

- (d) The surface on which eggs are to be collected must be ventral to the flies.

The details of the procedure I have followed are as follows: Young flies, not over 24 hours old, are transferred to fresh food without etherization (20-40% and over). Two or three days later they are transferred to fresh food. At this time the medium on which the eggs are to be collected is prepared. It consists of ordinary cornmeal-molasses-agar with lampblack added to give contrast to the white eggs. The cornmeal is sifted before cooking. The food mixture is poured onto the ordinary type of paper milk bottle caps, leaving a margin of 1 cm. all around. When cool, the surface is uniformly seeded with fresh yeast. (Caps for 24 hours are prepared at one time.) The next morning the surface of the food on the caps is scraped with a metal tissue lifter. The flies are transferred to empty half-pint bottles which are capped with the prepared paper caps. The bottles stand with the caps down. New caps are substituted at appropriate intervals.

Eggs have been collected by this method continuously for a week or more at intervals of 2, 4, 6, 8, 12 hours. If the rate of oviposition falls off after a few days it may sometimes be renewed by transferring the flies to regular food bottles for 2-3 days. Strains that do not reach their optimum rate of egg-laying as early as the fourth day may be kept on regular food longer before beginning the experiment. (*D. pseudo-obscura* does well after 7-10 days from hatching, *affinis* and *miranda* even later.)

If properly fitting caps are used (diam. = 1.625" for Bridges-type bottle, and 1.640" for most others), they may be washed and reused indefinitely. (Copied from DIS-4:65-66)

Schweitzer, Morton D. Handling eggs and larvae.

When eggs are collected in the manner outlined above the usual high mortality due to handling and yeast overgrowth may be minimized by several precautions. After counting, the entire slab of food (or a segment containing an appropriate number of eggs) may be transferred to the surface of regular unyeasted food. If the surface of the food on the cap is sliced off with a scalpel just before use, the danger of yeast overgrowth is much reduced. An alternative method of transfer, that has given high percentages of imagines, is to allow the eggs to hatch on the food while it is still attached to the cap. The young larvae are transferred with a fine scalpel. In transferring larvae, an efficient method is to gently touch the scalpel to a larva, then touch the larva to a second one, etc. until 25-75 are adhering to each other. In this way the larvae are subject to a minimum of direct handling. (Copied from DIS-4: 66-67)

Hoover, Margaret E. Eggs for larval observations.

In studies involving the embryological stages of *Drosophila*, the use of a synthetic medium may be found useful; especially if it is necessary to watch growing larvae day by day, a transparent food