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 (LD mapping) 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 LD mapping, 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 inbred 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
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...
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, may exhibit genetic variation for lifespan in natural populations. The finding that InR may harbor genetic variation for lifespan in D. melanogaster species for this gene, and that InR may be under selection (Palmer et al., 2003, 2002).

The study also identifies some interesting novel candidate ‘gerontogenses’. 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) have 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 (Tatar et al., 2003).

Table 1 presents the candidate gene, its biological function, whether it has been previously implicated in aging, and whether the QTL harbors natural genetic variation for lifespan.

<table>
<thead>
<tr>
<th>Candidate gene or gene region</th>
<th>Biological function</th>
<th>Involved in aging</th>
<th>Genetic variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol dehydrogenase (Adh)</td>
<td>Alcohol metabolism</td>
<td>Yes?</td>
<td>Yes</td>
</tr>
<tr>
<td>InR</td>
<td>Insulin signaling pathway</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mutagen sensitive 306</td>
<td>Oxidative stress</td>
<td>Yes?</td>
<td>No</td>
</tr>
<tr>
<td>Insulin-like receptor (InR)</td>
<td>Oxidative stress response</td>
<td>Yes?</td>
<td>No</td>
</tr>
<tr>
<td>Adh</td>
<td>Heat shock response</td>
<td>Yes?</td>
<td>Yes</td>
</tr>
<tr>
<td>Alcohol dehydrogenase (Pg)</td>
<td>Environmental stress response</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Presenilin (Pse)</td>
<td>Mating, reproduction</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Accessory protein 26A (Acp26A)</td>
<td>Cell proliferation, differentiation</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Heat shock response</td>
<td>DNA repair</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Sex peptide</td>
<td>Cytoplasmic organization</td>
<td>?</td>
<td>No</td>
</tr>
</tbody>
</table>

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

Tested together as Pgd and Zic (Gpdh), because no single mutant stocks were available. Data from Geiger-Thornsberry and Mackay (2004) and references cited therein.
Fig. 1. The principle of quantitative complementation testing (QCT). QCT requires that a minimum of two parental strains (P₁, P₂), 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 gene(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 (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/P₁; Df/P₂ or M/P₂; Bal/P₁ or W/P₁; Bal/P₂ or W/P₂). The resulting data are then analyzed statistically, using analysis of variance, to determine whether the phenotypic difference in the effect of the QTL alleles (P₁, P₂) 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 occurring 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. 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 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.

Acknowledgements

I thank Jan Vijg and Marc Tatar for helpful comments.

References


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