Molnár, I. and I. Kiss. Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary. An easy method for rearing axenic Drosophila lines.

Tissue culture work in our laboratory needed a constant supply of axenic Drosophila larvae with a relative developmental synchrony. The routinely used method of starting axenic cultures newly in each case with surface-sterilized eggs was found unreliable because great differences in the developmental synchrony from larva to larva appeared in the same culture. Instead, we maintain the lines under constant axenic conditions, and start the cultures with eggs collected from germ-free animals. The following method can produce large numbers of relatively synchronous animals with minimal effort.

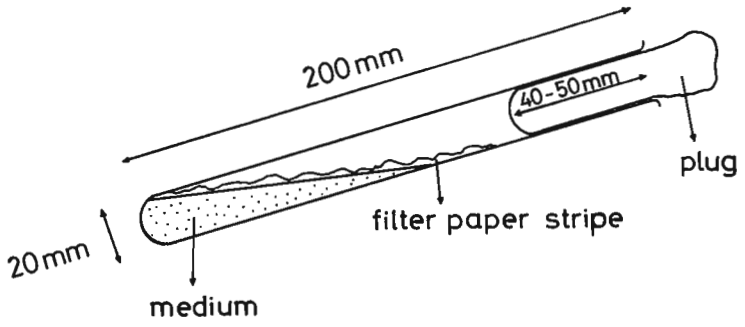
The axenic lines were originally initiated from surface-sterilized eggs (7 min in CaOCl solution saturated at room temperature, followed by rinsing 3 times in sterile distilled water). Once established, the line is maintained by serial transfers of the egg-laying axenic adults.

Medium:

$CaCl_2$	0.54	g
cornmeal	126	g
wet baker's yeast	220	g
agar	8.9	g
water	ad 1000	ml

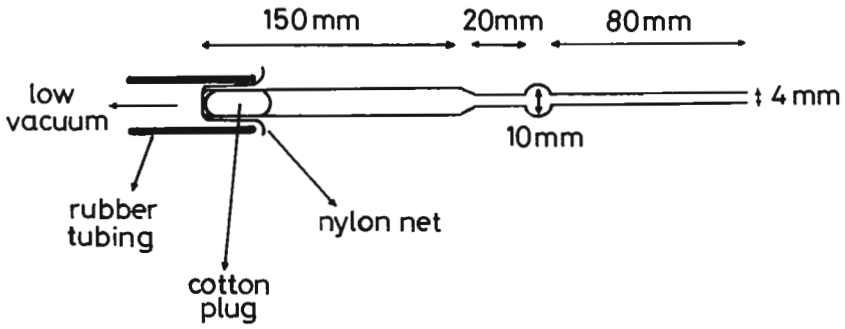
The agar is cooked in 3/4 l of water until it melts; the rest of the ingredients are mixed up in 1/4 l of water, added to the agar solution and cooked for another 5 min. Then the medium is distributed in 15 ml aliquots into large test tubes (20 mm x 200 mm) closed with tight plugs made of paper-tissue. Contamination of the test tube wall with medium should be avoided because the flies can stick to it. The media are sterilized by autoclaving at 121°C (2.1 at) for 30 min and then allowed to solidify in a slant agar form. Fresh media are kept at 25°C for a week before use; any contamination by bacteria, yeast or molds can be easily recognized and selected out during this time. Before use, a sterile strip of rough filter paper is placed onto the surface of the medium which sucks up the condensed water and prevents the flies' sticking to the surface (Fig. 1). The strip should not touch the plug, otherwise the plug becomes wet and contamination can easily get through it. The axenic larvae develop in this medium normally and start to make puparium at about 120 h after egg-laying at 25°C.

Fig. 1



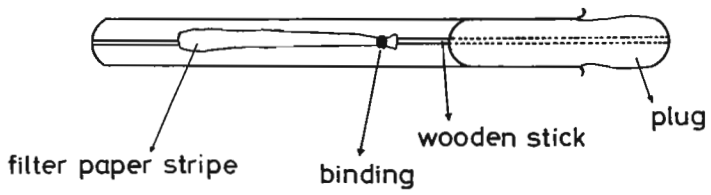
Transfer of flies: All the operations are made under a laminar-flow sterile hood. After shaking off the flies gently, the plug is removed from the test tube and the opening flamed. The test tube can be left open for minutes after this because the flies cannot escape through the hot opening until it cools down again. The flies are collected from the tube by sucking them up into a sterile Pasteur-pipette attached to a pump (Fig. 2); they cannot escape out of the pipette until the sucking is on. The medium should not be touched with the tip of the pipette because its inner wall will be contaminated and the flies stick to it. Then the sucking is stopped by detaching the rubber tubing and the pipette is put into a new test tube with fresh medium. (The opening of the new

Fig. 2



tube has also been flamed.) Holding the tube in a vertical position, the flies are shaken out of the pipette by knocking its tip against the wall.

Fig. 3



Collection of timed eggs: For this purpose, we use an empty test tube, the plug of which has an inserted wooden stick reaching down to the end of the tube. There is a strip of rough filter paper bound to and lying along the stick (Fig. 3). The tube and the insert are sterilized together, then the upper side of the filter paper strip is covered with a few drops of melted, sterile medium. When it has solidified, the plug with the insert is put into another empty, dry test tube into which the egg-laying flies have been transferred. The flies will lay their eggs onto the strip. The test tube is kept in the dark and the plug with the insert changed for new ones at convenient times, under sterile conditions. The paper strip with the eggs is cut off the stick and placed onto the surface of a sterile slant medium.

Sterility probes are not necessary to make for each subculture. However, it is advisable to avoid mixing the flies from different tubes and to separate, by marking, the tubes with eggs from different fly populations. In the case of any contamination, this helps in identifying and separating the possibly contaminated cultures. For checking yeast contamination, a small sample of the medium or a few flies are suspended in 5 ml of sterile broth (0.5 g yeast extract and 1 g glucose in 100 ml tap water, autoclaved) in a test tube and incubated at 29°C. Yeast growing in the settlings can be seen in 3 days after inoculation.

Parente, A. and R. Arking. Wayne State University, Detroit, Michigan. A convenient method of collecting moderately large numbers of eggs from D. melanogaster.

Previous techniques for collecting moderately large numbers of synchronous eggs have been tedious because they usually require large numbers of adults maintained in a population cage. We wish to present an alternative technique for collecting variable numbers (500-5000 eggs/collection) of highly synchronously developing embryos. Peak egg production in Drosophila occurs between 5-15 days after eclosion. To maintain this high level of efficiency it is essential to have an adequate supply of matured females available at all times.

In our laboratory this is accomplished by raising Drosophila in a rotational sequence under optimal conditions. Newly eclosed adults are placed on food and aged for 4 days at 22°C. On the 5th day, the adults are ready to be used for egg collection. This is done by placing 150 females and 75-100 males in a wide mouth glass bottle, the mouth of which is covered by a scored, yeasted, agar plate. The bottle is then inverted and placed in a totally dark incubator free from disturbances. The females are allowed to lay eggs, and at the end of the desired time periods the old plates are removed and new ones put in their places. The eggs may then be left on the agar plates to incubate for some predetermined time period or may be washed off the agar with a stream of water into a funnel containing an appropriately sized wire mesh. Adult females older than 10 days are discarded or used for stock and replaced by a new supply of mature females.

Table 1.

Collection period	No. females per bottle	No. eggs per plate Range	No. eggs per plate Mean	No. eggs per female per hr.	Percent synchrony	Percent abnormal*
30 min.	120	116-204	164	2.73	100	0
1 hr.	120	775-915	840	7.00	100	5-6
2 hr.	120	1000-2000	1500	6.25	81	10

*As measured 1-3 hours after collection.