only when cilia have grown beyond steady-state length, conditions resulting in shorter than wild-type length flagella should have unaffected disassembly rates in the absence of CNK2. This can be tested by using cells that have regenerated their flagella to half length in the presence of the protein synthesis inhibitor cycloheximide [14], or by using a variety of short flagella mutants [18]. Since overexpression of CNK2 results in shorter flagella and CNK2 knockdown results in long flagella, it is possible that the stimulation of disassembly requires modulation of CNK2 expression. Testing CNK2 expression levels during assembly and disassembly induced by environmental, genetic, and developmental perturbations can provide further information about its activation profile. Many Neks can also be regulated by autophosphorylation or phosphorylation by upstream kinases [19,20]. An analysis of putative phosphorylation sites on CNK2 and development of phospho-CNK probes could produce a more sensitive readout of CNK2 activation state. Upregulation of flagellar disassembly when flagella exceed steady-state length can explain why conditions that result in excessively long flagella are exceedingly rare compared with short flagella phenotypes, which can result from any defect in coordinated IFT. Evaluation of CNK2 expression or activation under conditions producing long flagella should help to determine whether inactivation of CNK2 is a prerequisite for the development of this phenotype.

Evaluating flagellar length under various conditions that induce shortening, as carried out by Hilton *et al.* [9], has yielded important insights about flagellar resorption kinetics. However, such experiments measure the net length change incorporating both assembly and disassembly events. Biochemical analyses have shown that the amount of anterograde and retrograde IFT proteins within flagella actually increase during drug-induced and meiotic resorption [15]. The increased IFT complexes are, however, devoid of cargo, leaving binding sites available for retrieval of flagellar material. An analysis of IFT dynamics in real time will be useful for identifying precisely how assembly and disassembly are linked during flagellar resorption and how molecular players, including CNK2 and LF4, can alter their dependence on one another.

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Aging: Why Do Organisms Live Too Long?

Fruit flies selected to reproduce on the fifth day of adult life for many generations remarkably keep on living for six weeks, showing no change in lifespan. A mutation-accumulation experiment suggests that the same genes confer high early-life fitness and long life.

Alexei A. Maklakov

> "We don't stop playing because we grow old; we grow old because we stop playing."
>
> George Bernard Shaw

Aging has been famously referred to as 'an unsolved problem of biology' by British immunologist Peter Medawar [1], who then proceeded to provide one of the most well-known accounts of how aging may evolve. Medawar was

Figure 1. A salmon is a salmon is not a fly.

The famed life-style of salmon can be considered the ultimate evolutionary solution for the post-reproductive lifespan — these large fish live for several years but then experience what appears to be an attack of accelerated aging and die within a few weeks following their final bout of reproduction. However, such life histories are rare exceptions rather than the rule. The new study in fruit flies, by Kimber and Chippindale [8], suggests why this may be the case. (Image courtesy of Thomas P. Quinn.)

among the first to realize that the strength of natural selection declines with age even in a population of theoretically immortal organisms, because intrinsic immortality does not keep out death from accidents, predation or disease. Therefore, the probability of survival will keep declining with age, rendering late-life events unimportant from the standpoint of natural selection. Later developments of this central idea, most notably by Williams [2] and Hamilton [3], form the basis of the classic evolutionary theory of aging and suggest that senescence should start creeping in immediately after reproductive maturation and increase dramatically when reproduction is no longer possible. From this angle, the famed life-style of the Pacific salmon (Figure 1) can be considered the ultimate evolutionary solution for the post-reproductive lifespan — these large fish live for several years but then experience what appears to be an attack of accelerated aging and literally disintegrate and die within a few weeks following their final bout of reproduction [4]. However, while there are other examples of such accelerated aging in animals, these are rather exceptions than the rule [5]. Many animals, including humans, exhibit prolonged post-reproductive lifespan when protected conditions shield them from the hardships of natural living [6]. In some species, such as whales, lions and baboons, post-reproductive lifespan has also been observed in the wild [6,7]. Why do animals live longer than expected under evolutionary equilibrium? A new study by Kimber and Chippindale [8] in this issue of *Current Biology* uses experimental evolution in *Drosophila melanogaster* fruit flies to address this question and offers a rare glimpse into the evolution of post-reproductive lifespan in real-time.

Back in 1982, fruit flies lived for about 40 days. Surprisingly, the new study [8] shows that when fly longevity was artificially curtailed to approximately five days of their adult life for many generations, sufficient to attain an evolutionary equilibrium in the population, their intrinsic lifespan did not change. Apparently, this is because the genes that confer high reproductive fitness in early life are the same genes that confer long life. Deleterious mutations in such genes are eliminated by selection, resulting in effective post-reproductive lifespan that is more than 800% longer than their reproductive lifespan. These results may have important implications beyond the evolution of post-reproductive lifespan and may affect how we see the evolution of aging as a whole.

The Enigma of Post-Reproductive Lifespan

The fact that life goes on after the end of reproduction has of course been known for a long time. However, early treatments dismissed it as an unimportant consequence of civilization, because it was believed to occur exclusively in humans and domestic animals. While it has since become known that also in nature animals continue to live after reproduction, the real problem of post-reproductive lifespan is that theory predicts an accumulation of deleterious mutations that will result in a 'wall-of-death' — a dramatic increase in mortality — following the end of reproduction [2,9]. Instead, mortality rates tend to decelerate in late life [10,11]. Empirical research on the evolution of post-reproductive lifespan in humans suggested that grandmaternal investment in kin can increase inclusive fitness and even account for the evolution of a menopause. However, kin selection is unlikely to provide a satisfactory explanation for species that lack parental and grandparental care [6]. The new study by Kimber and Chippindale [8] provides a refreshingly simple explanation for the prevalence of post-reproductive lifespan: most genes that confer high fitness early in life have positively pleiotropic effects on longevity, such that flies that are more fecund in early life are also long-lived. Individuals in good physiological condition are more likely to survive and reproduce when they are young and their machinery will continue to function long after the age at which the organism becomes invisible to selection. Post-reproductive lifespan may be best seen not as adaptation but as a genetic constraint, a useless by-product of adaptive evolution maximising early-life fitness. This, of course, does not preclude the possibility that inclusive fitness benefits may further shape the evolution of post-reproductive lifespan in species with complex social organization.

Positive Pleiotropy

While the phenomenon of post-reproductive lifespan is certainly intriguing, the study by Kimber and Chippindale [8] also sheds light on one of the big questions in biology — how does aging evolve? Since Medawar's time, evolutionary biologists relied on two key mechanisms to explain the pervasiveness of senescence: mutation accumulation [1] and antagonistic pleiotropy [2]. Mutation accumulation requires alleles with high to moderate age-specificity, which allows alleles that act late in life to accumulate deleterious mutations because they have no appreciable effect on fitness of young organisms — this is the so-called late-life 'selection shadow'. In contrast, antagonistic pleiotropy relies on alleles with broad pleiotropic effects spanning all ages and maintains that alleles with a positive effect on fitness in early life have a negative effect in late life. Both theories predict that increased extrinsic (non-aging) mortality, such as from accidents, predation or infectious disease, should invariably result in the evolution of accelerated aging and reduced intrinsic lifespan [1,2,9], with antagonistic pleiotropy having received most empirical support to date.

However, during the last decade, empirical findings inconsistent with this widely accepted line of reasoning started to accumulate [12–17]. First, it has become apparent that there could be many alleles with positively, rather than antagonistically, pleiotropic effects on early-life fitness and longevity that are segregating in the populations under mutation-selection balance [13–16]. This means that a substantial amount of alleles that increase early-life fitness also increase longevity. Second, higher extrinsic mortality can paradoxically result in increased lifespan [12,15] and slowed down late-life mortality rates [18], accompanied by increased reproductive performance [15]. How could this happen? A recent model suggested that condition-dependence can result in the evolution of decelerated aging under increased mortality, but this model does not explicitly predict a corresponding increase in reproduction [19]. One possibility is that we overlook the importance of positive pleiotropy between early-life and late-life fitness in shaping senescence. Positive pleiotropy across a wide range of ages provides a very simple and straightforward explanation for maintenance of genetic variation for lifespan and aging via mutation-selection balance. Moreover, this hypothesis has no problem accounting for the existing body of empirical evidence, including "non-classic" evolutionary responses to selection on age-specific fitness when longevity increases and ageing decelerates in response to increased extrinsic mortality [15,18]. Assuming positive genetic covariation between fitness and longevity, selection is maximizing intrinsic lifespan in the populations but failing to a different degree. Positive pleiotropy predicts that when increased mortality increases selection on organismal physiological condition, we will witness the evolution of decelerated aging and increased intrinsic lifespan [15]. Importantly, unlike previous models, positive pleitropy explicitly predicts that evolution of long life under increased condition-dependent mortality will be accompanied by increased fitness. Finally, positive pleiotropy suggests that organisms will routinely have relatively long post-reproductive lifespans simply because of selection on early-life fitness, as was found in Kimber's and Chippindale's study [8]. The stronger the selection on overall condition the longer the post-reproductive lifespan.

Extrinsic mortality results in a 'selection shadow' in late-life, which is the ultimate reason for evolution of senescence. However, the relative importance of different genetic processes remains unclear despite decades of research. It is quite likely that there are many classic mutation accumulation and antagonistic pleiotropy alleles segregating in a population [1,2,9,20], but this study adds to the recent body of evidence that positive pleiotropy may also play a major role [15]. Earlier evidence in favor of positive genetic covariation between fitness and longevity was dismissed based on methodological flaws [20]. However, Kimber and Chippindale's study [8] is a powerful demonstration of how positively pleiotropic alleles that affect both early-life fitness and longevity can maintain lifespans that stretch all the way deep into the 'selection shadow'. Future research will tell us whether positive pleiotropy across a wide range of ages is a key process shaping both reproductive and post-reproductive lifespan in different organisms.

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