

Evolution, Fruit Flies and Gerontology

Evolutionary Biology Helps Unravel the Mysteries of Ageing

Amitabh Joshi

In the past decade or so, genetic theories of the evolution of ageing and studies on populations of fruit flies (*Drosophila* spp.) in the laboratory have provided a new perspective on the phenomenon of ageing. These recent advances, very different in approach and methodology from traditional gerontological studies, have provided a wealth of knowledge about the mechanisms of ageing, as well as some answers to deeper, more philosophical questions, such as “why do organisms age at all?”.

Different Approaches to the Issue of Ageing

Since earliest times, people have been aware of, and indeed obsessed with, the phenomenon of ageing. Mythology and history abound with examples of mankind’s quest for a formula that would either ensure immortality, or at least postpone the general decline in health and well-being associated with ageing. By the nineteenth century, this interest had crystallised into a well defined approach followed by scientists, many of whom were medical doctors, who were interested in understanding the processes of human ageing with the ultimate goal of postponing ageing in humans. This approach, rooted in mammalian physiology, gave rise to modern gerontology, which is still characterised by a focus on physiological changes accompanying human (or at least mammalian) ageing.

On the other hand, nineteenth century thinkers like Alfred Russell Wallace (co-propounder, with Darwin, of the theory of evolution by natural selection) and August Weismann had already begun to speculate on the mechanisms causing the evolution of



Amitabh Joshi studies evolutionary genetics at the Jawaharlal Nehru Centre for Advanced Scientific Research. He obtained his Ph.D. from Washington State University in 1993, working on the evolutionary ecology and genetics of species interactions under John N Thompson. Subsequently, he was at the Univ. of California, Irvine, studying ageing and other aspects of life-history evolution in fruit flies.

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ageing as opposed to the actual mechanism of ageing in any particular species. However, lacking the foundation of modern evolutionary genetics, their theories tended to lapse into vague idealistic arguments. They thought of ageing as being for the long-term good of the species, rather than the immediate benefit of the individual. Consequently, it was not until the middle of the twentieth century, after the development of population genetic theory, that evolutionary theories of ageing were clearly enunciated by scientists like J B S Haldane and Sir Peter Medawar.

The principal difference between the gerontological and evolutionary approaches to the study of ageing lies in the nature of the questions asked, even though both approaches attempt to unravel the mechanisms of ageing. Gerontologists tend to focus on what are called *proximal causes* (e.g. looking at the effect of hormone levels on ageing), whereas the evolutionary biologist focuses on so-called *ultimate causes* (e.g. why ageing has evolved in certain species and not in others). As we shall see, the latter approach has ultimately led to a better understanding of proximal causes of ageing as well.

What is Ageing?

Ageing is an acceleration in the rate at which the probability of survival declines with age, relative to the rate associated with juveniles measured under conditions free of externally imposed sources of mortality.

At this juncture, it would be useful to point out that not all organisms age (can you think of any in light of the following definition of ageing?), and that the word ageing (or senescence) has a fairly precise meaning for biologists. A decrease in the likelihood of surviving with increasing age is not necessarily ageing. Even if the probability of death at any point in time, say from accidents, predation, or just wear and tear, is constant (or even decreasing), it will result in a decreasing probability of survival with age (see *Box 1*). A definition of ageing commonly used by evolutionary biologists and demographers is that *ageing is an acceleration in the rate at which the probability of survival declines with age, relative to the rate associated with juveniles measured under conditions free of externally imposed sources of mortality.*



Mortality Rates and Survival Probability

To see why a constant mortality rate (*i.e.* a constant probability of death during a given , or interval, of time) gives rise to a decrease in the likelihood of survival with increasing age, consider the following example. In your kitchen you have ten beautiful porcelain cups. You also have a very clumsy servant, whose job it is to wash these cups each day. Let us say that the probability that the servant breaks a cup while washing it is 1/10. Intuitively, one would imagine that after a few days you would have very few cups left, and that ultimately all the cups would get broken. In other words, the fraction of cups still intact at any given day will tend to decrease over time. This empirical observation about the decreasing proportion, over time, of intact cups can also be expressed as a decrease in the probability that a cup is still intact some arbitrary number of days after the point at which all ten were intact. The critical point in this example is that even though the probability of a cup getting broken each day is constant from day to day, the probability of a cup being intact on a given day is one minus the cumulative probability that it got broken on *any of the preceding days*. The cumulative probability of getting broken tends to increase with the passage of time, causing a concomitant decline in the probability of still being intact. The reader can, no doubt, generalise from cups to organisms and from clumsy servants to the vagaries of fate.

Evolutionary Theories of Ageing

There are two major genetic theories that suggest mechanisms by which the phenomenon of ageing might have evolved: antagonistic pleiotropy and mutation accumulation. Both these theories build upon a basic tenet of evolutionary biology, namely that the strength of natural selection acting on a gene tends to decrease with the age at which the gene is expressed in the organisms carrying it (see *Box 2*). In genetics, pleiotropy refers to a situation where one gene affects two or more traits. Antagonistic pleiotropy occurs when the effects of the same gene on two different traits are opposite in nature. For example, if a particular gene increased your height but simultaneously decreased your weight, it would be considered to show antagonistic pleiotropic effects on height and weight. One especially well documented case of antagonistic pleiotropy involves egg production and life-span. These two traits are inversely related in many organisms

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The Force of Natural Selection Declines with Age

In evolutionary biology, natural selection is often likened to a force that tends to change the genetic composition of a population over generations, thereby leading to adaptive evolution. A simple, but important, tenet of evolutionary theory is that the strength of natural selection acting on a gene tends to decrease with the age at which the gene is expressed in the organisms carrying it. As an example, consider two genes in humans, one of which kills its carriers around the age of 15 while the other kills its carriers around the age of 40. Clearly, the first gene will rather rapidly disappear from a population because individuals carrying it die before they are likely to have had children; the gene is not likely to be passed on to subsequent generations and is, thus, being eliminated by natural selection. The gene that kills its carriers around the age of 40, however, will be transmitted to the next generation almost as efficiently as a gene that has no harmful effect at all, because by 40 most people have had as many children as they are likely to have for life. Thus, even though both genes are equally harmful in a physiological sense (both are lethal), one escapes natural selection because its harmful effect is expressed only late in life at a time when it no longer really affects the number of offspring its carriers can bear. Both major genetic theories of the evolution of ageing (antagonistic pleiotropy and mutation accumulation) are based upon the fact that the strength of natural selection declines with age.

including *Drosophila*. Females that produce more offspring early in life tend to die earlier than those that produce less offspring. The genes resulting in the greater egg production of such females can, therefore, be considered to simultaneously reduce their life-span. These genes may be thought of as increasing the Darwinian fitness (*i.e.* the ability to contribute offspring to subsequent generations) of their carriers early in life because they enable the carriers to lay more eggs. At the same time, by reducing life-span, these genes are also reducing the fitness of their carriers, but only relatively late in life. Evolutionary theory predicts that genes that increase fitness early in life will be favoured by natural selection, even if they have harmful effects later on. This is a simple consequence of the fact that the force of natural selection declines with the age at which a gene is expressed. Selection against a gene that is harmful later in life will be relatively weak and will, therefore, tend to be overshadowed by selection in favour of the gene based on its beneficial effects early on. Consequently, the proportion of genes with beneficial effects

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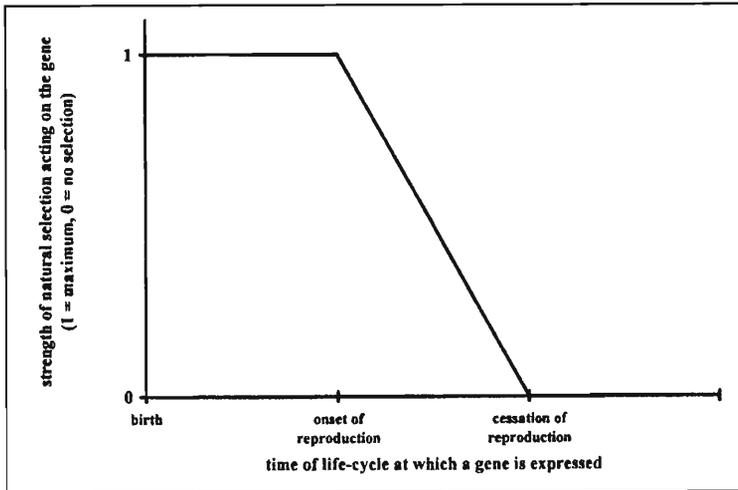


Figure 1 The force of natural selection declines with age. The strength of natural selection is at a maximum when acting on genes expressed before the onset of reproduction. It declines with respect to genes expressed at later and later ages during the reproductive phase of the organism's life-cycle. Genes expressed after the cessation of reproduction are not acted upon by natural selection at all. The steepness of the decline in the strength of natural selection during the reproductive phase increases when reproductive events tend to be more frequent earlier in the reproductive phase of the life-cycle.

early in life and harmful effects later on will tend to increase over generations in populations. In this view, then, ageing emerges as the manifestation late in life of the harmful pleiotropic effects of these genes, almost a by-product of natural selection for enhanced reproductive ability early in life.

The mutation accumulation theory of the evolution of ageing is very similar conceptually to that of antagonistic pleiotropy. In this theory, it is assumed that harmful mutant genes continually arise in populations. Some of these mutant genes exert their harmful effects early in life. Such genes are eliminated from populations by natural selection. Other mutant genes have no effects early on, but have harmful effects relatively late in life. Due to the declining force of natural selection with age, these genes escape elimination by selection and persist in populations. Some such genes may even become more common in a population over many generations entirely due to chance (a phenomenon referred to as random genetic drift). Thus, over a long span of time, many such genes with no effects early in life, but harmful effects later on, may accumulate in populations and contribute to the visible deterioration associated with ageing, because most individuals in such populations will be carrying a number of these genes that are harmful late in life. Note that the mechanisms

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invoked by the theories of mutation accumulation and antagonistic pleiotropy are not mutually exclusive: both phenomena could well be occurring together in populations.

Both the theories described above are based upon the decline in the force of natural selection with age. The principal differences between the two theories are in the types of genes causing ageing that are assumed to occur in populations, and in the evolutionary mechanism by which these genes become common in populations over the course of evolution. The antagonistic pleiotropy theory assumes that some genes are beneficial early on but harmful at later ages. Such genes become more common in populations over time through natural selection. In the mutation accumulation scenario, genes harmful later in life which, however, have no effects on fitness early on, become more common over time through the random process of genetic drift.

Testing Evolutionary Theories of Ageing with *Drosophila*

There is now empirical evidence demonstrating the occurrence of both these mechanisms of ageing in laboratory populations of the fruit fly *Drosophila melanogaster*.

Both of the evolutionary theories of ageing give rise to predictions that can be tested in the laboratory in a fairly straightforward manner. Indeed, there is now empirical evidence demonstrating the occurrence of both these mechanisms of ageing in laboratory populations of the fruit fly *Drosophila melanogaster*. If the antagonistic pleiotropy theory holds true, then genes increasing some aspect of fitness (e.g. egg production) early in life should have detrimental effects later on in life, and vice versa. By subjecting fruit flies to natural selection in the laboratory, Michael Rose (a Canadian evolutionary biologist, now at University of California, Irvine) and his colleagues were able to confirm this prediction (see *Box 3* for details on how this was done). Populations that evolved increased longevity and egg production late in life, as a response to selection, showed a correlated decline in their egg production early in life. On the other hand, populations that initially had high longevity and relatively low egg production early in life underwent a correlated decrease in



Natural Selection for Increased Life-Span in the Laboratory

In an evolutionary sense, selection is said to occur when different genotypes tend to produce different numbers of offspring, thereby resulting in some genotypes (the more fit ones) being better represented in the next generation than others (the less fit ones). In natural selection, whether in the wild or in a laboratory, the relative fitness of genotypes is determined entirely by how well adapted they happen to be to their environment. In artificial selection, as practised by plant and animal breeders, the genotypes of individuals are inferred from their phenotypes and the experimenter then determines which of those genotypes will be permitted to contribute offspring to the next generation. To select for increased life-span in the laboratory, Michael Rose and his colleagues started with five populations of *D. melanogaster* that had a mean life-span of about 35 days. In these populations, adult flies emerged from the pupae at about 9 days of age. The eggs laid by the flies when they were 14 days old were then used to start the next generation, and the adults were discarded. From these populations, Rose and his colleagues derived five new populations in which they pushed forward each generation the day on which eggs were collected for initiating the next generation. For example, in the first generation of selection, they may have collected eggs on day 19 rather than 14, and in the second generation, at day 24 rather than 19. By changing the day of egg collection, they ensured that those genotypes that happened to be better able to produce eggs at a slightly older age would be better represented in each subsequent generation: natural selection for increased egg-production later in life, which implies indirect selection for life-span, because to lay more eggs late in life an individual first must survive that long (Incidentally, can you think of how artificial selection for increased life-span would be done?).

longevity and late-life egg production when they were successfully subjected to selection for increased egg-laying early in life. This symmetric pattern of evolutionary change in opposite directions for early and late life fitness traits, respectively, provides experimental support for the antagonistic pleiotropy theory.

Under the mutation accumulation scenario, different populations are expected to harbour at least partly different sets of genes that have harmful effects later in life, as a consequence of the random nature of the accumulation of such genes in populations through random genetic drift. Given that most harmful mutations tend to be recessive (why that is so is a rather interesting issue but, unfortunately, beyond the scope of this article), one would expect that if two populations that exhibited ageing because of mutation



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accumulation were crossed, then the hybrids would perform better than the parents at advanced ages (see *Box 4*). This is because the hybrids would be heterozygous for at least some of the genes with harmful effects late in life that existed in the parental populations. Such an effect was observed by Laurence Mueller (now at University of California, Irvine) when he carried out all possible pair-wise crosses among three populations of *D. melanogaster* that showed a senescent decline in egg production from an age of about 3 weeks onward. The hybrids consistently produced more eggs than their parents at ages of 3 or 4 weeks, suggesting that the senescent decline in the parental populations was, indeed, a consequence of mutation accumulation. The fact that the hybrids did not lay more eggs than their parents at earlier ages rules out the possibility that the result was merely an expression of *hybrid vigour*, a situation where hybrids outperform their parents at all ages.

Drosophila with Postponed Ageing as a Model System

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We have seen earlier that in order to test evolutionary theories of ageing, Rose and his colleagues created extremely long-lived flies in the laboratory by subjecting them to natural selection for increased life-span and egg-laying at advanced ages. These five long-lived populations of flies have since provided what is perhaps the best system in the world for studying the behavioural, physiological, biochemical and genetic correlates of the ageing process. The reason for this is that these flies live up to three or four times longer than normal *D. melanogaster* would under laboratory conditions. These long-lived flies have an average life-span exceeding 120 days whereas flies from control populations typically live about 35 days. Comparison of the long-lived flies with control flies, therefore, allows the identification of those aspects of their biology that differ from the control flies, and are, consequently, likely to play a major role in ageing. The Ageing Group at University of California, Irvine, headed by Rose, has been studying these populations intensively for the last



Testing the Mutation Accumulation Theory

To see why crossing two populations and comparing the hybrids to their parents allows us to test the mutation accumulation theory of the evolution of ageing, consider the following scenario. We have two populations (lets call them X and Y) that show some pattern of ageing, say a decline in egg production at the age of 3 weeks. We assume that this is because certain genes that adversely affect egg production at week 3, but not before, have become very common in these populations through random genetic drift (this is just another way of saying that we assume that the pattern of ageing in these populations has evolved through mutation accumulation). Now, because genetic drift is a random process, we assume that at least some of these genes are unique to one or the other population. There may be other genes that have become common in both populations by chance, but that really does not make any difference to the argument. Given that most harmful mutations tend to be recessive, we can then write out a partial genotype for an average individual from each of these two populations as follows.

Average individual from population X: $AAbbCCdd$

Average individual from population Y: $AABBccdd$

Here, at the A locus, both populations lack the harmful allele a , whereas at the D locus, the harmful recessive d allele has become very common in both populations. The crucial loci for testing mutation accumulation, however, are the B and C loci. In population X, the harmful recessive allele b has become very common (that is why the average member of this population has the genotype bb at this locus). In population Y, on the other hand, the harmful recessive allele c has become very common. The four loci considered in this example represent four categories of loci that may be thought to occur in populations. The actual number of loci of each type will, of course, vary from one population to the next. All that is required for testing the theory of mutation accumulation is that there be at least some loci in the two populations being studied that show the pattern depicted here for the B and C loci. Now consider the hybrid arising from a cross of individuals from the X and Y populations.



The hybrids are heterozygous at the B and C loci. Because the harmful alleles b and c are recessive, these hybrids will escape their harmful effects. Consequently, at the third week the hybrids should be able to lay more eggs than flies from the parental populations X and Y. If such a pattern is indeed seen, we conclude that mutation accumulation was responsible for the pattern of ageing seen in populations X and Y. Had antagonistic pleiotropy been the sole cause of the observed pattern of ageing, we would not expect the hybrids to lay more eggs than their parents at week 3. This is because under the antagonistic pleiotropy scenario the genes causing ageing become common in populations by the directional force of natural selection rather than by a random mechanism such as drift. Therefore, we would expect the same genes to become common in both populations. The hybrids would then remain homozygous for those genes because both parents would be homozygous at those loci.



One of the principal early findings of this group was that their long-lived flies tended to have higher resistance to various stresses like starvation and desiccation.

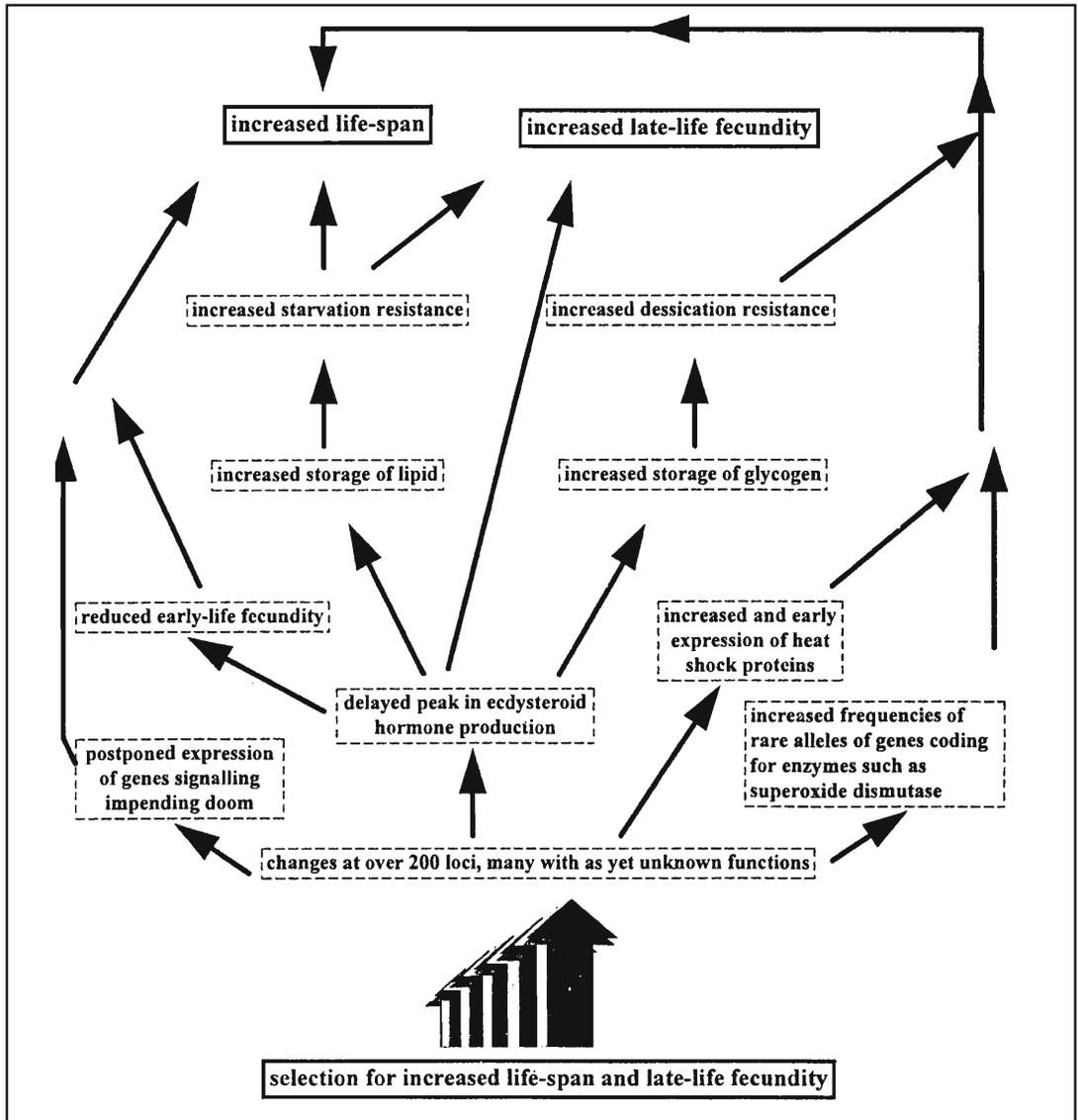
Differences between long-lived and control flies in patterns of lipid storage pointed to the involvement of hormones such as ecdysone in the ageing process.

several years. The group is a diverse collection of scientists with backgrounds in evolutionary biology, insect physiology, endocrinology and molecular genetics.

One of the principal early findings of this group was that their long-lived flies tended to have higher resistance to various stresses like starvation and desiccation. This was also confirmed by selecting flies for increased stress resistance, and observing that such flies also evolved a greater life-span. Moreover, the greater stress resistance of the long-lived flies was shown to be largely due to increased accumulation of food reserves, especially lipids, but also glycogen which acts as a reservoir for water in the body. Differences between long-lived and control flies in patterns of lipid storage pointed to the involvement of hormones such as ecdysone in the ageing process. These hormones are typically activated by mating and, among other things, cause a mobilisation of stored lipid reserves for egg production. As one might expect, the level of ecdysone-like hormones in young flies of the long-lived populations turned out to be considerably lower than in control flies of the same age. Electrophoretic studies have also revealed differences between long-lived and control flies in the relative frequencies of genes coding for alternative forms of several enzymes like superoxide dismutase (the rare *S* allele, coding for the more active form of the enzyme, has risen to high frequency in the *O* populations) and phosphoglucosmutase. Superoxide dismutase has long been thought to be linked to ageing because of its role, along with catalase, in preventing oxidative damage to cells by free radicals, and these observations lend empirical credence to this view. More detailed biochemical and genetic studies on these populations are currently underway to further elucidate the role of hormones and specific genes in the process of ageing. The strengths of this system are that direct comparisons can now be made, among populations of the same species, of individuals with normal and delayed patterns of ageing. This is a tremendous advance over the traditional gerontological approach of looking at small numbers of highly inbred individuals with accelerated ageing. A major problem



Figure 2 Multiple factors causing increased life-span in fruit flies. A schematic representation of the cascade of events underlying the successful response to selection for increased life-span in *D. melanogaster*. Many of the pathways shown are tentative and based on suggestive but, nevertheless, preliminary results. Many others are, undoubtedly, yet to be discovered.



with that approach was the inability to distinguish pathological effects causing early death from a true change in the rate of ageing. Moreover, conclusions drawn from highly inbred organisms are not easily extended to species showing

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crossbreeding, which is a much more common situation in nature.

Conclusion

The experimental validation of the evolutionary theories of ageing has some interesting implications for gerontology. Searching for a single underlying physiological cause of ageing across many species may well prove unfruitful. Given that ageing has evolved by the genetic mechanisms outlined above, there is no particular reason to expect physiological uniformity in ageing across species because it is unlikely that the same kinds of harmful genes would have become common in various species over the course of their evolution. Similarly, even within a species, there is likely to be a multitude of physiological processes contributing to the senescent decline associated with ageing. Indeed, in the words of Michael Rose, "*The only universal mechanism involved in senescence is that of its evolution*". Moreover, the evolutionary approach to ageing suggests that there is nothing about ageing that is in some sense intrinsic and fundamental to life itself. In the evolutionary theories, ageing, in fact, is seen to be a by-product of the way in which natural selection acts on organisms with certain types of life-histories. To answer a question raised earlier in this article, single-celled organisms do not age, neither do asexually propagating organisms such as corals and many plants. Moreover, organisms that die almost immediately after their first round of reproduction, such as annual plants and many insects, are also not considered to undergo ageing. Ageing, thus, is seen to be characteristic of multicellular, sexually reproducing organisms that continue to reproduce over a substantial part of their life cycle. It is only in such organisms that there is a considerable portion of the life-cycle during which the force of natural selection is declines with age.

The only universal mechanism involved in senescence is that of its evolution.

To conclude, I think the success of evolutionary biologists in understanding major aspects of the ageing process underscores

the dynamism and vast scope of current evolutionary biology, a discipline that is fundamentally different from all others in biology because, when faced with any phenomenon, it asks the question WHY?, rather than HOW? I hope that this article has left the reader with some feeling for the impact that evolutionary biology can have on fields not traditionally considered to be within its domain.

Suggested Reading

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Address for Correspondence
 Amitabh Joshi
 Animal Behaviour Unit
 Jawaharlal Nehru Centre for
 Advanced Scientific Research
 Jakkur P.O.
 Bangalore 560 064, India
 email: visitor@ces.iisc.ernet.in
 Fax: (080) 846 2766



Excuses for Not Doing the Math Homework

1. I accidentally divided by zero and my paper burst into flames.
2. I could only get arbitrarily close to my textbook. I couldn't actually reach it.
3. I have the proof, but there isn't room to write it in this margin.
4. I was watching the World Series and got tied up trying to prove that it converged.
5. I have a solar powered calculator and it was cloudy.
6. I locked the paper in my trunk but a four-dimensional dog got in and ate it.

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