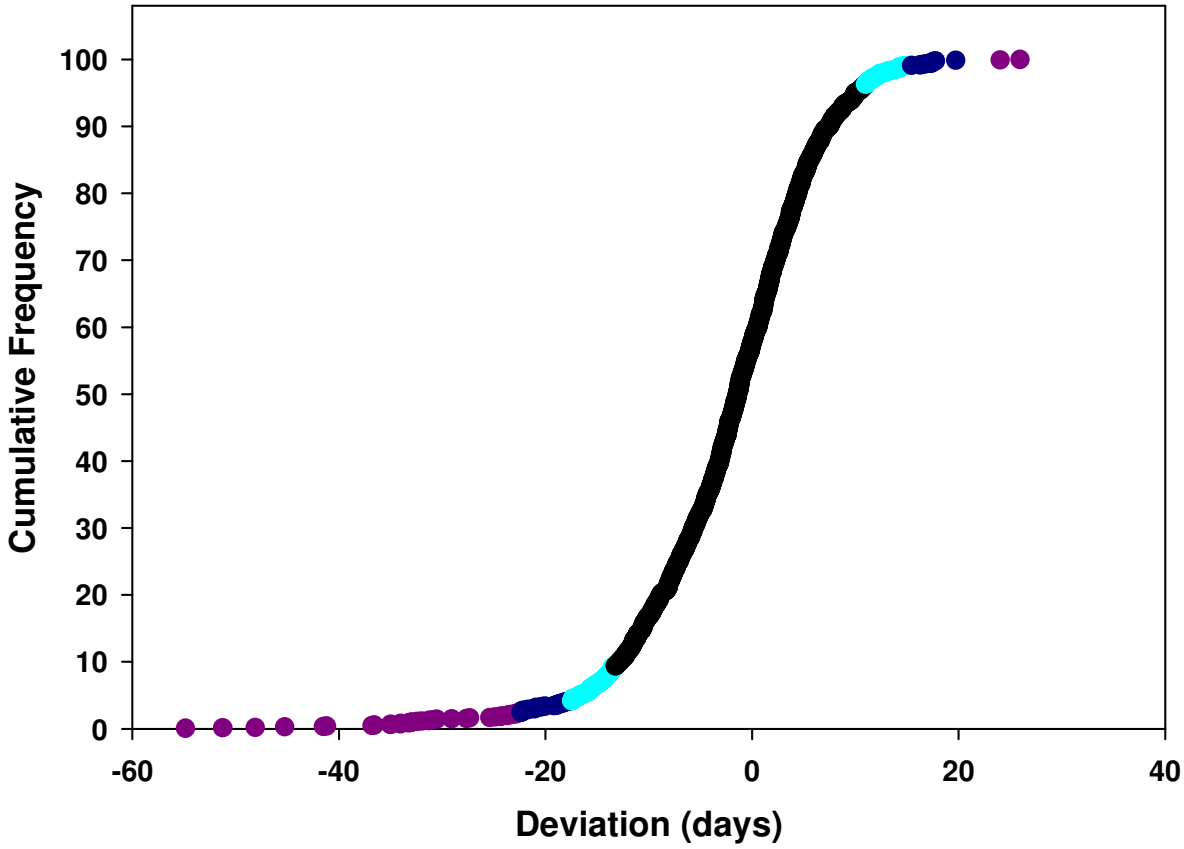
80
70
60
50
40
30
20
10
0
-60
-40
-20
0
20
40
Deviation (days)

B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90
80
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60
50
40
30
20
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0
-60
-40
-20
0
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40
Deviation (days)

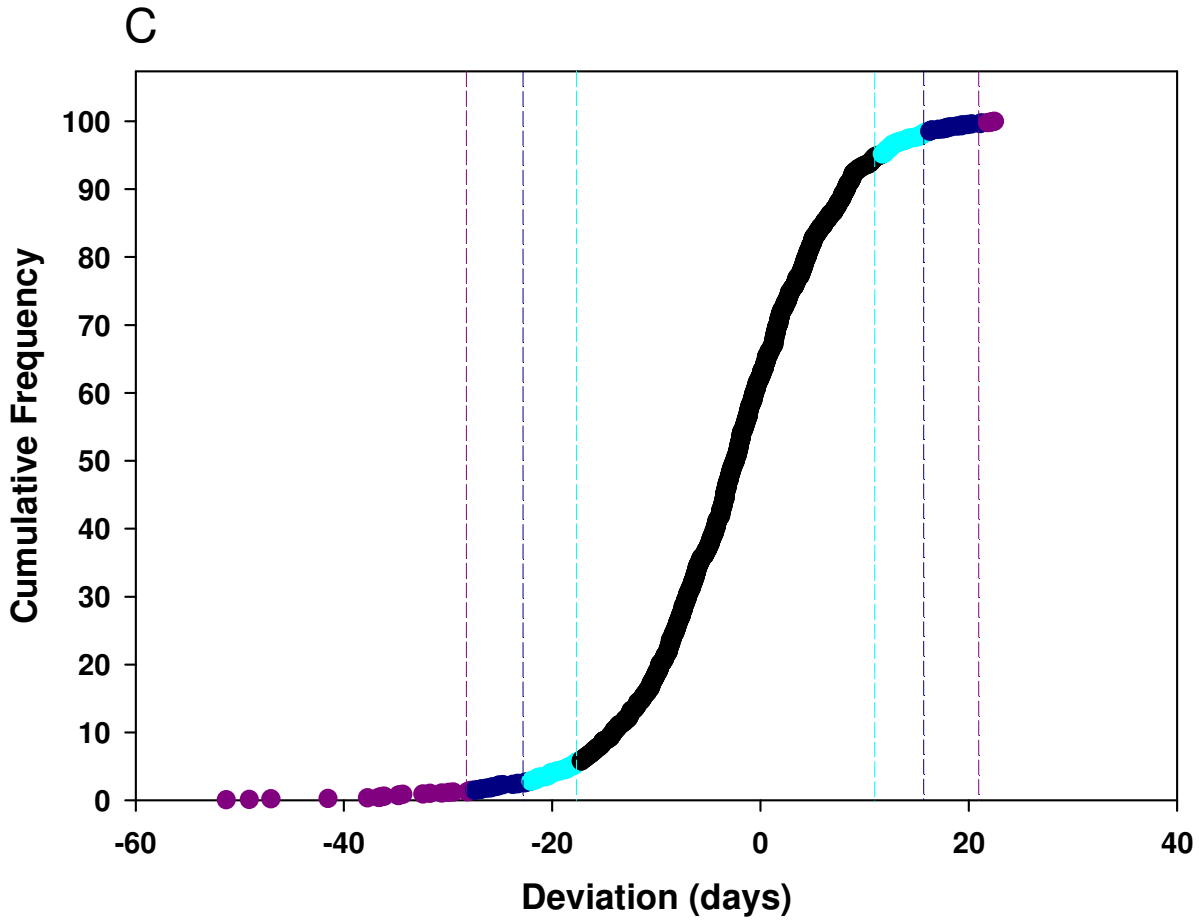
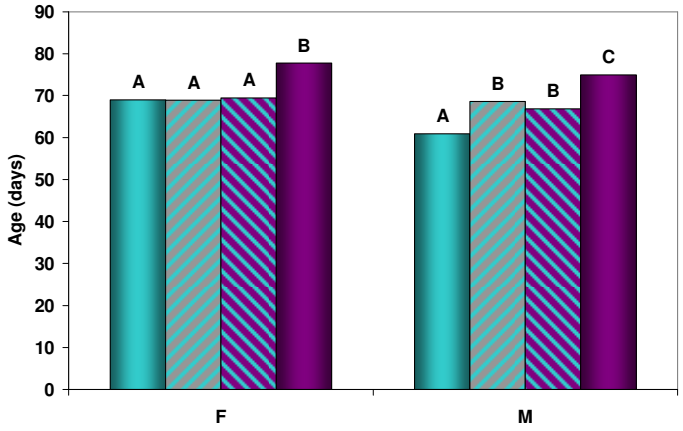

C
Cumulative Frequency
100
90
80
70
60
50
40
30
20
10
0
-60
-40
-20
0
20
40
Deviation (days)

Figure 3.2 – Analysis of revertant alleles. M denotes males and F denotes females. (A) Genotypes are *mub*^{BG00043} (dark pink), *mub*^{rev1} (precise revertant, cyan and grey hatched), *mub*^{rev3} (imprecise revertant, cyan and dark pink hatched), and Canton S F control (cyan). (B) Genotypes are *crol*^{BG00346} (dark pink), *crol*^{ev4} (precise revertant, cyan and grey hatched), and Canton S F control (cyan). (C) Genotypes are *esg*^{BG01042} (dark pink), *esg*^{rev3} (precise revertant, cyan and grey hatched), and Canton S F control (cyan).

A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80
70
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20
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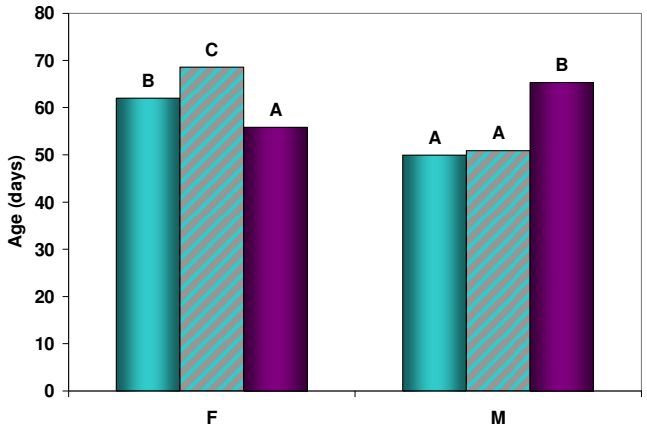
A
A
A
B
A
B
B
C
F
M

B


Age (days)
80
70
60
50
40
30
20
10
0
B
C
A
A
A
B
F
M

C

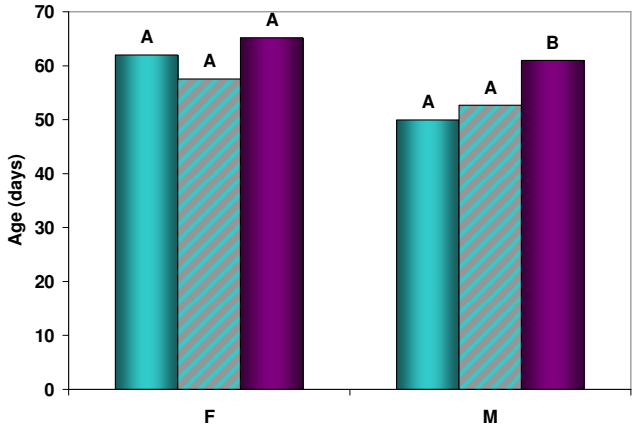

Age (days)
70
60
50
40
30
20
10
0
A
A
A
A
A
B
F
M

Figure 3.3 – Epistatic interactions between *P*-element insert lines associated with increased life span. Significant *SCA* effects that enhance the mutant phenotype (*i.e.*, are longer-lived than expected) are indicated by dark pink lines, and significant *SCA* effects that suppress the mutant phenotype (*i.e.*, are shorter-lived than expected) are indicated by cyan lines. (A) Sexes pooled. (B) Males. (C) Females.

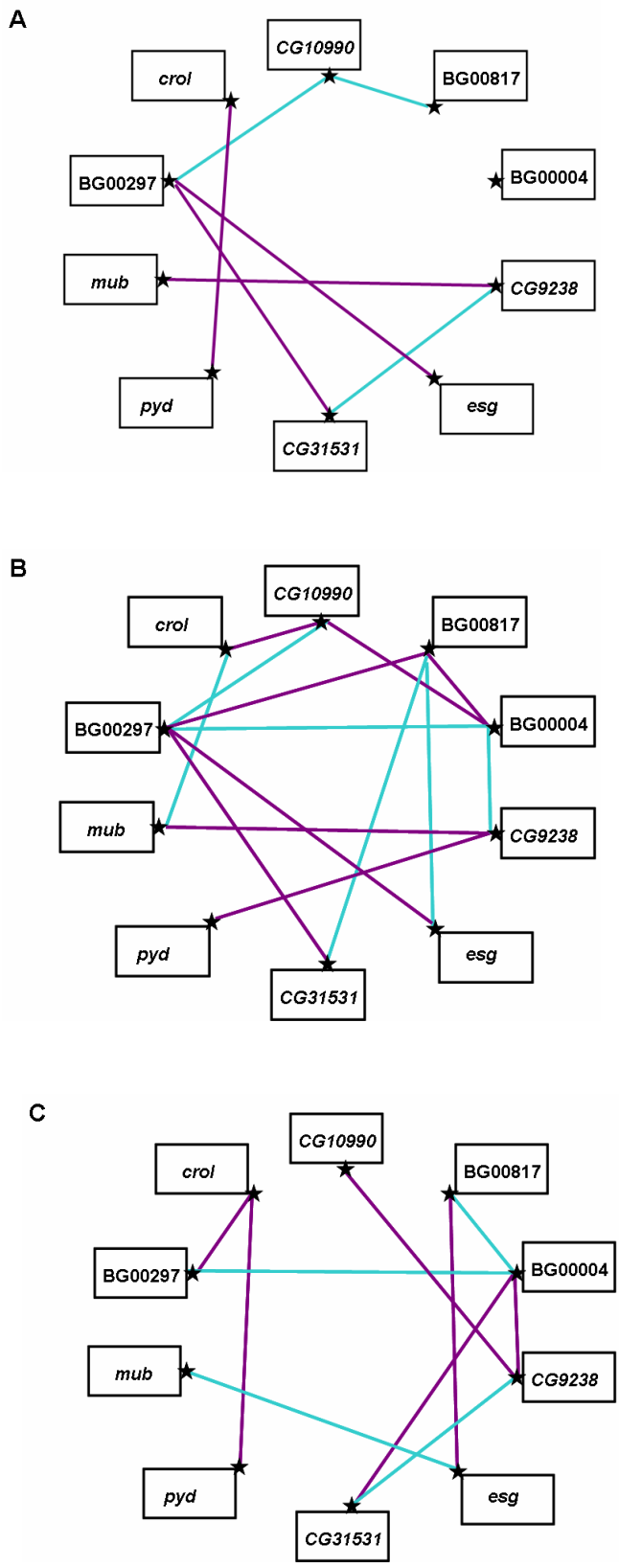
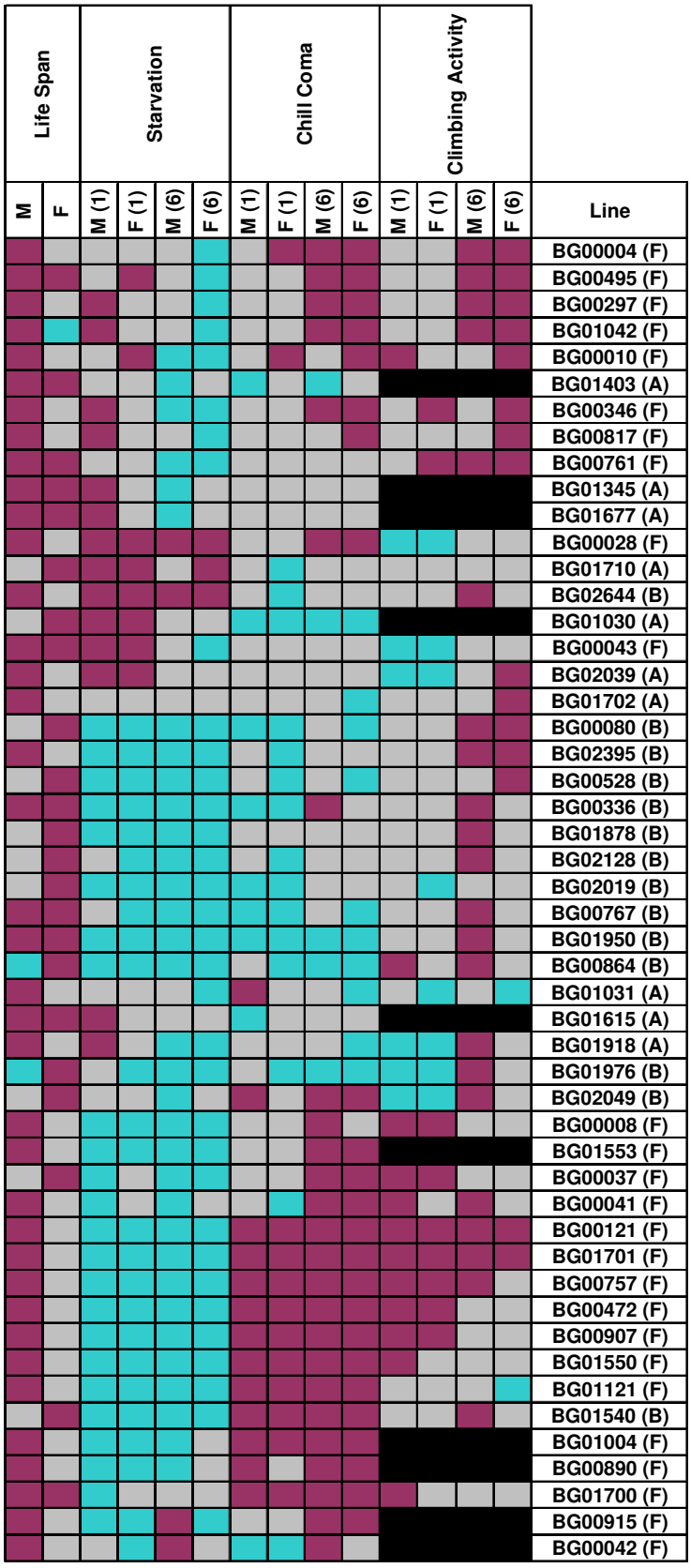

A
CG10990
crol
BG00817
BG00297
BG00004
mub
CG9238
pyd
esg
CG31531
B
CG10990
crol
BG00817
BG00297
BG00004
mub
CG9238
pyd
esg
CG31531
C
CG10990
crol
BG00817
BG00297
BG00004
mub
CG9238
pyd
esg
CG31531

Figure 3.4 – Pleiotropic effects of *P*-element insert lines associated with increased life span on starvation resistance, chill coma recovery and climbing activity, in males (M) and females (F) at one (1) and six (6) weeks of age. Dark pink indicates increased fitness and cyan indicates decreased fitness, relative to the co-isogenic control; grey indicates no significant difference from the control; and black indicates that the measurement was not done.


Life Span
Starvation
Chill Coma
Climbing Activity
M
F
M (1)
F (1)
M (6)
F (6)
M (1)
F (1)
M (6)
F (6)
M (1)
F (1)
M (6)
F (6)
Line
BG00004 (F)
BG00495 (F)
BG00297 (F)
BG01042 (F)
BG00010 (F)
BG01403 (A)
BG00346 (F)
BG00817 (F)
BG00761 (F)
BG01345 (A)
BG01677 (A)
BG00028 (F)
BG01710 (A)
BG02644 (B)
BG01030 (A)
BG00043 (F)
BG02039 (A)
BG01702 (A)
BG00080 (B)
BG02395 (B)
BG00528 (B)
BG00336 (B)
BG01878 (B)
BG02128 (B)
BG02019 (B)
BG00767 (B)
BG01950 (B)
BG00864 (B)
BG01031 (A)
BG01615 (A)
BG01918 (A)
BG01976 (B)
BG02049 (B)
BG00008 (F)
BG01553 (F)
BG00037 (F)
BG00041 (F)
BG00121 (F)
BG01701 (F)
BG00757 (F)
BG00472 (F)
BG00907 (F)
BG01550 (F)
BG01121 (F)
BG01540 (B)
BG01004 (F)
BG00890 (F)
BG01700 (F)
BG00915 (F)
BG00042 (F)

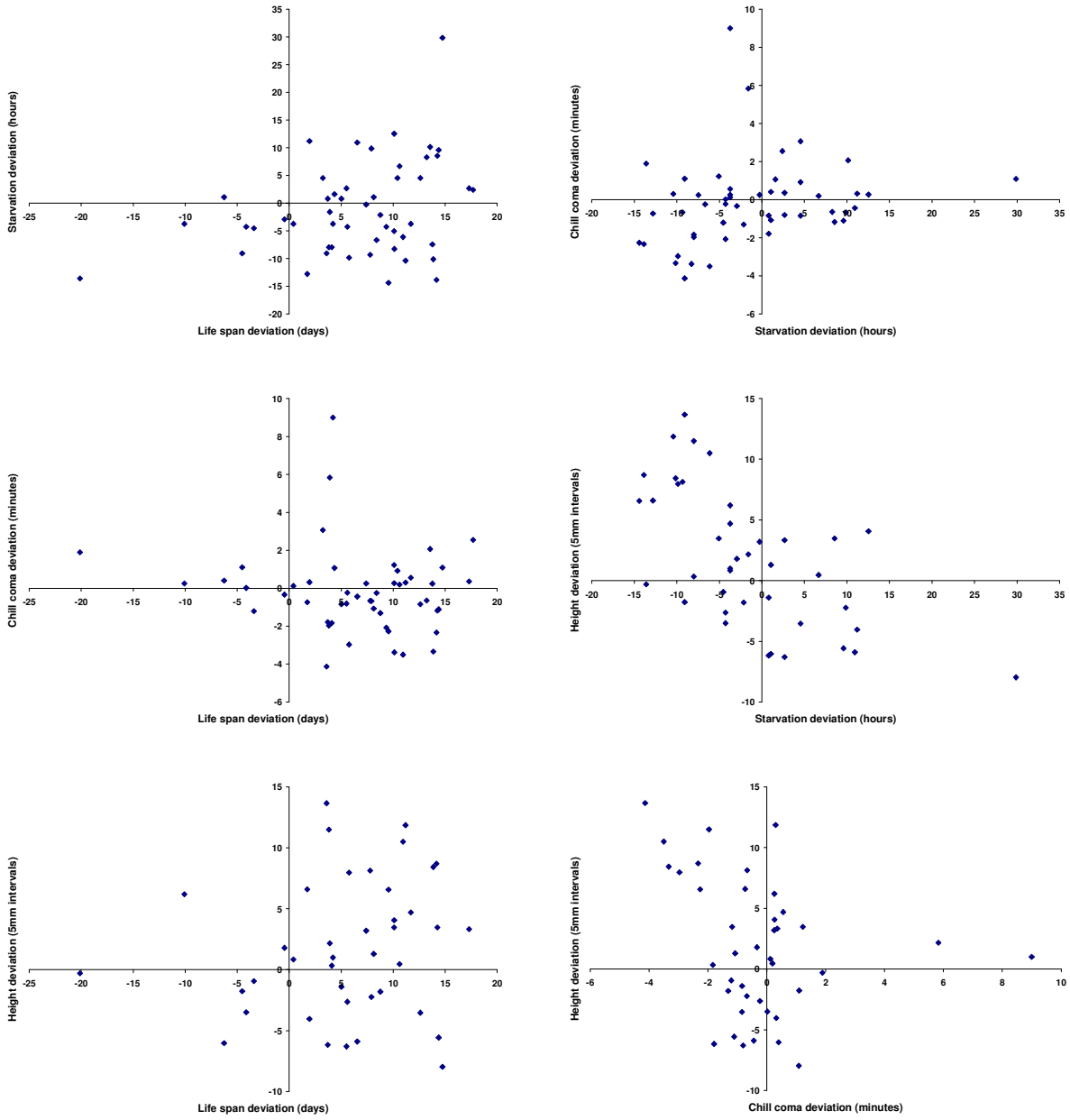


Figure 3.5 – Scatterplots of pleiotropic effects of *P*-element insertions associated with increased life span on starvation resistance, chill coma recovery and climbing activity, in males at one week of age. (A) Life span and starvation stress. (B) Life span and chill coma recovery. (C) Life span and climbing activity. (D) Starvation resistance and chill coma recovery. (E) Starvation resistance and climbing activity. (F) Chill coma recovery and climbing activity.

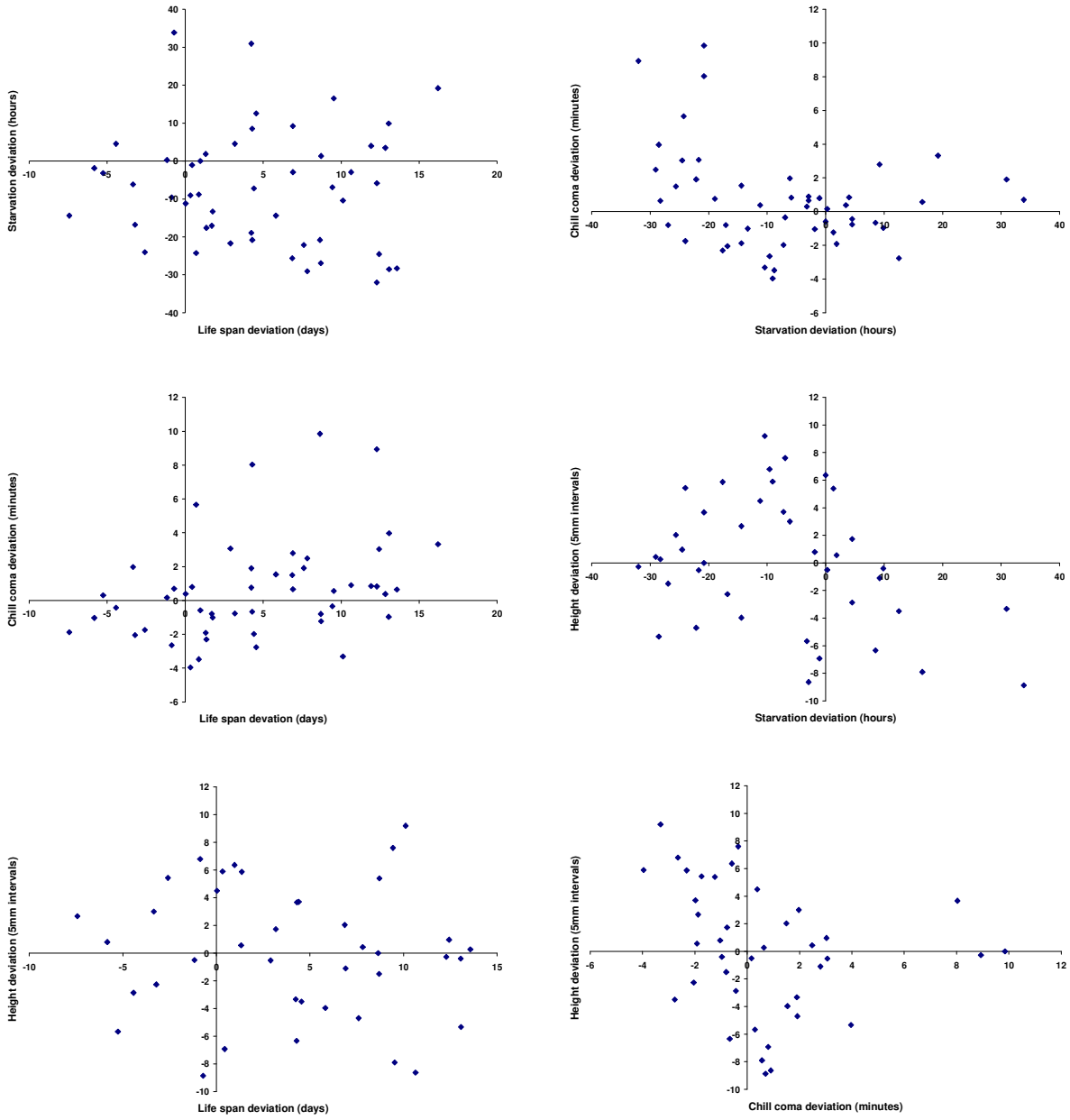


Figure 3.6 – Scatterplots of pleiotropic effects of *P*-element insertions associated with increased life span on starvation resistance, chill coma recovery and climbing activity, in females at one week of age. (A) Life span and starvation stress. (B) Life span and chill coma recovery. (C) Life span and climbing activity. (D) Starvation resistance and chill coma recovery. (E) Starvation resistance and climbing activity. (F) Chill coma recovery and climbing activity.

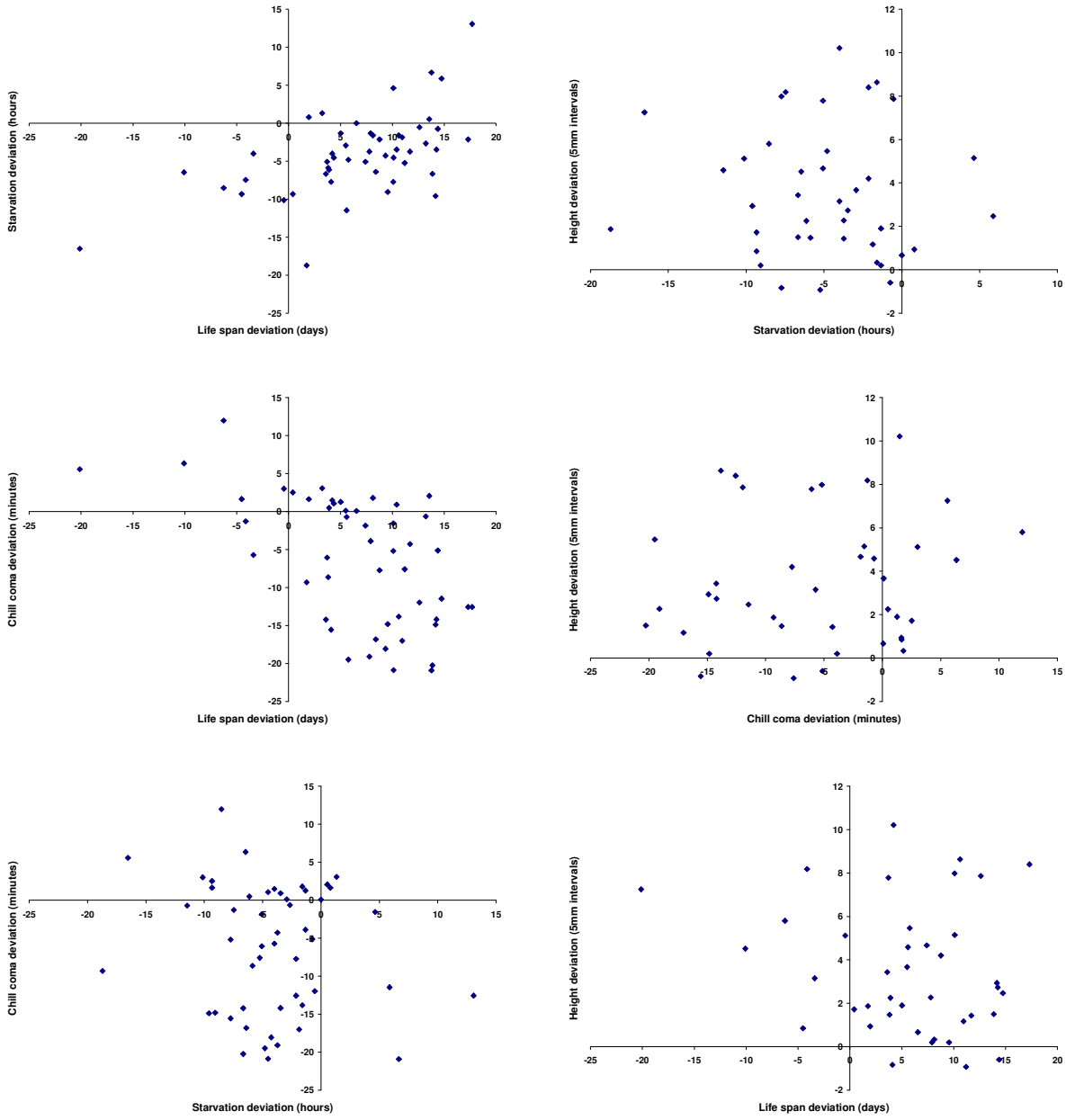


Figure 3.7 – Scatterplots of pleiotropic effects of *P*-element insertions associated with increased life span on starvation resistance, chill coma recovery and climbing activity, in males at sex weeks of age. (A) Life span and starvation stress. (B) Life span and chill coma recovery. (C) Life span and climbing activity. (D) Starvation resistance and chill coma recovery. (E) Starvation resistance and climbing activity. (F) Chill coma recovery and climbing activity.

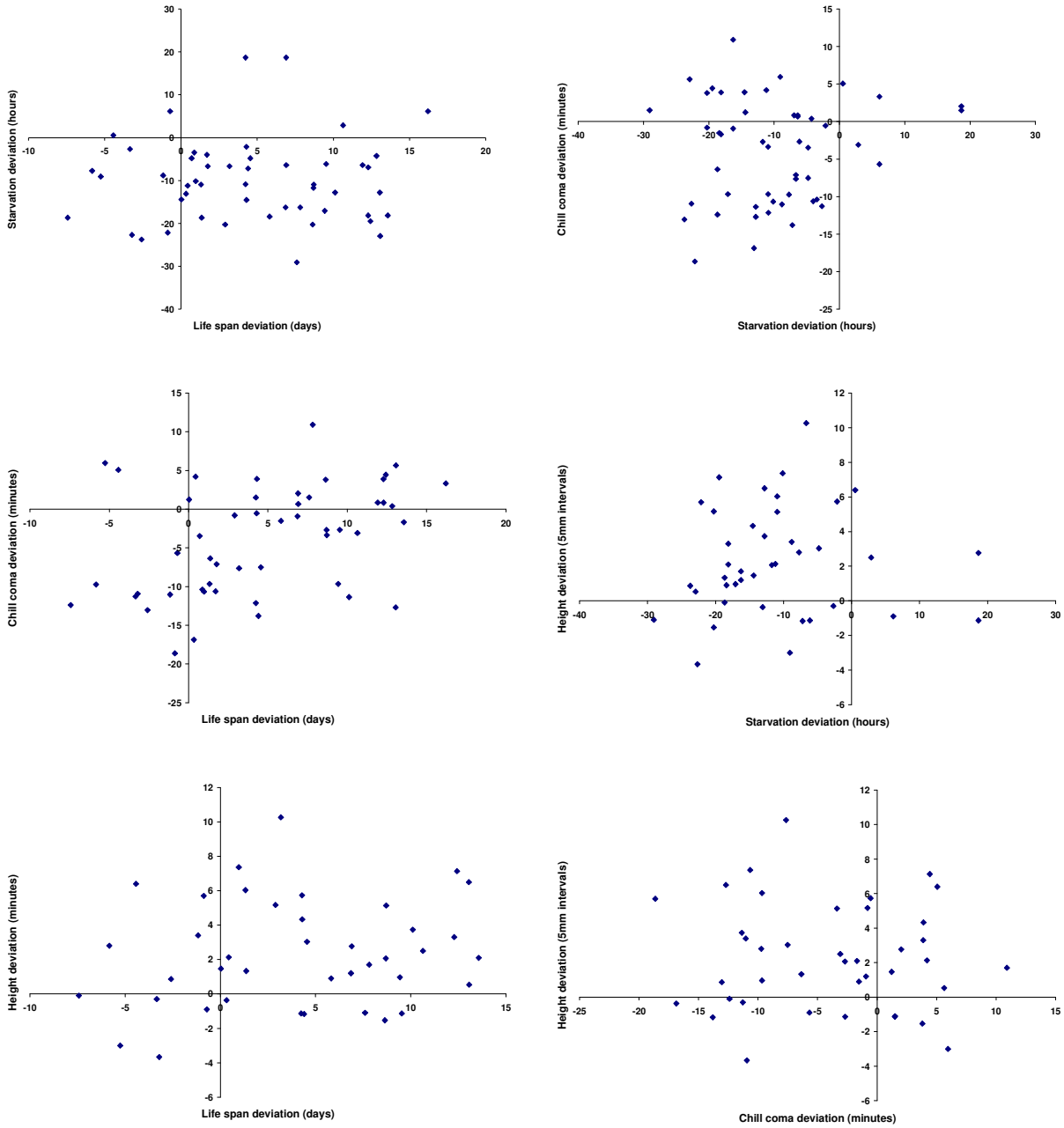
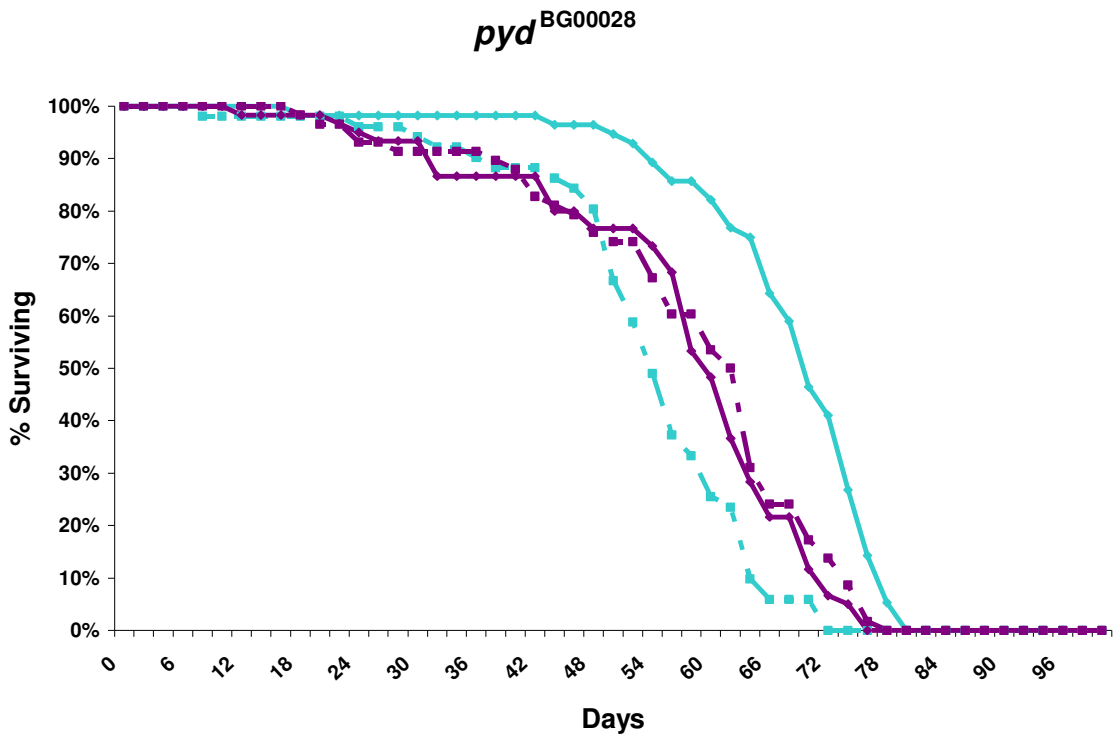


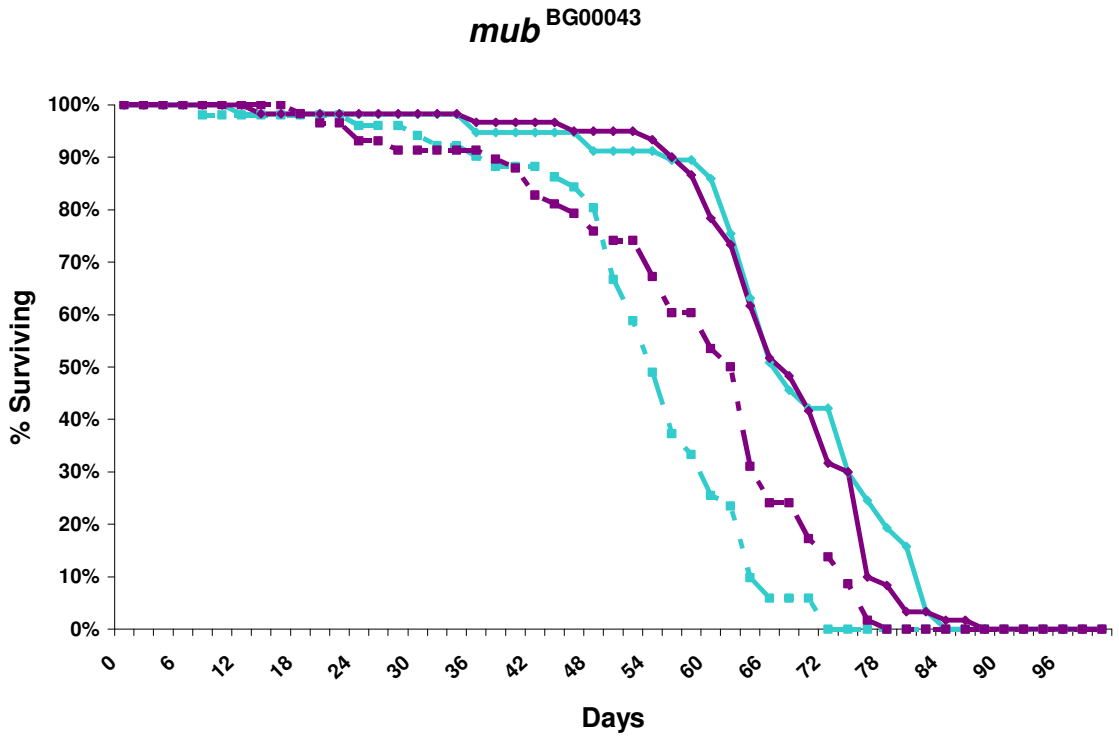
Figure 3.8 – Scatterplots of pleiotropic effects of *P*-element insertions associated with increased life span on starvation resistance, chill coma recovery and climbing activity, in females at six weeks of age. (A) Life span and starvation stress. (B) Life span and chill coma recovery. (C) Life span and climbing activity. (D) Starvation resistance and chill coma recovery. (E) Starvation resistance and climbing activity. (F) Chill coma recovery and climbing activity.

Figure 3.9 – Survival curves of *P*-element insertion lines associated with increased life span (diamonds and solid lines) and the co-isogenic control line (Canton S F, squares and dashed lines) used for whole genome microarray profiling. Cyan lines denote males; dark pink lines denote females. (A) *pyd*^{BG00028}. (B) *mub*^{BG00043}. (C) *crol*^{BG00346}. (D) *CG10990*^{BG00495}. (E) *CG9238*^{BG00761}. (F) BG00817. (G) *esg*^{BG01042}.

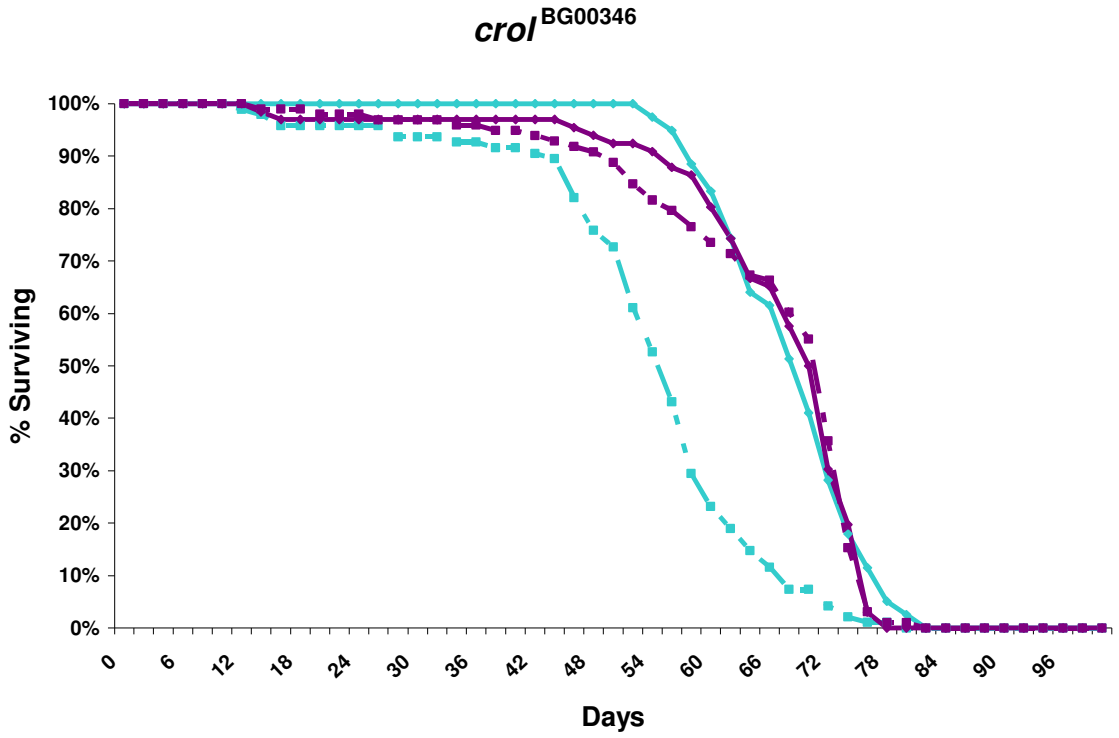
A


pyd BG00028
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

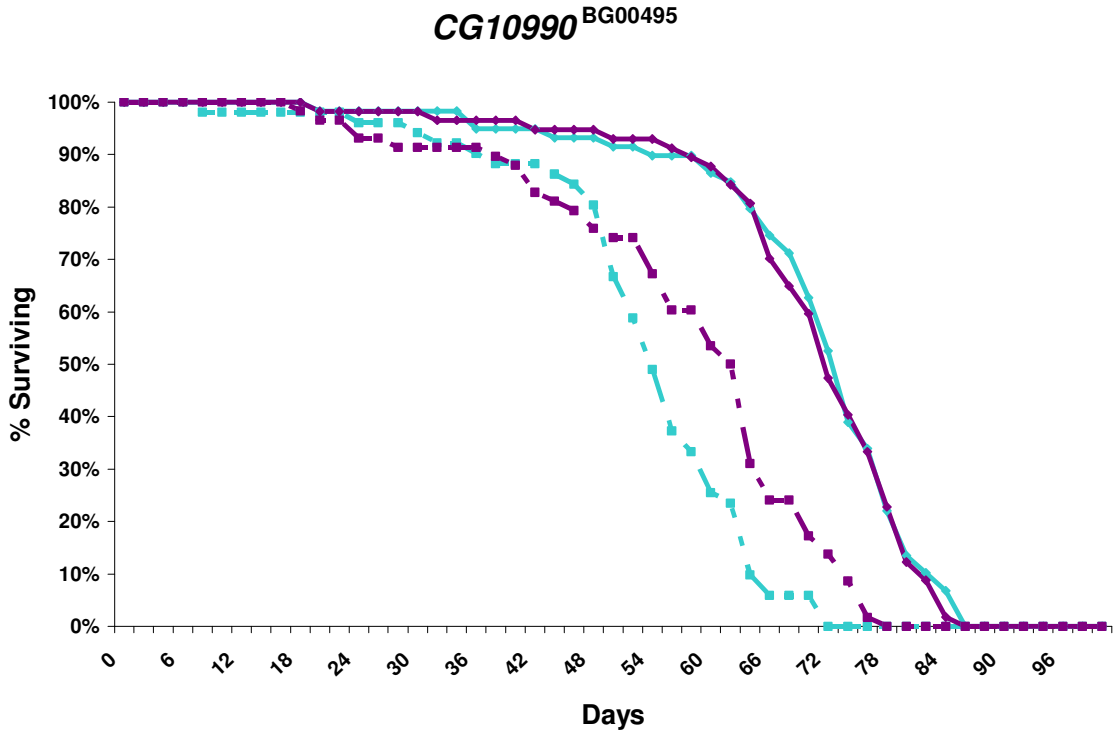
B


mub BG00043
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

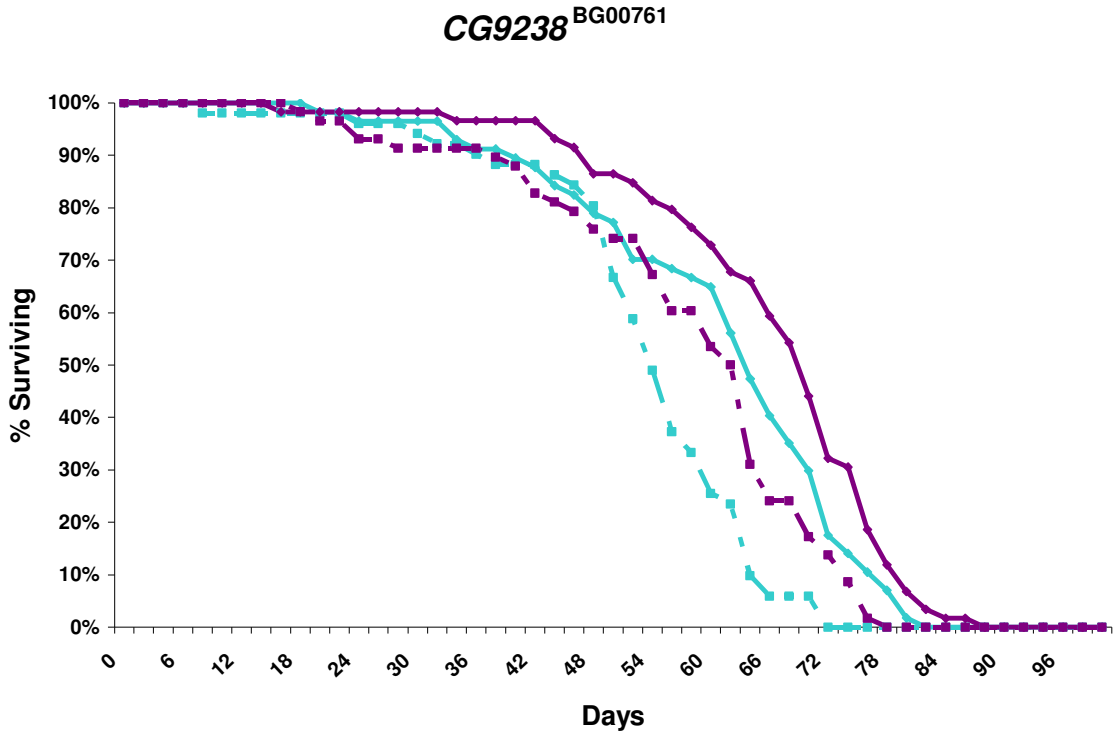
C


crol BG00346
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

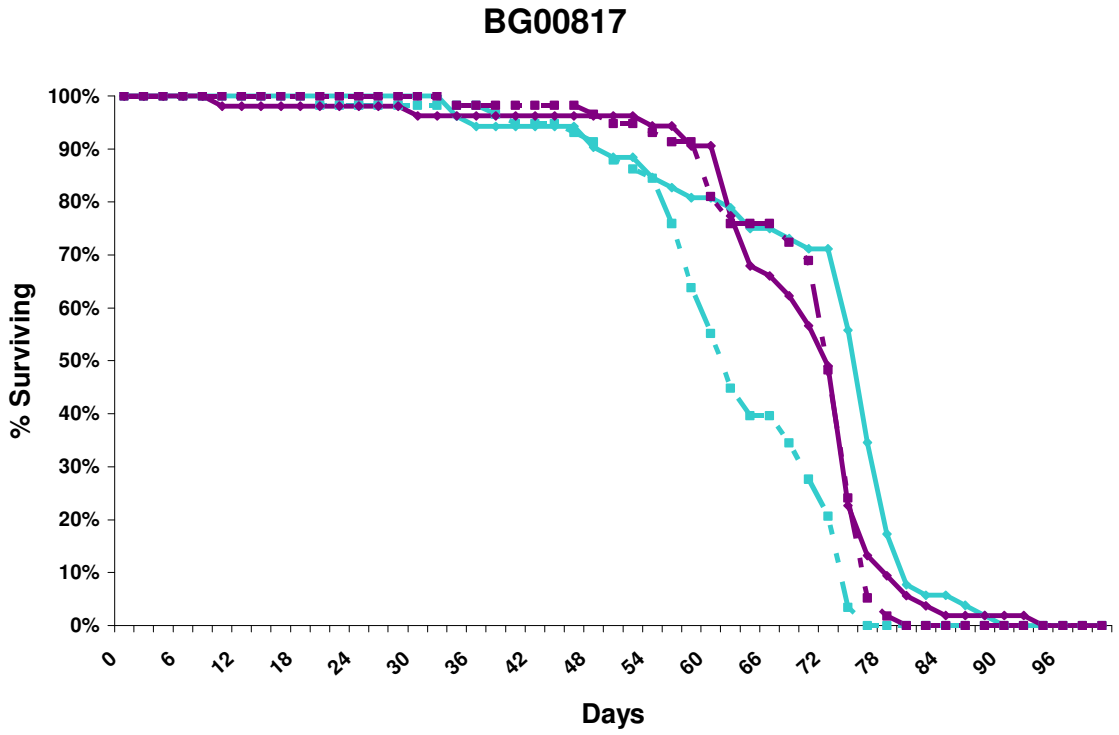
D


CG10990 BG00495
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

E


CG9238 BG00761
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

F


BG00817
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

G

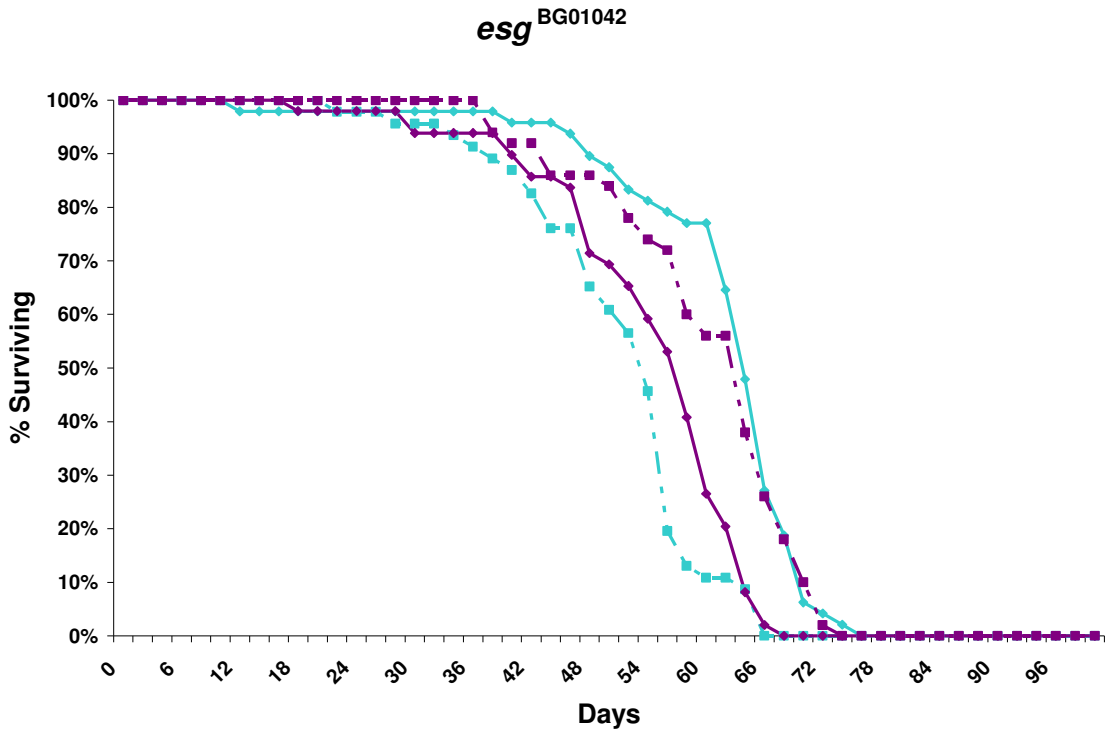
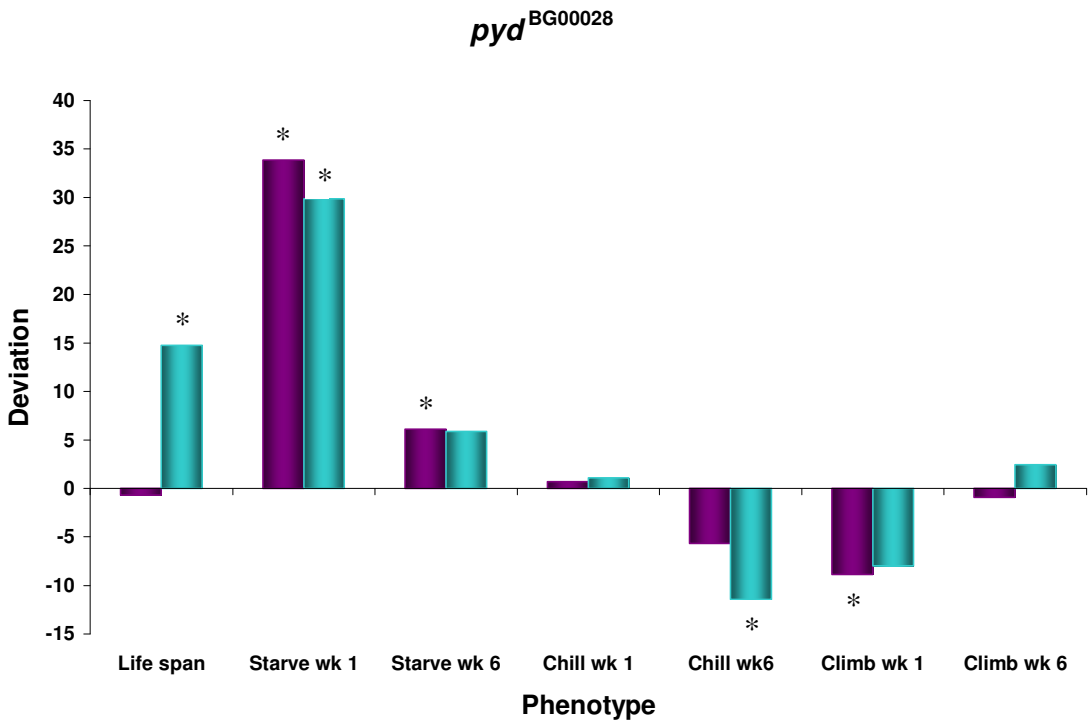
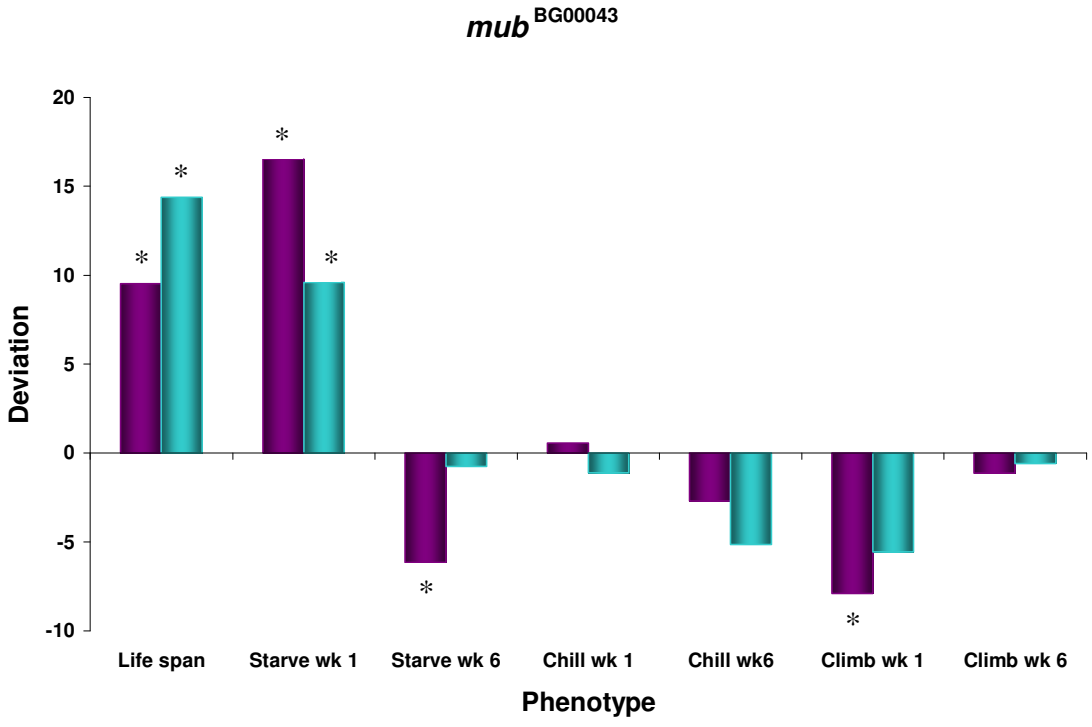

esg BG01042
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Surviving
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
Days

Figure 3.10 – Pleiotropic effects of *P*-element insertion lines associated with increased life span used for whole genome microarray profiling, expressed as deviations from the co-isogenic Canton S F control line. Cyan denotes males; dark pink denotes females. (A) *pyd*^{BG00028}. (B) *mub*^{BG00043}. (C) *crol*^{BG00346}. (D) *CG10990*^{BG00495}. (E) *CG9238*^{BG00761}. (F) BG00817. (G) *esg*^{BG01042}.

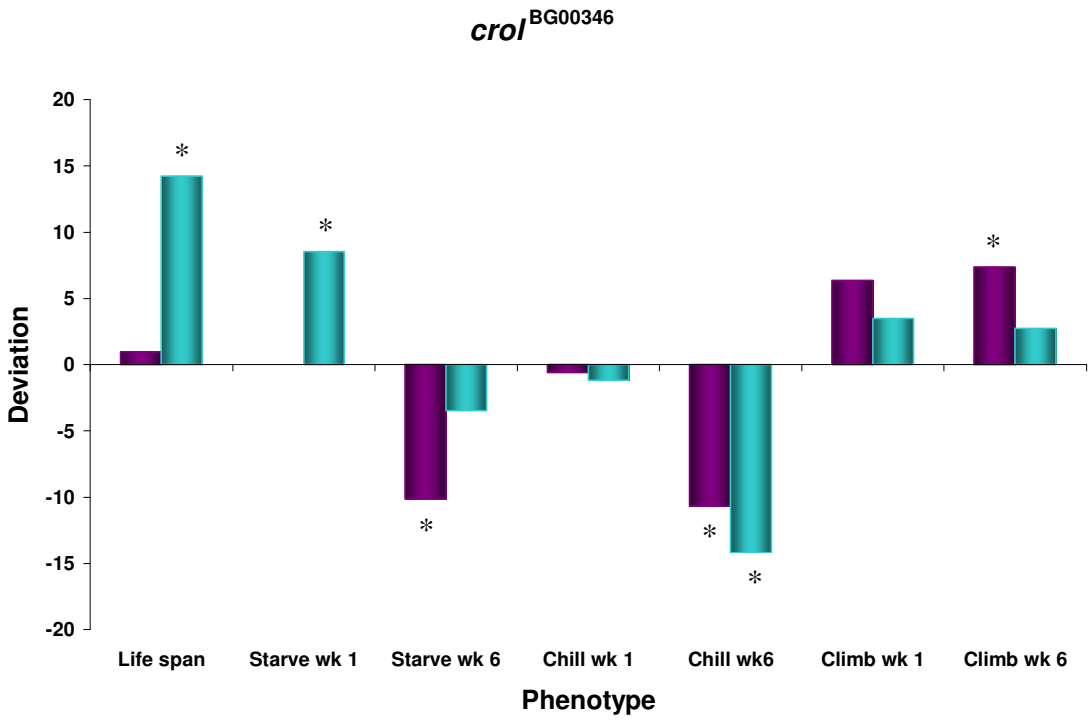
A


pyd BG00028
Deviation
40
35
30
25
20
15
10
5
0
-5
-10
-15
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

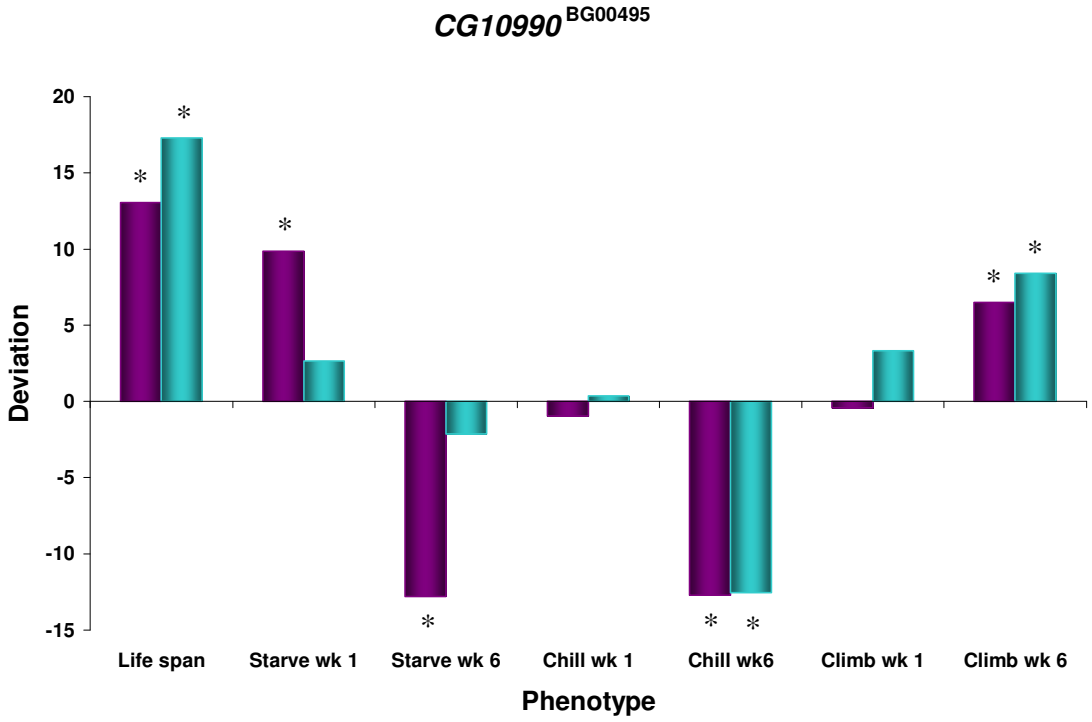
B


mub BG00043
Deviation
20
15
10
5
0
-5
-10
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

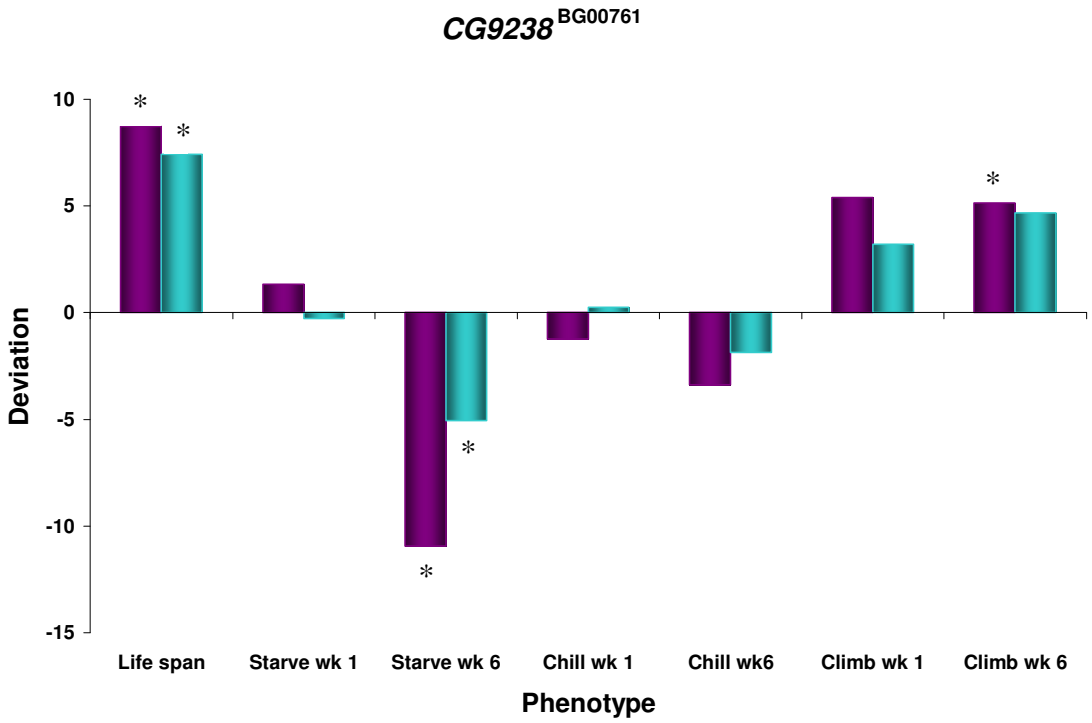
C


crol BG00346
20
15
10
5
0
-5
-10
-15
-20
Deviation
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

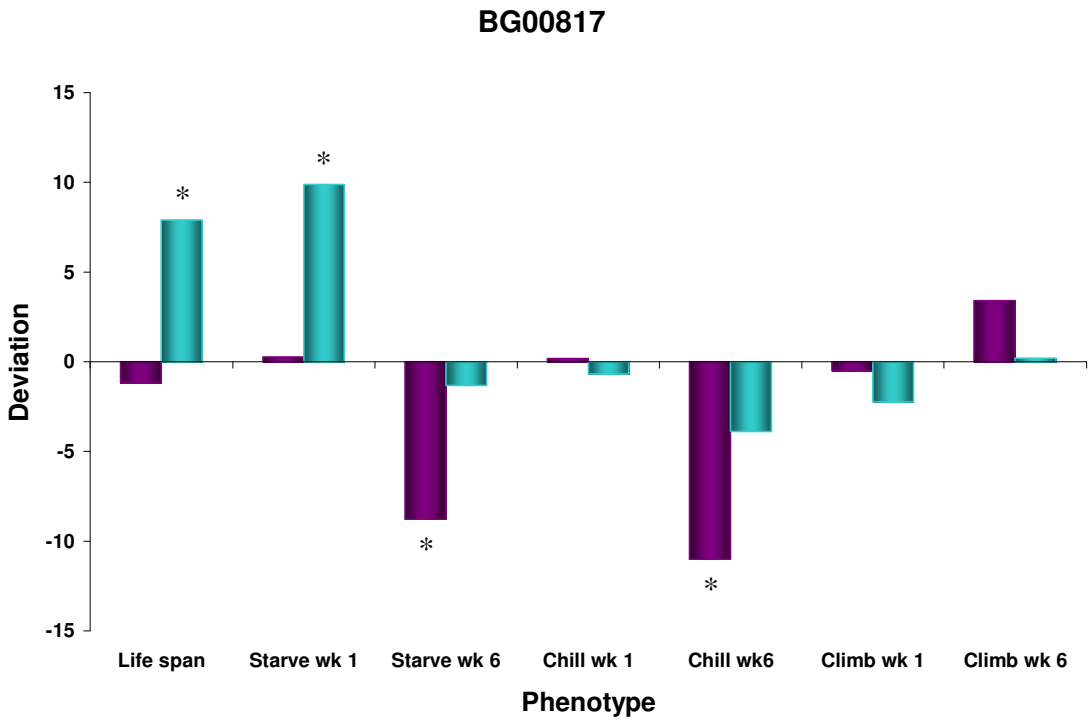
D


CG10990 BG00495
20
15
10
5
0
-5
-10
-15
Deviation
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

E


CG9238 BG00761
10
5
0
-5
-10
-15
Deviation
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

F


BG00817
15
10
5
0
-5
-10
-15
Deviation
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

G

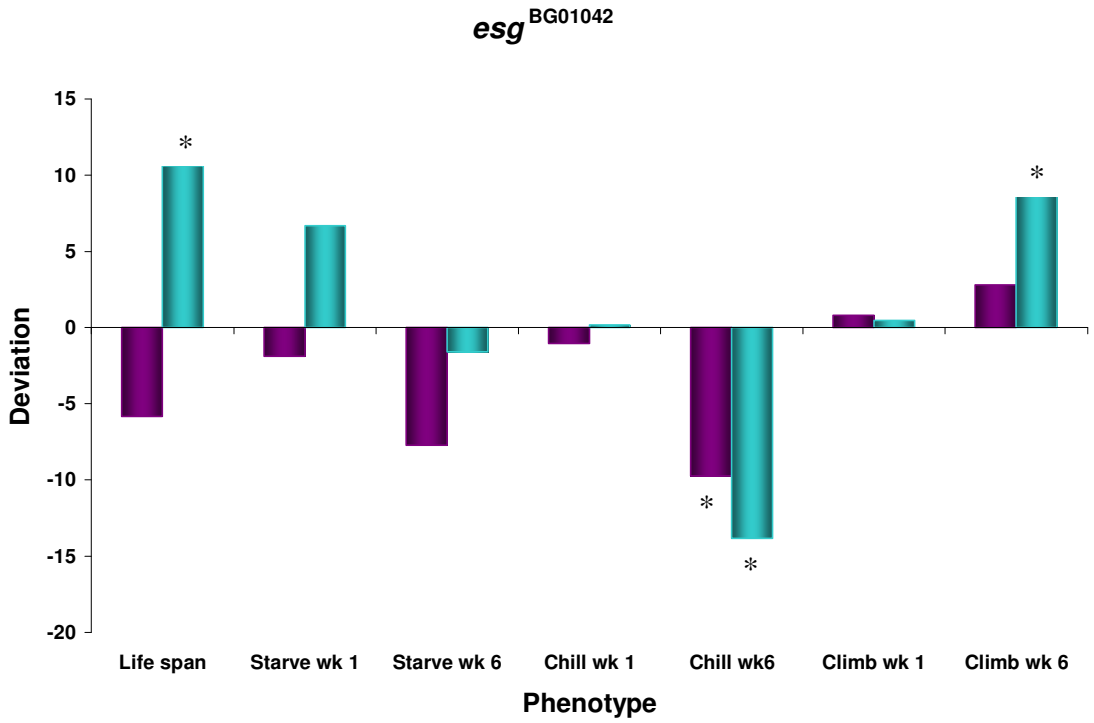
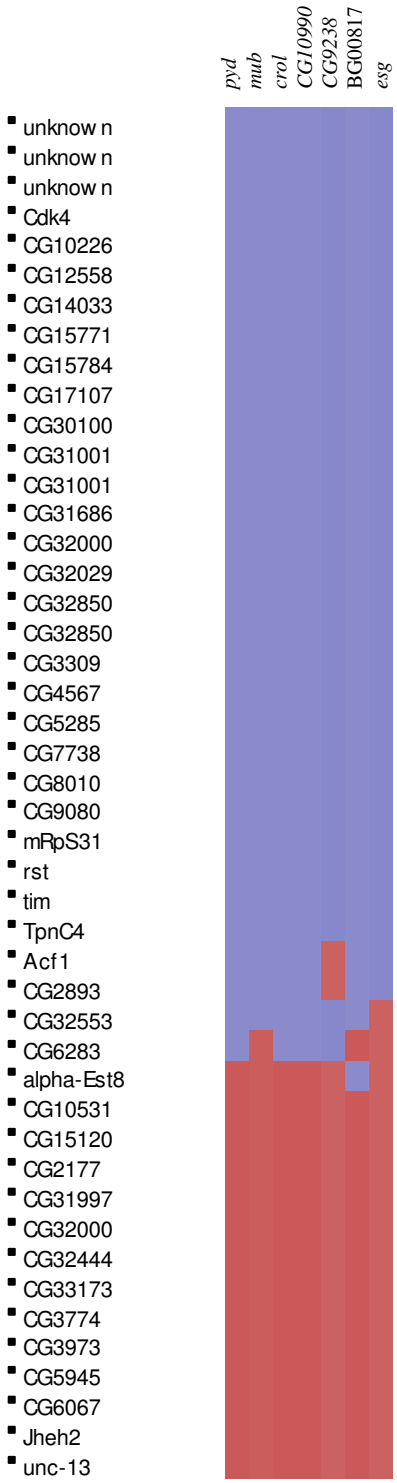
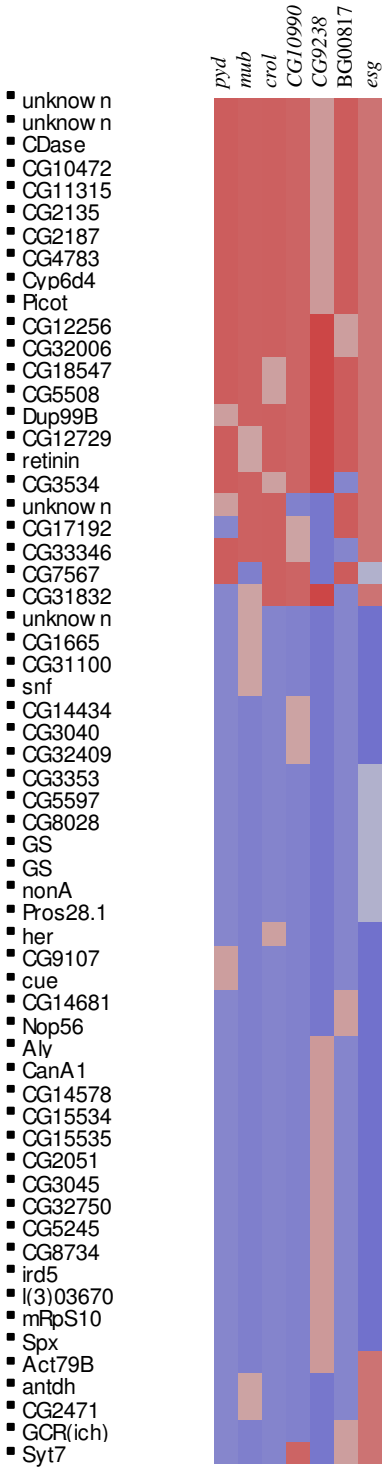

esg BG01042
15
10
5
0
-5
-10
-15
-20
Deviation
*
*
*
*
Life span
Starve wk 1
Starve wk 6
Chill wk 1
Chill wk6
Climb wk 1
Climb wk 6
Phenotype

Figure 3.11 – Cluster analysis of genes that are significant in all seven or six of the seven mutant lines. Red: increased expression relative to the control. Blue: decreased expression relative to the control.

Genes significant for all 7 lines



Genes significant for 6 of the lines



CHAPTER 4

Discussion

The Mutational Landscape of Longevity

We performed an unbiased, forward genetic screen of 1332 *P{GT1}* insertional mutations that were generated in one of six Canton S co-isogenic backgrounds for mutations affecting *Drosophila* longevity. In the initial screen, we identified 436 (32.7%) mutations with mean life spans that were significantly different from their co-isogenic control. Of these, 296 (67.89%) were associated with reduced life span, 135 (30.96%) were associated with increased life span, and 5 (1.15%) had sexually antagonistic effects on life span. The sample size per mutation in the initial screen was not large; therefore, many of the significant effects could be false positives. Nevertheless, if nearly one-third of mutations affect life span, the mutational target size for longevity must be large, consistent with the many mechanisms that are known to affect life span. We know the locations of the *P*-element inserts for 290 of the mutations associated with significant effects on longevity. Of these, 56 (19.3%) map to gene deserts (regions of the genome with no computationally predicted genes) and likely define novel un-annotated genes. With the exception of *foxo* (Giannakou *et al*., 2004; Hwangbo *et al*., 2004), none of the mutations tagged genes that have been previously associated with life span. Thus, forward genetic screens for mutations with subtle, quantitative effects on life span in a co-isogenic background is an efficient method for identifying novel genes affecting longevity and other complex traits (Anholt *et al*., 1996; Lyman *et al*., 1996; Norga *et al*., 2003; Harbison *et al*., 2004; Sambandan *et al*., 2006)

Substantially more mutations were associated with decreased than increased life span. It is generally assumed that mutations decreasing life span are less interesting than mutations increasing life span, since the former category of mutations could be generally deleterious and affect all aspects of fitness, while the latter are more likely to have specific effects on life span. Thus, we concentrated on confirming the effects of mutations associated with increased lifespan with larger sample sizes in a secondary screen, and identified 58 mutations associated with increased life span; the largest collection of such mutations described to date in *Drosophila*. The *P*-element insertion sites are known for 47 of these mutations: eight are in gene deserts; 12 are within 2kb of two genes; and 27 are within 2 kb of the

only annotated gene in the region. The mutations associated with significant increases in life span represent pathways known to affect life span (e.g., the insulin and metabolic pathways), as well as novel pathways involving taste, the nervous system and embryonic development (Table 3.2).

Mutations reducing life span are typically inferred to be in genes required for normal life span; over-expression of such genes may extend longevity. This has been observed in SOD and *catalase* (Orr and Sohal, 1994) and dFOXO (Giannakou *et al.*, 2004). Conversely, mutations increasing life span are thought to be in genes that normally function to limit life span; limiting expression of these genes thus extends longevity. This has recently been shown for a mutation in *Or83b* (Libert *et al.*, 2007) and *mth* in *Drosophila* (Mockett and Sohal, 2006). This logic presumes that all mutations in genes affecting life span have effects in the same direction. The proclivity of *P*-elements to insert in genomic hot-spots generated many insertions in the same genes, enabling us to observe directly the distribution of mutational effects in the same genes. While many mutations in the same genes did indeed have similar effects on life span, this was not always true. Specifically, the effects of mutations in the same gene on life span can vary according to the location and orientation of the *P*-element insertion, and genetic background. For example, four lines had *P*-element insertions in the *Tre1/Gr5a* intergenic region. *P*-elements in lines BG02514 and BG02257 are in the Canton S B background but are inserted in opposite orientations. Longevity is reduced in both sexes in BG02514, and increased in both sexes in BG02257 (Table 3.2, Rollmann *et al.*, 2006). Lines BG01272 and BG00218 contain *P*-element inserts in the same location and orientation, but the insert in BG01272 is in the Canton S B background and is associated with reduced longevity in males (Rollmann *et al.*, 2006), while the insert in BG00218 is in the Canton S F background and is associated with increased longevity in both sexes (Table 3.2, Rollmann *et al.*, 2006). Similarly, five mutations in the same location in *mub* in the Canton S F background were associated with increased life span, while two *mub* mutations in the Canton S B background were associated with decreased life span in the initial screen (one of which was in the same location as the mutation associated with increased life span in the Canton S F

background). A mutation 500 bp downstream of *esg* in the Canton S F background was associated with increased life span, whereas seven mutations 30-300 bp upstream of *esg* in the Canton S A and B backgrounds were associated with decreased life span in the initial screen. Two mutations 630 bp upstream of *pyd* were associated with increased life span in the Canton S F genetic background (Table 3.2), while a mutation 850 bp upstream of *pyd* in the Canton S A background and one in *pyd* in the Canton S B background were both associated with reduced life span in the initial screen. A mutation in the first intron of *Defense repressor 1* (*Dnr1*) was associated with increased life span in the Canton S A background, while two independent mutations in the same location in the Canton S B background were associated with reduced longevity in the initial screen. These observations highlight the inaccuracy of referring to genes that are required for normal life span or that normally limit life span. Mutational analysis identifies genes that are relevant to the regulation of life span, but variable allelic effects preclude inferring directionality of wild type function.

Epistasis

Mutations in different locations in the same gene could have variable effects on longevity if they interfere with different aspects of gene regulation, or if some are in regulatory regions and others directly affect the protein. Different mutational effects could also arise due to variation in the amount of the vector inserted into the genome or by partial genomic excision during the insertion process. Variable effects of mutations in the same location and orientation but different genetic backgrounds may be attributable to epistatic interactions with different alleles. Indeed, diallel crosses among just 10 of the mutations associated with increased life span revealed a complex network of epistatic interactions involving all 10 mutations, suggesting pervasive epistasis between alleles affecting life span. Mutations in the *Tre1/Gr5a* intergenic regions interact epistatically with mutations in genes affecting insulin signaling (Rollmann *et al*., 2006). It will be interesting to determine to what extent the other mutations interact with components of this well-established pathway, and to what extent the effects on life span are independent of insulin signaling. Epistasis

has repeatedly been observed between QTL alleles affecting variation in life span (Leips and Mackay, 2000; 2002) as well as between QTL alleles without main effects on life span (Mackay *et al*., 2006), although the identity of the genetic loci underlying the QTLs are not known.

Further evidence for the importance of epistasis in the genetic architecture of *Drosophila* life span comes from observations that the effects of transgene over-expression and single mutations on longevity vary according to genetic background. Over-expression of *Drosophila superoxide dismutase* resulted in increased life span in the background of a relatively short-lived strain, but decreased life span in a long-lived strain background (Sun and Tower, 1999). Similarly, the *Indy* mutation increased lifespan by 40-80% in the short-lived *Shaker*, *Hyperkinetic* and *drop dead* strains, but only by 15% in a strain selected for increased life span (Rogina *et al*., 2000). Over-expression of human *SOD* in *Drosophila* motorneurones increases life span (Parkes *et al*., 1998), but the magnitude of the increase varies in different wild type genetic backgrounds in a sex-dependent manner (Spencer *et al*., 2003). Likewise, introgression of each of three morphological mutations into seven wild-derived backgrounds showed considerable background-dependent effects on life span (Spencer and Promislow, 2005). These observations highlight the importance of future experiments to assess the effects of the mutations increasing life span in a range of naturally derived genetic backgrounds, and to identify the genes with which the mutations interact.

Sex-specificity

A striking feature of our screen is that the effects of mutations increasing life span are highly sex-specific, with a low, but significant, negative cross sex-genetic correlation of $r_{MF} \sim -0.3$. Epistatic effects were similarly sex-specific, and in three cases the direction of the epistasis was opposite in males and females. This is not the first report of sex-specific effects of genes affecting life span. As early as the middle of the last century, Maynard Smith (1958) noted that the genetic control of longevity was independent in *Drosophila subobscura* males and females. More recently, QTLs affecting variation in life span between two laboratory strains, Oregon

and 2b, have repeatedly been shown to have sex-specific effects (Nuzhdin *et al*., 1997; Vieira *et al*., 2000; Leips and Mackay, 2000; 2002; Pasyukova *et al*., 2000; Pasyukova *et al*., 2004). Perhaps surprisingly, most studies of aging examine only one sex (Burger and Promislow, 2004), but when both sexes are included, sex-specific mutational effects are common. For examples, the effects of mutations in the *Drosophila insulin-like receptor* (*InR*, Tatar *et al*., 2001), the insulin receptor substrate *chico* (Clancy *et al*., 2001) and *DTS-3*, a gene involved in ecdysone biosynthesis (Simon *et al*., 2003) had female-biased or female-specific effects on life span. As noted above, over-expression of human *SOD* in *Drosophila* motorneurones in different genetic backgrounds has sex-specific effects on life span (Spencer *et al*., 2003). Further, the benefits of dietary restriction on increased life span of *D. melanogaster* are greater in females than males (Magwere *et al*., 2004). The cause(s) of the sex-specific effects remain(s) mysterious (Burger and Promislow, 2004). However, it should be noted that sex-specific effects of mutations and QTLs are a common feature of the genetic architecture of complex traits in *Drosophila* and other organisms (Mackay, 2001), although such effects on life span are particularly extreme. It remains to be seen whether a common mechanism underlies sex-specificity for all traits.

Pleiotropy

The concept of trade-offs (antagonistic pleiotropy) is central to many evolutionary hypotheses for limited life span and senescence. Such trade-offs were historically envisioned to be governed by alleles with beneficial fitness effects early in life, when the force of natural selection is strong, but detrimental effects later in life, when natural selection is weak (Williams, 1957; Rose and Charlesworth, 1980). Kirkwood (1977) phrased this concept in terms of a physiological trade-off caused by organisms' need to optimally allocate resources to reproduction and somatic maintenance in the context of their ecology. Support for antagonistic pleiotropy comes from quantitative genetic studies documenting negative genetic correlations between early and late fitness components (Rose and Charlesworth, 1980; 1981; Luckinbill *et al*., 1984; Rose, 1984; Partridge and Fowler, 1992; Zwaan *et al*., 1995);

but these negative genetic correlations are not always found (Roper *et al*., 1993; Houle *et al*., 1994; Hughes, 1995; Tatar *et al*., 1996). Leips *et al*. (2006) found a negative genetic correlation between life span and early age fecundity in their QTL mapping population, and identified a QTL with antagonistic effects on early age fecundity. Oddly, in both cases the correlation was between female fecundity and male life span. Possible explanations for this phenomenon include sexually antagonistic alleles affecting different traits; a positive genetic correlation between female and male fecundity; and coincidental localization of separate QTLs affecting the two traits. *Drosophila* mutations affecting increased life span often exhibit antagonistic pleiotropy: mutations in *chico* and *InR* are dwarf and have reduced fecundity (Clancy *et al*., 2001, Tatar *et al*., 2001), and mutations of *Indy* have decreased fecundity under adult caloric restriction (Marden *et al*., 2003).

We have shown here that antagonistic pleiotropy is pervasive, in that all *P*-element insert lines associated with increased longevity were also associated with at least one deleterious pleiotropic effect on resistance to starvation stress, recovery after chill coma, and/or a general measure of health (climbing activity) at one week and/or six weeks of age (Figure 3.4). On average, the lines with increased longevity have overall decreased resistance to starvation stress and increased resistance to chill coma stress and increased general activity relative to the controls at six weeks of age. Mutations in genes in the insulin signaling pathway tend to have increased resistance to starvation and oxidative stress, accompanied by a trade-off in growth and fecundity (Holzenberger *et al*., 2003; Kramer *et al*., 2003; Fantin *et al*., 2000; Giannakou *et al*., 2004; Broughton *et al*., 2005; Tissenbaum and Ruvkun, 1998; *Gems et al*., 1998; Friedman and Johnson, 1987). Thus, our observation that resistance to starvation stress actually decreases in older flies from the long-lived strains runs counter to this theme. It will be interesting in the future to assess early and late age fecundity on these mutations. However, it should be noted that the negative genetic correlation between the sexes for longevity is itself a trade-off, and that patterns of pleiotropy are different for males and females. Mutations affecting female life span have antagonistic pleiotropic effects on resistance to chill coma stress. Mutations affecting male life span have positive pleiotropic effects on

resistance to starvation and chill coma stress, but there is antagonistic pleiotropy between male starvation stress resistance and climbing activity.

Candidate Genes

The power of the mutational screen is easily observed by the large variety of biological processes and molecular functions represented by the genes discovered. Not only do the candidate genes uncovered by this screen strengthen established life-extending hypotheses and introduce novel ones, but they also provide further evidence of the importance of gene networks.

An insertion in the first intron of *mushroom-body expressed* (*mub*) was identified in five separate cases (the insertion was in the same position for all five and in the same background, F). All but one of these inserts showed a highly significant ($P \leq 0.0006$) increase in life span for males, while females were only significant in three of the lines. *mub* is a transcription factor (Mi *et al*., 2003) thought to be involved in RNA processing (Mi *et al*., 2003; Park *et al*., 2004) neurodegeneration (Fernandez-Funez *et al*., 2000) and tauopathy (Shulman and Feany, 2003). *CG9238* is also thought to be involved in tauopathy (Shulman and Feany, 2003); two mutations in the same location in this gene were associated with increased longevity in our screen. Several of the genes are involved in immune response, including *Defense repressor 1* (*Dnr1*), *kayak* and *Ecdysone-induced protein 75B* (*Eip75B*) (Foley and O'Farrell, 2004; Kleino *et al*., 2005). *kayak* is also part of the wound healing process (Bosch *et al*., 2005).

CG9238 is also thought to be involved in metabolism as well as *foxhead box, sub-group O* (*foxo*), *kayak* and *Glucose transporter 1* (*Glut1*) (Mi *et al*., 2003; Crosby *et al*., 2007). *Phosphoglycerate kinase* (*pgk*) is another metabolic related gene that is part of glycolysis (Boeckmann *et al*., 2003). *CG10990* is thought to be involved in protein metabolism (Mi *et al*., 2003) and *Eip75B* regulates steroid metabolism (Bialecki *et al*., 2002). *escargot* (*esg*) and *polychaetoid* (*pyd*) both play a role in electron transport (Crosby *et al*., 2007).

While many of the candidate genes are involved in metabolic processes, even more are involved in transcription. *crooked legs* (*crol*) regulates transcription from

the RNA polymerase promoter (D'Avino and Thummel, 2000) as well as *esg*, *scalloped* (*sd*) *Eip75B*, *pipsqueak* (*psq*) and *mub* (Mi *et al*., 2003; Crosby *et al*., 2007). This characteristic made several of the candidates well suited to microarray studies in a search for their regulated genes that may be affecting the change in life span.

Another common area of regulation among the candidates is development. Several mutations are in genes that affect nervous system development. Development of the central nervous system is regulated in part by *kayak* (Giesen *et al*., 2003); *esg* is involved in the regulation of the peripheral nervous system (Prokopenko *et al*., 2000); *sd* regulates the development of sensory organs (Srivastava and Bell, 2003); and *crol* regulates mesoderm development (Crosby *et al*., 2007).

Stress resistances have previously shown to be correlated with an increase in life span (Luckinbill, 1998; Service *et al*., 1985; Orr and Sohal, 1994). Several candidate genes support this correlation. *crol* may be involved in a response to freezing (Crosby *et al*., 2007) as well as resistance to starvation (Harbison *et al*., 2004) and displays a locomotor response to mechanical stress (Jordan *et al*., 2006). *Fkbp13* is involved in protein folding (Mi *et al*., 2003; Crosby *et al*., 2007). *foxo* is also thought to be involved in stress response (Junger *et al*., 2003).

Some genes that have been shown previously to affect life span affect the cell cycle during meiosis and mitosis (Fukuyama *et al*., 2006; Dryden *et al*., 2003). Several of the candidate genes implicated by our screen also have a role in the cell cycle; e.g., *foxo*, *mub* and *Glut1* (Mi *et al*., 2003). These genes may aid in exploring the relationship between the cell cycle, telomere shortening and life span.

Two lines had an insertion 500 bp upstream from *polychaetoid*. *pyd* and *kayak* are both involved in the JNK cascade (Noselli and Agnes, 1999). JNK signaling has been shown previously to affect life span in Drosophila (Wang *et al*., 2003).

Several *P*-element insertions the *Tre1/Gr5a* intergenic region have variable and sex-specific effects on life span, starvation and heat stress resistance, and taste

(Rollmann *et al*. 2006). The insertions also interact epistatically with mutations in genes affecting insulin signaling (Rollmann *et al*., 2006).

Candidate genes are involved in many other processes that are linked to life span extension or have the potential to do so. *nervana 3* (*nrv3*) may be involved in the transport of several ions (Crosby *et al*., 2007). *CG32604* primarily regulates the splicing of nuclear mRNA (Mount and Salz, 2000). *Dek* is also thought to be involved in RNA processing (Crosby *et al*., 2007). *foxo* is known to be part of the insulin signaling pathway (Puig *et al*., 2003) and plays a role in extending life span (Hwangbo *et al*., 2004; Giannakou *et al*., 2004). *psq* regulates gene silencing via chromatin remodeling (Lehmann, 2004). *CG10990* and *mub* both play a role in apoptosis (Mi *et al*., 2003).

Transcriptional Profiling

Whole genome expression profiling of mutations that have been derived in the same co-isogenic background is a powerful tool for identifying networks of co-regulated genes that potentially affect the trait(s) affected by the mutations. Taken at face value, our analysis of gene expression of six week old adults in seven mutant lines associated with increased life span and the control strain indicate that many genes affect life span. We identified 4,481 transcripts that were differentially expressed among all eight genotypes using a false discovery rate criterion of $q \leq 0.001$ (Storey and Tibshiani, 2003). This is in contrast to comparable study of mutations affecting olfactory behavior, which identified an order of magnitude fewer genes (Anholt et al., 2003), and consistent with the intuitive expectation that longevity would have a larger mutational target size than olfaction. Transcripts from many of the candidate genes identified in the *P*-element screen and from genes that have been previously shown to affect life span were also differentially expressed in the background of the seven focal lines. This suggests that the co-regulated genes are indeed excellent candidate genes affecting life span. The fact that transcripts of three of the focal mutations were differentially expressed in the appropriate mutant background provides independent evidence that the *P*-element does affect the gene in which it has inserted. Further, mutations in co-regulated genes may interact

epistatically with mutations in the focal genes (Anholt *et al*., 2003), defining genetic networks affecting longevity. The large number of co-regulated genes in each mutant background is consistent with the large number of epistatic interactions we observed among just 10 mutations associated with increased life span. The mutations in *pyd*, *mub*, *crol*, *CG10990* and *esg* affected a diverse array of biological processes that were somewhat unexpected, given their functional annotations. For example, these genes were not expected *a priori* to affect metabolism and reproduction; yet these categories were over-represented overall. These observations suggest that these loci may interact with insulin signaling and other well-described pathways affecting life span.

Several other studies have reported whole genome changes in gene expression in aging *Drosophila* and *C. elegans*. Pletcher *et al*. (2002) examined both aging and caloric restriction, and found considerable over-representation for biological processes involving the cell cycle, metabolism, DNA repair and replication, transcription, RNA processing, gametogenesis and perception of light. Similarly, we observed over-representation of gene ontologies for metabolism, cell cycle, mating behavior and response to light. Unfortunately, the expression data of Pletcher *et al*. (2002) are not publicly available, precluding a direct comparison of the lists of genes that were co-regulated by mutation associated with increased life span and those implicated in this analysis of normal aging and prolonged life span through caloric restriction.

However, we were able to compare the genes that were co-regulated in the seven *P*-element lines associated with increased life span with the analysis of normal aging in two *Drosophila* strains recently published by Lai *et al*. (2007). We observe extensive overlap with the 48 candidate genes postulated by Lai *et al*. (2007), on the basis that they exhibited significant changes in transcript abundance with age and between the two strains, and that were located in known life span QTL (Nuzhdin *et al*., 1997; Vieira *et al*., 2000; Leips and Mackay, 2000, 2002). Almost 23% (11) of these genes were significantly different between our genotypes at $q < 0.0001$, 50% (24) were significant between our genotypes at $q < 0.001$. Three probe sets mapping to *Tequila* were represented on our array and all three were highly

significant for changes in expression from the control (two were down-regulated in the *pyd*, *crol* and BG00817 mutant backgrounds and one was up-regulated in the same three backgrounds). The probe set that was increased in all three also maps to *CG34104*, which may explain the increase in expression instead of a decrease. *Tequila* is thought to be involved in ATP synthesis coupled proton transport, chitin metabolism and proteolysis (Crosby *et al*., 2007; Ross *et al*., 2003). The results of Lai *et al*. (2007) indicate that it is expressed more in older females. Interestingly, our results would suggest that it is the lines that were significant for male-specific increases in life span that show large changes in *Tequila* expression, while the lines where both sexes display longer life spans have fewer changes in *Tequila* expression. This is an observation that requires more exploration to determine the underlying basis for co-regulation of *Tequila* in these lines.

There was also significant overlap of the genes that were co-expressed in *Drosophila* mutations associated with increased life span with many of the *C. elegans* orthologs that were co-regulated in the long-lived *daf-2* mutant background (Murphy *et al*., 2003). 30 of the 39 up-regulated genes and 11 of the 20 down-regulated genes identified by Murphy *et al*. (2003) had *Drosophila* homologs. 30% (9) of the up-regulated genes were significant in our study at $q < 0.0001$, and 63% (19) were significant at $q < 0.001$. For the down-regulated genes, only 27% (3) were significant at $q < 0.001$ (none were significant at $q < 0.0001$). These numbers are slightly inflated as several heat shock genes in *C. elegans* are homologous to a single *Drosophila* gene, *lethal (2) essential for life*.

Many genes that have been previously shown to affect life span showed differential expression in the mutant lines associated with increased life span. For example, *InR* was down-regulated in both the *CG9238* and *CG10990* backgrounds, consistent with the previously observed decrease of *InR* expression associated with increased life span (Tatar *et al*., 2001). *Alcohol dehydrogenase* (*Adh*) was up-regulated in the mutant *pyd*, *mub* and *esg* backgrounds. *Adh* expression has been shown to decrease with age (Zou *et al*., 2000) so an increase in expression could conceivably be associated with an increase in life span. The expression of *Sirt2*, a member of the *Drosophila* Sirtuin family (Frye, 2000), was strongly decreased in the

mub, BG00817 and *esg* mutant backgrounds. The *mub* mutant displayed an increase in *Sod* expression and a decrease in *chico* expression which mirrors previous reports of changes of the expression of these genes in association with increased life span (Orr and Sohal, 1994; Parkes *et al*., 1998; Clancy *et al*., 2001). Interestingly *mub* mutant males showed an almost 50% increase in expression of *Indy*, in contrast to the decrease of *Indy* expression previously associated with increased life span in the *Indy* mutant background (Rogina *et al*., 2000).

Future Prospects

The first concern to be addressed in any further work is to establish that the *P*-element disruption of the candidate gene is the cause of the change in longevity. Revertants have currently only been created for four of the lines with increased life span. It would be beneficial to find revertants for the other lines, in particular those used in the epistasis and microarray experiments, to further support that the *P*-element is in fact responsible for the change in phenotype. Revertants lead to fairly solid evidence for causality, but cannot always be obtained. Hence, another method to determine if the *P*-element is causing the change in life span may be necessary. Transgenic rescue would thus be a useful complementary approach, in which another *P*-element containing a functional form of the gene in question is crossed into the mutant line. Here, the expectation would be a decrease in longevity; thus one would need to be cautious in the interpretation of causality, as the insertion of the transgene may disrupt another gene that affects life span. Creation and testing of multiple insertion of the same transgene could minimize this problem. In addition to transgene rescue, one could test the effects of increased expression of the candidate gene above normal levels and observe whether or not it leads to a corresponding opposite change in longevity.

After causality has been established, it would be useful to explore the contribution of the candidate genes to naturally occurring variation in longevity, by associating molecular variation of a sample of alleles derived from a natural population with phenotypic variation in life span. Association mapping for quantitative trait nucleotides (QTNs) in longevity genes has previously led to SNPs

associated with an increased life span (De Luca *et al*., 2003; Carbone *et al*., 2006). Fine tuning of a longevity gene down to a causal SNP(s) is one of the ultimate goals of life span research. Furthermore, it leads to a better understanding of how the gene interacts with other genes and its local environment to extend longevity.

As discussed earlier, some work has already been done to establish interactions between the *Tre1*/*Gr5a* mutant lines and genes in the insulin signaling pathway (Rollmann *et al*., 2006) as well as some of the mutant lines with each other. This work needs to be extended to the other lines as well as some of the other established longevity pathways such as caloric restriction. The results of the microarray experiment suggest that *mub*, *CG9238* and *CG10990* could be involved in the insulin signaling pathway, which makes them optimal candidates for complementation testing with mutant genes involved in insulin signaling. It would also be interesting to do complementation tests with lines that have an insert in the same gene, but in a different location or genetic background that lead to vastly different effects. For instance, an insertion in *pyd* led to an increase in life span in the "F" Canton S background, but a decrease in life span in the "A" background. Similarly, mub mutants in the "F" background increased life span while decreasing it in the "B" background. There were also many sex-specific and sex-antagonistic effects that need to be investigated. Why do the genes act so differently in some mutants and backgrounds and not in others? This is a question that could be addressed using mosaics.

Mosaics are individuals, developed from a single fertilized egg, that have two or more populations of cells with different genotypes. They are typically created in *Drosophila* via mitotic recombination events caused by breaks in DNA due to ionizing radiation (Patterson, 1929) or a recombination system based on yeast FLP recombinase and the corresponding target FRT sequence (Golic and Lindquist, 1989). The FRT/FLP system works in male and female germ-line cells and promotes high frequency mitotic recombination at FRT sequence sites on homologous chromosomes (Golic, 1991). Xu and Rubin (1993) have created *Drosophila* lines with FRT sequences inserted near the centromeres on each chromosomal arm along with markers that allows for mosaic analyses of more than

95% of the *Drosophila* genes. The main drawback of this system is that wild-type cells are the ones containing the marker, sometimes making it difficult to observe mutant cells. Lee and Luo (1999) solved this problem using mosaic analysis with a repressible cell marker (MARCM) system that takes advantage of the GAL4-UAS system coupled with GAL80, a repressor of GAL4, where only the mutant cells are marked. Gynandromorphs, a specific class of mosaics where an individual has both male and female cells (typically a female with cells lacking an *X* chromosome), have been used to explore sex-specific expression since Morgan (1914) discovered them. Creating gynandromorphs using the MARCM system would be useful in identifying sex-specific expression as well as possible tissue-specific expression of candidate genes.

It would be interesting to explore each of the mutant lines for any reproduction trade-offs during both early and late life. As discussed previously, this has been an important part of life span research and many life span genes have not yet been investigated for a reduction in fecundity. Thus far it is not clear if a trade-off exists in certain life span pathways (such as the insulin signaling pathway). Building up a database on each of the lines would be valuable in understanding whether or not there is a reduction in fecundity associated with them or any of the pathways in which they are involved.

While the microarray experiment has been incredibly informative, there is still more that needs to be done. The results of the microarrays await verification using real-time reverse transcription polymerase chain reaction (RTPCR) (to measure relative expression levels). In addition to the one- and six-week time-points used here, it would be valuable to look at other time-points, especially at a time closer to the end of the mutant life span to see if expression levels reflect those of the control at six-weeks. Furthermore, it is necessary to determine where the genes are being differentially expressed. The microarrays used whole-bodies, making it impossible to know what tissues expression is occurring within. The *P*-element contains *Gal4* so mutant lines could be crossed to a line containing a UAS-driven *lacZ* gene to observe spatial expression. *In situ* hybridizations could also be used to identify tissues where the gene is expressed.

The Future of Life Span Genetics

Using multiple, complementary techniques to search for the genetic mechanisms underlying life span has become the norm and will continue to be necessary to tease apart the true factors from the false. Cross discipline collaborations will be vital in order to tie together the many aspects of aging. The human haplotype mapping project now provides hundreds of thousands of SNPs that may be used for whole-genome linkage mapping. This in turn may allow the prediction of drug response and disease susceptibility in individuals. Protein arrays will be a genetic goldmine if they become feasible for use with longevity. Currently they are too expensive for most labs and many pertinent proteins are unknown. As we further characterize the genes known to have an effect on longevity, particularly the interactions between them, we will be able to model interactions to estimate the effect a change in one will have on the others as well as the life span. Continued examination of alternate forms of gene regulation such as methylation and gene silencing will be necessary to fully understand the aging process. Finally, it is important that in the rush to use the modern, genome-wide methods of gene analysis that the classical techniques are not forgotten. They often provide the links necessary for the understanding of the big picture of aging.

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