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Progress Report
Research in the Biology of Ageing

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Introduction

The biology of ageing is finally becoming tractable science. After millennia of dead-ends and extravagant promises, we can now see our way to understanding the mechanisms of ageing. And there is now the genuine prospect of substantially intervening in the process so as to postpone it. These developments have taken place quite rapidly over the last twenty years, and may not be widely known. Here we introduce some of the basic features of this intellectual revolution, along with its more portentous implications for the human predicament. At the centre of this reformation has been evolutionary genetics. Mathematical research has revealed the evolutionary necessity of ageing in most organisms. Experimental research has shown that evolutionary forces readily alter patterns of ageing. The evolutionary research has been allied with genetic and molecular investigations as well, allowing a broad-based attack on the diverse problems of ageing. This attack has now penetrated far enough that the postponement of ageing has become a problem limited by 'technological' restraints rather than shortcomings in our general understanding of the process.

We now understand the evolution of ageing

Among the most important initial advances in understanding ageing were the theoretical developments of the 1960s and 1970s. Hamilton (1966) and Charlesworth (*e.g.* 1980) mathematically developed what is now known as the evolutionary theory of ageing. Ageing can arise through two mechanisms which are not mutually exclusive. First, deleterious variants of genes with late ages-of-action rise to higher frequencies than deleterious gene variants with early ages-of-action.

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This is an inevitable consequence of the declining force of natural selection with increasing age. Imagine, to use a simplistic example, two mutations in humans, each of which causes a fatal breakdown in some key biochemical pathway. In one individual the mutation's age-of-action is thirteen years of age. In another, the same deleterious effect is specified but it does not have this effect until one hundred years of age. One generation hence, the mutation with the early age-of-action will not be present because the individual carrying the fatal trait dies before giving birth. The mutation with the late age-of-action, on the other hand, will be present in all of the offspring of the original carrier, since they will be born long before the mutation has its fatal effect. The later a deleterious trait is expressed, the less likely that it will reduce its carrier's fitness; indeed the less a mutation will have *any* effect on its carrier's fitness. Consequently, harmful gene variants with later ages-of-action will accumulate in the genome of each species. This is often referred to as the *mutation-accumulation* mechanism.

Alternatively, senescence may arise from selection for individual genes if those genes have both positive effects with an early age-of-action and negative effects with a later age-of-action, a mechanism known as *antagonistic pleiotropy*. As a possible example, consider estrogen production in human females. While estrogen is critical for reproduction, cumulative exposure is linked to increased mortality risk from breast cancer (McManus and Welsch 1984) and endometrial cancer (Henderson *et al*. 1982). Taken together, these two mechanisms give us a satisfying evolutionary explanation for why we age. The puzzle of why we age has been solved.

The evolutionary theory of ageing might seem to imply the universality of ageing. On the contrary, an interesting prediction that follows directly from the theory is that any species that reproduces by symmetrical fissioning should be free from ageing. These species, which include bacteria, do not have adult age-classes that might differ in the intensity of selection acting upon them. Consequently, each time a bacterium divides, a new generation begins. Consistent with this prediction is the fact that at present no senescing prokaryote species are known. Because vertebrates have no such reproduction, on the other hand, the evolutionary theory predicts that all vertebrates should exhibit ageing.

We now understand how to manipulate ageing

Selection

Empirical support for the evolutionary theory of ageing has been found in both the laboratory and the wild, particularly through comparisons of populations evolving under conditions of high *versus* low environmental mortality. Populations living in relatively safe habitats, that is, those with low rates of age-independent mortality, are predicted to age more slowly than those evolving in habitats with higher-age-independent mortality rates (Edney and Gill 1968). In the more hazardous environment, the power of natural selection to weed out harmful late-acting gene variants is reduced – organisms suffer mortality at the hands of predators, disease and environmental extremes. On the other hand, individuals living in a safer environment are more likely to reach the age at which their reproduction will be curtailed by a late-acting harmful allele's (forms of a gene) expression. Consequently over many generations, the frequency of such late-acting harmful gene variants is decreased.

Austad (1993) compared two genetically isolated populations of opossums. The populations lived in island and mainland habitats with substantially different rates of externally-imposed mortality, *e.g.* predation. Consistent with the predictions of Edney and Gill (1968), the island opossums, evolving in an environment free from their major predators, exhibited increased longevity relative to opossums from the mainland. These ageing-rate and longevity differences remained when opossums from the two populations were raised under identical conditions in the laboratory.

In laboratory selection experiments, the force of natural selection in later ages can be experimentally varied in a more controlled fashion. Rose (1984), for example, found that stocks of fruit flies perpetuated by permitting individuals to reproduce only at later ages showed a postponement of senescent changes – including increases in the mean and the maximum longevity observed – when compared with stocks of early reproducing flies (see also Luckinbill *et al.* 1984; Partridge and Fowler 1992). In essence, these late reproducing lines have an experimentally reduced mortality risk: the only flies contributing to the gene pool are those with a genome sufficiently devoid of late-acting deleterious gene variants to allow the individual to survive to advanced ages. Using a similar protocol, Nagai *et al.* (1995) selected for late-life fertility in outbred mice. After only 16 generations of selection, they found an almost 20 per cent increase in mean lifespan and a doubling of the reproductive lifespan. This laboratory selection is continuing;

subsequent observations will indicate whether there is a continual increase in mean life span or whether the process reaches a limit. Relative to the alternative models based on inbred animals, this may be the best available rodent model of human ageing (*cf.* Phelan 1992).

Diet

The ageing rate also can be slowed using non-genetic physiological manipulations. Since the 1930s (McCay *et al.* 1935, 1939), it has been observed that caloric restriction increases both the mean and maximum longevity in a wide range of taxa, including rodents (Comfort 1979; Weindruch and Walford 1988). Such rodents are also well-known to have greatly reduced fertility and reduced levels of circulating reproductive hormone, significantly depressing reproductive performance (Ball *et al.* 1947; Nelson *et al.* 1985; Bronson 1989; Holehan and Merry 1985; Sisk and Bronson 1986). Recent caloric restriction studies using rhesus and squirrel monkeys have also shown promising results (Ingram *et al.* 1990; Kemnitz *et al.* 1993; Lane *et al.* 1996).

Numerous hypotheses have been suggested to explain the mechanism by which caloric restriction increases longevity. As with rodents on restricted calorie diets, the primates studied have exhibited a slight but significant decrease in body temperature, possibly slowing the rate of DNA damage and the formation of certain types of tumours (Lane *et al.* 1996). Given the common evolutionary antagonism between survival and reproduction (*e.g.* Rose 1991), dietary restriction may increase life span as a consequence of the physiological enhancements that result from the concomitant reproduction in reproductive effort (Phelan and Austad 1989).

We now are investigating genetic mechanisms of ageing

Wild and laboratory populations evolved under differing mortality regimes provide strong support for the evolutionary theory of ageing and are the most promising models of human ageing. Their utility as models for postponing human ageing, however, is tied closely to the degree to which the mechanistic approach of molecular and cellular biology can be incorporated into the evolutionary biology of ageing. Work with the nematode *Caenorhabditis elegans* uses brute force genetics to reveal the hereditary machinery underlying ageing in this parasitic worm. At present, it provides a state-of-the-art probe into the outer limits of the possibilities for ageing. Recent discoveries have included several different classes of genes that affect life span. The *daf* group of

genes controls whether individuals enter a dormant larval stage, the dauer. Mutations to such genes can force individuals to enter this state, which is otherwise triggered by environmental cues such as food shortages or population crowding. They can also increase the normal life span by as much as four-fold (*e.g.* Larsen *et al.* 1995). A different set of genes, known as 'clock' genes, appears to control the rate at which a whole suite of bodily processes occurs (Lakowski and Hekimi 1996). Mutations in one or more of the three identified genes (*clk-1-2-3*) can lengthen the stages of embryonic and post-embryonic growth as well as retarding the rate of swimming and defecation. Worms with the combination of a *clk* mutation and a *daf* mutation exhibit mean longevities up to five times greater than normal.

Despite these dramatic results, it is difficult to see how we are going to apply these findings to human ageing. Of particular difficulty is the great genetic dissimilarity between nematodes and humans. Our ancestries are separated by at least a billion years, during which time our respective genetic determinants of ageing are likely to have diverged dramatically. Further, although *daf* and *clk* mutations seem to support the idea that the 'rate of living' is somehow relevant to longevity (Pearl 1928), the demonstration that longer-lived, caloricallyrestricted rodents have higher metabolic rates (per gram of lean body mass) than *ad libitum* fed controls surely qualifies the importance of this idea in mammals (McCarter *et al.* 1985). Still, to the extent that caloric restriction alters the number and type of cells per gram of lean-body mass, the significance of this observation as a criticism of the rate-of-living idea is diminished. The importance of genes controlling the rates of metabolism and specific physiological processes as determinants of mammalian longevity remains to be seen.

Among fruit-flies *Drosophila melanogaster*, selected for postponed ageing, electrophoretic analyses have indicated significantly higher levels of the more active superoxide dismutase (SOD) variant (Tyler *et al.* 1993). Enzymes such as SOD effectively 'detoxify' free radicals – highly unstable molecule fragments containing an unpaired electron, produced during normal enzymatic reactions – and are consequently of great interest to gerontologists (*cf.* Sohal 1987). If left unchecked, free radicals can propagate chain reactions, just as atoms steal electrons from one another, damaging molecules important to normal cell functioning in the process. The genetic 'engineering' of fruit flies has also shown that SOD activity enhancement can increase lifespan (Reveillaud *et al.* 1991). These findings indicate that it is possible simply to compare postponed-ageing populations with normally-ageing control populations to find particular gene loci that

provide lifespan enhancements. One hopeful prospect is that these same methods could be applied to mice with genetically-postponed ageing, such as those of Nagai *et al.* (1995). As we will discuss below, this could be a critical step towards postponing human ageing.

We now see that some research is not related to ageing

Before the development of today's genetic-evolutionary paradigm for ageing research, several alternative approaches were tried but with little success in the manipulation or understanding of ageing. Indeed, most of this research has had no impact on the ageing of *any* organism. Much of this research has however been of interest in relation to cell proliferation and genetic disease. One of the more interesting distractions for ageing research has been 'diseases of ageing', such as progeria, Werner's syndrome and Huntington's Disease. These are dramatic genetic disorders that typically cause early death by hideous pathology. Children with Hutchinson-Gilford's progeria, for instance, largely cease growing, lose their hair, wrinkle, and die – usually of cardiovascular disease – before the age of 20 years (DeBusk 1972). Individuals with Huntington's Disease suffer spectacular nervous system disease beginning in middle-age, and end their lives incontinent and demented. Research on these disorders, such as the recent discovery of the gene responsible for Werner's syndrome (Yu *et al.* 1996), seems to offer us keys to the processes of normal ageing, particularly in their dramatic acceleration of key symptoms.

But that hope is chimerical, for there is no logical guarantee that genetic disorders which cause early death arise from mechanisms that cause normal ageing. Considering that ageing arises from an increase in the frequency of deleterious gene variants with late ages-of-action, ageing is almost certainly controlled not by one or even a few genes but a large number. Consequently ageing is expected to be expressed as a large number of different, often unrelated, mechanisms. In other words, some individuals will succumb to cardiovascular disorders, others to cancer, and still others to complications arising from Alzheimer's Disease. Thus the intensive study of diseases of apparently accelerated human ageing, while worthwhile for their victims, is not likely to produce interventions in normal ageing. An identical argument applies to laboratory animals that have accelerated ageing, like the senescence-accelerated mice (SAM; Takeda *et al.* 1994; Finch 1994). Further, these animals are inbred and consequently their applicability as models of human ageing is weakened in the same

manner as other inbred systems (Phelan 1992). Unlike natural populations of outbreeding mice, they exhibit the smaller litter sizes, decreased vitality and decreased viability associated with inbreeding depression.

A greater distraction for ageing research has been work with laboratory cultures of mammalian cells grown out of the body. Leonard Hayflick discovered more than thirty-five years ago that such cells are incapable of indefinite proliferation (Hayflick and Moorhead 1961). Scientists have spent the intervening years understanding this phenomenon in detail. At present, it is thought that the key factor controlling the failure of cell growth is at the ends of the chromosomes, called the telomeres. It appears that with each successive cell division the gene-free region at the end of the chromosome becomes shorter and shorter (Harley *et al.* 1991). However, there are reasons to doubt that this work is particularly germane to ageing. Cells taken from very old humans can still proliferate. Moreover, many cell types are capable of living and functioning efficiently for many decades after their proliferation has ceased, the best example being the cells of the human brain. No one has managed to connect firmly any fatal disorder of normal human ageing to the failure of cell proliferation. For these reasons, we doubt the value of this type of cell research for ageing. It does, of course, have great value for cell biology in general.

We now know how in principle to postpone human ageing

It is now trivial to create organisms with inherently postponed ageing in the laboratory. However, the methods that we use to do this, selection and genetic mutation, are not practical for humans – they require tens of generations or unacceptably invasive procedures to accomplish. The technical question, then, is how can we extend the fruits of this laboratory research to postponing ageing in humans. Two broad strategies might be used.

The first assumes the existence of genes that act as universal controls on ageing. If the genes that help postpone ageing in parasitic worms or fruit flies can also control ageing mechanisms in humans, then the postponement of human ageing should be relatively easy. We need only accumulate knowledge of the control of ageing in laboratory animals, and then apply that knowledge to humans. Such applications might take the form of 'phenetic engineering', by taking cells from a person's body, altering them genetically so as to ameliorate ageing mechanisms, and then re-supplying those cells to the person of origin. Alternatively

we may discover that particular cellular signals, hormones for example, are altered with postponed ageing, and attempt to mimic that alteration in humans. As yet, no such hormones systems are well-established. The assumption behind this entire strategy is one of the physiological universality of ageing genetics.

The second strategy does not presume such universality. However, it does exploit the physiological similarity of man and mouse. We can do many, though not all, of the same experiments with mice as with flies and nematodes. Scientists can and have created longer-lived mice. Mice are very closely related to humans, with most of the same genes, cell types, organs, and diseases. Physiological changes that can postpone ageing in mice have an excellent, though perhaps not certain, chance of working in humans. Otherwise, we would follow the same specific approaches as those outlined for the previous strategy. It would just take much longer to have first to accumulate the required knowledge of mice. In either case, the postponement of human ageing is no longer a prospect confined to mystics and charlatans. We have a firm scientific foundation for approaching the problem. It certainly won't be easy to crack, but it is at least now clear how we proceed.

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