



Raising *Drosophila melanogaster* in the laboratory

Collecting *D. melanogaster* virgins

To ensure consistency with the expected progeny genotype, *D. melanogaster* females to be used for crosses must not have previously mated. At 25°C, adult females do not mate for about 6 hours, while at 18°C this time increases to about 16 hours. These females that have never mated are called virgins and can be collected using the following three procedures:

Removal method

Remove all flies from the container 8-10 hours before collection. This removal must be done very carefully, checking the surface of the medium well. All females present 8-10 hours after removal will be virgins. Collect them in small groups in independent containers filled with fresh medium and leave them for 2-3 days to ensure that larvae do not hatch. If they do, the flies are not usable because they are not virgins and must be discarded. Because *D. melanogaster* females are photoperiod sensitive, they tend to hatch early in the morning; therefore, early collections will ensure the greatest number of virgins collected; however, it is possible to collect them throughout the day.

Visual method

Recently hatched, and therefore virgin, females are larger than older females and are lighter in color. In addition, in the first hours after exiting the pupal case they have a dark dot on their abdomen (the meconium, the remains of their last meal before pupating). Virgins can be collected under a microscope using these recognition parameters. This method is recommended if you only need a few virgins.

Temperature cycling

This procedure requires two collections per day using temperature cycles and can maximize the number of virgins collected in a day. At a temperature of 18°C, development is slowed and females do not mate for at least 16 hours after eclosion from the pupal case. Remove flies in the late afternoon and bring the containers to 18°C; the next morning, 98% of the females will be virgins. Gather and return the containers to 25°C for 6 hours; the females, which are virgin by definition, are collected and the containers are again brought to 18°C overnight; the next morning, the females/virgins are collected again. The 2% of non-virgin females are eliminated on the basis of the presence of newly-hatched larvae in the containers in which individual independent groups of collected virgins are left for a couple of days.