COMMENTARY

Benchmarks for ageing studies

The hopes for improving human health during ageing are largely based on studies with animal models. But **Linda Partridge** and **David Gems** ask if we are learning the right lessons from ageing research.

Ageing is complex. Diverse molecular and cellular damage accumulates over time, causing functional failure in different tissues. The process can seem to be intractable to experimental analysis or medical intervention — a pessimistic view overturned by the discovery of genetic mutations that can extend healthy lifespan in laboratory model organisms[1]. Perhaps even more surprising, these genetic effects seem to be conserved over large evolutionary distances, because mutations in related genes can extend lifespan in the yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and the mouse[2,3].

Thus, despite their very different physiology and lifestyles, the simpler and shorter-lived yeast, worm and fruitfly can be used to help understand mammalian ageing. Mutations that extend lifespan can also reduce the impact of ageing-related diseases, including cancer, cardiovascular disease and neurodegeneration[1]. This raises the prospect that a single, underlying ageing process may act as a common risk factor for multiple diseases. Drugs that slow down ageing could therefore reduce the impact of many of the ageing-related diseases simultaneously.

Yet the biology of ageing is a young field with emerging pitfalls (see table, page 167). Genetic mutations that increase longevity will generate exciting headlines, but experimental findings are only as good as the experimental design. For example, neglect of genetic background effects can lead to misleading or hard-to-interpret results[4]. If researchers are rescuing a lab strain from the effects of a disadvantageous environment or genetic background, then we may be learning less about human ageing than we think. It is critical to learn the right lessons from this work if the field is to flourish and provide real insights into human ageing.

"Ultimately, the findings of ageing research need confirmation in humans."

The model-organism approach has already told us much about other processes, such as development, immunity and behavioural traits. But compared to many developmental traits, such as the number of eyes or limbs, lifespan is more sensitive to the genetic make-up of different strains and to the environment. For instance, a mutation always occurs in a genetic background — the rest of the genome — and in some cases lifespan differences vanish when the background is made identical in mutants and controls[4]. Often, organisms of the same strain, but from different laboratories, actually differ in lifespan. A goal of longevity research is to identify gene products that control lifespan and that could be good drug targets; however, reported effects of drugs on lifespan are themselves not always reproducible[5].

Understanding the reasons for all this variability is a key challenge for the field. We need to investigate the robustness and repeatability of findings, to better control experimental variables and to establish some benchmarks for experimental work on lifespan.

One immediate problem for measurements of lifespan is that death can be a surprisingly slippery endpoint. When measuring population lifespan, it is critical to discriminate between accidental and ageing-related deaths, and to exclude the former, which can be tricky. Flies can meet with accidental death from sticky food products and worms can die prematurely when young worms hatch inside their parent and devour it from within.

Identifying the cause of death is therefore a somewhat subjective process, vulnerable to potential bias. So it is wise to work in 'blind' conditions — ignorant of the identity of the experimental treatment and according to stated criteria. Different observers should be able to obtain similar results. For mice, animals can be excluded from a study for veterinary reasons, and this should also be done according to a standard set of criteria, and reported in the literature. Such precautions might seem obvious, but they are routinely overlooked.

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The lifespan of model organisms (such as fruitflies) is affected by the lab environment.

Variance within strains

The most pervasive problem in lifespan research is the presence of uncontrolled genetic differences between strains under study. Substantial natural genetic variation can exist across the genome among different individuals and strains. A mutant gene arose, at some point, in a single individual. That individual had a particular genetic background, which will be passed to progeny along with the mutant gene.

To detect the influence of a specific mutant gene on lifespan, it is essential to place it in a genome that is otherwise identical to that of the strain used as the control. This is best done by repeatedly crossing carriers of the mutation with a standard genetic strain (Fig. 1, overleaf). In flies and mice it is often necessary to bring two different mutations together to produce the desired gene expression, thereby also crossing the two genetic backgrounds in which the mutations reside. This will introduce uncontrolled genetic variation unless both strains have been previously backcrossed to the same control, and hence have the same genetic

background as each other. For mice, where new mutants are usually introduced into a mixed genetic background for technical reasons, it can take years of backcrossing to establish the mutation in the control strain.

An unfortunate and under-appreciated fact is that backcrossed strains can diverge genetically from each other quite rapidly. Spontaneous mutations occur regularly, causing gradual genetic divergence between strains. In addition, the presence of a mutation can lower reproductive success, so that natural selection acts on genetic variation elsewhere in the genome, causing it to diverge, often rapidly, from the genome of the control strain. The effects of a mutation can therefore lessen with time. For example, the yellow mutation in *Drosophila* makes the fruitfly a golden colour and also greatly impairs the courtship behaviour of male fruitflies. Female fruitflies from long-standing yellow stocks are more receptive to the courtship of yellow males, presumably because natural selection has acted to prevent the females from failing to mate.

Strains can also diverge genetically in the lab because low numbers of individuals lead to inbreeding. This allows natural harmful genetic variants to become more common, resulting in lowered survival and fertility. This is particularly problematic whenever inbred strains are crossed with each other, for instance to produce targeted changes of gene expression, because hybrid offspring typically have a longer lifespan than their parents. Thus, one should not assume that strains that were backcrossed several generations ago have remained genetically homogeneous; regular backcrossing is required to achieve this.

An imperfect world

Genetic divergence between strains over time is also problematic when it comes to comparing results from different laboratories. In a perfect world, laboratories would all use genetically identical strains to ensure direct comparability of results and to eliminate uncontrolled sources of genetic variation. However, the reality is that laboratory stocks can differ substantially in lifespan, even when ostensibly from the same strain. For instance, the *C. elegans* strain N2, originally isolated from mushroom compost, is treated as the wild-type by convention. Yet a comparison of 'N2' strains from different laboratories revealed median lifespans ranging from 12 to 17 days[5]. This problem can be partly addressed by freezing strains that are not in use, to minimize divergence, but this is a major weakness of using *Drosophila*, for which attempted methods of cryo-preservation have been largely unsuccessful. To improve repeatability in fruitfly studies, laboratories may therefore need to share strains immediately before undertaking measurements.

The variable longevity of strains raises a question that also applies to human ageing. What constitutes a 'normal' lifespan? In the N2 worm study described above, only the longest-lived of the laboratory strains had a lifespan resembling that of wild strains[6]; the rest had accumulated life-shortening mutations during laboratory culture. This shortening of lifespan in lab strains is also seen in *Drosophila*[7]. The results for mice are less clear-cut, but suggestive of slower-maturing lab strains[8]. An additional problem with mice is that inbreeding can shorten lifespan, and the standard lab stock are deliberately inbred to produce genetic uniformity within strains. This problem can be avoided by the use of mice that are the product of a standard cross between multiple strains, which produces a standard but outbred mouse[9]. For the invertebrates, backcrossing to wild strains and the use of husbandry methods that maintain wild-type lifespans could help avoid declines in lifespan in the laboratory.

More indirectly, genetic variation among strains can cause them to respond differently to mutations and to lab conditions. It is important to understand whether this genetic variation is essentially normal, and if so, whether it is part of natural ageing processes, or instead the result of some laboratory artefact. For instance, in most strains of yeast, over-expression of the *SIR2* gene extends lifespan, but one strain is completely unresponsive[10], for reasons that are not understood.

In other cases the interpretation is clearer. A meta-analysis of studies of *Drosophila* in which genes encoding antioxidants had been over-expressed showed a clear pattern: the lifespan-extending effects were greatest in experiments with the shortest-lived control strains, with no effect seen with the longest-lived controls[11]. These data suggest that over-expression of the antioxidant gene restored normal lifespan in strains whose lifespan had been shortened by laboratory culture. Similarly, dietary restriction can produce substantial increases in mean and maximum lifespan in laboratory-adapted rodents. But a recent study found no increase in mean lifespan in wild-derived mice, which had longer lifespans and lower food intake — although there was a small increase in maximum lifespan and a decreased incidence of tumours[12]. Disentangling the effects of natural genetic variation from the effects of laboratory culture is an important task for the future.

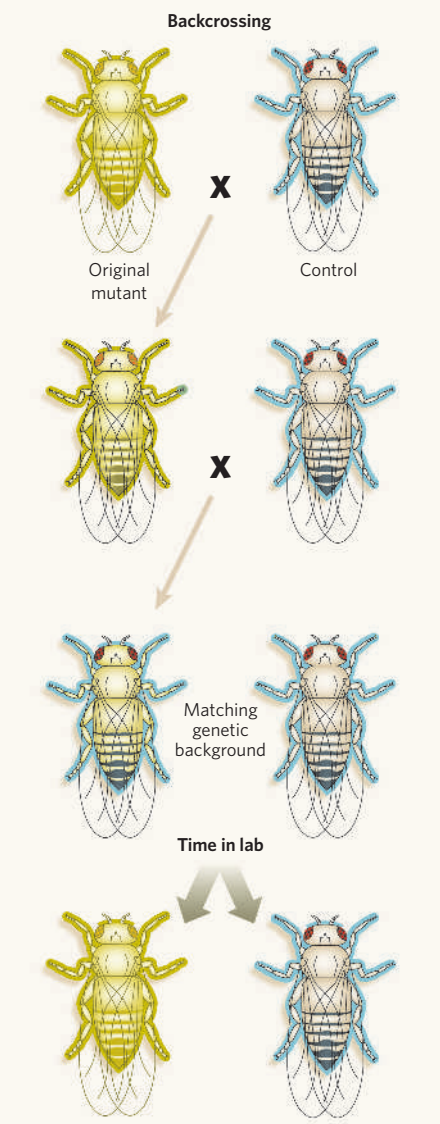
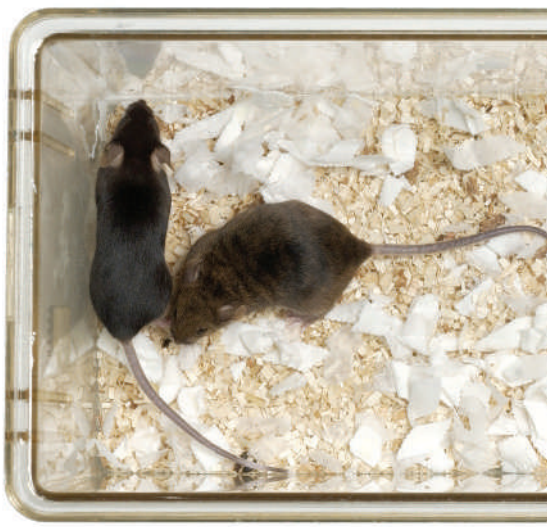


Figure 1 | Backcrossing model organisms. Repeatedly backcrossing mutants with controls will prevent genetic divergence and create individuals with identical genetic backgrounds.

External variation

Environmental sources of variation are equally problematic. Laboratory environments are in some ways more benign and in others more dangerous than those in nature. Study conditions can cause the outcome to vary, and understanding this source of variation is hugely important for applying results from model organisms to humans. For example, the impact of natural enemies, including pathogens, is greatly reduced in the lab, and there is a superabundance of food and little opportunity for exercise.

Food can sometimes be harmful. *C. elegans* is generally fed on another model organism, the bacterium *Escherichia coli*. However, in nature the worm eats soil microbes, such as slime moulds, and *E. coli* has been shown to be mildly



EXPERIMENTAL FACTORS AFFECTING LIFESPAN STUDIES

Factors affecting lifespan	Yeast	*C. elegans*	*Drosophila*	Mouse
Subjective decisions about age at death	Problems assessing if mother cell divided once or twice	Premature deaths e.g. caused by rupture of uterus	Accidental death caused by sticking to food products	Variable veterinary criteria for excluding mice
Genetic background can affect lifespan	Strains differ in lifespan	Lifespan shorter in some lab strains than in wild strains	Strains differ in lifespan. Flies cannot be frozen	Strains differ in lifespan
Reduced lifespan through inbreeding	Unknown	Not applicable. Worms are self-fertilizing hermaphrodites	Shorter lifespan for inbred offspring; longer lifespan for subsequent hybrids	Shorter lifespan for inbred offspring; longer lifespan for subsequent hybrids
Genetic adaptation to laboratory conditions	Unknown	Unknown	Lifespan shortens during lab culture	Some evidence that lab strains are shorter-lived
Laboratory environment	Variations in nutrient supply can affect lifespan	Bacterial food supply can be pathogenic and vary between laboratories	Variation in food supplies or recipes and use of mixed-sex populations	Variations in bedding, food supplies and husbandry can affect lifespan

KEY: Moderate problem Significant problem Important problem. Weightings reflect our impressions of the extent to which these factors have posed potential or actual problems for existing studies.

pathogenic to *C. elegans*. If the worms are fed a different bacterium, *Bacillus subtilis*, which is arguably more similar to the natural bacterial diet of the worms, their lifespan is extended and they respond less strongly to life-extending mutations[13]. At least some of the increase in lifespan seen following dietary restriction in worms could therefore result from reduced exposure to food pathogens. Some laboratories use 'disabled' bacteria that are alive but cannot divide, to avoid this complication.

Reproductive behaviour can also affect lifespan. In *Drosophila*, the presence of males can greatly shorten the lifespan of females and vice versa. It is thus important that any mating regime is standardized when measuring lifespan, unless mating effects are specifically being studied, and the simplest way to achieve this is to work with the sexes separately.

Given these many factors, we recommend that environmental variables are clearly specified when describing methods, and wherever possible, procedures should be standardized, both during the rearing of strains and the measurement of their lifespan. We should also be aware that variables that seem unimportant to researchers may still affect the experimental subjects. For example, mice are highly sensitive to smell and to noise, which will vary between laboratories and are difficult to control.

Ultimately, the findings of ageing research need confirmation in humans. The main goal of studies with model organisms is to generate hypotheses about the mechanisms of human ageing. Humans in many industrialized societies have some similarities to laboratory model organisms. They are largely freed from the burden of infectious diseases, they are surrounded by a superabundance of food, many of them take little exercise and they voluntarily restrict their reproductive rate. Unlike laboratory model organisms, they have not yet undergone many generations of adaptation to this regime. So, rather than studying only lab-adapted strains, wild strains of model organisms examined under laboratory conditions may provide one relevant comparison to human ageing. For mice, which are likely to undergo considerable stress during adaptation, work under controlled, but semi-natural, conditions could be revealing.

"Experimental findings are only as good as the experimental design."

Working on humans

Work has also started in human populations on genetic associations with lifespan, ageing-related diseases and other late-life traits. Studying the genetic basis of lifespan in humans presents some peculiar challenges beyond those normally associated with determining the genetic basis of complex traits. The characteristics of older people, by definition, do not become apparent until they are old. By that time, many of the obvious control groups (siblings, spouses) are no longer available for study. The accumulation of environmental exposures during a long life — including the pre-natal environment — could be important, but unknown, factors in determining late-life health and survival. The genetic composition of populations in specific geographic areas can also change with time through immigration, resulting in a mixture of sub-populations with multiple genetic differences, making careful choice of control groups essential.

Studies of twins, combined with long-term studies of the health of individuals, offer one way of circumventing some of these difficulties. They have shown that genetic influence on mortality before the age of 60 is small and increases after that age, with genetic differences accounting for about 25% of the variation in lifespan[14]. This natural genetic variation is comparable to what has been observed in model organisms. But we know that in some animal studies we can get a doubling of lifespan from a mutation in a single gene, and there may therefore be untapped potential for modifying healthy lifespan in humans.

As the research field of genetic effects on ageing and lifespan starts to mature, the pitfalls and their remedies are becoming apparent. Aside from the need to make new discoveries, the key future challenges are to understand sources of variation, deliver robust and repeatable findings and to make the studies of model organisms as relevant as possible to humans. Understanding sources of variation is challenging, but once understood in context, they will enrich our knowledge of the complex process of ageing. ■

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WELLCOME IMAGES

Like humans? Lab mice have abundant food, take little exercise and are safe from most infectious diseases.

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Acknowledgements We thank Brian Kennedy, Richard A. Miller and Scott D. Pletcher for helpful comments.