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Editorial

Assessing natural variation in genes affecting *Drosophila* lifespan

Longevity is a complex QUANTITATIVE TRAIT (see Box 1) contributing to Darwinian fitness, and dissecting its genetic architecture is a fundamental problem in life history evolution, evolutionary genetics, and molecular gerontology. Quantitative genetic experiments indicate that lifespan is determined by many loci and that many populations harbor substantial amounts of additive and nonadditive GENETIC VARIATION for longevity, with HERITABILITIES between 10 and 30% (e.g. Tower, 1999; Mackay, 2002). Ultimately, to understand how evolution shapes senescence, the age-dependent functional decline of survival and reproduction, and how aging leads to the onset of late-life diseases such as Alzheimer, we need to track down the genes involved in aging. Although we still have an only limited understanding of the molecular mechanisms affecting longevity, the field has recently witnessed rapid progress in identifying candidate genes affecting aging in model organisms such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*.

For example, while the number of loci contributing to lifespan may be high, several individual loci with major effects on *Drosophila* longevity have been found. Transgenic manipulation experiments reveal that overexpression of genes such as *Cu/Zn Superoxide dismutase* (SOD), the heat shock protein *Hsp70*, and the scaffold protein *DPOSH* can extend the lifespan of flies (Tower, 2000; Aigaki et al., 2002). Similarly, mutations at loci such as *Insulin-like receptor* (*InR*), the insulin-receptor substrate *Chico*, the sodium dicarboxylate transporter *I'm not dead yet* (*Indy*), the putative G-protein coupled receptor *Methuselah* (*mth*), and the histone deacetylase *rpd 3* have been found to prolong adult lifespan in *Drosophila* (Helfand and Rogina, 2003). Yet, despite the rapidly expanding list of candidate genes for aging, molecular genetic analyses are not informative about whether standing genetic variation at these loci contributes to phenotypic variance for lifespan in natural populations.

While developmental geneticists typically focus on major effects of induced mutations or transgenes, evolutionary geneticists work on much more subtle phenotypic differences caused by standing natural genetic variation, the substrate on which evolutionary change by natural selection is based upon. Although it is becoming increasingly clear that both developmental and evolutionary geneticists have been studying qualitatively different forms of genetic variation at the same loci (Stern, 2000), it is still unclear whether this also holds for genes affecting lifespan. For example, not all candidate loci with major effects on longevity may exhibit segregating allelic variation in natural populations. Thus, while the major lifespan effects identified by molecular gerontology may be of biomedical interest, they may be of only limited relevance for our understanding of the evolution of aging in natural populations. Yet, as nicely illustrated by the work of Geiger-Thornsberry and Mackay published in this issue, the gap between the molecular and evolutionary genetics of aging is about to be closed, thanks to recent advances in quantitative genetics.

The major challenge for evolutionary quantitative genetics is to map QUANTITATIVE TRAIT LOCI (QTL), i.e. chromosomal regions containing one or several loci affecting a trait, to the level of a molecularly characterized gene. Indeed, at least 19 QTL affecting variation in longevity have been mapped in *Drosophila* by the group of Trudy Mackay (Mackay, 2002). Once those QTL have been mapped down to a genetic locus, LINKAGE DISEQUILIBRIUM MAPPING (LDM) can be used to determine the actual molecular POLYMORPHISMS that are responsible for the phenotypic variation. This would not be possible by conventional sequencing efforts. Sequencing candidate regions for polymorphisms between QTL strains is hindered by the fact that the *Drosophila* genome is extremely polymorphic. Thus, de facto *all* polymorphic sites within the candidate region, typically between 1 and 4 sites per kilobase, are associated with phenotypic differences between QTL strains, even if they do not functionally contribute to them (Mackay, 2002). However, before embarking on LDM, the most important task is to identify high-priority candidate genes, harboring functional, segregating genetic variation in natural populations. This is exactly what Geiger-Thornsberry and Mackay have now accomplished for *Drosophila* lifespan.

Using QUANTITATIVE COMPLEMENTATION TESTS (QCT), the authors have examined a total of sixteen candidate chromosomal regions and genes for longevity in inbreed strains derived from a natural population of fruit flies (see Table 1). Whereas fine-scale mapping QTL to the level of the gene is typically very difficult for most organisms, requiring dense maps of molecular markers and large sample sizes, *Drosophila* is amenable to COMPLEMENTATION mapping, using either DEFICIENCIES or NULL MUTATIONS of candidate

Box 1. Concepts in evolutionary quantitative genetics

This glossary explains some of the evolutionary quantitative genetic concepts and terms used in the text.

BALANCER: A chromosome containing several inversions and markers. Markers on the balancer chromosomes facilitate crossing schemes, while the inversions prevent recombination between homologs.

COMPLEMENTATION: A complementation test is a genetic test of allelism. A mutant allele of a candidate gene is placed in heterozygous state with another allele, and the genotype is then evaluated for a normal phenotype (complementation) or for an abnormal phenotype (failure of complementation). In the case of complementation, the two alleles belong to different genes: the effect of the mutant allele is 'complemented' (or 'compensated for') by a functional allele at the candidate locus. In the case of failure to complement, the two alleles are likely to belong to the same gene (allelism). The strong phenotypic effect of the mutant allele cannot be complemented by the presence of a functional allele at the same locus.

DEFICIENCY: A chromosomal rearrangement in which a piece of the chromosomal genome is missing. Sometimes also called a deletion.

GENETIC VARIATION: Phenotypic variation among individuals that can be attributed to genetic differences. Technically, genetic variation is measured as genetic variance, the variance of values for a phenotypic trait that is caused by genetic differences among individuals.

GERONTOGEN: A gene that affects lifespan is sometimes called a 'gerontogene'. Note, however, that genes that affect longevity are often pleiotropic, i.e. also affect other traits than lifespan. Thus, classifying genes as 'gerontogenes' is somewhat ambiguous.

HERITABILITY: The proportion of the phenotypic variance attributable to additive genetic variance, i.e. a measure of how much of the phenotypic variation among individuals is due to genetic differences among them.

HYPOMORPHIC: A hypomorph is a mutation which results in a reduction or decreased activity of a gene product.

LINKAGE DISEQULIBRIUM (LD): Linkage disequilibrium or gametic disequilibrium is the nonrandom statistical association of alleles at different loci into gametes. That is, under LD, some alleles of different genes are more likely to occur together than one would expect by chance. LD can be caused by several processes, for example, if loci are tightly physically linked, i.e. closely together on the same chromosome, or if selection favors a particular combination of alleles.

LINKAGE DISEQUILIBRIUM MAPPING (LDM): Linkage disequilibrium mapping is a quantitative trait loci (QTL) fin-scale 'association' mapping method which screens for LD, i.e. an association, between the QTL alleles and polymorphic molecular markers. While a new mutation affecting a quantitative is initially in complete LD with all polymorphic alleles in that population, recombination will after some time restore LD of the mutant allele with all other but the most closely linked genes. Consequently, a molecular marker will be in LD with the allele affecting the quantitative trait only if the marker and the QTL allele are closely linked. LDM screens for such linkages.

NULL ALLELE: A null allele or 'amorph' is a mutation that inactivates a gene by producing either no or only nonfunctional gene product.

POLYMORPHISM: Polymorphism refers to the presence of allelic variation for a single gene. By convention, a polymorphic gene is a locus for which the most common allele has a frequency of less than 0.95. Thus, in contrast, for a monomorphic gene, the most common allele in the population has a frequency of larger than 0.95.

QUANTITATIVE TRAIT: A quantitative trait, in contrast to a categorical, discrete or 'Mendelian' trait, is measured on a continuous, numerical scale. For instance, height, weight, and number of offspring are quantitative traits, whereas sex or the presence or absence of wings are Mendelian traits. The values of a given quantitative trait often follow a Gaussian (normal) distribution.

QUANTITATIVE TRAIT LOCUS (QTL): A quantitative trait locus is a chromosomal region containing one or several genes affecting a quantitative trait.

QUANTITATIVE COMPLEMENTATION TEST (QCT): A quantitative complementation test is a genetic test of allelism for quantitative traits. QCT provides a systematic test to examine whether and which genetic candidate locus or loci contribute to the QTL. The method (see also Fig. 1) requires a mutant (null), a wildtype, and a minimum of two QTL alleles. In the F1 generation, the phenotypes of the hybrids of the QTL alleles with both the mutant and the wildtype allele are measured to compare the effects of the two or more QTL alleles across the mutant versus wildtype genetic background.

genes that uncover the candidate gene or region of interest. Classically, complementation tests only work for recessive mutations with large effect, but the Mackay group has been at the forefront in developing complementation tests for quantitative traits such as bristle number and longevity. Although the method is subject to some caveats, the QCT approach can be used to examine whether a given gene or small chromosomal region contributes to the QTL effect (see Fig. 1; Long et al., 1996; Pasyukova et al., 2000; Mackay, 2002). Thus, QCT holds great promise for studying how specific candidate genes affect the phenotype of interest.

The study of Geiger-Thornsberry and Mackay significantly advances our understanding of the genetics of lifespan in *D. melanogaster*. Their work shows for the first time that several candidate genes for aging may be variable in natural populations, and thus potentially subject to selection. Two of the QTL, exhibiting genetic variation for lifespan, contain genes that have been previously implicated

Table 1
Candidate genes examined by Geiger-Thornsberry and Mackay (2004)

Candidate gene or gene region	Biological function	Involved in aging	Genetic variation
Phosphoglucomutase (*Pgm*)	Metabolic energy storage	?	No
Glucose-6-phosphate dehydrogenase (*G6pd*)[a]	Pentose phosphate shunt pathway	?	No
Phosphogluconate dehydrogenase (*Pgd*)[a]	Pentose phosphate shunt pathway	?	No
Glycerol-3-phosphate dehydrogenase (*Gpdh*)	Glycerophosphate shuttle	?	No
Alcohol dehydrogenase (*Adh*)	Ethanol metabolism	Yes?	Yes
Insulin-like receptor (*InR*)	Insulin signaling pathway	Yes	Yes
Superoxide dismutase (*Sod*)	Oxidative stress	Yes?	No!
Catalase (*Cat*)	Oxidative stress response	Yes?	No!
Rosy (*ry*)	Oxidative stress response	Yes	No!
Hsp22–Hsp28	Heat shock response	Yes?	Yes
Punch (*Pu*)	Environmental stress response	?	No
Accessory protein 26A (*Acp26A*)	Mating, reproduction	?	Yes
Acp70A (*Sex peptide*)	Mating, reproduction	?	Yes
Period (*per*)	Circadian rhythm	?	No
Epidermal growth factor (*Egfr*)	Cell proliferation, differentiation	?	No
Mutagen sensitive 306 (*mus306*)	DNA repair	?	No
Presenilin (*psn*)	Cytoskeleton organization	?	No

The table shows the candidate gene, its biological function, whether it has been previously implicated in aging, and whether the QTL harbors natural variation for lifespan.

[a] Tested together as *Pgd* and *Zw* (*G6pd*), because no single mutant stocks were available. Data from Geiger-Thornsberry and Mackay (2004) and references cited therein.

in the aging process: the *Alcohol dehydrogenase* (*Adh*) locus, whose expression is downregulated during aging, and the *Insulin-like receptor* (*InR*) locus, a gene involved in insulin signaling, some mutations of which dramatically extend adult lifespan in fruit flies. The finding that *InR* may exhibit genetic variation for lifespan in natural populations is of particular interest to both the molecular and the evolutionary gerontologist. *InR* is homologous to the *C. elegans Daf-2* gene which affects lifespan in worms, and is a central component of insulin signaling, a major metabolic pathway regulating cell size and proliferation, growth, reproduction, and aging in worms, flies, and mice (Tatar et al., 2003). Thus, insulin signaling may be a major, evolutionarily conserved regulator of lifespan in various organisms, and has been implicated in diseases such as diabetes, obesity, and cancer. Yet, despite rapid advances in our understanding of insulin signaling, Geiger-Thornsberry and Mackay are among the first to show that *InR* may actually contribute to segregating genetic variation for aging in natural populations. Interestingly, the finding of Geiger-Thornsberry and Mackay is corroborated by recent genetic association studies surveying sequence polymorphisms at *InR*. These studies suggest that there is indeed ample genetic polymorphism among wild *D. melanogaster* populations at the *InR* locus, evolutionary divergence among several *Drosophila* species for this gene, and that *InR* may be under selection (Palmer et al., 2001, 2002).

The study also identifies some interesting novel candidate 'GERONTOGENES'. The authors present evidence suggesting that the *Heatshock protein* cluster *Hsp22–Hsp28*, molecular chaperones involved in heat stress, and the *Accessory proteins 26A* (*Acp26A*) and *70A* (*Acp70A*; also called *Sex peptide*), involved in reproduction, exhibit standing genetic variation for longevity. Although these genes are currently unknown to directly affect lifespan, the findings by Geiger-Thornsberry and Mackay strongly suggest them as candidate loci for aging. Indeed, several of the heatshock protein genes examined (*Hsp22*, *23*, *26*, *27*) change their expression during the aging process, and overexpression of *Hsp70* extends lifespan in flies (Tower, 1999). Remarkably, a new paper, just published online while this editorial is being written, suggests that overexpression of small mitochondrial *Hsp22* indeed extends Drosophila life span (Morrow et al., 2004)—as one would predict from the findings of Geiger-Thornsberry and Mackay. Furthermore, the authors also tested *Accessory protein* genes (*Acp26A*, and *Acp70A*) for segregating variation affecting lifespan. While the effects of these two genes on lifespan are currently unknown, at least one *Accessory protein* gene (*Acp62F*) has been shown to directly affect aging, and two others (*Mst57dc*, *Acp36DE*) to change their expression levels during senescence. Interestingly, the finding that the *Sex peptide* locus (*Acp70*), not previously implicated in aging, harbors genetic variation for lifespan opens up some intriguing possibilities. Sex peptide, which is transferred to females upon mating, is known to reduce the receptivity of female flies while increasing their oviposition. Yet, sex peptide is also known to stimulate juvenile hormone (JH) synthesis (Kubli, 2003); JH itself is a neuroendocrine hormone with major stimulatory effects on oogenesis and negative effects on lifespan in many insects (Tatar et al., 2003). Intriguingly, HYPOMORPHIC mutants of *InR* are JH-deficient, sterile, and long-lived, but treatment with JH restores fecundity while decreasing lifespan to wildtype levels (Tatar et al., 2001). Thus, remarkably, Geiger-Thornsberry and

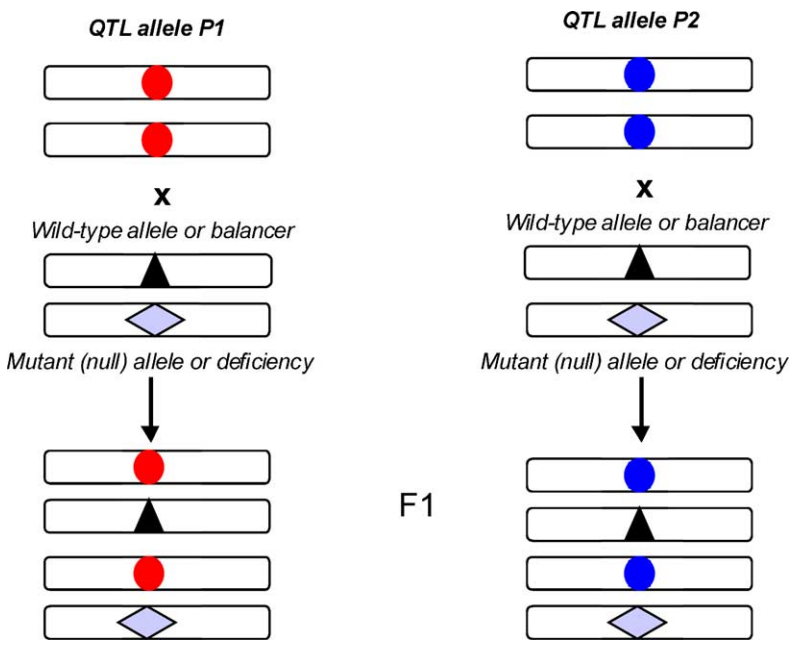

Fig. 1. The principle of quantitative complementation testing (QCT). QCT requires that a minimum of two parental strains (P_1, P_2), containing different QTL variants, are crossed to a *deficiency* stock, which is maintained over a *balancer* (*Df*/*Bal*). Deficiency stocks contain a chromosome which lacks a part of the genome, so that the deficiency uncovers the candidate genes(s) of interest. Yet, since the deficiencies usually uncover more than a single gene, more precision can be achieved by crossing the parental QTL strains to a *null mutant allele* stock, maintained either over a balancer or a wildtype allele (*M*/*Bal* or *M*/*W*) at the candidate locus. In either case, the quantitative trait phenotype is then measured for a number of F1 individuals of each of the four genotypes (*Df*/*P1* or *M*/*P*1; *Df*/*P2* or *M*/*P*2; *Bal*/*P1* or *W*/*P*1; *Bal*/*P2* or *W*/*P*2). The resulting data are then analyzed statistically, using analysis of variance, to determine whether the phenotypic difference in the effect of the QTL alleles (P_1, P_2) is (a) either the same between the deficiency (or mutant) and balancer (or wildtype) genetic background (quantitative complementation) or (b) different between the deficiency (or mutant) and balancer (or wildtype) genetic background (quantitative failure to complement). Failure to complement suggests a genetic interaction between the candidate gene QTL allele and the naturally occuring QTL and can either be attributed to allelism (i.e., the deficiency or null allele encompasses a QTL in the parental strains with different allelic effects) or epistasis (the QTL in the parental strains interacts with other QTL on the *Df*, *M* or on the *Bal*, *W* chromosome). QCT is a powerful tool for suggesting candidate genes for further study, but cannot ultimately prove that the QTL is allelic to the candidate gene. Also see the text box for definitions of key concepts and terms.

Mackay have found natural variation at two loci that affect the metabolism of a hormone which is known to affect lifespan.

Although the evidence is still rather mixed, genes involved in the response to oxidative stress, such as *Catalase* (*Cat*) and *Rosy* (*ry*), probably affect lifespan, with the clearest effects so far found for overexpression of *Superoxide dismutase* (SOD; Tower, 2000). Interestingly, however, Geiger-Thornsberry and Mackay did not find segregating variation for these genes, suggesting that not all 'gerontogenes' harbor alleles that contribute to variation in longevity in natural populations. Thus, while these loci may proximately regulate lifespan, they may have been subject to strong purifying selection, eliminating or reducing allelic variation in natural populations.

Yet, as is appreciated by Geiger-Thornsberry and Mackay, the QCT method is subject to some caveats. While QCT analysis of candidate genes with null mutations is simplified by knowing the exact location of the mutation, using chromosomal deficiencies is much less precise because they uncover a whole chromosomal region, not only a single candidate gene. For example, the deficiency uncovering the *InR* locus also uncovers some 90 other genes. Thus, while QCT is a powerful tool for suggesting candidate genes for further study, the method cannot ultimately prove that the QTL is allelic to the candidate gene. Ultimately, unambiguously demonstrating allelism will require further fine-scale mapping using LDM or confirmation by transgenic analysis. A large-scale QCT analysis of candidate genes, as the one of Geiger-Thornsberry and Mackay, is a major step towards that goal.

Together with the study of Schmidt et al. (2000), showing adaptive evolution of *Methuselah* in natural populations, the work by Geiger-Thornsberry and Mackay represents a major advance for the evolutionary genetics of aging. To some, however, these findings may not be surprising: there is no reason why major 'gerontogenes', as identified by molecular genetics, should not also play a role, in the form of more subtle allelic variants, in shaping lifespan in natural populations. Yet, both studies nicely bridge the still existing gap between molecular genetics, focussing on strong phenotypes with little evolutionary relevance, and evolutionary biology, typically treating the molecular mechanisms and the actual genes affecting traits in natural populations as a blackbox. Very soon we will be able to unambiguously relate variation in molecular properties to variation in whole-organism traits

such as lifespan. This is good news for Darwinian gerontologists.

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