Podger, R. N. and J. S. F. Barker. University of Sydney, Australia. Collection of large numbers of larvae of homogeneous age and development.

In recent experiments, where we have been studying the effect of variation in larval density on various fitness components, up to 30,000 newly hatched larvae were required on any one day. Larvae were to be used at up to 2 hours post-hatching, and it was important that they be as homogeneous as possible. However, previous experience had shown that crowding and disturbance of adults causes some females to delay laying of eggs subsequent to fertilization. Thus, the larvae hatching from even a short period egg collection will be at quite variable stages of development.

The technique described here allowed a steady supply of larvae throughout a day, and ensured that larvae of advanced development were excluded. Large numbers of 1 to 2 day old flies raised under uncrowded conditions on dead yeast fortified medium were collected and placed in 9x9x3 inch polythene population cages; 5000 to 6000 per cage. The food available to these adults comprised approximately equal amounts of a heavy live yeast suspension and ordinary cornmeal- treacle medium. Instead of the regular medium jars, bark corks (1 1/2 inch diameter) with a circular depression cut in the narrow end, were used as food receptacles. For egg collection, all 9 food containing corks were replaced by ones containing thin discs of 1.5% agar (1/16-1/8 inch thick). These discs remained in the cage for one hour, and when on any one day more than two egg samples were to be collected from a population, the discs were lightly smeared with a 5% dead yeast suspension before use. Up to 8 consecutive hourly egg samples were obtained each day. Provided food was available to the adults overnight, a population continued to produce large numbers of eggs for several days. On removal from a cage, egg collection discs were stored on 7x4 1/2 inch metal trays with a fibreglass mesh base. The trays were stacked in a plastic box and covered with calico to reduce drying out and to prevent contamination.

We had examined the hatching pattern of eggs of the strains being used and found the distribution to be markedly bimodal. For example, for D. melanogaster Oregon-R-C, eggs hatched from a few hours after laying, with a small peak at 19 hours. Hatching of the bulk of eggs commenced at about 20 hours, so that those hatching from about 19 to 20 hours comprise a mixture of "held" eggs and early-hatching normally laid eggs. The numbers hatching increased rapidly from 20 hours to a sharp mode at 21 hours, and hatching was essentially complete by 23 hours. The beginning of the peak hatching period was therefore known, and at this time after the mean of an egg sampling period, all hatched larvae were washed from the discs using a plastic squeeze bottle. Excess water was removed by gentle application of fine paper tissue. All larvae were readily removed in this way without disturbing unhatched eggs. All washing water and equipment used was held at 25° C before use, to allow equilibration to this standard environment.

Collection of larvae commenced one hour after washing, but of course, could follow sooner if younger larvae were required. Larvae were collected, using a dissection needle, for a one hour period fron each sample of discs, so that they were up to 2 hours old when used to initiate experimental cultures. The numbers of larvae available in this period from the 9 discs of a one hour cage sample varied from about 2000 to 4000. An experienced operator could collect between 1000 and 2000 larvae per hour, depending on the numbers of larvae and strain mixtures being placed in individual cultures.

Counce, S. J. Yale University, New Haven. Whole mounts of *Drosophila* embryos.

This technique for whole mounts of insect embryos is not original with me, but is easy, relatively quick, and gives good results with several species of *Drosophila*. Developmental details are so clear the technique could be used for screening for mutants affecting specific embryonic stages.