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.

1. Dechorionate eggs in diluted Clorox and wash.
2. Fix in formol-alcohol-acetic acid, pricking the eggs with tungsten needles sharpened in melted sodium nitrite.
3. 30% alcohol, 15 minutes; 70% alcohol, 15 minutes.
4. Stain 24-48 hours in alcoholic borax carmine.* Best results were obtained with pre-World War I German stains kindly given me by Kenneth Cooper, but carmine obtained from G. T. Gurr gives good results. Superficial staining occurs within a few hours but nuclear details are better in embryos stained for longer periods.
5. Destain with acid alcohol (a few drops of concentrated HCl in 70% alcohol) until desired contrast is obtained, usually 24-48 hours. Embryos should be a pale cherry red and will deepen in color when placed in xylol.
6. Dehydrate through absolute alcohol, clear in xylol, and mount. To prevent fragmentation of embryos, the coverslip is supported on two sides by small pieces broken from no. 1 coverslips. Sealing with fingernail polish prevents shrinkage of the mounting medium away from the sides of the coverslip.

*Alcoholic borax carmine (after ROMEIS): Grind together finely 2-3 grams carmine with 4 grams borax. Dissolve by slowly heating in 100 ml distilled water. Cool. Add 100 ml 70% alcohol. Let stand for a week shaking frequently. Filter before use.

Spieth, Herman T.[1] University of California. A method for transporting adult Drosophila.[2]

In the course of recent investigations of the endemic Hawaiian drosophilids, some difficulties arose with the transportation via motor vehicles and/or inter-island airplanes of field captured adult Drosophila from the cool, wet rain forests to the much warmer coastal area of Honolulu. Not only are the insects sensitive to heat and desiccation, but also they are likely to become "stuck" in the food and to the walls of containers more often than is true for individuals of species from other parts of the world. The following method was devised and has proven eminently successful for the transportation of the flies not only from the field to the laboratory at the University of Hawaii in Honolulu, but also from Hawaii to the University of Texas via air freight.

Eight-dram vials are lined with water-dampened, 6.5 x 6.5 cm. pieces of chromatography paper that has approximately the same texture and thickness as that of a common desk blotter.

The following media is then prepared:

15 gm. Bactoagar
1000 ml. Water
50 ml. Karo syrup (dark)

The agar is added to the water and the mixture is heated sufficiently to dissolve the agar. The Karo syrup is then added and the resulting mixture is simmered for 2 to 3 minutes.

Into each paper-lined vial the hot mixture is poured to a depth of approximately 8 mm. (0.25 in.), the exact amount to be determined by the absorbency of the paper being used. The vials are tightly stoppered with non-absorbent cotton, and are then autoclaved for 15 to 20 lbs. pressure, after which the autoclave is slow-exhausted. When removed from the autoclave, all of the liquid medium should have been absorbed into the paper lining and only a very thin film should remain on the bottom of the vial.

The vials should be prepared 1 to 2 days before use but since they are sterile they do not need to be refrigerated. If they are to be kept a longer time before flies are introduced into them, they should be stored in a tight container to reduce evaporation via the cotton plug.

When the flies are introduced into the vials, they feed readily upon the surface of the media-impregnated paper and cling to the surface easily. Fecal materials are absorbed into the paper and condensation does not form upon the paper surface when the vials containing flies are placed in a cold insulated shipping container that is kept cooled by means of 2 or 3 frozen containers of "Scotch Ice," "Magic Cold," or other chilling agent. The individuals of all species tested to date have remained healthy for 4 to 7 days before it was necessary to change them to a fresh vial.