MY FAVOURITE PAPER

Adam Rudner, Ramon Tabtiang and Cynthia Kenyon (1993)

A mutation which doubles *C. elegans* life span (imagine being 140). *Worm Breeder's Gazette* 12 (5): 94.

Genetics Society News (2005) 52, 53-55

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A source of fascination for historians is the way that particular cultural movements or traditions, conflicts and so forth spring onto the scene, seemingly out of nowhere. I remember wondering as a boy during the 1960s where rock and roll music had come from. From watching television it seemed to me that after a long pre-history of Bing Crosby and Frank Sinatra, by some extraordinary process there had suddenly appeared, *ex nihilo*, the Beatles and Herman's Hermits.

Such miraculous births are a popular topic for scientific historians. Arthur Koestler, for example, wondered about the renaissance of cosmology after the dark ages, and the origins of modern physics: how did the process begin that led to Isaac Newton and Albert Einstein? Koestler dismissed Copernicus as a medievalist who just happened to be right with his heliocentric theory. For Koestler the first recognisably modern physicist was Johannes Kepler, who devised his three laws of planetary motion in the early 1600s. Much of Koestler's book *The Sleepwalkers* is dedicated to exploring how someone like Kepler could exist, a man so much a child of the dark ages (he narrowly rescued his mother from being burned as a witch). How was it that he wandered out of the medieval darkness?

The history of science is pock-marked with such sudden appearances of new fields of research. One such is the recent emergence of lifespan genetics – the topic of my own research. Perhaps at some future time, when the biology of ageing is long since fully elucidated, and treatments for ageing-related diseases such as cardiovascular disease and ageing-related cancers and dementias is routine, historians will wonder how the process of understanding ageing really began.

While the biology of the ageing process remains largely mysterious, it has always been obvious that longevity is largely under genetic control: different animal species have very different lifespans, even when protected from external causes of mortality. This has to be a function of their genome. If lifespan is genetically determined in the same way as, say development, morphology, behaviour, why is it that classical genetic studies of lifespan only really began in the 1990s? After all, ageing is an interesting topic, since for most of us it represents our death. On the face of it, in failing to examine the genetics of ageing sooner, geneticists seem guilty of a serious failure of imagination.

I first became aware of the possibility of a classical genetics of ageing by accident. One day in 1989, as graduate student at the University of Glasgow, I was eating a sandwich in the library on the top floor of the Institute of Genetics, and leafing through what I thought was the latest issue of *Genetics*. I came across a paper with the title "A mutation in the *age-1* gene in

Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility" from Tom Johnson's lab at the University of Colorado (Friedman and Johnson, 1988). In fact, the issue of *Genetics* was an old one… left there, I suspect by Gordon Lithgow, another graduate student in the Institute at that time (now also working on ageing in *C. elegans,* at the Buck Institute near San Francisco).

I was entranced by this paper: a single gene mutation could result in a major increase in lifespan, suggesting that one could apply classical genetics to study ageing. Yet it seemed that the increase in lifespan had a relatively trivial explanation. Reproduction reduced lifespan, and the *age-1* mutation also reduced reproduction, which appeared to explain the increase in lifespan. Nobody at that time expected a single gene mutation to extend lifespan by acting on the ageing process itself, since the evolutionary theory of ageing predicted that ageing should be controlled by a large number of genes.

But still, if ever there was a topic for the future of biology, this surely was it. Yet the problem was: How could one study the genetics of a trait predicted to be controlled by a very large number of genes. It seemed that in this case the obvious short-lived model animals, *C. elegans* and Drosophila, would not be suitable. What one needed was an organism which had evolved more than one pattern of ageing, more than one lifespan. One could then use classical genetics to identify genes that regulated the switch between lifespans, and study differential gene expression to identify the large number of lifespan genes. But such organisms are rare in nature (or so it seemed). In *C. elegans* there is a long-lived form, which lives much longer than the adult: the dauer larva. This is a developmentally arrested third stage larva, a non-feeding dispersal stage. Yet dauers seemed uninteresting with respect to ageing, since they appeared to represent a dormant stage, akin to a seed or spore. Their long lifespan was attributed to their hypometabolic state.

Subsequently, while working as a postdoc at Imperial College, I searched the literature for a suitable model organism, and settled on another nematode: *Strongyloides ratti*. This strange creature has two adult forms, one free-living and the other parasitic. The important thing was that these two adult forms were reported to have very different lifespans. *S. ratti* would not be a convenient model organism to work with, but if it represented the only route to understand the biology of ageing, it would be worth the effort. So, I applied for a fellowship to develop *S. ratti* as a model for studying ageing – and it was turned down.

It was at that point that I was struck by a bombshell. In February 1993 I was sitting in the communal office of Rick Maizels's lab when Mark Blaxter (then working on *C. elegans*) handed me the latest issue of the *Worm Breeder's Gazette* (now sadly defunct). He pointed to an abstract from Cynthia Kenyon's lab at UCSF. She had discovered that a mutation in the gene *daf-2* doubled adult lifespan. I realized that had my fellowship application been approved, I would not have wanted it.

The gene *daf-2* had been discovered a decade earlier by Don Riddle at the University of Missouri. His work focused on the genetic control of dauer larva formation. *daf* stands for abnormal in dauer formation. Now, dauers normally form when food levels are low and population density high.

Riddle discovered several classes of *daf* mutant… some that form dauers constitutively (Daf-c) even in non-dauer inducing conditions, and others that were dauer defective (Daf-d), i.e. unable for form dauers under otherwise dauer-inducing conditions. It turned out that Cynthia Kenyon had wanted to screen for long-lived mutants, and had wanted to do this using a mutant which left no fertile progeny. She had opted to use a *daf-2(e1370)* mutant, since it has a temperature-sensitive dauer constitutive phenotype. At the permissive temperature it develops normally, but at the non-permissive temperature it forms 100% dauer larvae. A necessary test prior to her planned mutant screen was to check that the *daf-2* mutant itself is healthy and exhibits a normal wild-type lifespan. To her surprise, *daf-2(e1370)* doubled adult lifespan.

Kenyon at once saw this implications of this. The great longevity of dauer larvae had been attributed to hypometabolism, and dormancy. But the fact that *daf-2* mutant adults were long-lived, yet active and fertile, implied that it was possible to switch on dauer larva longevity processes in the adult – i.e. to uncouple the dauer developmental arrest from their increased longevity. This suggested that the *C. elegans* genome was able to specify two distinct longevity programmes, that of the dauer larva and that of the adult.

These preliminary findings were developed in a paper entitled "A *C. elegans* mutant that lives twice as long as wild type", published in *Nature* in December 1993 (Kenyon et al., 1993). The genetics of dauer larva formation was already well characterized by the labs of Don Riddle and, latterly, Jim Thomas. Dauer control genes had been organized by epistasis analysis into a series of branching pathways. The Kenyon lab began to test these genes, focusing in particular on the gene *daf-16*. Here mutants are dauer defective, and *daf-16*(-) suppresses the Daf-c phenotype of *daf-2*. *daf-16(-)* also proved to suppress the extended lifespan resulting from *daf-2(-)*, suggesting that the effect of *daf-2*(+) on ageing involved antagonism of *daf-16* activity. Kenyon *et al*. surmised that the really interesting gene here was *daf-16*, since it is a powerfully promoter of longevity.

Some *daf-2* alleles showed slight decreases in fertility. To test the possibility that this was the cause of their increased lifespan, Kenyon *et al*. examined worms that were sterile either due to surgical removal of their gonad, or mutation of a gene called *fem-3*. In neither case was lifespan increased, suggesting that the increased lifespan of *daf-2* was not attributable to any effects on fertility. In fact, it turned out that the longevity of *age-1* mutants was not the result of reduced fertility either; instead *age-1* mutants proved to be dauer constitutive and *age-1* to act with *daf-2*.

The real importance of this study is that it established that one could use classical genetics to understand the genetic determinants of ageing and lifespan. Before it, to try to understand ageing through the study of long-lived mutant would have been considered naïve. The biology of ageing seemed an unassailable, impregnable fortress. The *daf-2* finding let down the draw-bridge, initiated a dazzling cascade of discoveries (including many more from the Kenyon laboratory), and stimulated a rapid growth of the field of lifespan genetics. *daf-2*, *age-1* and *daf-16* proved to encode elements of an insulin/IGF-1 signalling pathway. It then transpired that the role of this pathway in ageing is

evolutionarily conserved, controlling ageing in fruitflies and rodents; there are hints that it may also control human ageing. Currently the processes regulated by *daf-16* are the subject of intense investigation. Meanwhile numerous other genes and pathways have been identified which control lifespan in yeast, nematodes, fruitflies and mice.

After reading the gazette abstract, I began to look for a job in one of the *C. elegans* labs in the U.S. that worked on ageing, to learn how to work with the Worm. Luck was on my side: I obtained a fellowship to work with Don Riddle at the University of Missouri-Columbia, and began work there in the Autumn of 1993.

Friedman, D.B. and Johnson, T.E. (1988) A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. Genetics 118, 75-86.
Kenyon, C., Chang, J., Gensch, E., Rudener, A. and Tabtiang, R. (1993) A *C. elegans* mutant that lives twice as long as wild type. Nature 366, 461-464.