Maroni, G. & S.C. Stamey. University of North Carolina, Chapel Hill, North Carolina. Use of blue food to select synchronous, late third-instar larvae.

Hatching of the first instar and the two larval molts are events when it is possible to collect D.melanogaster larvae of a well defined developmental age. The larval molts are not practical for this purpose because the larvae are buried in the food, at this time, and they require close inspection to be classified. The harvest of developmentally synchronous late third-instar larvae presents a special problem because this is so far removed from hatching, the most convenient collection stage, that synchrony is deteriorated: in our experience, larvae that had hatched over a one-hour period reached pupariation with an asynchrony of ten to fifteen hours. Larvae stop feeding late in the third instar and here we report a method that makes use of this fact to identify and collect synchronous larvae two and four hours before puparium formation.

Eggs from several thousand flies were collected on a yeasted agar dish from a population cage. Newly hatched first instar larvae were transferred to 236 ml (half-point) culture bottles at a density of 100 larvae per bottle. The medium used was the standard corn meal, molasses and agar to which was added, while soft, the dye bromphenol blue, at a concentration of 0.05%.

Larvae that feed on medium containing bromophenol blue show a distinctly blue alimentary canal. This color persists for a while after they cease feeding and enter the wandering stage that precedes pupariation. After three to four hours the color becomes noticeably lighter and finally it disappears completely just before pupariation. The process of color loss is probably continuous, but one can distinguish larvae of three classes with respect to gut color: dark, light and clear. Figure 1 shows plots of the cumulative number of larvae which have reached different stages of development. In this particular experiment, the lightening of gut color was evident approximately 3.75 hr after the initiation of the wandering stage and pupariation followed 2.0 hr later. When observing a large number of larvae as in this experiment, it is impractical to keep track of the clear larvae because pupariation ensues shortly afterwards. In an experiment in which the individual progress of a few larvae was followed, we found that the time between clearing of the gut color and pupariation is 10-15 minutes.

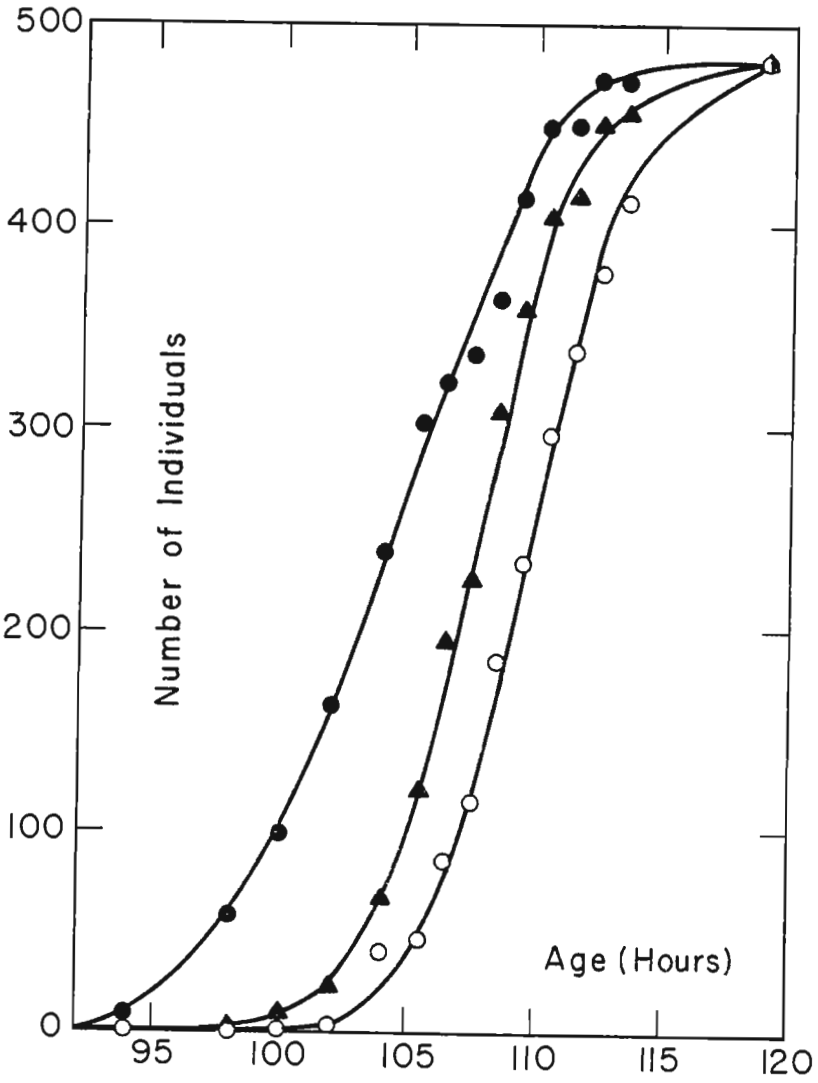


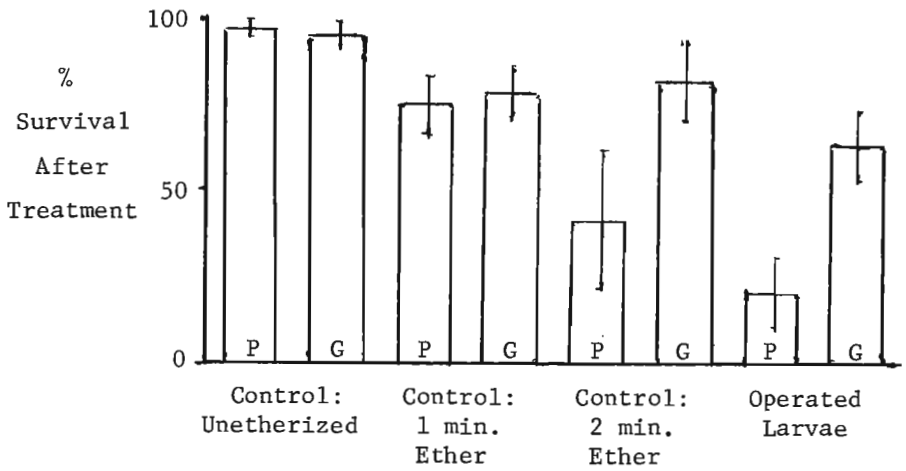
Figure 1. Cumulative points of the number of Samarkand larvae which have reached three different stages in late larval development: ●, wandering stage, dark blue; ▲, wandering stage, light blue; ○, prepupae. The data are pooled from four bottles grown concurrently and with approximately equal numbers of larvae. Time was measured from the mid-point of the hatching period which was one hour.

The length of the wandering stage may be quite variable in different cultures; however, this variability can be substantially reduced by keeping the number of individuals per culture constant and by incubating cultures in a high humidity atmosphere. The presence of bromophenol blue in the medium has no detectable effect on development time and it does not interfere with the assays for such enzymes as alcohol dehydrogenase, alpha glycerophosphate dehydrogenase, aldehyde oxidase or with protein assays.

References: Maroni & Stamey 1983, DIS this issue; Maroni et al. 1982, Genetics 101:431-446.

McCrady, E. University of North Carolina, Greensboro, North Carolina. Possible detrimental interaction between etherized larvae and polystyrene culture vials.

Survival of larval hosts injected with disc parts reached an unacceptably low level in a recent series of experiments. After reducing the period of etherization to its lowest practical length, the possibility arose that the high mortality might be due to interaction of larval cuticle retaining ether molecules with the surfaces of polystyrene culture vials (Carolina Biological) in which the operated larvae were isolated. Dead larvae were frequently found stuck to the walls of such vials, while the few surviving animals appeared to have remained in the food until pupariation. To test this possibility, the control series summarized in the graph was carried out, comparing the survival of operated and control animals in glass and polystyrene vials after differing amounts of etherization.



P= Polystryene Vials (Carolina Biological)

G= Glass Vials

Data on control etherizations are averages of four wild-type stocks, including the one routinely used in our experimental work. No significant differences were found in the survival of the different stocks. The results indicate strongly that if etherization of larvae exceeds one minute, the use of polystyrene vials for subsequent culture should be avoided. Experimental operations performed since change over to glass vials have routinely averaged over 60% survival, and we have adopted one minute, 25 seconds as the optimal length of etherization for larvae in a saturated chamber.

McRobert, S.P. & L. Tompkins. Temple University, Philadelphia, Pennsylvania. Stalking the wild Drosophila.

We have developed a simple and efficient procedure for collecting Drosophila in the field. Instead of a paper cup suspended from a branch with string (e.g., Spencer 1950), our trap is a clear plastic cup, available wherever disposable picnic supplies are sold, which is hung by a loop of red yarn. We bait these traps with mashed banana topped with active dry yeast. Flies are collected by being shaken into a plastic sandwich bag which has been quickly placed over the open end of the cup. The flies are