Title
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METHOD FOR COLLECTING LARGE NUMBER OF FERTILIZED
D. MELANOGASTER EGGS IN MEIOTIC STAGES

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Technical Notes

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Method for collecting large numbers of fertilized D. melanogaster eggs in meiotic stages.

The method to be described permits the transfer of females from one egg collection dish to another with no loss of females and with a minimum of agitation to the females thus disturbing the egg-laying pattern very little. Although we have used the method for collecting large numbers of eggs it would also be satisfactory for collections from single females.

Two days before the eggs are needed virgin females and males are collected and stored separately with approximately 40 flies in each ½-pint culture bottle which contains yeasted standard cornmeal-agar-molasses medium. Also on this day, 600 cc of the same type culture medium should be mixed with 10 gm of live Baker's yeast and allowed to ferment for two days. In the morning, two days after having been collected, males from each bottle are shaken (without etherization) into the bottles with the females, giving about 40 pairs per bottle. After about three hours the flies are shaken, without etherization, into tubes used as egg-laying chambers (one bottle of flies per tube) and the tubes are placed immediately on blotting paper for collection of eggs.

The tubes are clear plastic, about 45 mm long and 22 mm in diameter. One end of the tube is covered with a single layer of dacron gauze and the other end is plugged with cotton after the flies have been shaken in. Dacron gauze is used because the fibers do not absorb moisture and do not shrink or expand with moisture changes. The gauze may be held on the tube with rubber bands or the gauze may be glued to or embedded in the plastic with the proper solvent. We have found the latter method to be most satisfactory.

The collection dish consists of Petri dish lid or base which has been filled with the previously mentioned fermented culture medium. On the surface of this food is placed a Kimwipe or Kleenex-type tissue on which the...
blotting paper will be placed. Dark green blotting paper cut into rectangles about three inches by four inches is found to be satisfactory for the egg collection as the eggs are readily visible against the dark background. The blotting paper is first soaked in a vinegar solution (nine parts water to one part commercial white vinegar) before it is placed on the collection dish. Both the fermented food and the vinegar solution are necessary to stimulate rapid egg laying. At the end of the egg-laying interval the tubes may be gently lifted, the blotting paper with the eggs removed, a new piece of blotting paper placed on the fermented food and the tubes placed on the fresh paper for another collection. The eggs may be treated while on the paper, or removed easily with a needle or small brush, or the paper and eggs may be inserted into a bottle containing culture medium and permitted to develop.

During collecting intervals of five minutes we have been able to collect over 200 eggs occasionally (using nine tubes), and average about 100 eggs. In our experiments we normally make 30 to 40 such collections in an afternoon. In a sample of 190 eggs collected in this manner and then prepared with Feulgen's stain it was observed that slightly more than 75% of the eggs were in meiotic stages. Fixation in some cases did not occur until about 20 minutes after the eggs were laid so the percentage of eggs in meiotic stages at the end of the five-minute collection period would be higher than is indicated. If small quantities of very young eggs are desired it would be best to use fewer flies in each tube and then to select those tubes in which the eggs are being laid rapidly.

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