

<p style="text-align: center;"><b>Life span experiment</b> <b>Per H. Scholtz, 1999; updated by L. Tsai, 2004</b></p>
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**Setting up your fly crosses**

Day -12: Set up crosses of fly lines to be tested as you would for your behavioral assay. Cross 20 males to 20 females in bottles and incubate at 25° C.

Day -10: Clear parents from the bottles set up on Day 0.

Day 0: Collect the males needed for this assay from the bottles set up on Day -12, and proceed with the assay.

**Life span assay**

1. For a large-scale assay, test 1000 males (20 vials of 50 males/vials) per genotype. The total number of males per genotype is optional, depending on the scale of your assay.
2. Keep the flies at 25° C in a humidified incubator.
3. Transfer the flies twice a week (e.g. every Monday and Friday) to new vials of fly food. (Low-filled food vials are sufficient.) Use the freshest possible food at each transfer.
4. At each transfer, count and record all flies that are stuck on the bottom of the vials as dead. *Note: This may not necessarily mean that they are dead and motionless, but the chances that they will free themselves within a 3- to 4-day period out of the food is low. When flies lying motionless and presumably dead are transferred to a new vial, they have a very high likelihood to get stuck in the food at the next transfer and be counted dead at that time. If this system is used consistently across all genotypes and your control(s), results will be comparable despite the few exceptions that are likely to occur.*
5. Continue with transfers until all flies are dead (>90 days). After around Day 60, start checking the vials more often and see if they need to be transferred more frequently. (Per LT, older flies seem to produce something that makes the food goopier in a shorter amount of time.)
6. Graph your results.