

Science Education Collection

# Invertebrate Lifespan Quantification

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## Abstract

Many animals naturally stop growing upon reaching adulthood, after which they undergo aging or "senescence" until dying. The amount of time between an organism's birth and death is called its lifespan, which can be influenced by various biological and environmental factors. By exposing organisms to different growth conditions, scientists can better understand the factors affecting lifespan. Flies and worms are ideal organisms to perform such experiments, given their short generation time and simple culture requirements.

This video provides a brief overview of the factors affecting aging, and goes on to describe basic protocols for invertebrate lifespan quantification experiments. Finally, three research applications of lifespan quantification will be discussed. These experiments explore the effects of diverse factors, such as temperature, drugs, pathogens, and diet, on lifespan.

## Transcript

Lifespan quantification experiments allow scientists to examine the genetic and environmental influences on an organism's lifespan. Invertebrate model organisms, such as the fruit fly *Drosophila melanogaster* and the roundworm *Caenorhabditis elegans*, have proven extremely useful in this regard. By performing experiments that measure and manipulate lifespan in these organisms, scientists have begun to work out the factors affecting the aging process.

In this video, you will learn about some principles behind lifespan and aging, basic protocols for measuring lifespan in the worm and the fruit fly, and ways in which these experiments are being applied.

Before looking at the experimental procedures for measuring lifespan, it's important to first understand what is lifespan and aging.

Lifespan is the amount of time that an organism lives and functions, between birth and death. Some organisms, like many plants, might theoretically live forever. Meanwhile, most animals would naturally stop growing, even in ideal conditions, after which they will age or "senesce" until dying.

Scientists don't yet completely understand why organisms age. Diverse factors, including radiation and chemical damages from the environment, and toxic byproducts from our own metabolic processes, have all been suggested to play a role in aging.

To study these factors, researchers have been taking advantage of the short generation times of invertebrate model organisms, such as worms and flies, which allow scientists to observe multiple generations over days or weeks, rather than months, as in the case of mammalian models such as mice. They are also quite amenable to genetic manipulation, with many available tools that allow specific genes to be turned on or off easily to observe their effects on the aging process.

Now that we've learned why invertebrate models are highly amenable to aging studies, let's look at how experiments to measure lifespan are performed in worms. Briefly, these experiments involve collecting parent worms, synchronizing the age of their progeny, treating larval worms with a drug to inhibit reproduction, and finally transferring worms to test plates and counting worms that are dead or alive.

At the start of the procedure, it is necessary to obtain a sufficient number of age-matched animals by synchronizing a batch of worms to all lay eggs at the same time. To do this, first worms are left on the same culture plate for approximately one week, allowing them to consume all the available food, and thereby inducing starvation. Some of the worm larvae that are starved will enter a hardy, growth-arrested state called "dauer." Then, these dauer larvae are moved to a fresh plate. On this food-rich environment, the dauer larvae will resume their life cycle and become reproductively mature young adults.

Finally, after two days these adult worms are transferred onto another fresh plate and allowed to lay eggs for up to 24 hrs. Once a sufficient number of eggs are acquired, the adults are removed from the plate, and eggs are incubated at 20°C for 2-3 days to allow the worms to hatch and grow to the L4 larval stage.

To measure worm lifespan, these larvae are transferred to plates containing the drug FUDR, which suppresses worm reproduction without affecting adult lifespan. Over the course of the experiment, to determine if worms are alive or dead, the plate can be tapped to observe worm movement. For older worms, it might be necessary to gently prod the worm's head to elicit a response. Remove dead worms and record the number of dead and live worms. The animals should be transferred to fresh plates every 2-3 days to avoid starvation.

Now that we have shown you lifespan quantification in worms, let's examine how it is done in fruit flies. Briefly, the procedure involves allowing females to lay eggs in a special cage, collecting eggs, allowing larvae to develop into flies, sorting flies based on sex, and finally counting dead flies that appear over time.

To obtain age-matched flies, adult flies are placed into an egg collection cage, which contain fruit juice-agar plates streaked with yeast paste. The collected eggs are washed, placed into larval growth bottles, and incubated for about 10 days for the flies to develop. One-day old adult flies are then transferred to adult food bottles and incubated for two days to allow them to reach sexual maturity.

These flies are then collected, anaesthetized, and sorted into males and females to avoid confounding the lifespan measurements with sex-specific factors. Next, the single-sex flies are placed into growth vials. Every two days, flies should be "flipped" into new vials with fresh food. Dead flies are scored by counting those that are left in the old vial, as well as those that have dropped into the new vial.

Now that you have learned the basic protocols for measuring lifespan in invertebrate models, let's look at how scientists are adapting these techniques to studying the biology of aging and longevity.

Researchers are studying the effects of different factors, such as temperature and drugs, on lifespan. In this particular study, scientists cultured worms in liquid media in 96-well microtiter plates, so that the effects of multiple substances and growth conditions can be tested at once. They found that, for example, growing the worms at 25°C, rather than 20°C, decreased worm lifespan, as did high concentrations of the antidepressant drug Mirtazepine.

Lifespan assays can also be combined with techniques such as RNA interference, or RNAi, to identify genes that affect animal survival. RNAi is a powerful experimental technique that uses short RNAs to decrease, or "knockdown," the expression level of a target gene. Here, scientists treated worms with RNAi against the *bec-1* gene, then infected them with the pathogenic bacteria *Salmonella* and performed a survival assay. Worms with *bec-1* knocked down showed a marked decrease in lifespan compared to worms where the gene is expressing normally.

Lastly, lifespan quantification experiments are being used to assess the effect of different growth conditions on the longevity of animals. In this experiment, scientists cultured age-matched worms on a series of plates with varying amount of bacterial food. This growth regimen, called caloric or dietary restriction, has been observed to have a significant effect on worm lifespan.

You've just watched JoVE's video on lifespan quantification in invertebrate models. This video discussed some of the principles behind aging and lifespan, the protocols for measuring lifespan in worms and flies, and a few applications of lifespan measurement experiments. Ultimately, the hope is that these experiments will help scientists find the factors and pathways that control aging, and allow the development of therapies to address age-related diseases, like Alzheimer's and cardiovascular diseases. Thanks for watching!