[Fly 3:1, 29-38; January/February/March 2009]; ©2009 Landes Bioscience

Review

Dissecting the genetics of longevity in *Drosophila melanogaster*

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Abbreviations: IIS, insulin/insulin-like signaling; JNK, jun-N-terminal kinase; QTL, quantitative trait locus; RIL, recombinant inbred line; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; TOR, target of rapamycin

Key words: lifespan, longevity, aging genes, mutation screen, QTL, genetic variance, natural populations, evolution

***Drosophila melanogaster* has been an historically important system for investigating the genetic basis of longevity, and will continue to be valuable as new technologies permit genomic explorations into the biology of aging. The utility of *D. melanogaster* resides in two resources: its powerful genetic tools as a model system, and a natural ecology that provides substantial genetic variation across significant environmental heterogeneity. Here we provide a review of the genetics of longevity in *D. melanogaster*, in which we describe the characterization of individual aging genes, the complexity of the genetic architecture of this quantitative trait, and the evaluation of natural genetic variation in the evolution of life histories.**

Introduction

Organism longevity is a quantitative trait determined by both environmental and genetic components. *Drosophila melanogaster* has proved one of the most useful model systems for exploring the genetic determination of lifespan, both by identification of candidate aging genes by classical genetics approaches, and by characterization of the contribution of natural genetic variation to longevity phenotypes, artificial selection responses, and natural selection responses. The laboratory lifespan of *D. melanogaster* is on the order of eight weeks, and is highly responsive to manipulations like induced mutations or artificial selection regimes—long-lived strains can show twice the lifespan of short-lived strains. But despite the tractability of this system to experimentation, a few critical questions regarding the genetics of aging remain largely unanswered. Is the genetic determination of longevity principally governed by many genes of small effect, or by a few genes of large effect? And are the aging genes discovered by mutational analyses the same genes that contribute to differences in longevity phenotypes in natural populations?

Although these questions persist, work in *D. melanogaster* has led to substantial advances in our understanding of the biology of aging. Identification of the same candidate aging genes by independent approaches demonstrates the efficacy of these methods and suggests that comprehensive characterization of the most important genetic determinants is possible. Furthermore, the rapid development of genomic techniques will facilitate exploration of the complex genetic architecture of lifespan, which, as a highly quantitative trait, can only be fully understood on a genome-wide scale. Identification of specific aging genes has also permitted evaluation of how these loci contribute to the observed genetic variance for lifespan in natural populations. Comparison of the subset of genes shown to affect natural variation in lifespan to the subset of genes shown to extend lifespan by genetic manipulation will yield critical insights into the utility of these different approaches to characterize the genetics of lifespan. For example, a gene that extends lifespan by laboratory induced mutation might regulate aging, but not necessarily contribute to observed differences in lifespan in the wild: such a gene may be under strong natural selection constraint and vary little at the nucleotide level; it may harbor substantial allelic variation that does not affect the phenotype and is segregating neutrally; or, alternately, it may harbor functionally significant allelic variation that responds to natural selection. Characterizing the natural genetic variation that affects lifespan can reveal subtleties in the mechanisms of lifespan mediation, and will help us better understand how mutational analyses identify and describe genetic determination of quantitative traits.

In addition to dissecting the genetics of longevity, *D. melanogaster* has been invaluable in exploring the physiology of aging.[1-4] This research, along with characterization of the types and rates of senescence and the importance of genes and gene classes in hypothesized mechanisms of aging regulation, is beyond the scope of this review. Instead, we describe the identification of specific aging genes in *D. melanogaster* that show general effects on organismal longevity, or lifespan, and discuss the complexity of the genetic architecture of lifespan as it affects genetics research and the evolution of longevity phenotypes. We also describe the specific cases where allelic variation has been shown to affect lifespan, and provide a hypothetical framework for the maintenance of longevity phenotypes by natural selection in the wild.

Identification of Aging Genes

Identification of specific genes that regulate lifespan in *D. melanogaster* has been achieved by two processes: mutational analysis,[5] in which manipulation of gene or pathway function has demonstrated lifespan extension, and quantitative trait locus (QTL) analysis,[6] in

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Submitted: 12/11/08; Revised: 01/02/09; Accepted: 01/06/09

Previously published online as a *Fly* E-publication:
http://www.landesbioscience.com/journals/fly/article/7771

Table 1 **Characterization of genes in *D. melanogaster* that extend lifespan when gene activity is decreased**

Gene	Characterization	Reference
chico	Females homozygous for $chico^{1}$ show a median lifespan increase of 43%; heterozygous females and males show median lifespan increases of 13% and 36%, respectively; homozygous males do not live longer, but age at a slower demographic rate	4, 23
dilp genes	Ablation of cells producing *dilp2, dilp3* and *dilp5* increases median lifespan by 10.5% in males, 18.5% in virgin females and 33.5% in mated females	26
dS6K	Inhibition of *TOR* pathway signaling by ubiquitous overexpression with the *da*-Gal4 driver of the dominant-negative UAS-$dS6K^{STDETE}$ produces a mean lifespan increase of 12–34%	30
dTOR	Inhibition of *TOR* pathway signaling by ubiquitous overexpression with the *da*-Gal4 driver of the dominant-negative UAS-$dTOR^{FRB}$ produces a mean lifespan increase of 24–26%	30
DTS-3	Heterozygous *DTS-3* females, but not males, show a mean lifespan increase of 42% at 29°C; females show increases at lower temperatures if also exposed to higher temperature early in life	38
EcR	Males and females heterozygous for multiple alleles disrupting the *EcR* locus show lifespan increases of up to 50%	38
Indy	Enhancer trap lines heterozygous for P-element insertions at *Indy* show a doubling of mean lifespan in males and females, but these lifespan extensions are likely artifacts of genetic background and *Wolbachia* infection	40, 41
InR	Heteroallelic, hypomorphic InR^{p5545}/InR^{E19} females show a lifespan extension of 85%; after survival to day 10, males show a lifespan extension of 43%	22
mth	Males and females homozygous for a P-element insertion at *mth* show an average lifespan increase of 35%; UAS/Gal4-mediated expression of an antagonist peptide extended mean lifespan by 38% at 29°C	39, 134
ovo	Females heterozygous for ovo^{D1} show significant lifespan extension; extension is greater in lines selected for short life than lines selected for long life	28
puc	Flies heterozygous for loss-of-function alleles $puc^{A251.1}$ or puc^{E69} show extensions of median and maximum lifespan	32
rpd3	Males heterozygous for hypomorphic $rpd3^{P\text{-}UTR}$ or null $rpd3^{def24}$ alleles show lifespan extensions of 33% or 41–47%, respectively; heterozygous $rpd3^{P\text{-}UTR}$ females show extensions of 52%	34
sun	Females heterozygous for sun^{EM67} or sun^{Y6} mutations show increases in average lifespan of 25–51%; *sun* resides on the X chromosome and mutations in males are lethal	42

which genic elements affecting natural variation in longevity have been mapped to specific positions along the chromosomes. Tables 1 and 2 summarize the characterization of specific genes shown to extend lifespan in *D. melanogaster* by decreased or increased gene activity, respectively, and Table 3 summarizes the characterization of genes for which allelic variation is associated with variation in longevity.

Mutational analysis. Genes involved in stress response**.** The association between stress and lifespan has motivated the identification of many aging genes. The hypothesis that reactive oxygen species (ROS) cause aging[7] led to tests for lifespan extension by increasing activity of genes that promote antioxidant defenses. Overexpression of both *Catalase* (*Cat*) and *Superoxide dismutase* (*SOD*) has demonstrated increased organismal longevity,[8,9] although these effects are highly dependent upon the sex and genetic background of the strains being tested.[8,10,11] The enzyme peptide methionine sulfoxide reductase A counteracts oxidative damage by catalyzing the repair of oxidized methionine, and overexpression of *msrA* in the nervous system can extend lifespan.[12] Enhancement of the redox process by overexpression of *glucose-6-phosphate dehydrogenase* (*G6PD*) also increases lifespan.[13] Accumulation of isoaspartyl residues in cellular proteins is a degenerative process that affects protein function. Carboxyl methyltransferase (*Pcmt*) counteracts this aging process by modifying isoaspartyl residues, and ubiquitous overexpression of this protein can extend lifespan at elevated temperatures.[14] A screen for genes that show differences in gene expression between normal and stress conditions identified several loci that had already been shown to affect lifespan, as well as two additional candidates, heat-shock protein (hsp) genes *hsp26* and *hsp27*; independent overexpression of both genes extends lifespan.[15] Tests on other molecular chaperones that are induced in response to stress have shown that increasing copy numbers of *hsp70* reduces the mortality rate after a non-lethal induction of stress,[16] and that overexpression of *hsp22*, either ubiquitously or in motor neurons, also extends lifespan.[17] Similarly, an extra copy of *meiotic-41* (*mei-41*), which may repair DNA damaged by oxidative stress, increases longevity.[18]

Genes involved in insulin signaling**.** Examination of members in the insulin/insulin-like signaling (IIS) pathway has identified a suite of genes that can extend lifespan by reduction of insulin signaling.[19] The role of IIS in nutrient sensing, metabolism and determination of body size, processes which may regulate the well-characterized lifespan extensions by dietary restriction,[20] and the identification of the nematode insulin receptor homolog *daf-2* as an aging gene,[21] led to tests for lifespan extensions via this pathway in *D. melanogaster*. Independent disruption of the *Insulin-like Receptor* (*InR*) or the *InR* substrate, *chico*, or overexpresion of *dFOXO*, a downstream transcription factor, or *PTEN*, which promotes nuclear localization of endogenous dFOXO, all reduce insulin signaling and extend lifespan.[22-25] Ablation of cells that produce insulin-like peptides (encoded by *dilp* genes) also increases longevity.[26]

Genes in other pathways**.** Other potential aging genes have been evaluated based on their role in hypothesized mechanisms of aging, including members of a number of pathways that appear to interact with IIS. The elongation factor *EF-1*α is required for protein synthesis, and reduction of EF-1α is associated with senescence; ubiquitous overexpression of *EF-1*α can extend lifespan.[27] The tradeoff between longevity and reproduction prompted evaluation of *ovo*, the disruption

Table 2 **Characterization of genes in *D. melanogaster* that extend lifespan when gene activity is increased**

Gene	Characterization	Reference
Cat	Transgenic flies with P-element insertions contributing one extra copy of *Cat* and one extra copy of *SOD* show median lifespan increases of 6–33%	8
Cctl	Conditional overexpression via the DOX-dependent P{PdL} system produces an average lifespan increase of 7%	43
dFOXO	Conditional overexpression of *dFOXO* in the adult fat body via the mifepristone inducible-Gal4 system and via induction of UAS-*dFOXO* by P{Switch}S1106 increases median lifespan in both sexes by 35–56% and in females by 22–52%, respectively, but demographic error may account for lifespan extension via the UAS-dFOXO by P{Switch}S1106 system	24, 25, 135
DPOSH	Neural-specific overexpression of *DPOSH* extends mean lifespan by 14% at 25°C	33
dTsc1	Inhibition of *TOR* pathway signaling by ubiquitous overexpression with the *da*-Gal4 driver of a UAS construct containing *dTsc1* extends mean lifespan by 14%	30
dTsc2	Inhibition of *TOR* pathway signaling by ubiquitous overexpression with the *da*-Gal4 driver of a UAS construct containing *dTsc2* extends mean lifespan by 12%	30
EF-1α	Ubiquitous overexpression of *EF-1α* extends male lifespan by 18% at 25°C and 41% at 29.5°C; females were not tested	27
filamin	Conditional overexpression via the DOX-dependent P{PdL} system produces an average lifespan increase of 8.5%	43
fwd	Conditional overexpression via the DOX-dependent P{PdL} system produces an average lifespan increase of 8%	43
G6PD	Overexpression of *G6PD* via the UAS-Gal4 system increases mean lifespan by 16–38% among multiple driver and responder genotypes in males and females	13
hebe	Conditional overexpression via the DOX-dependent P{PdL} system increases lifespan in males and females; multiple strains show median lifespan increases of 2.2–31%	44
hep	Constitutive overexpression of *hep* in neuronal tissue via the UAS-Gal4 system extends lifespan in males; females were not assayed	32
hsp22	Overexpression using the UAS-Gal4 system, either ubiquitously or in motor neurons, increases mean lifespan by approximately 32%	17
hsp26	Overexpression using the UAS-Gal4 system increases mean lifespan by 30–31%	15
hsp27	Overexpression using the UAS-Gal4 system increases mean lifespan by 27–31%	15
hsp68	Constitutive overexpression of *hsp68* via the UAS-Gal4 system extends lifespan in males; females were not assayed	32
hsp70	Expression of *hsp70* 10–15% above normal reduces the mortality rate two weeks after non-lethal heat stress, but does not necessarily extend total lifespan	16, 136
magu	Conditional overexpression via the DOX-dependent P{PdL} system increases lifespan in males and females; multiple strains show median lifespan increases of 2.2–18%	44
mei-41	Transgenic flies with one extra copy (but not two extra copies) of wild-type *mei-41* conferred by a P-element transposon show an increase in lifespan	18
msrA	Overexpression of *msrA* in the nervous system using the UAS-Gal4 system produces a median lifespan increase of 70%	12
Pcmt	Ubiquitous overexpression of *Pcmt* via the UAS-Gal4 system extends average lifespan by 32–39% at 29°C, but not at 25°C	14
PTEN	Overexpression of *dPTEN* in the adult fat body via UAS-*dPTEN* induction by the P{Switch} strain S132 increases lifespan by 19.6% in females and 17.4% in males	24
Sir2	Ubiquitous overexpression of multiple *dSir2* constructs via the UAS-Gal4 system increases lifespan by 18–57%; neuronal overexpression increases lifespan by 20–52%	35
SOD	Overexpression of *SOD* by multiple gene copies or by expression of human *SOD1* in adult motor neurons increases mean adult lifespan up to 40%, but lifespan extension by *SOD* is highly dependent upon sex and genetic background	8–11, 137
Sug	Conditional overexpression via the DOX-dependent P{PdL} system produces an average lifespan increase of 6%	43
VhaSFD	Conditional overexpression via the DOX-dependent P{PdL} system produces an average lifespan increase of 8%	43

of which confers female sterility and lifespan extension.[28] The target of rapamycin (TOR) pathway interacts with IIS and also regulates body size,[29] and inhibition of TOR signaling by single gene manipulation also extends lifespan, including by overexpression of *dTsc1* or *dTsc2* or by expression of dominant-negative forms of *dTOR* or *dS6K*.[30] The Jun-N-terminal Kinase (JNK) pathway, which is activated in response to stress, antagonizes IIS and causes nuclear localization of dFOXO.[31] Extension of lifespan by increased JNK signaling, which is dependent upon *dFOXO*,[31] has been demonstrated independently by overexpression of *hemipterous* (*hep*), a JNK kinase, by overexpression of *hsp68*, which is induced by JNK signaling, and by disruption of *puckered* (*puc*), a JNK phosphatase.[32] Overexpression of *Drosophila Plenty of SH3s* (*DPOSH*) activates *puc* and also extends lifespan.[33]

Lifespan extension has been demonstrated by two other genes that may operate through mechanisms related to IIS and dietary restriction, *rpd3* and *Sir2*. Reduction in levels of the histone deacytlase Rpd3 extends lifespan, but not under dietary restriction, suggesting a common mechanism between these two processes.[34] Reduction of *rpd3* expression also increases RNA levels of the histone deacetylase Sir2,[34] the direct overexpression of which also extends lifespan.[35] The activity of these histone deacytlases are further linked together and to IIS: a decrease in Sir2 levels prevents lifespan extension by either *rpd3* or caloric restriction,[35] lifespan extension in *C. elegans* by *sir-2.1* requires the ortholog to *dFOXO*, *daf-16*, and the mammalian ortholog of *Sir2* regulates the activity of *FOXO* family members.[36] IIS is also a likely regulator of the synthesis of secondary hormones

Table 3 **Characterization of genes in *D. melanogaster* for which natural allelic variation is associated with variation in longevity**

Gene	Characterization	Reference
Catsup	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *Catsup*; complementation tests using $Catsup^{1}$, $Catsup^{cs1}$ and $Catsup^{cs2}$ mutant alleles demonstrate that allelic differences at *Catsup* affect variation in male lifespan; naturally-occurring polymorphisms at *Catsup* demonstrate association with variation in longevity	6, 61
Ddc	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *Ddc*; three natural polymorphisms segregating at *Ddc* account for 15.5% of the genetic contribution to lifespan from chromosome 2	47, 54, 59
Dox-A2	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *DOX-A2*; complementation tests using $Dox\text{-}A2^{1}$, $Dox\text{-}A2^{2}$ and $Dox\text{-}A2^{mfs1}$ mutant alleles demonstrate that allelic differences at *Dox-A2* affect variation in male lifespan	6
Lim3	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *Lim3*; complementation tests using $Lim3^{1}$ and $Lim3^{2}$ mutant alleles demonstrate that allelic differences at *Lim3* affect variation in male lifespan	6
ms(2)35Ci	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *ms(2)35Ci*; a complementation test using the $ms(2)35Ci^{02316}$ mutant allele demonstrates that allelic differences at *ms(2)35Ci* affect variation in male lifespan	47, 62
stc	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *stc*; complementation tests using stc^{6} and stc^{11112} mutant alleles demonstrate that allelic differences at *stc* affect sex- and allele-specific variation in lifespan	47, 62
tup (isl, l(2)37Aa)	Deficiency complementation tests between strains *Ore* and *2b* identify a lifespan QTL containing *tup*; a complementation test using the $tup^{isl\text{-}1}$ mutant allele demonstrates that allelic differences at *tup* affect variation in male lifespan	6

like juvenile hormone and ecdysone, which are well-evidenced determinants of insect life history.[37] Mutations that disrupt the ecdysone receptor, *EcR*, and *DTS-3*, which is involved in ecdysone biosynthesis, also extend lifespan.[38]

Genes in uncharacterized pathways. Mutation screens for longevity genes have identified a handful of candidates not otherwise characterized as members of known pathways that mediate lifespan. P-element insertion screens have shown that disruption of the G-protein coupled receptor *methuselah* (*mth*) and the Krebs cycle cotransporter *I'm not dead yet* (*Indy*) extend lifespan,[39,40] although the effect at *Indy* appears to be an artifact of genetic background and Wolbacia infection, not activity at the gene itself.[41] Mutations at *stunted* (*sun*), which encodes endogenous peptide ligands of Mth, have also been shown to increase lifespan.[42] Generation of a P-type transposable element with a doxycycline-inducible promoter has permitted, in genome-wide screens, the identification of genes that extend lifespan by overexpression; aging genes identified by this method include *CTP:phosphocholine cytidylyltransferase-I* (*Cctl*), *filamin*, *four wheel drive* (*fwd*), *Sugar baby* (*Sug*), *VhaSFD*,[43] *hebe* and *magu*.[44] Forced misexpression via a genome-wide P-element gene search has also identified 23 genic elements, out of a total of 646 inserts, that are correlated with relatively longer lifespan;[45] this method may also prove useful in the characterization of specific genes that mediate longevity.

QTL analysis. QTL mapping has identified a substantial number of specific genes that affect longevity, and even more genic regions that contain unexamined candidate loci (reviewed in ref. 6). This approach identifies aging loci by localizing differences in longevity between natural strains to chromosomal regions, either flanked by known molecular markers (recombination mapping),[46] or uncovered by deficiency chromosomes containing genomic deletions (deficiency mapping).[47] For example, QTLs have been identified in recombinant inbred lines (RILs) derived from laboratory strains selected for different lifespans, including redundant identification of the same regions by either recombination or selection mapping;[48-50] in RILs derived from recently collected wild strains;[51,52] and by deficiency mapping to localized chromosomal regions.[47,53-55] Complementation tests, using chromosomal deficiencies spanning aging genes already identified by mutational analysis, have demonstrated the efficacy of deficiency mapping in identifying longevity QTL.[56] RILs derived from *Oregon* (*Ore*) and *2b* strains have also been used to identify dozens of lifespan QTL via recombination mapping.[53,57-60] Between these same strains, deficiency complementation mapping has uncovered more than a dozen QTL affecting lifespan.[47,53,54] While this approach yields much higher resolution, the average deficiency QTL still contains about 50 genes.[6] However, for genes within these QTL for which mutant alleles are available, gene-specific complementation tests have identified seven genes that contribute to longevity variation between *Ore* and *2b*: *Catsup*,[61] *Dopa decarboxylase* (*Ddc*),[54] *Diphenol oxidase A2* (*Dox-A2*), *Lim3*,[6] *ms(2)35Ci*, *shuttle craft* (*stc*)[62] and *tailup* (*tup*).[6]

Like many insects, *D. melanogaster* is capable of expressing a form of diapause, a neuroendocrine-mediated physiological syndrome that results in reproductive quiescence and organismal persistence over long periods of suboptimal conditions.[63] Diapause expression extends lifespan and delays senescence,[64] but the propensity to express diapause is variable within and among *D. melanogaster* populations, and diapause genotype, independent of diapause expression, is associated with natural variation in longevity.[65] QTL and complementation mapping identified *couch potato* (*cpo*) as a major determinant of diapause, and consequently as a likely candidate for determining lifespan phenotypes in natural populations.[66] Variation in diapause expression has also been linked to allelic variation at both *timeless* (*tim*), a light-dependent component of the circadian

clock,[67] and the insulin-regulated phosphatidylinositol 3-kinase (PI3-kinase) gene *Dp110*.[68] Diapause in the house mosquito *Culex pipiens* is also regulated by *Dp110*,[69] and the homolog of *Dp110* in *C. elegans*, *age-1*, is well established as a gene that affects aging and dauer formation in worms.[70] This suggests that insect diapause and the dauer phenotype in *C. elegans* may be in part regulated by homologous pathways,[71] and while direct mutational and functional tests of diapause genes on longevity phenotype are lacking, such analyses have the potential to confirm *tim*, *cpo* and *Dp110* as aging genes in Drosophila.

Complexity of Genetic Architecture

Epistasis and background effects. The characterization of single genes that affect longevity has been invaluable in identifying underlying pathways and mechanisms. However, because lifespan is a highly quantitative trait, its genetics can only be characterized comprehensively on a genome-wide level. Aging genes span many functional classes and implicate diverse biochemical and physiological processes in the mediation of longevity; future identification of aging genes will broaden these lists, and it is likely that a substantial fraction of the genome participates in the regulation of lifespan.[6] Screens that have evaluated genome-wide patterns of gene expression have also identified a wide range of gene classes that are differentially expressed between young and old ages, including several specific loci that have already been shown to mediate lifespan;[72-74] these approaches should prove useful in the continued identification of aging genes and the characterization of genetic interactions. The broad range of loci, pathways and processes that affect longevity means that investigation of any subset of these mechanisms will always be influenced by other factors, and the importance of background effects has been demonstrated by every approach used to explore lifespan. In addition to genetic background (the interaction of any one gene with the rest of the genome, or epistasis), these effects also include sex, age and environment. Consequently, the complexity of genetic determination of lifespan is not only compounded by epsistatic interactions among genes, but also by interactions between those genes and the sex and age of the organism, and between genes and environmental conditions like diet quality and temperature. This complexity can frustrate the identification and characterization of genic elements that affect longevity, and make comparisons between experimental findings difficult.

The importance of genetic background in the expression of lifespan phenotypes is not surprising given the quantitative nature of the trait, and its relevance has been well-demonstrated in the specific cases for which it has been tested. The original characterization of *Indy* as an aging gene showed that the robust lifespan extension in short-lived strains is weaker in strains selected for long life;[40] lifespan extension by *ovo* is also shorter in lines selected for long life compared to lines selected for short life;[28] and lifespan extension by overexpression of *SOD* was also shown to be dependent upon the laboratory strain used.[10] Additional work has further characterized longevity expression by *SOD* as highly dependent on genetic background and sex: overexpression of human *SOD* in the motorneurons in ten lines extends lifespan in some but not others, and these effects are different between males and females.[11] Evaluation of the functional significance of allelic variation at *mth* has also shown that the contribution of different alleles to longevity is significantly affected by epistasis and sex.[75] Epistatic interactions in the expression of longevity have also been demonstrated among lifespan QTL and among marker pairs within inbred lines, some of which show sensitivity to mating status.[6]

Individual aging genes identified by mutational[14,22-24,26,27,34,38] and QTL[47,53,57-59,62] analysis also routinely show effects that are differentiated by temperature and/or sex. For example, lifespan is extended by overexpression of *Pcmt* at 29°C, but not 25°C;[14] allelic variation at *ms(2)35Ci* affects male, but not female, longevity.[62] The cross-sex genetic correlation (r_{GS}) describes the degree to which one locus affects both sexes in a quantitative analysis; r_{GS} values range between -1 (if the same genes affect variation in longevity in males and females, but in opposite directions), 0 (if different genes affect variation in longevity between the sexes) and 1 (if the same genes affect the sexes in the same direction). Estimates of r_{GS} from virgin RILs derived from *Ore* and *2b* strains were approximately 0.2, indicating some, but not complete, consistency between the sexes; when these same lines were mated, and/or reared in stressful conditions, estimates of r_{GS}increased.[6] Age also appears to affect the expression of genes that mediate longevity: QTL that affect lifespan have also been shown to affect other traits in an age-dependent manner, including fecundity[59,60] and metabolic rate;[76] QTL that directly affect mortality also show dependence on organism age.[52] These results underscore the as yet uncovered complexity of interactions at loci that, in the majority of cases, have only been narrowly explored. Furthermore, the sensitivity of individual genes to background effects means that detection of candidate aging genes can be obscured or spurious, depending upon conditions: screening for lifespan effects in laboratory strains that have evolved high early fecundity and shortened lifespan may be biased towards the identification of genes that restore normal lifespan, or show disproportionate effects in those backgrounds.[77]

Correlations and pleiotropy. Just as the contribution of any one gene to the expression of longevity may be dependent upon other factors, the expression of longevity itself is complicated by pleiotropic effects of its determinants. Two major correlations underly the biology of aging: the negative correlation between lifespan and reproduction,[78,79] and the positive correlation between lifespan and stress tolerance.[80] While comprehensive characterization of these correlations is beyond the scope of this review, consistent demonstration of associations between these traits clearly implicates shared genetic and physiological mechanisms and provides a likely foundation for the evolution of longevity phenotypes. In fact, the ability to identify aging genes by extended longevity mutant phenotypes underscores the existence of antagonistic pleiotropy at these loci: gene disruption that extends life must also compromise fitness, or else functional copies would not persist in populations. Despite some evidence that lifespan and reproduction can be decoupled,[44,61,81] multiple analyses have revealed previously undetected tradeoffs under specific conditions.[82,83] Furthermore, the close association between stress tolerance and longevity has permitted the use of this trait as a proxy for long-lived phenotypes in studies that examine the genetic basis of lifespan.[15]

Artificial selection experiments have been instrumental in demonstrating genetic correlations between lifespan, reproduction and stress tolerance,[84-89] and single-gene manipulations have routinely demonstrated decreases in reproductive success and/or increases in stress tolerance with longevity extension.[22-24,39,42] Gene expression assays

have also demonstrated correlations between stress and longevity: genes associated with relatively longer lifespan in a misexpression screen were positively correlated with oxidative stress tolerance, and of those with known function, half are involved in stress resistance or redox balance;[45] of genes showing differential expression between young and old ages, about one third of those expressed at old ages also respond to oxidative stress.[74,90] Longevity QTL have also been mapped to the same regions as QTL for fertility and stress tolerance.[57,59,60] Pleiotropy at genic elements that mediate lifespan should exist so long as mechanisms are shared among traits. Parsing those mechanisms into discrete components that behave singularly may be possible in some cases, but identification of elements that induce shorter lifespan should only be possible under two conditions: (1) pleiotropy, where the element antagonistically affects another trait, and is maintained by positive selection for that trait; (2) neutrality, where the element does not affect fitness, and is never subject to natural selection. Given the persistence of many functional aging genes over historical time, the importance of pleiotropy is clear in the maintenance of genetically mediated longevity.

Variation in Natural Populations

Phenotypic variation. *D. melanogaster* shows significant variation in longevity within and among natural populations. Between populations, patterns of longevity correlate with latitude, and are possibly driven by differential selection imposed by variation between tropical and temperate climates. Isofemale lines derived from high latitude populations from the United States east coast show longer lifespan than lines from low latitude populations, and lines along this gradient show covariance between longevity and other life history traits, including incidence of reproductive diapause, triglyceride content, oocyte development, ovariole number and fecundity.[91] Recently derived lines from temperate European populations and tropical Central American and African populations also show differences in mean lifespan, and mean lifespan under different thermal environments.[92] Differences in longevity have also been observed between inbred lines recently derived from natural populations near Ankara, Turkey.[93] Longevity also varies significantly within populations: although lifespan varies predictably with geography in North America, diapause genotype explains the majority of the variation in longevity and other associated traits, including variation between individuals from the same population.[65] While longevity estimates from recently collected wild lines provide useful measures for experimentation in the laboratory, they do not necessarily represent actual age distributions in the wild. Mueller et al.[94] demonstrate a method for determining age-specific survival and mortality in natural populations by marking individuals sampled from the wild at unknown age and subsequently constructing life tables from recorded times-of-death. This technique has been used to describe the survival schedule of the medfly *Ceratitis capitata*, and could be of great utility in describing the age structures of natural populations of *D. melanogaster*.

Genetic variation. *D. melanogaster* exhibit robust genetic variance for lifespan, or evidence that nucleotide variation affects longevity phenotypes. For example, phenotype means for lifespan and other life history traits from inbred lines derived from North American populations show genetic variance for and genetic correlations among these traits.[91] QTL analyses and artificial selection regimes have also demonstrated that flies derived from the wild harbor allelic variation that affects lifespan: the QTL approach to the identification of aging genes, which has produced more candidates than can be easily tested,[6] is predicated upon alleles for longevity segregating in natural populations, and laboratory populations of *D. melanogaster* have shown robust responses to myriad artificial selection regimes affecting longevity, indicating the existence of sufficient additive genetic variance for these traits.[95] Artificial selection on multiple traits has demonstrated phenotypic responses in longevity, including selection on time of reproduction,[84,85,96-100] stress tolerance,[86,87] and response to diet quality.[101] While inconsistencies have been observed,[102-105] these studies clearly demonstrate that wild-derived lines harbor genetic variation at loci that affect aging, and that longevity tends to correlate negatively with reproduction and positively with stress and starvation tolerance. One study has performed direct artificial selection on lifespan in *D. melanogaster*,[106] and results support these same correlations between longevity, reproduction and stress tolerance.

Identification of aging genes by induced mutation, transgenics or QTL analysis has begun to improve our understanding of the number, type and magnitude of effect of genes that affect lifespan. However, these approaches do not test whether natural populations harbor segregating allelic variation, the substrate upon which natural selection acts, at these identified loci.[107] Evaluating these specific loci for natural allelic variation can test the importance of candidate aging genes in the evolution of longevity, and also reveal subtleties in the mechanisms of lifespan mediation that laboratory-induced mutations cannot. To date, natural allelic variation has been characterized at a handful of loci identified as aging genes. The G-protein coupled receptor *mth*, identified by mutational screen,[39] shows a cline in frequency of the most common haplotype across the latitudinal gradient of the U.S. east coast.[108] This haplotype is comprised of five SNPs across the coding region; no individual SNP shows this pattern across geography, but decay of linkage disequilibrium 5' and 3' of the *mth* locus[109] suggests that selection is acting on one or more unidentified polymorphisms within the gene. Allelic variation at *mth* is functionally significant: in a modified quantitative complementation scheme, wild-derived *mth* alleles showed significant differences in lifespan, fecundity and resistance to oxidative stress.[75] These results support the conclusion that some natural genetic variation at *mth* is adaptive, and current work is exploring sequence variation in UTRs in an effort to identify the functional polymorphism(s) within the locus.

Sequence polymorphism data are also available for genes in the insulin signaling pathway, one of which shows evidence of recent adaptive evolution. *InR* and *chico* were sequenced in wild lines from North America, and while both exhibit substantial polymorphism, only *InR* shows a significant pattern of allele frequency across latitude.[110] Specifically, an indel polymorphism in the first exon, which disrupts a region of glutamine-histidine repeats, shows significantly increasing and decreasing frequencies across latitude for the two most common alleles. Additional sequencing and polymorphism screening in Australia shows a nearly identical pattern on that continent.[110] These results suggest that this polymorphism is under direct selection across heterogeneous environments, and current work is testing the effect of this polymorphism on levels of insulin signaling and life history phenotypes. Although hypomorphic mutations at both *InR* and *chico* reduce insulin signaling and produce similar mutant

phenotypes,[22,23] only *InR* demonstrates an adaptive response at the nucleotide level in natural populations: analysis of wild-derived *chico* sequences show evidence of neutral evolution.[110] This suggests that while reduction of insulin signaling at almost any point in the pathway can extend lifespan,[19] different pathway members may be under different constraints. The downstream transcription factor *dFOXO* is an important component of the insulin signaling pathway: overexpression of *dFOXO* extends lifespan,[24] upregulation of *dFOXO* may be required for lifespan extension by other genes in the pathway, and the *C. elegans* ortholog *daf-16* is essential for lifespan extension by insulin signaling (reviewed in ref. 19). Natural variation at the human ortholog of *dFOXO*, *FOXO3A*, contributes significantly to differences in longevity in human populations,[111] and characterization of natural genetic variation at *daf-16* and other IIS pathway members in *C. elegans* is underway (Jovelin R and Phillips P, personal communication). Variation at this key transcription factor in other species is unknown, but future characterization and comparisons across taxa could yield insight into how a highly pleiotropic pathway can respond to natural selection. Specifically, evaluation of allelic variation at *dFOXO* in natural populations of *D. melanogaster* will deepen our understanding of how this pathway mediates longevity, especially since other pathway members have already been characterized in this system.

Transgenic experiments have shown that *Dp110*, another member of the insulin signaling pathway, affects the expression of reproductive diapause.[68] In *D. melanogaster*, diapause produces a significant extension in lifespan and is associated with genetic variance for longevity, fecundity, development time, lipid content and stress tolerance.[65,91,112,113] Two natural *Dp110* alleles which differentially affect diapause have been sequenced, though there is an absence of pronounced polymorphism between them: of 20 polymorphisms detected, none affect the amino acid sequence nor levels of RNA.[68] However, a single nucleotide polymorphism at *cpo* shows strong control over the ability of *D. melanogaster* to enter diapause.[66] The SNP shows a significant cline in frequency across latitude in the eastern U.S., suggesting a functional response to heterogeneous selection pressure. While neither of these genes have been demonstrated as aging genes in the laboratory, the association of *Dp110* with the insulin signaling pathway, a major mediator of lifespan, and the association of both genes with diapause, a process that directly mediates lifespan and is strongly genetically correlated with natural variation in longevity,[64,65] suggests that they may play an important role in determining lifespan phenotypes in wild populations.

Ddc was identified as a candidate gene for aging by QTL mapping and complementation tests, and linkage disequilibrium mapping shows that it is associated with natural variation for lifespan.[54] Sequence data from natural lines revealed high levels of polymorphism at the locus, including at the promoter, and high linkage disequilibrium between sites that is suggestive of balancing selection or a recent selective sweep. Frequency spectra of polymorphic sites also support balancing selection, with apparent selection on both long- and short-lived phenotypes. These data demonstrate that natural allelic variation is segregating at *Ddc*, and that specific polymorphisms within the gene are likely targets of selection in the wild. The gene *Catsup* is an aging gene that has pleiotropic effects on multiple traits, but individual polymorphisms within the locus show an absence of pleiotropy: identified from wild-derived *Catsup* alleles, the polymorphisms show independent effects on longevity, locomotor behavior and sensory bristle number.[61] In addition to identifying *Catsup* as a potentially important contributor to genetic variance for lifespan in the wild, these results also expose details in mechanisms of trait determination that may only be revealed by the subtle variations exhibited by natural mutations.

Evolution in natural populations. Two major theories have been proposed to explain the evolution of lifespan, and they have been extensively reviewed in the literature.[1,114-118] These theories are not mutually exclusive, and they rely on the assumption that the strength of natural selection decreases with organism age.[119,120] This decrease is evident even in populations which show no age-related decline in reproductive fitness or other traits: Medawar's imaginary population of non-senescing test tubes, for example, will experience weakest selection on the oldest age class. The weak selection is a function of age structure in the population, as the oldest age class will always be the smallest.[119]

The mutation accumulation theory posits that while mutations that deleteriously affect reproduction and survival will be quickly eliminated from a population if they are expressed early in life, similar mutations with late-life expression may escape natural selection.[119] Consequently, an accumulation of late-acting deleterious mutations may be responsible for the senescent phenotypes observed in most metazoans. Multiple predictions accompany this model (reviewed in ref. 116), including an increase in additive genetic variance for fitness traits with age and an effect by induced or accumulated mutations on patterns of longevity. *D. melanogaster* has been a major empirical system in testing these predictions. Increases in additive genetic variance at later ages has been demonstrated for several traits, including lifespan,[57,121,122] fecundity,[60,123,124] and male mating ability.[125,126] However, these results are not definitive. Other studies have failed to find increases in additive genetic variance with age,[127-129] or suggest that high estimates may be artifacts of statistical methodology.[116,124] Experiments that have induced mutations or permitted the accumulation of natural mutations in laboratory populations show some evidence of mutational variance in age-related traits,[130,131] but these results do not appear sufficient to fully explain the persistence of senescence. However, the characterization of many lifespan QTL that only show late age-of-onset effects provides strong evidence that the accumulation of late-acting mutations contributes to the evolution of senescence.[57]

The theory of antagonistic pleiotropy[120] differs from the theory of mutation accumulation in the expectation that late-acting, deleterious mutations may have beneficial, rather than neutral, effects early in life. For example, an allele that promotes senescence might experience positive selection if it produces a fitness benefit at early age, such as increased reproductive success. The identification of aging genes by mutational analysis supports the theory of antagonistic pleiotropy: functional, wild-type copies of aging genes would not persist in nature if null copies produce only putatively beneficial lifespan extensions. In fact, as previously discussed, most lifespan extension mutations produce additional costs to fitness. The observation of additive genetic variance for lifespan also supports the theory of antagonistic pleiotropy. Pleiotropic alleles that produce only beneficial or only deleterious effects should be fixed or purged in populations, reducing variation, but different alleles that affect fitness both positively and negatively may be maintained, preserving variation.[120] Even then, it is likely that variation in

selection pressure, across temporally or spatially heterogeneous environments, plays a role in the maintenance of this allelic variation at longevity loci. Many studies have contributed positive evidence for the theory of antagonistic pleiotropy, including genetic correlations among life history traits by artificial selection,[84-89] QTL analyses,[57,59,60] and characterization of wild lines.[65,112] However, characterization of the functional effects of allelic variation at individual loci and specific nucleotides within those loci can offer precise examples of how longevity genes evolve in natural populations.

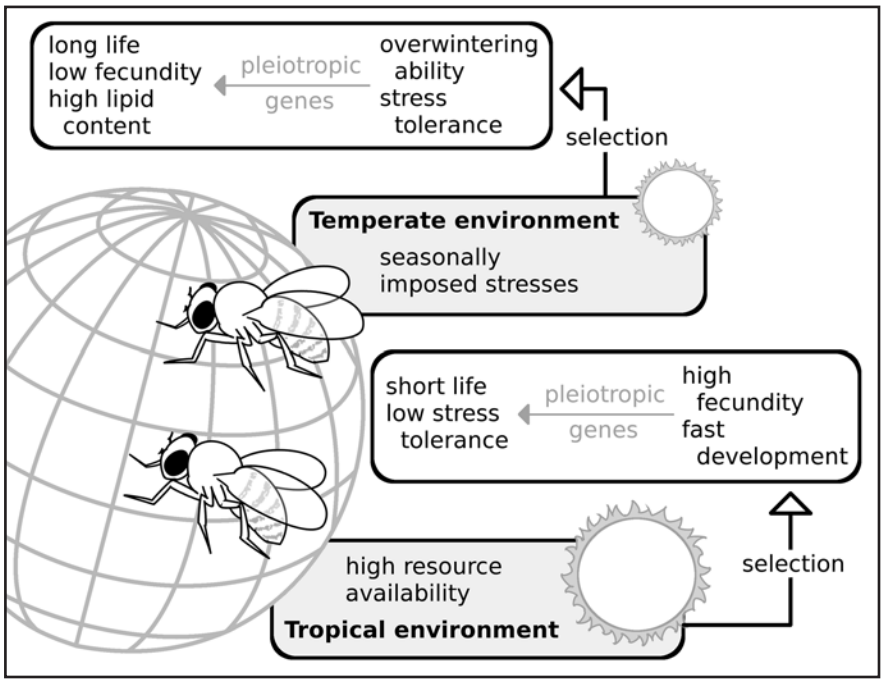

Figure 1. Hypothesized selection regime maintaining alternate life histories in *D. melanogaster*. Alleles that confer stress resistance are favored at high latitudes, where seasonal stress imposes strong selection; alternate alleles that confer high fecundity and faster development time are favored at low latitudes. Genetic correlations within this suite of life history traits, determined by pleiotropic loci, permit indirect evolution of lifespan, producing long-lived phenotypes at high latitudes and short-lived phenotypes at low latitudes.

Conclusion

The utility of *D. melanogaster* for investigating the genetic basis of longevity lies in its complementary resources: it offers both powerful genetic tools and a natural ecology that effectively provides a grand naturalistic experiment. *D. melanogaster* originated in tropical Africa and has colonized temperate regions, including the European, American and Australian continents, within the last several thousand years.[132] Surveys at multiple loci have revealed patterns of variation along latitudinal clines in which the frequencies of the derived alleles increase with latitude, suggesting adaptation to temperate habitats.[133] Moreover, the changing patterns of longevity, fecundity, stress resistance, development time and other life history variables exhibited by natural populations of *D. melanogaster* along such latitudinal gradients co-occur with hypothesized changes in environmental selection pressures.[112] A hypothetical selection regime, which imposes seasonal stresses at high latitudes, may favor stress resistant alleles in some environments and highly fecund alleles in others, indirectly driving the evolution of longevity and maintaining the distribution of lifespan phenotypes that we observe (Fig. 1). The identification of aging genes by mutational or QTL analysis offers an opportunity for fine-scale characterization of genetic variation for longevity using a gene-targeted approach. The demonstration of allelic patterns of variation at *mth*,[108] *InR*[110] and *cpo*[66] across heterogeneous environments shows how individual genes may contribute to the determination of these divergent life histories. Moreover, evaluation of how polymorphic alleles[75] or individual polymorphisms[61] affect phenotype can elucidate both how life histories evolve in natural populations, and how genotypes translate into phenotypes.

Acknowledgements

We thank one anyonymous reviewer for helpful comments on the manuscript.

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