Finnerty, V., D.L. Baillie and A. Chovnick. University of Connecticut, Storrs. A chemical system for mass collection of virgin females or males.

A purine selector system has been devised to kill flies lacking xanthine dehydrogenase (XDH) activity (Glassman, E., Fed. Proc., 24: 1243, 1965). The purine (Sigma Chem. Co., P6880) is used as an aqueous solution, generally 0.2%. Parental flies are allowed to remain in fresh culture bottles for 2-3 days. Immediately after transfer, 1-2 ml. of 0.2% purine is evenly distributed over the surface of the already formed culture. This method is useful since it allows stocks to be maintained indefinitely by the usual transferring. When one sex is required the chemical selector is simply applied to the required number of cultures in the manner just described.

1. For attached-X virgin females: such females, with any desired combination of markers (except those with drastically reduced or no XDH activity) are kept with ma-1 males. The purine system will kill ma-1 males before eclosion leaving only virgin females.

2. For virgin males: method 1 is reversed so that desired males are kept with homozygous ma-1 attached-X females.

3. For free-X heterozygous virgin females: a variation of method 1 is potentially useful where virgin females are needed for (X or autosomal) fine structure analysis. Where the heterozygous female, a^X/a^Y, are required, virgin females of the type a^X/a^X, homozygous for ma-1, are crossed to a^Y males. After treatment, the daughters, being ma-1/ma-1+ having normal levels of XDH activity, will survive. The sons, being ma-1, will be eliminated.

Similar selector systems employing ry with X-translocations may be utilized in situations where ma-1 would be undesirable.

Since the purine system may be used for a variety of genotypes and culture conditions, the concentration of purine may have to be adjusted to maximize the results. We have noted that dilute aqueous purine is subject to destruction by mold and therefore make up fresh solution with clean glassware as required. Any unused solution is kept refrigerated. The purine concentrations described have been successful with our medium (cornmeal, agar, molasses, karo, brewers yeast, tegosept) used in half-pint creamers, but may well need adjusting when used with different media or with different volumes of media.

Leuthold, U. and Würgler, F.E. Swiss Federal Institute of Technology, Zürich, Switzerland. Egg collection from individual females of D. melanogaster.

As a standard procedure for the registration of X-ray induced damage in stage 14 oocytes virgin females are irradiated. The females are then mated with unirradiated males and allowed to lay eggs for 24 hours. With this procedure two difficulties arise: (a) variation in control mortality resulting from eggs deposited by non-inseminated females and (b) heterogeneity of oocyte stages tested if some females deposit large numbers of eggs. To avoid these difficulties the following modified method is used:

Females from uncrowded standard cultures[1] are collected as virgins and kept for 4 days in "feeding bottles" with well-yeasted medium[1]. On the 5th day the females are irradiated and mass mated with about 2 days old males in empty bottles in a dark room. About twice as many males as females are used. After 2 to 3 hours the females (which do not lay eggs in the empty bottles) are separated from the males and put individually into special egg collection arrangements in a room of 25°C. and 96% relative humidity. Each egg collection arrangement consists of a glass beaker (5 cm diameter, 9 cm high) standing upside down on a thick blotting paper and a small plastic bowl (1.5 cm diameter, 1 cm high) placed in a central position beneath the beaker. The bowl is two-thirds filled with fermenting egg laying medium[1]. A large area of the smoothed surface of this medium is covered with black paper soaked previously in 1% acetic acid. Since most of the liquid will be absorbed by the medium, more acetic acid is dropped into the paper. Females anaesthetized by CO_2 are brought individually under each beaker. As the black paper in the bowl is the only wet place in the arrangement the flies will deposit most of the eggs on it. Occasionally some eggs may be found on the free surface of the medium or on the wall of the bowl. During the egg collection period groups of 24 of these arrangements are brought under a light-tight cover which prevents the flies from disturbance by light changes in the experimental room. With this method an average of about 20 eggs per female are deposited within 3 hours. From

these eggs the rate of radiation induced dominant lethals can be determined.[2]

A test for insemination of the females is done in the following way: At the end of the collection period the females are put individually into small culture tubes with standard medium. Because younger stages of oogenesis are much less sensitive to the induction of dominant lethals by X-rays, even strongly irradiated females, which have been inseminated, will deposit viable eggs after some time. Examining these cultures for progeny after 6 to 7 days allows for the detection of non-inseminated females by the lack of larvae or pupae in the tubes.

This egg collection method initially developed for the stock "Berlin wild" has been successfully adapted to a strain (XY/XY) with retarded maturation of the flies and reduced rate of oviposition. In this case 6 days old virgin females were used and the mating period as well as the egg collection period have been prolonged to 4 hours. With similar modifications the method has been used for experiments with a triploid strain and for tests where inseminated females were irradiated (Lütolf, Graf, unpublished).

The method can also be adopted for dominant lethal tests after irradiation of mature sperms in males. In this case single irradiated males are mated for a few hours with single females in small empty tubes. Then the females are put individually into the egg collection arrangements. Egg collection can be extended to many hours (e.g. overnight) since the cells to be tested have been transferred by a single copulation to the females, and no difficulties from differential radiosensitivity of various cell stages can appear.

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References: 1. Würgler, F.E., Ulrich, H. and Spring, H.W. Experientia 24: 1082, 1968. 2. Würgler, F.E., Petermann, U. and Ulrich, H. Experientia 24: 1293, 1968.

Williamson, J.H. and P. Stubblefield. University of California, Riverside, California. An efficient method of collecting homogeneous samples of stage 14 oocytes.

A cursory review of the pertinent literature will convince anyone that previously used methods of collecting samples of stage 14 oocytes are based on hearsay. Usually females are aged several days, mated, and the number of eggs per female limited to a maximum of 24. This practice is based on the assumption of twelve ovarioles per ovary and one stage 14 oocyte per ovariole. Our technique (borrowed from D.R. Parker) is to rear females in uncrowded cultures, collect virgins at twelve-hour intervals, and to store females for four days on new culture medium sprinkled with live dry yeast. Females are then lightly etherized, put into gelatin capsules, allowed to recover, irradiated and mated without etherization to males that had also been aged on yeasted medium. Matings were made on food warmed to room temperature and held at 25° C. with lights for twelve hours at which time all flies are discarded. Egg counts from individual females revealed that many produced more than 24 eggs in twelve hours. Subsequently two samples of 30 C(1)RM, y v bb / B^{S}Yy+ females, one group aged for four days, the other five days, were dissected and the number of stage 14 oocytes per female determined. The 4-day old females averaged 43.4 stage 14's (range: 22-68) and the 5-day old females averaged 45.8 stage 14's (range: 24-74). In most cases each ovariole contained two or three stage 14's and all ovaries were made up of 16 or 18 ovarioles. Ovarioles with three stage 14's contained no additional oocytes of intermediate stages, and only a few very early stages. A third group of thirty females of the same genotype, 4 days old, produced an average of 25 eggs per female in a twelve-hour interval (range:0-68).

Wild type females from a cross of Canton-S and Guasti-36-10 were collected and aged 4 days as described above. Fifty-nine females were dissected and averaged 84.4 stage 14's per female (range: 52-111). Sixty-three females from a cross of Oregon-R and Guasti-36-10 averaged 70.2 stage 14's per females (range: 39-104). Apparently strains differ in the rate of egg production and each experimental strain should be analyzed accordingly. It seems reasonable that as long as the number of eggs laid in a twelve-hour interval does not exceed the number of "stored" stage 14 oocytes, one can assume that a homogeneous sample is obtained.