

(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 ♂). 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

becomes essential. We have found the following technique satisfactory for egg-larvae counts.

Eggs collected from spoons containing a level amount of cornmeal-agar food blackened by finely powdered charcoal and painted with yeast are placed in a row upon the surface of Pearl's synthetic medium in a watchglass. If each individual is to be accounted for throughout larval life, 10-12 eggs in each watchglass will probably be found a sufficient number. The eggs are easily transferred by needle to the watchglass without injury. Enough yeast is carried along in the transfer so that the addition of yeast is unnecessary. Each day every watchglass can be inspected as often as desired and each larva can be accounted for. We have most satisfactorily used a Greenough binocular with 9 X oculars, 2.3 X objectives, 150 Watt light placed horizontally to the mirror so that the reflected light passes up through the glass stage and through the transparent food giving intense illumination.

If it is necessary to study the individuals further, the pupae can be transferred from the watchglasses to regular food vials where pupation will occur normally and the adult flies can be collected in routine fashion.

Danner, Edwin C. Methods for obtaining Drosophila eggs for embryological study.

In obtaining Drosophila eggs for fixation and sectioning or for study in vitro, the homopathic

vials (3/4 X 3-1/2 inch) were found to give good results. (Shell vials of similar size may be better.) Approximately one half inch of banana agar medium is placed in each sterilized vial and allowed to solidify. Immediately upon solidifying, a strip of toweling (1 X 4 inch) with one end cut rounded to fit the vial is inserted so that the rounded end lies upon the medium and at right angles to the remainder of the strip which is pressed against the side of the vial. Moisture from the medium is absorbed by the toweling. Powdered yeast is then sprinkled upon the moist toweling and allowed to stand twelve to twenty-four hours.

In preliminary experiments, single pair matings resulted in good egg production. Virgin females approximately four and a half to five days old, when mated, usually laid eggs shortly after being fertilized. The eggs were laid on the growth of yeast on the strips of toweling. The parents may easily be shaken from the vial and the strip of toweling with the eggs upon it easily removed. Upon the removal of the eggs, the strip may be reinserted and the parents returned to the vial for further egg laying.

In collecting the eggs for study, the moist toweling was made to adhere to a piece of cardboard or filing card to facilitate handling. With the aid of a binocular microscope, the eggs were easily removed with a needle and were free from medium.

To remove the opaque chorion, the eggs were placed in a drop of distilled water upon a glass slide. The excess water