A New Method for Rearing *Drosophila*[1]

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The familiar half-pint bottle containing a culture medium has provided an extraordinarily simple and generally satisfactory means of rearing species of *Drosophila*. In fact, it is conceded that there would be no particular advantage in making a change if the maintenance of stock cultures were the sole or principal purpose in mind. However, in attempting to determine the important variables involved in population growth of *Drosophila*, the authors found it necessary to devise a new technique that would provide a better chance for the manipulation and control of these variables. The method to be described satisfied this need beyond expectation, and, in addition, offers many other advantages for research and teaching in the fields of genetics, physiology, ecology, toxicology, and general biology.

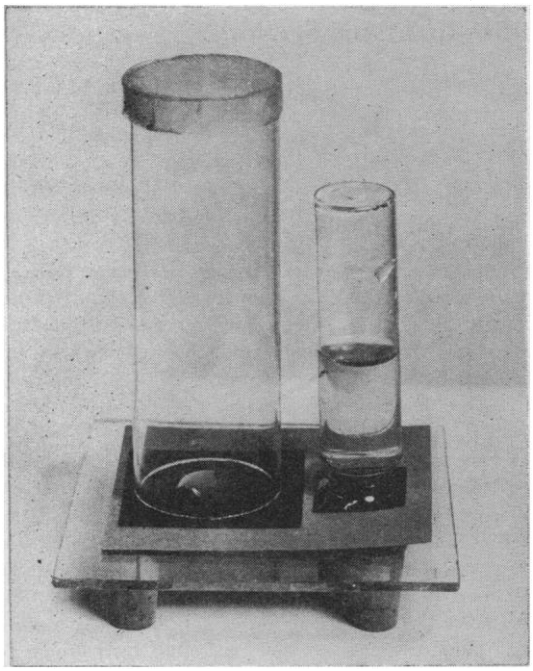

Fig. 1. Oviposition cage assembly.

The equipment used for the purpose of obtaining eggs is shown in Fig. 1. It consists of a base plate of glass, 4″ square, supported by cork-stopper legs held in place with Duco cement. A piece of seed-germinating blotting paper[2] is placed on the glass plate, two smaller pieces of the same paper (shown in the illustrations as black squares for contrast) being placed on the larger sheet. The oviposition cage consists of a glass cylinder, 4½″ high by 1½″ in diameter, covered with a silk bolting-cloth cap held in place with a double celluloid collar. The blotting paper assembly is kept moist, to a highly satisfactory degree, by water which is drawn from a 6-dram homeopathic vial used as a reservoir. A small notch filed in the lip of the vial permits an uninterrupted flow of water to replace that lost by evaporation.

The materials necessary for rearing larvae are the same as those described for oviposition with the exception of the rearing chamber illustrated in Fig. 2. This chamber is made from a glass cylinder 1½″ in both height and diameter. The bottom is sealed to prevent the escape of larvae by dipping the lower rim of the cylinder in melted beeswax; the cylinder is then pressed firmly on a separate square of blotting paper until the wax hardens.

Ordinary desk blotting paper can be substituted for germination blotting paper. The latter is better, however, particularly for use as the floor of the rearing chamber. Any loosely woven cloth that will prevent fly escape can be used instead of silk bolting cloth.

When eggs are desired, the first step in the process is to draw a circle, of the same diameter as that of the glass cylinder, on a square of blotting paper a little larger than the diameter of the cylinder. Then a line,

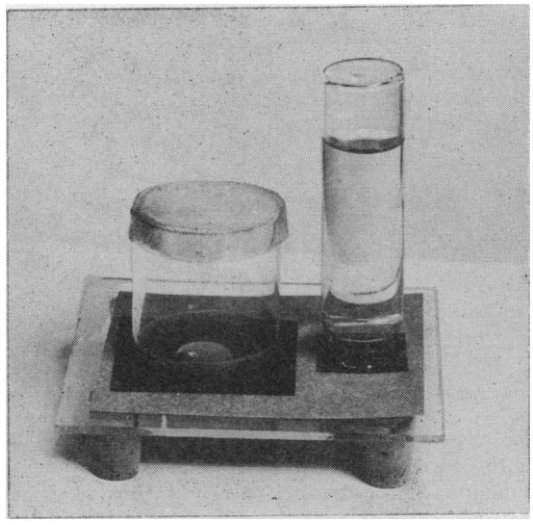

Fig. 2. Larval rearing chamber assembly.

marking off about one-third of the circle area, is scratched on the paper with a needle. Several other lines are scratched lightly across the surface of the remaining two-thirds of the circle drawn on the blotting paper. About 2 cc of a food consisting of equal parts by weight of cake yeast and honey is smeared on the surface of the paper in the smaller area marked out as shown in Fig. 3. Following this preparation of the feeding and oviposition surface, flies are introduced into the glass cylinder and the cylinder placed over the circle drawn on the blotter. They become adjusted very quickly and soon begin to oviposit, principally in the multiple scratches made on the free blotter surface. In the example illustrated in

[1] Paper No. 2367, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Manufactured by the Rochester Paper Company, Rochester, Michigan.

Fig. 3, 58 female flies of the species *D. melanogaster* were allowed to feed and oviposit for 3 hrs. In this short time they produced a total of 1,946 eggs, 94.6% of which were laid along the scratches, 3.1% on the food, and the remaining 2.3% on the free blotter surface between scratches. When eggs are laid in this way, they are easy to count and to remove.

The rearing of larvae is accomplished by the use of the set up shown in Fig. 2. Before eggs are placed in the rearing chamber a quantity of food consisting of equal parts of cake yeast and tap water is dropped on the blotter surface. This food is used instead of the yeast-honey mixture because it does not become sticky or ferment, and yet it produces large, vigorous flies. Again the homeopathic vial water reservoir serves to keep the entire system moist. When eggs are to be used, the oviposition chamber, with its blotter base, is inverted, and the top is tapped gently to force the flies downward. Then the floor of the chamber can be removed and replaced with a fresh oviposition surface. That portion of the blotting paper bearing eggs is cut off and placed on edge in the rearing chamber so that the lower margin

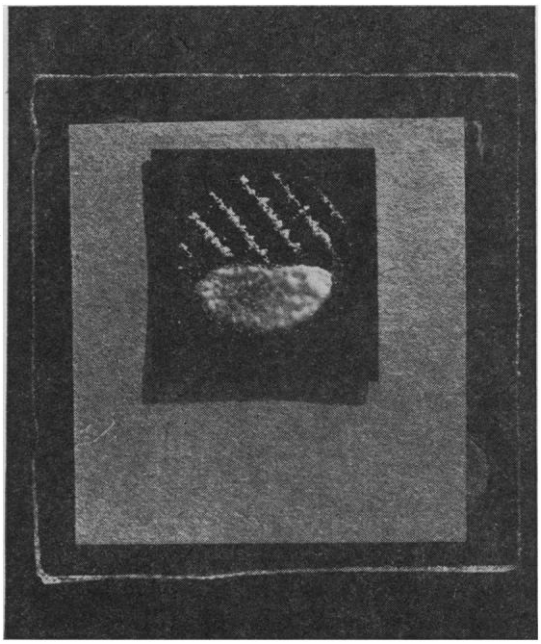

FIG. 3. Surface view of floor of oviposition cage showing food smears and rows of eggs deposited in scratches made on blotter surface.

makes a good contact with the moist floor of the chamber. This position of the egg-bearing piece of blotter is important to insure a thorough wetting and, further, to prevent the eggs from becoming submerged in the food mixture. Upon hatching, the larvae can move easily to the pool of food. Additional food should be added when necessary or, more specifically, at any time when the larvae start wandering around the chamber. If an adequate amount of food is supplied, there will be very little larval wandering and little tendency for them to bore through the blotting paper floor. Pupation is completed successfully without the necessity of providing a special surface. Frequently 1,000 or more flies of good size emerge in one of these small rearing chambers.

After emergence of the adults in the rearing chamber they can be removed by using either one of two methods. First, if the flies are to be anesthetized, the rearing chamber is inverted over an ether bottle with a mouth of about the same diameter. Second, when active flies are to be transferred, they are forced to the bottom by striking the rearing chamber sharply on the table top, after which the cap is removed and a glass oviposition cylinder set over the opening with a minimum of loss from escaped flies. Finally, the blotter prepared for feeding and oviposition can be slipped between the two chambers, and the cylinder moved back to the platform.

One of the important things upon which the success of the method depends is the maintenance of a supply of water to keep blotter surface and atmosphere above moist. The 6-dram vial used by the authors has supplied sufficient water to accomplish this purpose for about 24 hrs at ordinary room temperature and over a considerable range of relative humidity. The use of a small separate square of blotting paper under the water reservoir makes it possible to refill the reservoir without inverting the entire platform. The vial can be inverted easily by lifting it after a spatula has been slipped under the small square of paper. After the vial has been refilled and the paper square replaced, the vial can be inverted again without spilling water if a little care is exercised.

Among the most important advantages afforded by this new method is the fact that a ventilated system is provided which prevents the accumulation of gaseous products of metabolism; at the same time it is possible to maintain nearly constant moisture conditions in both the atmosphere and substrate. Within this open, yet humid, environment there is still another advantage over the standard milk bottle method. The food and substrate are easier to prepare and to maintain in a condition favorable for both oviposition and development. In addition, both the size of the system and the character of the food can be modified readily.[3]

As a result of these general advantages, with this technique, it is easier to measure such biological constants as fecundity, fertility, and survival and to isolate and place any growth stage under close examination or experimentation. It is also possible to produce large numbers of standard flies and to recover filial generations more easily, but it should be understood, that this method does not take the place of the usual *Drosophila* rearing method when only maintenance of stocks is desired.

References

1. ALPATOV, W. W. *J. exp. Zool.*, 1932, **63**, 85–111.
2. HARNLY, M. H. *J. exp. Zool.*, 1929, **53**, 141–170.

[3] Modifications of the standard method for the purpose of obtaining eggs have been developed by Alpatov (*1*), Harnly (*2*), and others. Although these methods greatly simplified the task of counting eggs, they still involve a tightly closed system and a variable substrate.