

Ageing: The Many-Headed Monster

Dispatch

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Ageing involves numerous pathways that are not functionally coordinated. The genome-wide profiling of transcription during *Drosophila* ageing is proving to be a powerful new tool for identifying these pathways.

Ageing is a biological puzzle of long standing, particularly because it manifests itself over a wide range of biological systems, tissues and functions. For some time, the outstanding task has been to find experimental strategies that make sense of the complexity of ageing. There is now reason to hope that such experimental strategies have been found. As reported in this issue of *Current Biology*, Pletcher *et al.* [1] profiled the transcripts of *Drosophila* on a genome-wide scale over a range of adult ages, in cohorts raised on either a standard diet or a restricted diet (see Figure 1; caloric restriction is well-known to extend the life span of most experimental animals [2]). They have identified genes that show changes in expression with age over both nutritional regimes, as well as genes with transcriptional profiles that differ between these two nutritional regimes. Using good replication and stringent statistical criteria, approximately 6–7% of the 13188 fly genes assayed — those on the Affymetrix fly chip — were found to show changes in transcription with age. Surprisingly, about half of these genes were found to be common to both treatments, about 400 in total. Is this a reasonable result for ageing research, and what does it indicate for our future understanding of the problem?

Ageing — An Unusual and Difficult Problem

Some of the earliest experimental research on ageing was that of Raymond Pearl [3] on the duration of life in *Drosophila* during the 1920s. But before 1980, little progress was made in the study of ageing in *Drosophila*. The problem was that most of the tools of the genetics trade did not give useful results. The large-effect mutants studied by Pearl and colleagues almost always reduced life span. The *Drosophila* mutants that enhanced life span most eliminated the reproductive organs of females [4], making such mutants of doubtful relevance. A new crop of mutants that extend *Drosophila* life spans have been produced ([5] for example), but it is not yet clear whether any of these will reveal the physiology of normal ageing.

Most biological research attempts to uncover functional pathways, whether specific biochemical reactions or large-scale developmental processes. Such pathways are built by natural selection, and thus have attributes that are ‘well-designed’, even though

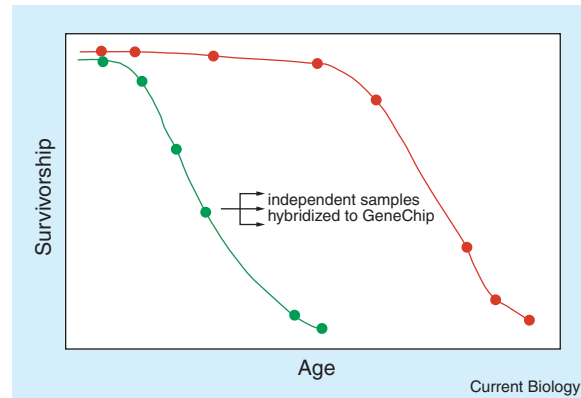


Figure 1. Plot showing survivorship curves for fruit flies under normal (green) and reduced calorie (red) conditions.

The graph shows the declining survivorship of *Drosophila* populations, with time points at which flies were sampled for microarray analysis by Pletcher *et al.* [1]. At each time point, three independent RNA samples were prepared and hybridized to the array.

there is no designer. Mutation and other genetic tricks that modify functional pathways usually impair their operation, and in so doing they reveal how those pathways operate. This is why genetics is perhaps the most powerful tool in the biologist’s toolbox. It exposes functions, and teases them apart.

But ageing is not a function. Ageing can be defined as an endogenous progressive deterioration in age-specific components of fitness [6]. It is not actively selected for. It is instead a secondary effect of the decline in the force of natural selection with age [7,8]. From such theory it follows that many loci, and many biochemical pathways, are expected to produce the deleterious effects of ageing, because it is a secondary side-effect of normal evolution. Earlier, less definitive work using selected stocks [9] indicated that many loci contribute to ageing in *Drosophila* [10,11]. So Pletcher *et al.* [1] are probably correct to implicate hundreds of loci in the control of *Drosophila* ageing. But the question remains, how can we slay this many-headed monster?

A New Tool for Ageing

The findings of Pletcher *et al.* [1] bode well for serious attempts to understand the many-headed monster of ageing. They identify a laundry list of gene pathways which are upregulated or downregulated with ageing, including many of the usual suspects as well as a few new pathways. Pletcher *et al.* [1] carried out a novel form of annotation using the Gene Ontology database (<http://www.geneontology.org>). For every gene ‘category’ in the Ontology, they tested whether the fraction of genes in that category showing age-dependent gene expression is greater than the fraction of genes significant at a genome-wide level. Functional categories of genes whose members are greatly enriched above the general level of expression are then offered as candidates for further ageing research.

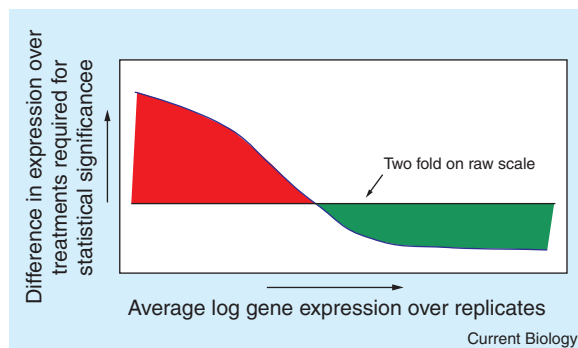


Figure 2. Hypothetical plot of how big a difference in gene expression would have to be in order to be statistically significant as function of the average level of gene expression over the entire genome.

This plot is based on the observation that, on a log scale, the variation in gene expression is often a function of the mean, generally being much greater for less-expressed genes than more-expressed genes (blue line). Statistical tests based on fold change implicitly assume a constant variance on a log scale. Thus hypothesis tests based on fold change are overly liberal for less-expressed genes (red) and overly conservative for more expressed genes (green). The cross-over point is dependent on the details of the experiments carried out.

In this way, Pletcher *et al.* [1] identified a number of such functional classes that were not previously thought to be important in ageing, including genes involved in chorion formation, serine protease inhibition, antibacterial peptides and the mitochondrion. The caloric restriction treatment further identified genes encoding nuclear components involved in cell growth and maintenance. The effort of the authors to identify functional classes of genes important in ageing is a service to the community, as these may open more fruitful avenues of research than an unannotated list of genes showing change.

The statistical rigor Pletcher *et al.* [1] employed in their study should also be applauded. It is still lacking from many published microarray experiments. The authors sampled six to eight time points in the control and caloric restricted lines, and at each time point they replicated their microarray experiment using three independent samples of flies. While there are ways to compensate for a lack of replication [12], the replication employed by Pletcher *et al.* [1] has been shown to increase the power of microarray experiments and reduce the rate of false positives [13,14]. It is important that microarray experiments are replicated at the level of the experimental organism or extracted tissue, because the biological variation at these levels is often much larger than the variation engendered by microarray technology. Specifically, replicate hybridizations of the same RNA pools to arrays tend to make detected differences appear more significant than they are in reality.

Pletcher *et al.* [1] also employed statistical tests of significance, as opposed to merely considering 'fold change' over experiments. Again, this is an important consideration because the variance in gene expression over replicate experiments is generally a function of how highly a gene is expressed (see Figure 2).

Statistical analyses based on fold change will tend to be overly liberal for less expressed genes (red in Figure 2) and overly conservative for more expressed genes (green in Figure 2). A proper statistical framework, with *p* values corresponding to tests of specific models, provides better comparisons of gene expression patterns over heterogeneous classes of genes. In this respect, the data analysis of Pletcher *et al.* [1] raises the bar for the ageing research community and the microarray community in general.

There are problems, of course. Both age and caloric intake are known to regulate aspects of adult physiology other than ageing, especially reproduction. While reproduction exhibits ageing also, it substantially complicates the analytical problem to have effects on survival confounded with effects on reproduction. In particular, it will be hard to associate most of the differences in gene expression found by Pletcher *et al.* [1] specifically with either survival or reproduction. But against this criticism, there is a great deal of evidence that indicates that survival trades-off against reproduction in the genetics of ageing [6], a trade-off that is part of a phenomenon called antagonistic pleiotropy. Hopefully, future work using microarrays will be able to sort out these complexities. Therefore, despite some ambiguities and limitations, the work of Pletcher *et al.* [1] opens a new door for the study of ageing, a problematic field that may now be ready for rapid progress, after a century of slow going.

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