The Genomic Basis of Postponed Senescence in *Drosophila melanogaster*

Megan Ulmer Carnes¹, Terry Campbell¹, Wen Huang¹,², Daniel G. Butler¹, Mary Anna Carbone¹,²,³, Laura H. Duncan¹, Sasha V. Harbajan¹, Edward M. King¹, Kara R. Peterson¹, Alexander Weitzel¹, Shanshan Zhou¹,²,³, Trudy F. C. Mackay¹,²,³ *

¹ Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina, 27695, United States of America, ² Program in Genetics, North Carolina State University, Raleigh, North Carolina, 27695, United States of America, ³ W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina, 27695, United States of America

* trudy_mackay@ncsu.edu

Abstract

Natural populations harbor considerable genetic variation for lifespan. While evolutionary theory provides general explanations for the existence of this variation, our knowledge of the genes harboring naturally occurring polymorphisms affecting lifespan is limited. Here, we assessed the genetic divergence between five *Drosophila melanogaster* lines selected for postponed senescence for over 170 generations (O lines) and five lines from the same base population maintained at a two week generation interval for over 850 generations (B lines). On average, O lines live 70% longer than B lines, are more productive at all ages, and have delayed senescence for other traits than reproduction. We performed population sequencing of pools of individuals from all B and O lines and identified 6,394 genetically divergent variants in or near 1,928 genes at a false discovery rate of 0.068. A 2.6 Mb region at the tip of the X chromosome contained many variants fixed for alternative alleles in the two populations, suggestive of a hard selective sweep. We also assessed genome wide gene expression of O and B lines at one and five weeks of age using RNA sequencing and identified genes with significant (false discovery rate < 0.05) effects on gene expression with age, population and the age by population interaction, separately for each sex. We identified transcripts that exhibited the transcriptional signature of postponed senescence and integrated the gene expression and genetic divergence data to identify 98 (175) top candidate genes in females (males) affecting postponed senescence and increased lifespan. While several of these genes have been previously associated with *Drosophila* lifespan, most are novel and constitute a rich resource for future functional validation.

Introduction

Lifespan and senescence (the post-reproductive decline in survival and fertility with advancing age) vary enormously within and among taxa, with some organisms attaining exceptional longevity and negligible senescence [1–3]. The question of why lifespan is limited and why there is...
variation in aging within species is addressed in general terms by evolutionary theory. Natural selection declines with age [4], so mutations with late age-specific deleterious effects are nearly neutral with respect to natural selection and can accumulate in populations at appreciable frequencies [5]. In addition, mutations with beneficial effects early in life but detrimental effects later in life will accumulate [6,7]. Both classes of mutations, in addition to unconditionally deleterious mutations affecting survival and fertility at all ages, will result in segregating genetic variation for lifespan in natural populations.

Indeed, estimates of the heritability of lifespan range from 10–30% in humans and other organisms [1,2,8–16], and quantitative trait loci (QTLs) affecting lifespan have been mapped in C. elegans [17–19], Drosophila [20–31], mice [32–34] and humans [35–39]. However, with a few exceptions [40–42], naturally segregating loci affecting lifespan have not been mapped to the level of individual genes. In contrast, studies assessing changes in gene expression with age, transgenic manipulation of candidate genes and direct screens for mutations affecting lifespan, largely in model organisms, have identified multiple genetic mechanisms affecting lifespan, some of which are ‘public’ [3,43] and common to multiple taxa. One such general mechanism of aging is the regulation of metabolism, in particular components of the insulin or insulin-like signaling pathway [44–59]. The increase in lifespan associated with decreased insulin signaling may be mechanistically related to the increase in life span conferred by dietary restriction [60–64]. Other general mechanisms of aging inferred from analyses of model organisms include genes affecting the ability to detoxify reactive oxygen species [65–69], reproduction [21,48,70–77], gene silencing [78–80], telomere integrity [81], DNA repair and replication [82–84], mitochondrial [85,86] and membrane function [87], resistance to heat, starvation and other environmental stressors [88–94], sensory perception [95,96], and immune response [97–100]. Despite these insights, we do not know to what extent these genes harbor polymorphisms affecting naturally segregating variation in lifespan within populations.

A powerful alternative to mapping QTLs by linkage and association analysis is to track changes in allele frequency between replicated populations undergoing laboratory evolution via natural or artificial selection [101–103]. This approach has the potential to identify causal genes and even variants affecting the trait of interest that are in common between all evolved populations under favorable experimental conditions (sufficient replication, large and genetically diverse populations, many generations of evolution, and genome wide variant detection by whole genome sequencing) [104–107]. Combining genetic divergence analyses with changes in gene expression can further aid in identifying causal genes and provide biological context to genome wide polygenic divergence [104,105]. D. melanogaster populations respond rapidly to laboratory selection for increased lifespan [15,104,108–110], facilitating the application of the ‘evolve and re-sequence’ approach to this trait. Here, we combine pooled DNA sequencing with RNA sequencing at two chronological ages to quantify the genetic and genomic responses of five replicate lines selected for postponed senescence via later reproduction (Old, or O lines) relative to five unselected control lines (Base, or B lines) [109]. We identify many novel candidate genes affecting increased lifespan and postponed senescence, many of which are orthologous to human genes.

**Materials and Methods**

**Drosophila stocks**

Ten D. melanogaster lines generated by Rose [109] were used in this study. Five lines were selected for delayed reproductive senescence (O1, O2, O3, O4, O5) and five were unselected (B1, B2, B3, B4, B5). The B and O line stocks were maintained in 14-day and 70-day generations, respectively, as described previously [111]. All lines were maintained at 25°C on cornmeal-
molasses-agar medium (cornmeal, 65 g/L; molasses, 45 ml/L; yeast, 13 g/L) under a 12:12 hour light:dark cycle. These flies were a generous gift of Dr. Philip Service.

Life history phenotypes and statistical analyses

**Lifespan.** To minimize larval density effects, experimental flies were produced for each line by allowing 6 males and 6 females to mate and lay eggs for one day in vials containing 10 ml culture medium. Offspring from these vials were collected at 1–3 days post-eclosion for lifespan assays. Lifespan was assessed for each population using 50 replicate vials, each containing 3 males and 3 females and 5 ml culture medium. Flies were transferred without anesthesia to new vials containing 5 ml of fresh food every 2–3 days; dead flies were removed from the vials upon observation. Deaths were recorded every 1–3 days until all individuals were deceased. We performed two-way factorial mixed-model analyses of variance (ANOVA) separately for males and females of form: $Y = \mu + P + L(P) + Rep(P \times L) + \epsilon$, where $P$ is the fixed effect of population (B vs. O), $L$ and $Rep$ are the random effects of line and replicate, respectively, and $\epsilon$ is the residual (error) variance. Parentheses indicate nested effects. ANOVAs were performed using SAS software version 9.4 [112].

**Phototaxis.** Parental flies were reared as described above for lifespan. Offspring used in phototaxis assays were maintained in bottles with 50 males and 50 females per bottle and transferred to fresh bottles every 2–3 days. Phototaxis was assessed in the countercurrent apparatus [113] for three replicates of 50 flies per sex per line at one, two, three and four weeks of age. Flies were allowed to recover overnight from CO2 exposure, and dark-adapted for 30 minutes prior to performing the assay in a dark room between 9:00–11:30 am. To assess phototaxis, flies were tapped to the bottom of the first start tube and the apparatus was laid horizontally with the distal tubes 5 cm away from a 15 W fluorescent light. The flies were given 15 seconds to reach the distal tube, and the procedure was repeated 7 more times per trial. Thus, each fly received a score between 1 (did not move toward the light in the first tube) and 8 (moved towards the light 7 times). We performed two-way factorial mixed-model ANOVAs of phototaxis separately for males and females of form: $Y = \mu + P + A + P \times A + L(P) + A \times L(P) + Rep(A \times P \times L) + \epsilon$, where $P$, $A$, $L$ and $Rep$ are the fixed effects of population and age, $L$ and $Rep$ are the random effects of line and replicate, respectively, and $\epsilon$ is the residual (error) variance. Parentheses indicate nested effects.

**Capillary feeding (CAFÉ) assay.** Parental and experimental flies were reared as described above for lifespan. Food consumption was assessed using a modified version of the CAFÉ assay [114] at one, two, three and four weeks of age. Eight flies of the same sex, line and age were anesthetized with CO2 and placed into each of six replicate vials containing 2 ml of 1.5% non-nutritive agarose and three 5 μL capillary tubes (Kimble Glass Inc.) containing a 4% (weight/volume) sucrose solution inserted through a foam plug. The capillary tubes were capped with mineral oil to minimize evaporation. The vials were placed in a transparent plastic container in which high humidity is maintained with open containers of water at 25°C and the flies were allowed to feed on the sucrose solution for 24 hours. After the first 24 hours of acclimation, the capillaries were removed and three fresh capillaries with sucrose solution were added to each vial. The flies were then given another 24 hours to feed on the sucrose solution after which the capillaries were marked to indicate the amount of food consumed and removed and the number of surviving flies was recorded. The amount of sucrose solution consumed was measured in millimeters to the closest 0.5 mm and adjusted to μl per fly per vial after correction for evaporation using control vials containing no flies in the same humidity chamber. We performed two-way factorial mixed-model ANOVAs of food consumption separately for males and females of form: $Y = \mu + P + A + P \times A + L(P) + A \times L(P) + \epsilon$, where $P$, $A$, $L$ and $\epsilon$ are as defined above for phototaxis. Parentheses indicate nested effects.
**Chill coma recovery time.** Parental and experimental flies were reared as described above for phototaxis. Chill coma recovery time \[115\] was assessed at one, two, three and four weeks of age with 50 flies per sex, line and age. We anesthetized flies using CO\(_2\) and let them recover for 24 hours prior to the assays. We then quantified chill coma recovery by transferring (without anesthesia) flies to empty vials, and placing them on ice for three hours. We transferred the flies to room temperature, and recorded the time it took for each individual to right itself and stand on its legs. We performed two-way mixed model ANOVAs as described for food consumption.

**Productivity.** Parental and experimental flies were reared as described for phototaxis. Productivity was assessed at one, two, three and four weeks of age with 10 vials containing 10 ml culture medium and 3 males and 3 females per line and age. Experimental animals were anesthetized using CO\(_2\) and given 24 hours to recover prior to setting up the experimental vials. Flies were allowed to lay eggs for 24 hours, and the total number of emerging progeny was counted every day for a total of 16 days. We performed two-way mixed model ANOVAs as described for food consumption.

**B and O sequence divergence**

Genomic DNA was extracted from 100 females of each line using Genomic-Tip 100/G columns (Qiagen Inc.), following homogenization using liquid nitrogen with a mortar and pestle. Genomic DNA was sequenced on the Illumina GAIIx platform by 68bp paired-end sequencing at the Genome Sciences Laboratory, North Carolina State University. Sequence reads were aligned to the BDGP5 reference genome using BWA (version 0.6.2) \[116\]. Alignments were locally realigned, marked for PCR duplicates, and base qualities were recalibrated using GATK (version 2.4) \[117\] and Picard Tools (version 1.89) \[117\]. Subsequently, uniquely mapped reads were piled up at each genomic position to identify putative SNPs. A polymorphic site was considered for further analysis if it passed the following filters: (1) at least 10X coverage by bases with Phred scale quality > 13; (2) bases passing the quality filter constituted at least 80% of all bases at the site; (3) no more than 250X coverage; (4) maximally 5% reads at the site represented indels; (5) the two most frequent alleles accounted for more than 95% of all alleles; (6) the minor allele frequency was at least 5% in at least one of the ten samples; (7) the Chernoff bound of the P-value for testing polymorphism versus sequencing error was smaller than 10\(^{-5}\); and (8) the Fisher’s exact test for strand bias had P > 10\(^{-5}\). Divergence of allele frequency between O and B was tested using t-tests at each site individually, requiring that at least three O lines and three B lines had estimable allele frequencies.

**Gene expression analysis**

**RNA extraction and sequencing.** Flies were reared exactly as described for lifespan. We collected 80 samples for RNA sequencing, all between 1–3 pm. There were two replicate samples of 50 3–5 day old males and females (week 1 samples) and 50 33–35 day old males and females (week 5 samples) from each line, with one exception. The B1 females are shorter-lived than the other B lines; therefore the old B1 females were 26–28 days old (4 weeks) in order to obtain sufficient flies. Flies were flash frozen over dry ice and stored at -80°C. Total RNA was extracted with Trizol (Life Technologies, California, USA) and an RNAeasy Mini Kit (Qiagen, Limburg, Germany). Ribosomal RNA (rRNA) was removed using a Ribo-Zero™ Gold Kit (Epicentre, Wisconsin, USA) with 5 ug total RNA input. Depleted mRNA was fragmented and converted to first strand cDNA. During the synthesis of second strand cDNA, dUTP instead of dTTP was incorporated to label the second strand cDNA. cDNA from each RNA sample was used to produce barcoded cDNA library using NEXTflex™ DNA Barcodes (Bioo Scientific,
Texas, USA) with an Illumina TrueSeq compatible protocol. Library size was selected using Agencourt Ampure XP Beads (Beckman Coulter, Indiana, USA) and centered around 250 bp with an approximate insert size of 130 bp. Second strand DNA was digested with Uracil-DNA Glycosylase before amplification to produce directional cDNA libraries. Libraries were quantified using Qubit dsDNA HS kits (Life Technologies) and a 2100 Bioanalyzer (Agilent Technologies, California, USA) to calculate molarity. They were then diluted to an equal molarity, re-quantified, and 16 libraries were pooled. Pooled library samples were quantified again to calculate the final molarity, denatured, and diluted to 14 pM. Pooled library samples were clustered on an Illumina cBot and sequenced on an Illumina Hisseq2500 using 125 bp single-read v4 chemistry.

**Transcriptome assembly and analysis.** RNA sequences were demultiplexed using the Illumina bcl2fastq program (version 1.8.4) and summarized as follows: there was an average of 17,447,402 reads generated per sample (min. 14,194,039 reads/sample, max. 24,652,714 reads/sample), and 93% of reads had a base call accuracy of ≥ 99.9%. Read quality was assessed using the FastQC program (version 0.11.2) (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The reads were pre-processed using Cutadapt (version 1.6) [118] to remove residual adapter sequences. Ribosomal RNA (rRNA) sequences were removed by aligning the sequencing reads using TopHat (version 2.0.13) [119] to known rRNA sequences downloaded from GenBank [120] and FlyBase (Dmel Release 5.57) [121]. The remaining reads were processed following the Tuxedo suite pipeline [122]. The sequencing reads were aligned to the D. melanogaster reference transcriptome and reference genome (FlyBase Dmel Release 5.57) using default parameters, allowing for up to 5 mismatches per read. Alignment rates were highly variable with an average alignment rate of 86%. We ruled out sample degradation, human contamination, and sequencing lane errors as the cause for the observed mapping rate variability, and inferred that natural sources of bacteria and other environmental components were the most likely explanation. After alignment, reads were assembled against the FlyBase reference transcriptome for gene-level analysis of annotated genes using Cuffquant (version 2.2.1) and default parameters, allowing for an increased number of fragments per locus (-max-bundle-frags increased to 10,000,000). The resulting expression values were geometric mean normalized using Cuffnorm (version 2.2.1) [123]. For the analysis of unannotated transcripts, transcriptome assembly was first performed using Cufflinks (version 2.2.1) without providing the annotated Flybase transcriptome; all other parameters were maintained. The resulting transcriptomes, from all 80 samples, were merged using Cuffmerge (version 2.2.1) to generate a high-confidence, sample-specific, reference transcriptome. Sample transcriptomes were then re-assembled against the generated reference transcriptome and expression levels were quantified using Cuffquant. The expression values were then geometric mean normalized using Cuffnorm. Finally, unannotated genes were identified for analysis by selecting expressed loci classified as ‘unknown, intergenic’ (transfrag class code u). After normalization, any gene (annotated or unannotated) whose mean expression value was 0 or whose maximum expression value was < 1 was dropped from the analysis.

We performed a factorial mixed effect ANOVA of the normalized gene-level count data using the following model: \( Y = \mu + A + S + P + A \times S + A \times P + S \times P + A \times S \times P + L(P) + \varepsilon, \) where \( S \) denotes the fixed effect of sex and all other terms are as defined above. We used the Benjamini and Hochberg method [124] of controlling the false discovery rate (FDR), and considered an FDR of < 0.05 for any term in the ANOVA to be significant. Over 94% of genes had a significant sex main effect or interaction term. Therefore, we performed ANOVAs separately for males and females for each gene expression trait using the model: \( Y = \mu + A + P + A \times P + L(P) + \varepsilon. \) All statistical analyses were conducted using SAS software [112]. Gene annotation and identification of human orthologs was done using Ensembl databases [125].
Gene ontology (GO) and functional annotation analyses

We performed gene ontology and functional annotation analyses for genetically divergent genes and genes significant for the fixed effects in the ANOVAs of gene expression using the functional annotation cluster tool in DAVID (version 6.7) [126,127]. If a gene subset of interest was larger than 3,000 (a DAVID-set threshold), then the top 3,000 most significant genes were used in the analysis. An enrichment score of 5 (corresponding to a geometric mean normalized P-value threshold of 1E-5) was used as the significance cutoff.

Results

The O and B lines were derived from an outbred laboratory population founded by wild-caught animals from South Amherst, MA, USA in 1970, and maintained at large population size for 130 generations prior to establishing the experimental populations [109]. Thus, the base population had been adapted to laboratory conditions and should have reached global quasi-linkage equilibrium before the establishment of the B and O populations in February 1980 [109]. At the time the experiments described here were conducted, the B populations had been continually maintained at 14 day discrete generation intervals for over 850 generations and the O populations at 70 day generation intervals for over 170 generations, since 1996 in the Mackay laboratory. Population sizes each generation are of the order of one to two thousand individuals for all lines. Thus, these lines have many desirable properties for analysis by the ‘evolve and re-sequence’ approach. The populations are large and genetically heterogeneous, well-replicated, and have undergone many generations of evolution under controlled environmental conditions.

Phenotypic characterization of O and B lines

The O and B populations are highly divergent for lifespan (Fig 1A and 1B; S1 Table). On average, O lines live 70% longer than B lines (62.1 days vs. 35.7 days, respectively). Since the O lines were selected for postponed reproductive senescence, we assessed productivity and other fitness-related traits of all lines at one, two, three and four weeks of age (Fig 1C–1I, S1 Table). The productivity of O lines is higher than that of B lines averaged over all ages, and the B lines exhibit marked reproductive senescence while the O lines do not (Fig 1I). Averaged over all ages, the B lines consume more sucrose in the CAFÉ assay than the O lines, and food consumption generally declines with age. However, this decline is greatest for the B lines (Fig 1C and 1D). The O lines respond robustly in the phototaxis assay and this tendency declines with age, but surprisingly, the B lines barely move towards light, even at week one (Fig 1E and 1F). With respect to the CAFÉ and phototoaxis assays, B lines have a ‘couch potato’ phenotype, eating a lot and moving little, while the O lines eat less and are more motile (at least in response to a light stimulus). The time to recover from a chill induced coma increases with age, but there is little differentiation between the O and B lines averaged over all ages. However, the B lines display more senescence for this trait than do the O lines, particularly in females (Fig 1G and 1F). Thus, response to continuous selection for postponed senescence has persisted, and is accompanied by correlated responses in lifespan and other fitness traits, and delayed senescence for traits other than reproduction.

B and O sequence divergence

We performed population sequencing of pools of individuals from all B and O lines. We identified 432,580 single nucleotide polymorphisms (SNPs) segregating in these lines, and performed simple t-tests to assess differences in allele frequency between the populations (Fig 2, S2 Table).
At a lenient reporting \(P\)-value threshold of \(P \leq 10^{-3}\), we identified 6,394 variants in or near 1,928 genes (for a false discovery rate of FDR = 0.068). A total of 450 variants in or near 413 genes exceeded a rigorous Bonferroni correction for multiple tests. The majority of the highly significant variants were located in a 2.6 Mb region at the tip of the X chromosome, which contained many variants fixed for alternative alleles in the two populations, suggestive of a hard selective sweep and long range linkage disequilibrium (LD) [128]. Several of the genes with differences in SNP allele frequencies between the B and O populations had previously been implicated to affect lifespan (e.g., \(\text{Cat} [65]\), \(\text{Cct1} [87]\), \(\text{cpo} [129]\), \(\text{Dhc64c} [130]\), \(\text{Eip75B} [131]\), but the vast majority are novel candidates. We performed Gene Ontology (GO) enrichment analyses [126,127], excluding genes in the 2.6 Mb region of the X chromosome because the high degree of LD in this region precludes identifying candidate genes. The genetically divergent genes are highly enriched for biological process categories involved in development and differentiation, in particular the development and function of the nervous system (S3 Table).
Gene expression analysis

We assessed genome wide gene expression of O and B lines at one and five weeks of age, separately for males and females, using RNA sequencing (S4 Table). Week one corresponds to 11% and 20% of the average lifespan of O and B lines, respectively; while week five corresponds to 56% of the average lifespan of O lines and 98% of the average lifespan of B lines. We performed a three-way factorial analysis of variance (ANOVA) on each gene expression trait to partition the variation in gene expression between the main effects of sex, age, population and all interactions. We used a false discovery rate of FDR < 0.05 to account for multiple tests. We found that sex was significant for 94% of genes as a main effect or interaction term, consistent with previous analyses documenting substantial sexual dimorphism of gene expression [23,130,132,133]. Therefore we performed two-way ANOVAs to partition variance of gene expression between the main effects of age, population and the age by population interaction, as well as among lines within each population, for all annotated genes with detectable expression (15,586 in females and 16,174 in males). A summary of the number of significant genes for each term in the ANOVAs is provided in Table 1 and full ANOVA results are given in S5 and S6 Tables.

We first examined the genomic distribution of genes with significant main effects of age, population, and the age by population interaction (Fig 3). We plotted the fraction of significant genes in each category in non-overlapping 0.5 Mb windows, and found they were largely uniformly distributed across the genome, with a few notable exceptions. In females, there is strong enrichment of genes significant for the age by population interaction near the centromeres of chromosomes 2L and 2R and also near the centromere of chromosome 3L. In males, the enrichment is near the centromere and telomere of chromosome 2R. The genes in these regions do not have anything obvious in common, nor are they members of gene families (S7 Table).

Genes whose expression changes with age are candidate genes affecting lifespan as well as biomarkers of aging. A large fraction of the genome—27% in females and 61% in males—changes expression with age (Table 1), consistent with previous studies [23,130,134–136]. We performed GO enrichment analyses for the genes that were up- and down-regulated with age, separately for females (S8 Table) and males (S9 Table). In females, genes up-regulated with age were highly enriched for GO terms associated with immune response, stress response, defense response and detoxification of xenobiotics, while genes that were down-regulated with age were highly enriched for GO terms associated with mitochondrial function and oxidative phosphorylation. Genes that were up- and down-regulated with age in males were enriched for the same GO categories as for females, as expected since 74% of the genes that changed with age in females and males were the same (Table 1, S9 Table). In addition, genes that were up-regulated with age in males were highly enriched for GO categories associated with morphogenesis and development, including development of the nervous system; regulation of metabolism, gene expression and protein synthesis; signal transduction, mitosis, DNA repair, and programmed cell death. In addition, 41 up-regulated genes were significantly enriched for the GO terms “determination of adult life span”, “aging” and “multicellular organismal aging” (S9 Table). Genes that were down-regulated with age in males were also enriched for GO terms associated with metabolism and catabolism (S9 Table).

Genes with significant changes in expression between the B and O populations are candidate QTLs affecting variation in lifespan. In contrast to the large fraction of the genome associated with expression changes with age, only ~5% of the genome fell into this category, and these genes were largely different in females and males (Table 1). In females, genes down-regulated in B lines were enriched for GO terms associated with gene expression, protein synthesis, mitosis, metabolism, RNA binding and mitochondrial function; while genes up-regulated in B
lines were enriched for GO terms associated with immune, defense and stress responses and detoxification of xenobiotics (S8 Table). In males, genes down-regulated in B lines were enriched for GO terms associated with detoxification of xenobiotics while genes up-regulated in B lines were enriched for GO terms associated with morphogenesis and development (S9 Table).

Transcripts exhibiting the signature of postponed senescence are those for which there is a change in gene expression of B lines with age but this change is attenuated but in the same

Table 1. Numbers of significant (FDR < 0.05) annotated genes for each term in the ANOVA models of gene expression.

| Term                        | Female | Male  | Both sexes |
|-----------------------------|--------|-------|------------|
| Age                         | 4,145  | 9,853 | 3,085      |
| Age up-regulated (W1 < W5)  | 1,999  | 4,231 | 1,300*     |
| Age down-regulated (W1 > W5)| 2,146  | 5,622 | 1,360*     |
| Population                  | 780    | 1,011 | 178        |
| Population B < O            | 568    | 438   | 115*       |
| Population B > O            | 212    | 573   | 58*        |
| Age × Population            | 3,171  | 4,998 | 1,276      |
| Total number of tested genes| 15,586 | 16,174| 15,563     |

W1: Week 1; W5: Week 5.

* These numbers do not sum to the total numbers in both sexes because of differences in the directionality of effects across sexes.

3,085 genes have a significant age effect in both the male and female dataset but only 2660 have an effect in the same direction. The remaining 425 genes are up-regulated in one sex and down-regulated in the other. Likewise, 5 genes have opposite effects in the B and O populations.

doi:10.1371/journal.pone.0138569.t001

3.085 genes have a significant age effect in both the male and female dataset but only 2660 have an effect in the same direction. The remaining 425 genes are up-regulated in one sex and down-regulated in the other. Likewise, 5 genes have opposite effects in the B and O populations.

doi:10.1371/journal.pone.0138569.t001

Fig 3. Genome-wide distribution of genes showing significant age, population, and age x population interaction effects on gene expression. The proportions of genes showing significant age, population or age x population interaction effects within 0.5Mb non-overlapping windows are plotted along the chromosome arms. Genes are located by their mid-gene coordinates. The top and bottom panels are the distributions for females and males, respectively.

doi:10.1371/journal.pone.0138569.g003
direction in the O lines, and further the gene expression of older O lines remains similar to that of young B lines. Genes exhibiting this signature of postponed senescence will be among those with a significant age by population interaction term. The population by age interaction term was significant for 3,171 genes in females and 4,998 genes in males (Table 1). In females, genes with significant age by population interactions were enriched for GO terms involving the plasma membrane and development and function of the nervous system (S8 Table). In males, genes with significant age by population interactions were enriched for GO terms associated with mitochondrial function, oxidation reduction and amine metabolism (S9 Table).

Finally, we extended the analysis to previously unannotated genes, of which there were 1,950 in females and 2,299 in males (S10 and S11 Tables). Many of the novel genes were located in heterochromatic regions: 1,104 (57%) in females and 1,311 (57%) in males. Very few of the female novel genes were significantly associated with age (100, 5.1%), population (0) and the age by population interaction (29, 1.4%) (S10 Table). In contrast, many of the male novel genes were significantly associated with age-specific gene expression changes (1,037, 45.1%), although very few were associated with population (15, 0.65%) and the age by population interaction (50, 2.17%) (S11 Table).

**Candidate genes for postponed senescence**

Causal variants affecting postponed reproductive senescence, increased lifespan and other traits that are among the list of variants that are divergent in allele frequency between the B and O populations. However, these variants often occur in local LD blocks leading to poor resolution of individual genes—particularly within the 2.6 Mb region at the tip of the X chromosome. Further, the genes with these divergent variants may be associated with other traits that have evolved as a correlated response to selection for postponed selection in the O lines and are not necessarily associated with differences in lifespan between the B and O lines. Candidate genes affecting increased lifespan and postponed senescence are also among the transcripts with significant age by population interactions. However, gene expression analyses alone cannot distinguish between expression changes causing phenotypic divergence in lifespan from those that are a consequence of phenotypic divergence, and a cis-regulatory change in expression of gene can cause trans-regulatory changes in gene expression of other genes, leading to correlated gene expression modules [132,137,138]. Therefore, we hypothesized that integrating results from sequence divergence gene expression divergence would enable us to identify the top candidate genes affecting postponed senescence and increased lifespan as those significant in both analyses.

Not all significant age by population interactions in the gene expression analyses are consistent with the pattern of gene expression difference between the O and B lines expected from postponed senescence. We therefore filtered these genes by requiring that they fall into one of eight interaction groups consistent with postponed senescence (Fig 4). We defined interaction groups 1 and 2 as those for which there was no significant difference in expression between the B and O populations at week 1, expression in the B populations significantly increases in week 5, and expression in the O populations either remains constant (group 1) or changes marginally at week 5 (group 2). Similarly, we defined interaction groups 3 and 4 as those for which there is again no significant difference in expression between the B and O populations at week 1, expression in the B populations is significantly decreased in week 5, and expression in the O populations either remains constant (group 3) or changes marginally at W5 (group 4). Interaction groups 5 and 6 are those for which there is a significant difference in expression between the B and O populations at both weeks, but the difference is larger at week 5 than week 1, expression in the B lines is significantly increased at week 5, and expression in the O lines either
Fig 4. Interaction plots showing signatures of postponed senescence. Each plot shows the mean gene expression levels in normalized fragments per kilobase of exon per million fragments mapped (FPKM) at week 1 (W1) and week 5 (W5) in the B population (blue) and the O population (red). All examples are for male gene expression. See text for the description of the groups. (A) Group 1: CG6188 (interaction FDR = 5.57E-04). (B) Group 2: CG2233 (interaction FDR = 9.89E-06). (C) Group 3: Sfp53D (interaction FDR = 4.83E-06). (D) Group 4: CG31198 (interaction FDR = 8.72E-05). (E) Group 5: CR45054 (interaction FDR = 5.01E-05). (F) Group 6: CG6910 (interaction FDR = 3.63E-06). (G) Group 7: CG2930 (interaction FDR = 2.10E-05). (H) Group 8: Vap-33B (interaction FDR = 4.22E-03).

doi:10.1371/journal.pone.0138569.g004
remains constant (group 5) or changes marginally at week 5 (group 6). Finally, interaction
groups 7 and 8 are those for which there is a significant difference in expression between the B
and O populations at both weeks, but this difference is larger at week 5 than week 1, expression
in the B populations is significantly decreased at week 5, and expression in the O populations
either remains constant (group 7) or changes marginally at W5 (group 8). Applying these fil-
ters reduced the number of candidate genes to 687 in females (S12 Table) and 1,459 in males
(S13 Table). The vast majority of these genes were in interaction groups 1–4 (94.5% in females
and 94.3% in males).

We next required that a variant from the divergence analysis occurred within 1 kb of the
genes with expression changes consistent with postponed senescence and increased lifespan. A
total of 98 genes in females and 175 genes in males remained after applying this filter (S14 and
S15 Tables). These genes were not enriched for any GO terms. Rather, they spanned the diver-
sity of biological functions previously associated with aging, including the regulation of metabo-
lism and macromolecule biosynthesis, immune/defense response, stress resistance,
reproduction, mitochondrial function, oxidation-reduction, DNA repair and replication, and
regulation of gene expression. Many of these genes affect developmental processes, including
development and function of the nervous system, none of which have previously been associated
with lifespan. Possibly the same genes that act early in development to build organisinal struc-
tures are also responsible for the long-term maintenance of these structures in adults. Approxi-
mately one-third of the candidate genes associated with postponed senescence have no known
biological functions. Finally, only 20 of these genes are in common between males and females.

**Discussion**

We have characterized the phenotypic, genetic and genomic divergence of lines subjected to
long-term laboratory evolution for postponed reproductive senescence [109]. The O lines live
nearly twice as long as the B lines, have increased productivity at all ages, and exhibit post-
poned senescence for other phenotypes not under direct selection. We determined the genomic
DNA sequences of the O and B lines to assess the pattern and magnitude of genetic differentia-
tion between them. Of the ~18,000 genes in the *Drosophila* genome, ~11% have a variant that
is genetically divergent between the O and B populations at our reporting threshold of
$P < 10^{-3}$ (FDR = 0.068), many of which are in a 2.6 Mb region of the X chromosome that
appears to have undergone a hard selective sweep [128].

We performed RNA sequencing of the O and B lines at one and at five weeks of age, and
used ANOVA to partition the variation in gene expression for each expressed transcript into
the main effects of age and population, and the age by population interaction, separately for
each sex. Consistent with previous studies [23,130,134–136], we found that a substantial fra-
c tion of the genome exhibits significant (FDR < 0.05) changes in gene expression with age.
Intriguingly, transcriptional divergence between the O and B populations was modest (5–6% of
the genome) with respect to the main effect of population, but much greater (20–31%) for the
age by population interaction. This suggests that transcriptional divergence between the O and
B populations is age-specific, as would be expected for transcripts with less change with age in
the O lines relative to the B lines (*i.e.*, postponed senescence). We identified the subset of genes
with significant age by population interactions with gene expression signatures consistent with
postponed senescence, and the subset of those genes that are candidates for postponed senes-
cence because they are also genetically divergent between the B and O population– 98 genes for
females and 175 genes for males.

Several of these candidate genes have been previously associated with *Drosophila* lifespan.
We identified CG10383, which encodes a hydrolase, as a candidate gene for postponed
senescence and increased lifespan in both sexes. Over-expression of CG10383 in the nervous systems extends lifespan [139]. Catalase (Cat) and Autophagy-related 7 (Atg7) were among the candidate genes affecting lifespan in females. Null mutations of Cat [65] and Atg7 [140] reduce lifespan. Candidate genes in males included methuselah-like 8 (mthl8), molting defective (mld, also known as DTS-3), CTP:phosphocholine cytidylyltransferase 1 (Cct1), Peptidoglycan recognition protein LF (PGRP-LF) and brummer (bmm). mthl8 is annotated to affect lifespan by virtue of its homology with mth (methuselah), which encodes a G-protein coupled receptor that, when down-regulated, increases lifespan [114]. mld (DTS-3) encodes a protein with Krüppel Zn-finger domains and is involved in ecdysone biosynthesis. A dominant temperature sensitive allele of mld has been associated with a female-specific increase in lifespan [141]. The observation that mld is a male-specific candidate gene in this study is not surprising, since we have previously observed that the same mutation affecting lifespan can have different sex-specific effects depending on the genetic background, and that different mutations in the same gene and genetic background can have variable and sometime opposite effects on longevity [131,142]. Cat1 [87] and PGRP-LF [139] were both identified in large scale screens for genes with increased lifespan. bmm, which encodes a triglyceride lipase, was found to be up-regulated on starvation in a genome wide transcriptome analysis comparing fed and food-deprived flies [143]. Subsequent analyses showed that a mutation in bmm had a context-dependent effect on lifespan, with decreased lifespan under fed conditions, but increased lifespan when flies were starved [143].

Several of the candidate genes for postponed reproductive senescence and increased lifespan were associated with a variant from the divergence analysis with a P-value exceeding a Bonferroni correction for multiple tests: CG18031, CG42340, CG11378, CG3699 and mus81 in females; Insulin-like peptide 6 (Ilp6), Cytochrome P450-4d1 (Cyp4d1), silver (svr), female sterile (1) Nasrat (fs(1)N), CG2854, CG13868, CG6428, CG7713, Centaurin gamma 1A (CenG1A) and CG33307 in males. This study represents the first biological functional annotation for the majority of these genes (CG18031, CG42340, CG11378, CG3699, Cyp4d1, CG2854, CG13868, CG6428, CG7713, CG33307). While the others have not been implicated to affect lifespan previously, most are plausible candidates because they perform similar roles as other genes known to affect aging. mus81 plays a role in DNA repair [144]. Ilp6 is thought to be an insulin receptor and affects adult body size by regulating post-feeding growth [145] as well as growth under conditions of nutritional deprivation [146]. svr encodes Carboxypeptidase D [147] and has pleiotropic effects on imaginal disc-derived wing morphogenesis, long term memory, stress response and phagocytosis [148,149]. CenG1A is a member of the gamma subgroup of the Centaurin superfamily of small GTPases and is a Phosphoinositide-3-kinase enhancer (PIKE) protein thought to regulate ecdysone signaling-dependent second to third instar larval transition [150]. fs(1)N is an unexpected candidate affecting male postponed senescence since it is involved in oogenesis and highly expressed in female reproductive tissues [121]. However, it does have a low level of expression in testes [121].

The genetic architecture of Drosophila lifespan is highly sex-specific [24–27,29,130,131,142], as indeed is the majority of the candidate genes identified in this study. However, 20 of our top candidate genes (including the above-mentioned CG10383) were found in both sexes. Again, this study represents the first biological functional annotation for most of these genes (CG12253, CG5991, CG2233, CG3604, Organic anion transporting polypeptide 30B (Oatp30B), CG6357, CG11395, CG30098, CG32061, CG42557, CG43175, CR45054, CR45272); and the other genes common to males and females are plausible candidates. Cecropin A2 (Ceca2) is involved in antibacterial humoral response [151–153], as is Ras-like protein A (RalA) [154]. RalA also regulates polar-cell differentiation during oogenesis [155]. fat (ft) is involved in cadherin and calcium ion binding and acts to regulate planar cell polarity in the
wing [156] and body hair [157]. It is also a tumor suppressor and as such acts to regulate growth [158]. *multiple wing hairs (mwh)* encodes a G protein binding domain-formin homology 3 (GBD-FH3) domain protein that acts downstream of the planar cell polarity pathway to regulate wing hair development [159,160]. *exit protein of rhodopsin and TRP (Xport)* is a chaperone for the transient receptor potential (TRP) channel and its G-protein coupled receptor, rhodopsin (Rh1) and interacts with both Trp and Rh as well as the small heat shock proteins Hsp27 and Hsp90 [161]. *shrub (shrb, also known as ESCRT)* has pleiotropic effects on multiple biological process, including autophagy [162], nervous system development [163–166], negative regulation of mycobacterial growth [167] and growth of the female germ line [168,169].

The candidate genes affecting postponed senescence and increased lifespan identified in this study are a rich resource for future functional validation. Many of these genes have human orthologs and may advance our understanding of ‘public’ mechanisms of aging [3,43]. Although we used the lowest $P$-value from the genetic divergence analysis as a filter to integrate with the gene expression data, these polymorphisms are not necessarily the causal ones. However, many are indeed in transcriptional start and end sites of the genes exhibiting transcriptional signatures of postponed senescence, a hallmark of *Drosophila cis*-eQTLs [170]. Determining which polymorphisms are causal will be a critical step towards functional analyses of their pleiotropic effects on fitness and insights about why they remain segregating in nature, providing specific examples of general evolutionary explanations.

**Supporting Information**

**S1 Table.** Mixed model analyses of variance of lifespan and senescence for feeding behavior in the CAFÉ assay, phototaxis, chill coma recovery time and productivity in the B and O lines. Population and Age are fixed effects, the rest are random. df: degrees of freedom; MS: Type III mean squares, F: F ratio statistic; $P$: $P$-value.

(DOCX)

**S2 Table.** Divergence in allele frequency between O and B lines. All frequencies are estimated with respect to the allele that is more frequent in the O lines, which is indicated by the column "Allele for frequency estimation".

(XLSX)

**S3 Table.** Biological Process Gene Ontology (GO) enrichment analysis of genes divergent in O and B lines (excluding the first 2.6Mb of the X chromosome). An enrichment score of 5 (corresponding to a geometric mean normalized $P$-value threshold of 1E-5) was used as the significance cutoff from the functional annotation cluster analysis.

(XLSX)

**S4 Table.** Gene expression data for all samples. B1-B5 are the five B lines and O1- O5 the five O lines. W1 and W5 denotes weeks one and five, respectively. F indicates females and M males, 1 and 2 are the two biological replicates per population, week and sex. Tab A gives raw expression data in FPKM and Tab B the geometric mean normalized expression counts.

(XLSX)

**S5 Table.** $P$-values and FDR from ANOVA of female gene expression and summary statistics. The variant with the smallest $P$-value from the genetic divergence analyses is also given.

(XLSX)

**S6 Table.** $P$-values and FDR from ANOVA of male gene expression and summary statistics. The variant with the smallest $P$-value from the genetic divergence analyses is also given.

(XLSX)
S7 Table. Gene in regions enriched for transcripts with significant Age and/or Population × Age terms.
(XLSX)

S8 Table. Gene Ontology (GO) enrichment analyses of female gene expression data. An enrichment score of 5 (corresponding to a geometric mean normalized P-value threshold of 1E-5) was used as the significance cutoff from the functional annotation cluster analysis. (A) Age analyses. (B) Population analyses. (C) Population and age and population × age analyses.
(XLSX)

S9 Table. Gene Ontology (GO) enrichment analyses of male gene expression data. An enrichment score of 5 (corresponding to a geometric mean normalized P-value threshold of 1E-5) was used as the significance cutoff from the functional annotation cluster analysis. (A) Age analyses. (B) Population analyses. (C) Population and age and population × age analyses.
(XLSX)

S10 Table. P-values and FDR from ANOVA of female gene expression of novel genes and summary statistics. W1: Week 1; W5: Week 5.
(XLSX)

S11 Table. P-values and FDR from ANOVA of female gene expression of novel genes and summary statistics. W1: Week 1; W5: Week 5.
(XLSX)

S12 Table. Transcripts exhibiting signatures of postponed senescence in females.
(XLSX)

S13 Table. Transcripts exhibiting signatures of postponed senescence in males.
(XLSX)

S14 Table. Candidate genes for postponed senescence and increased lifespan in females.
(XLSX)

S15 Table. Candidate genes for postponed senescence and increased lifespan in males.
(XLSX)

Acknowledgments
We thank Robert R. H. Anholt for comments on the manuscript. This is a publication of the W. M. Keck Center for Behavioral Biology.

Author Contributions
Conceived and designed the experiments: TFCM TC. Performed the experiments: TC DGB MAC LHD SVH EMK KRP AW SZ. Analyzed the data: MUC WH TC TFCM. Wrote the paper: MUC WH TC TFCM.

References
1. Finch CE, Ruvkun G. The genetics of aging. Annu. Rev. Genomics Hum. Genet. 2001; 2: 435–462. PMID: 11701657
2. Finch CE, Tanzi RE. Genetics of aging. Science 1997; 278: 407–411. PMID: 9334291
3. Pitt JN, Kaeberlein M. Why Is aging conserved and what can we do about it? PLoS Biol. 2015; 13: e1002131. doi: 10.1371/journal.pbio.1002131 PMID: 25923592
4. Charlesworth B. Evolution in age-structured populations. Cambridge Eng.; New York: Cambridge University Press; 1980.
5. Medawar PB. An unsolved problem of biology. London.: Published for the college by H. K. Lewis; 1952.
6. Kirkwood TBL, Rose MR. Evolution of senescence—late survival sacrificed for reproduction. Phil. Trans. Roy. Soc. Lond. B 1991; 332: 15–24.
7. Williams GC. Pleiotropy, natural selection, and the evolution of senescence. Evolution 1957; 11: 398–411.
8. Herskind AM, McGuie M, Holm NV, Sorensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. Hum. Genet. 1996; 97: 319–323. PMID: 8786073
9. McGuie M, Vaupel JW, Holm N, Harvald B. Longevity is moderately heritable in a sample of Danish twins born 1870–1880. J. Gerontol. 1993; 48: B237–244. PMID: 8227991
10. Gudmundsson H, Gudbjartsson DF, Frigge M, Gulcher JR, Stefansson K. Inheritance of human longevity in Iceland. Eur. J. Hum. Genet. 1996; 97: 319–323. PMID:8786073
11. Ljungquist B, Berg S, Lanke J, McClearn GE, Pedersen NL. The effect of genetic factors for longevity: A comparison of identical and fraternal twins in the Swedish twin registry. J. Geront. A Biol. Sci. Med. Sci. 1998; 53: M441–M446.
12. Perls T, Kunkel L, Puca A. The genetics of aging. Curr. Opin. Genet. Dev. 2002; 12: 362–369. PMID: 12076681
13. Perls T, Shea-Drinkwater M, Bowen-Flynn J, Ridge SB, Kang S, Jpoyce E, et al. Exceptional familial clustering for extreme longevity in humans. J. Am. Geriatr. Soc. 2000; 48: 1483–1485. PMID: 11083328
14. Promislow DEL, Tatar M, Khazaai AA, Curtsinger JW. Age-specific patterns of genetic variance in Drosophila melanogaster. 1. Mortality. Genetics 1996; 143: 839–848. PMID: 8725232
15. Rose M, Charlesworth B. A test of evolutionary theories of senescence. Nature 1980; 287: 141–142. PMID: 6776406
16. Tatar M, Promislow DEI, Khazaai AA, Curtsinger JW. Age-specific patterns of genetic variance in Drosophila melanogaster. 2. Fecundity and its genetic covariance with age-specific mortality. Genetics 1996; 143: 849–858. PMID: 8725233
17. Ayyadevara S, Ayadevara R, Hou S, Thaden JJ, Shmookler Reis RJ. Genetic mapping of quantitative trait loci governing longevity of Caenorhabditis elegans in recombinant-inbred progeny of a Berge-bo x RC301 interstrain cross. Genetics 2001; 157: 655–666. PMID: 11556986
18. Reis RJS, Kang P, Ayadevara S. Quantitative trait loci define genes and pathways underlying genetic variation in longevity. Exp. Gerontol. 2006; 41: 1046–1054. PMID: 16919411
19. Shook DR, Brooks A, Johnson TE. Mapping quantitative trait loci affecting life history traits in the nematode Caenorhabditis elegans. Genetics 1996; 142: 801–817. PMID: 8849889
20. Curtsinger JW, Khazaai AA. Lifespan, QTLs, age-specificity, and pleiotropy in Drosophila. Mech. Ageing Dev. 2002; 123: 81–93. PMID: 11718803
21. Durham MF, Magwire MM, Stone EA, Leips J. Genome-wide analysis in Drosophila reveals age-specific effects of SNPs on fitness traits. Nat. Commun. 2014; 5: 4338. doi: 10.1038/ncomms5338 PMID: 25000897
22. Forbes SN, Valenzuela RK, Keim P, Service PM. Quantitative trait loci affecting life span in replicated populations of Drosophila melanogaster. I. Composite interval mapping. Genetics 2004; 168: 301–311. PMID: 15454544
23. Lai CQ, Parmell LD, Lyman RF, Ordovas JM, Mackay TFC. Candidate genes affecting Drosophila longevity identified by integrating microarray gene expression analysis and QTL mapping. Mech. Ageing Dev. 2007; 128: 237–249. PMID: 17196240
24. Leips J, Mackay TFC. Quantitative trait loci for life span in Drosophila melanogaster: interactions with genetic background and larval density. Genetics 2000; 155: 1773–1788. PMID: 10924473
25. Leips J, Mackay TFC. The complex genetic architecture of Drosophila lifespan. Exp. Aging Res. 2002; 28: 361–390. PMID: 12227919
26. Nuzhdin SV, Pasyukova EG, Dilda CL, Zeng ZB, Mackay TFC. Sex-specific quantitative trait loci affecting longevity in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 1997; 94: 9734–9739. PMID: 9275193
27. Pasyukova EG, Vieira C, Mackay TFC. Deficiency mapping of quantitative trait loci affecting longevity in Drosophila melanogaster. Genetics 2000; 156: 1129–1146. PMID: 11063689
28. Valenzuela RK, Forbes SN, Keim P, Service PM. Quantitative trait loci affecting life span in replicated populations of Drosophila melanogaster. II. Response to selection. Genetics 2004; 168: 313–324. PMID: 15454545
29. Vieira C, Pasyukova EG, Zeng ZB, Hackett JB, Lyman RF, Mackay TFC. Genotype-environment interaction for quantitative trait loci affecting life span in Drosophila melanogaster. Genetics 2000; 154: 213–227. PMID: 10628982

30. Wilson RH, Morgan TJ, Mackay TFC. High-resolution mapping of quantitative trait loci affecting increased life span in Drosophila melanogaster. Genetics 2006; 173: 1455–1463. PMID: 16702433

31. Ivanov DK, Escott-Price V, Ziehm M, Magwire MM, Mackay TFC, Partridge L, et al. Longevity GWAS Using the Drosophila Genetic Reference Panel. J. Gerontol. A Biol. Sci. Med. Sci. 2015; Apr 28. pii: glv047. [Epub ahead of print]

32. Doria G, Barattini P, Scarpaci S, Puel A, Guidi L, Frasca D. Role of immune responsiveness and DNA repair capacity genes in ageing. Ageing Res. Rev. 2004; 3: 143–151. PMID: 15177051

33. Jackson AU, Galecki AT, Burke DT, Miller RA. Mouse loci associated with life span exhibit sex-specific and epistatic effects. J. Gerontol. A Biol. Sci. Med. Sci. 2002; 57: B9–B15. PMID: 11773201

34. Lang DH, Gerhard GS, Griffith JW, Vogler GP, Vandenbergh DJ, Blizard DA, et al. Quantitative trait loci (QTL) analysis of longevity in C57BL/6J by DBA/2J (BXD) recombinant inbred mice. Aging Cell 2010; 22: 8–19. PMID: 20305363

35. Beekman M, Blanche H, Perola M, Hervonen A, Bezrukov V, Sikora E, et al. Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study. Aging Cell 2013; 12: 184–193. doi: 10.1111/acer.12039 PMID: 23286790

36. Deelen J, Beekman M, Capri M, Franceschi C, Slagboom PE. Identifying the genomic determinants of aging and longevity in human population studies: progress and challenges. Bioessays 2013; 35: 386–396. doi: 10.1002/bies.201200148 PMID: 23423909

37. Deelen J, Beekman M, Uh HW, Broer L, Ayers KL, Tan Q, et al. Genome-wide association meta-analysis of human longevity identifies a novel conferring survival beyond 90 years of age. Hum. Mol. Genet. 2014; 23: 4420–4432. doi: 10.1093/hmg/ddu139 PMID: 24688116

38. Deelen J, Beekman M, Uh HW, Helmer O, Kuningas M, Christiansen L, et al. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. Aging Cell 2011; 10: 686–698. doi: 10.1111/j.1474-9726.2011.00705.x PMID: 21418511

39. Puca AA, Daly MJ, Brewster SJ, Matise TC, Barrett J, Shea-Drinkwater M, et al. A genome-wide scan for linkage to human exceptional longevity identifies a locus on chromosome 4. Proc. Natl. Acad. Sci. USA 2001; 98: 10505–10508. PMID: 11526246

40. Carbone MA, Jordan KW, Meliska CJ, Bartke A. Dwarf mice and the ageing process. Nature 1996; 384: 106.

41. De Luca M, Roshina NV, Geiger-Thornsberry GL, Lyman RF, Pasyukova EG, Mackay TFC. Dopa decarboxylase (Ddc) affects variation in Drosophila longevity. Curr. Biol. 2006; 16: 912–919. PMID: 16682353

42. De Luca M, Roshina NV, Papaconstantinou J, Miller RA, Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc. Natl. Acad. Sci. USA 2001; 98: 6736–6741. PMID: 11371619

43. Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc. Natl. Acad. Sci. USA 2001; 98: 6736–6741. PMID: 11371619

44. Giannakou ME, Goss M, Junger MA, Hafen E, Leevers SJ, Partridge L. Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science 2004; 305: 361. PMID: 15192154

45. Gil EB, Malone Link E, Liu LX, Johnson CD, Lees JA. Regulation of the insulin-like developmental pathway of Caenorhabditis elegans by a homolog of the PTEN tumor suppressor gene. Proc. Natl. Acad. Sci. USA 1999; 96: 2925–2930. PMID: 10077613

46. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Géloën A, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 2003; 421: 182–187. PMID: 12483226

47. Hwangbo DS, Gersham B, Tu MP, Palmer M, Tatar M. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature 2004; 429: 562–566. PMID: 15175753
52. Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and age-related disease. Nature 2013; 493: 338–345. doi: 10.1038/nature11861 PMID: 23325216

53. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R, A. C. elegans mutant that lives twice as long as wild type. Nature 1993; 366: 461–464. PMID: 8247153

54. Kenyon CJ. The genetics of ageing. Nature 2010; 464: 504–512. doi: 10.1038/nature08980 PMID: 20336132

55. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science 1997; 277: 942–946. PMID: 9252323

56. Morris JZ, Tissenbaum HA, Ruvkun G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. Nature 1996; 382: 536–539. PMID: 8700226

57. Paradis S, Ruvkun G. Caenorhabditis elegans Akt/PKB transduces insulin-receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev. 1998, 12: 2488–2498. PMID: 9716402

58. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. Science 2003; 299: 1346–1351. PMID: 12610294

59. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science 2001; 292: 107–110. PMID: 11292875

60. Hamilton DE, Archer JR. Genetic differences in effects of food restriction on aging in mice. J. Nutr. 1987; 117: 376–382. PMID: 3559752

61. Lakowski B, Hekimi S. The genetics of caloric restriction in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 1998; 95: 13091–13096. PMID: 9789046

62. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, et al. Calorie restriction extends longevity and diapause in Caenorhabditis elegans. Science 1997; 277: 942–946. PMID: 9252323

63. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, et al. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 1998; 394: 694–697. PMID: 9716135

64. Kharade SV, Mittal N, Das SP, Sinha P, Roy N. Mrg19 depletion increases S. cerevisiae lifespan by augmenting ROS defence. FEBS Lett. 2005; 579: 6809–6813. PMID: 16336970

65. Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL. Extension of Drosophila longevity and diapause by overexpression of human SOD1 in motorneurons. Nat. Genet. 1998; 19: 171–174. PMID: 9620775

66. Sun J, Folk D, Bradley TJ, Tower J. Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult Drosophila melanogaster. Genetics 2002; 161: 661–672. PMID: 12072463

67. Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. Cost of mating in Drosophila melanogaster females is mediated by male accessory gland products. Nature 1995; 373: 241–244. PMID: 7816137

68. Fowler K, Partridge L. A cost of mating in female fruit flies. Nature 1989; 338: 760–761.

69. Friedman DB, Johnson TE. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics 1988; 118: 75–86. PMID: 3860934

70. Gems D, Riddle DL. Longevity in Caenorhabditis elegans reduced by mating but not gamete production. Nature 1996; 379: 723–725. PMID: 8602217

71. Mockett RJ, Sohal RS. Temperature-dependent trade-offs between longevity and fertility in the Drosophila mutant, methuselah. Exp. Gerontol. 2006; 41: 566–573. PMID: 16677788

72. Partridge L, Farquhar M. Sexual activity reduces lifespan of male fruitflies. Nature 1981; 294: 580–582.

73. Tissenbaum HA, Ruvkun G. An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans. Genetics 1998; 148: 703–717. PMID: 9504918

74. Westendorp RG, Kirkwood TB. Human longevity at the cost of reproductive success. Nature 1998; 396: 743–746. PMID: 9874369
78. Defossez PA, Lin SJ, McNabb DS. Sound silencing: the Sir2 protein and cellular senescence. Bioessays 2001; 23: 327–332. PMID: 11268038

79. Kim S, Benguría A, Lai CY, Jazwinski SM. Modulation of life-span by histone deacetylase genes in Saccharomyces cerevisiae. Mol. Biol. Cell 1999; 10: 3125–3136. PMID: 10512855

80. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 2001; 410: 227–230. PMID: 11242085

81. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science 1998; 279: 349–352. PMID: 9454332

82. de Boer J, Andressso JO, de Wit J, Huijmans J, Beems RB, van Steeg H, et al. Premature aging in mice deficient in DNA repair and transcription. Science 2002; 296: 1276–1279. PMID: 11950998

83. Woodhead AD, Mery BJ, Cao EH, Holehan AM, Grist E, Carlson C. Levels of O6-methylguanine acceptor protein in tissues of rats and their relationship to carcinogenicity and aging. J. Natl. Cancer Inst. 1985; 75: 1141–1145. PMID: 3865014

84. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, et al. Positional cloning of the Werner's syndrome gene. Science 1996; 272: 258–262. PMID: 8602509

85. Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, et al. Rates of behavior and aging specified by mitochondrial function during development. Science 2002; 298: 2396–2401. PMID: 12471266

86. Lee SS, Lee RY, Fraser AG, Kamath RS, Ahniger J, Ruvkun G. A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. Nat Genet 2003; 33: 40–48. PMID: 12447374

87. Landis GN, Bhole D, Tower J. A search for doxycycline-dependent mutations that increase telomerase activity. Proc. Natl. Acad. Sci. USA 1995; 92: 7540–7544. PMID: 7638227

88. Khazaeli AA, Tatar M, Pletcher SD, Curtsinger JW. Heat-induced longevity extension in Drosophila. I. Heat treatment, mortality, and thermotolerance. J. Gerontol. A Biol. Sci. Med. Sci. 1997; 52: B48–52. PMID: 9008657

89. Service PM, Hutchinson EW, Rose MR. Multiple genetic mechanisms for the evolution of senescence in Drosophila melanogaster. Inst. 1999; 402: 804–809. PMID: 10617200

90. Rose MR, V, Park SU, Graves JL. Selection on stress resistance Increases longevity in Drosophila melanogaster. Exp. Gerontol. 1992; 27: 241–250. PMID: 1521597

91. Khazaeli AA, Tatar M, Pletcher SD, Curtsinger JW. Heat-induced longevity extension in Drosophila. I. Heat treatment, mortality, and thermotolerance. J. Gerontol. A Biol. Sci. Med. Sci. 1997; 52: B48–52. PMID: 9008657

92. Service PM, Hutchinson EW, Rose MR. Resistance to environmental stress in Drosophila melanogaster selected for postponed senescence. Physiol. Zool. 1985; 58: 380–389.

93. Service PM, Hutchinson EW, Rose MR. Multiple genetic mechanisms for the evolution of senescence in Drosophila melanogaster. Evolution 1988; 42: 708–716.

94. Apfeld J, Kenyon C. Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature 1999; 402: 804–809. PMID: 10617200

95. Libert S, Zwiener J, Chu X, Vanvoorhees W, Roman G, Pletcher SD. Regulation of Drosophila life span by olfaction and food-derived odors. Science 2007; 315: 3113–1137. PMID: 17272684

96. Felix TM, Hughes KA, Stone EA, Dmevich JM, Leips J. Age-specific variation in immune response in Drosophila melanogaster has a genetic basis. Genetics 2012; 91: 989–1002.

97. Horn L, Leips J, Starz-Gaiano M. Phagocytic ability declines with age in adult Drosophila hemocytes. Aging Cell 2014; 13: 719–728. doi: 10.1111/acel.12227 PMID: 24828474

98. Felix TM, Hughes KA, Stone EA, Dmevich JM, Leips J. Age-specific variation in immune response in Drosophila melanogaster has a genetic basis. Genetics 2012; 91: 989–1002.

99. Zerofsky M, Harel E, Silverman N, Tatar M. Aging of the innate immune response in Drosophila melanogaster. Aging Cell 2005; 4: 103–108. PMID: 15771614

100. Burke MK, Dunham JP, Shahrestani P, Thornton KR, Rose MR, Long AD. Genome-wide analysis of a long-term evolution experiment with Drosophila. Nature 2010; 467: 587–590. doi: 10.1038/nature09352 PMID: 20844486

101. Keightley PD, Bullfield G. Detection of quantitative trait loci from frequency changes of marker alleles under selection. Genet. Res. 1993; 62: 195–203. PMID: 8157717
103. Teotonio H, Chelo IM, Bradic M, Rose MR, Long AD. Experimental evolution reveals natural selection on standing genetic variation. Nat. Genet. 2009; 41: 251–257. doi: 10.1038/ng.289 PMID: 19136954

104. Remolina SC, Chang PL, Leips J, Nuzhdin SV, Hughes KA. Genomic basis of aging and life-history evolution in Drosophila melanogaster. Evolution 2012; 66: 3390–3403. doi: 10.1111/j.1558-5646.2012.01710.x PMID: 23106705

105. Schlotterer C, Kofler R, Versace E, Tobler R, Franssen SU. Combining experimental evolution with next-generation sequencing: a powerful tool to study adaptation from standing genetic variation. Heredity 2015; 114: 431–440. doi: 10.1038/hdy.2014.86 PMID: 25269380

106. Schlotterer C, Tobler R, Kofler R, Nolte V. Sequencing pools of individuals—mining genome-wide polymorphism data without big funding. Nat. Rev. Genet. 2014; 15: 749–763. doi: 10.1038/nrg3803 PMID: 25246196

107. Turner TL, Stewart AD, Fields AT, Rice WR, Tarone AM. Population-based resequencing of experimentally evolved populations reveals the genetic basis of body size variation in Drosophila melanogaster. PLoS Genet. 2011; 7: e1001336. doi: 10.1371/journal.pgen.1001336 PMID: 21437274

108. Luckinbill LS, Arking R, Clare MJ, Cirocco WC, Buck SA. Selection for delayed senescence in Drosophila melanogaster. Evolution 1984; 38: 996–1003.

109. Rose MR. Laboratory evolution of postponed senescence in Drosophila melanogaster. Evolution 1984; 38: 1004–1010.

110. Zwaan B, Bijlsma R, Hoekstra RF. Artificial selection for developmental time in Drosophila melanogaster in relation to the evolution of aging—Direct and correlated responses. Evolution 1995; 49: 635–648.

111. Service PM, Vossbrink RE. Genetic variation in "first" male effects on egg laying and remating by female Drosophila melanogaster. Behav. Genet. 1996; 26: 39–48. PMID: 8852730

112. SAS Institute Inc. SAS 9.2 Software. Cary, NC: SAS Institute Inc.; 2002–2008.

113. Benzer S. Behavioral mutants of Drosophila isolated by countercurrent distribution. Proc. Natl. Acad. Sci. USA 1967; 58: 1112–&. PMID: 16578662

114. Ja WW, Carvalho GB, Mak EM, de la Rosa NN, Fang AY, Liong JC, et al. Prandiology of Drosophila and the CAFE assay. Proc. Natl. Acad. Sci. USA 2007; 104: 8253–8256. PMID: 17494737

115. Morgan TJ, Mackay TFC. Quantitative trait loci for thermotolerance phenotypes in Drosophila melanogaster. Heredity 2006; 96: 232–242. PMID: 16404413

116. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009; 25: 1754–1760. doi: 10.1093/bioinformatics/btp324 PMID: 19451168

117. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 2011; 43: 491–498. doi: 10.1038/ng.806 PMID: 21478889

118. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 2012; 7: 562–578. doi: 10.1038/nprot.2012.016 PMID: 22383036

119. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010; 11: R106. doi: 10.1186/gb-2010-11-10-r106 PMID: 20979621

120. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 2011; 43: 491–498. doi: 10.1038/ng.806 PMID: 21478889

121. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBO Journal 2011; 17: 1: 10–12.

122. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 2009; 25: 1105–1111. doi: 10.1093/bioinformatics/btp120 PMID: 19289445

123. Benson DA, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2014; 42: D32–37. doi: 10.1093/nar/gkt1010 PMID: 24217914

124. Drysdale R, FlyBase C, FlyBase: a database for the Drosophila research community. Methods Mol. Biol. 2008; 420: 45–59. doi: 10.1007/978-1-59745-583-1_3 PMID: 18641940

125. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 2012; 7: 562–578. doi: 10.1038/nprot.2012.016 PMID: 22383036

126. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010; 11: R106. doi: 10.1186/gb-2010-11-10-r106 PMID: 20979621

127. Benjamini Y, Hochberg Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 1995; 57: 289–300.

128. Cunningham F, Amode MR, Barrell D, Beal K, Billis K, Brent S, et al. Ensembl 2015. Nucleic Acids Res. 2015; 43: D662–669. doi: 10.1093/nar/gku1010 PMID: 25352552

129. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 2009; 4: 44–57. doi: 10.1038/nprot.2008.211 PMID: 19131956

130. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009; 37: 1–13. doi: 10.1093/nar/gkn923 PMID: 19033963
128. Smith JM, Haigh J. The hitch-hiking effect of a favourable gene. Genet. Res. 1974; 23: 23–35. PMID: 4407212

129. Schmidt PS, Zhu CT, Das J, Batavia M, Yang L, Eanes WF. An amino acid polymorphism in the couch potato gene forms the basis for climatic adaptation in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 2008; 105: 16207–16211. doi: 10.1073/pnas.0805485105 PMID: 18852464

130. Wilson RH, Lai CQ, Lyman RF, Mackay TFC. Genomic response to selection for postponed senescence in Drosophila. Mech. Ageing Dev. 2013; 134: 79–88. doi: 10.1016/j.mad.2012.11.003 PMID: 23262286

131. Magwire MM, Yamamoto A, Carbong MA, Roshina NV, Symonenko AV, Pasyukova EG, et al. Quantitative and molecular genetic analyses of mutations increasing Drosophila life span. PLoS Genet. 2010; 6: e1001037. doi:10.1371/journal.pgen.1001037 PMID: 20686706

132. Ayroles JF, Carbone MA, Stone EA, Jordan KW, Lyman RF, Magwire MM, et al. Systems genetics of complex traits in Drosophila melanogaster. Nat. Genet. 2009; 41: 299–307. doi:10.1038/ng.332 PMID: 19234471

133. Jin W, Riley RM, Wolfinger RD, White KP, Passador-Gurgel G, Gibson G. The contributions of sex, genotype and age to transcriptional variance in Drosophila melanogaster. Nat. Genet. 2001; 29: 389–395. PMID:11726925

134. Rockman MV. Reverse engineering the genotype-phenotype map with natural genetic variation. Nature 2008; 456: 738–744. doi: 10.1038/nature07633 PMID: 19079051

135. Sieberts SK, Schadt EE. Moving toward a system genetics view of disease. Mamm. Genome 2007; 18: 389–401. PMID: 17653589

136. Okamoto N, Yamanaka N, Yagi Y, Nishida Y, Kataoka H, O'Connor MB, et al. A fat body-derived IGF-like peptide regulates postfeeding growth in Drosophila. PLoS Genet. 2011; 7: e1002315. doi: 10.1371/journal.pgen.1002315 PMID: 22022278

137. Slaidina M, Delanoue R, Gronke S, Partridge L, Leopold P. A Drosophila insulin-like peptide promotes growth during nonfeeding states. Dev. Cell 2009; 17: 874–894. doi:10.1016/j.devcel.2009.10.009 PMID: 2005956

138. Sidyelyeva G, Baker NE, Fricker LD. Characterization of the molecular basis of the Drosophila mutations in carboxypeptidase D. Effect on enzyme activity and expression. J. Biol. Chem. 2006; 281: 13844–13852. PMID: 16556608

139. Sidyelyeva G, Wegener C, Schoenfeld BP, Bell AJ, Baker NE, McBride SM, et al. Individual carboxypeptidase D domains have both redundant and unique functions in Drosophila development and behavior. Cell Mol. Life Sci. 2010; 67: 2991–3004. doi: 10.1007/s00018-010-0369-8 PMID: 20386952
149. Stroschein-Stevenson SL, Foley E, O'Farrell PH, Johnson AD. Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol. 2006; 4: e4. PMID: 16336044

150. Gundner AL, Hahn I, Sendscheid O, Aberle H, Hoch M. The PIKE homolog Centaurin gamma regulates developmental timing in Drosophila. PLoS One 2014; 9: e97332. doi: 10.1371/journal.pone.0097332 PMID: 24845618

151. Kyistinen P, Samakovlis C, Hultmark D. The cecropin locus in Drosophila; a compact gene cluster involved in the response to infection. EMBO J 1990; 9: 217–224. PMID: 10942760

152. Samakovlis C, Kimbrell DA, Kyistinen P, Engstrom A, Hultmark D. The immune response in Drosophila: pattern of cecropin expression and biological activity. EMBO J 1990; 9: 2969–2976. PMID: 23907977

153. Verleyen P, Baggerman G, D'Hertog W, Vierstraete E, Husson SJ, Schoofs L. Identification of new immune induced molecules in the haemolymph of Drosophila melanogaster by 2D-nanoLC MS/MS. J Insect Physiol 2006; 52: 379–388. PMID: 16510152

154. Cronin SJ, Nehme NT, Limmer S, Liegeois S, Pospisilik JA, Schramek D, et al. Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. Science 2009; 325: 340–343. doi:10.1126/science.1173164 PMID: 19520911

155. Ghiiglione C, Devergne O, Cerezo D, Noselli S. Drosophila RalA is essential for the maintenance of Jak/Stat signalling in ovarian follicles. EMBO Rep. 2008; 9: 676–682. doi:10.1038/embor.2008.79 PMID: 18552769

156. Matakatsu H, Blair SS. Interactions between Fat and Dachsous and the regulation of planar cell polarity in the Drosophila wing. Development 2004; 131: 3785–3794. PMID: 15240556

157. Matakatsu H, Blair SS. Separating the adhesive and signaling functions of the Fat and Dachsous protocadherins. Development 2006; 133: 2315–2324. PMID: 16687445

158. Feng Y, Irvine KD. Fat and Expanded act in parallel to regulate growth through Warts. Proc. Natl. Acad. Sci. USA 2007; 104: 20362–20367. PMID: 18077345

159. Issman-Zecharya N, Schuldiner O. The PI3K class III complex promotes axon pruning by downregulating a Ptc-derived signal via endosome-lysosomal degradation. Dev. Cell 2014; 31: 461–473. doi:10.1016/j.devcel.2014.10.013 PMID: 25458013

160. Massouras A, Waszak SM, Albarca-Aguilera M, Hens K, Holcombe W, Ayroles JF, et al. Genomic variation and its impact on gene expression in Drosophila melanogaster. PLoS Genet. 2012; 8: e1003055. doi: 10.1371/journal.pgen.1003055 PMID: 23189034