Stocker, A.J. and J. Jackson. University of Texas (Southwestern) Medical School at Dallas, Texas. A technique for the synchronization of Drosophila for developmental studies.

During our studies on different aspects of the development of Drosophila pseudoobscura we have formulated a synchronization technique which provides uniform results as evidenced by puffing and electrophoretic patterns. To implement this technique we have used a type of population cage which was designed and built by us and is shown in figure 1. This cage consists of a 7" diameter cylindrical plexiglass chamber which is mounted upon a 8" by 15" base containing two 3 3/4" diameter holes for exact insertion of 95 mm Petri dishes. The Petri dishes are supported from beneath by three pieces of spring steel. The fly-containing upper chamber was constructed so that it could be easily rotated to fresh food and the used food removed. Thus, the flies can be changed to fresh food with a minimum of work and disturbance.

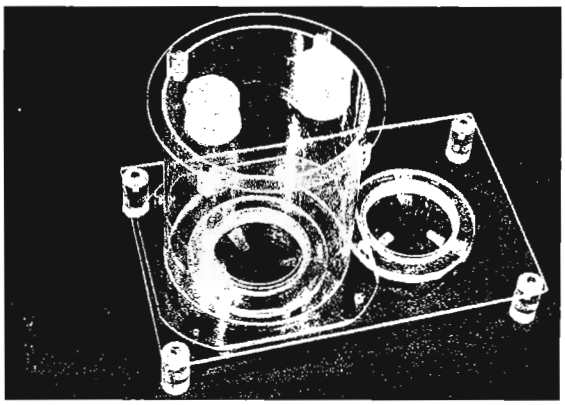

Figure 1. Population chamber designed to facilitate mass egg collections.

The egg collection and synchronization techniques were modified from those of Mitchell and Mitchell (1964), Berendes (1965) and Ashburner (1967). Several hundred newly emerged parent flies are put into the chamber and aged for one week on cream of wheat-molasses media. (These parents go through their larval stage under the same conditions as described for the larvae used in post-spiracle eversion studies). Egg collections are then carried out during the following two weeks on Ohba media (Ohba, 1961) blackened with charcoal and brushed with fermented yeast. This medium is provided for the flies when eggs are to be collected. It is a rich medium and larvae grown on it reach a uniformly large size. The Petri dishes containing the eggs are capped with 5 1/2" high plastic food containers and are incubated at 25°C for the required time.

Under the above conditions, many spiracle eversions take place at about 165 hours after egg laying. Therefore, for work done at and post spiracle eversion, newly everted larvae are easily recognized and either used or aged from that point.

For studies during the third instar, the flies are allowed to lay for a three hour period. The eggs produced during this period are incubated for 25 hours. At this time all newly hatched larvae are removed from the food by rinsing with Drosophila Ringer's and subsequent removal by hand. The remaining eggs are then incubated for an additional hour and the larvae hatching during this period rinsed from the food and collected by filtration. These larvae are placed on fresh food and aged to 72 hours after hatching, a time at which the majority have just entered the third instar. They are then rinsed from the food again and those of approximately the same size are retained on fresh food for experimentation. (Approximately 100 larvae are placed in each petri dish of food.) For mass experiments, the second sorting out is probably not necessary as most of these early third instar larvae are of the same size. The majority of spiracle eversions among larvae collected in this manner take place between 138 and 146 hours from the midpoint of the hatching period. All studies done during the third instar use the midpoint of the hatching period as a base.

References: Ashburner, M., 1967 Chromosoma (Berl.) 21: 398-428; Berendes, H.D., 1965 Chromosoma (Berl.) 17: 35-77; Mitchell, H.K. and A. Mitchell, 1964 DIS 39: 135-137; Ohba, S, 1961 Okayama Univ. Biol. J. 7: 87-125.

Work supported in part by Grant no. GM 16736-02 from the National Institutes of Health.