# DROSOPHILA CULTURE WITH A MINIMUM OF AGAR

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Since the early days of *Drosophila* culture, agar has served as an important ingredient of standard Drosophila media. At present agar stocks in many *Drosophila* laboratories are rapidly nearing depletion. Reduced laboratory personnel, the prohibitive price of agar and the prior claims on it for important bacteriological work constitute a serious problem. However, it seems patent that the Drosophila work should be continued at least to the extent of preserving valuable stock cultures which represent many years of careful work and which could in many cases not be replaced if once lost. For years the author has been particularly interested in culture methods, and therefore makes some suggestions here which he hopes may be helpful.

Stock cultures of the various *Drosophila* species are best kept in containers at least as large as quarter pint bottles and preferably in half pint bottles rather than in vials. The larger containers with larger food mass provide a margin of safety where reculturing is not done strictly on schedule. It is important to use enough adult flies in making up a fresh culture so as to eliminate chance of losing the culture through the accidental death or sterility of a few of the parent flies. As the adult flies feed on solid or semi-solid surfaces and do not burrow into media their food supply is determined by total exposed surface and not by volume of food. However, as flies easily become mired in soft food a medium with an agar base is by far the most convenient and satisfactory. Some laboratories have with some degree of success increased the corn-meal and oat-meal content of the medium and reduced or eliminated the agar content. However, such media are difficult to pour and not entirely satisfactory. For stock cultures we have reduced the agar content somewhat, but pour only a thin layer of medium about one fourth inch deep in half pint bottles. This gives the same feeding surface for adults as medium poured to a depth of an inch and a half. In this way we can pour from 125 to 150 half pint culture bottles with the use of 25 grams of agar. Then after a week or somewhat longer in case of slow breeding species a plentiful supply of food is added for the young larvae. This food contains no agar. It may consist of chunks of corn-meal, Karo, yeast mixture or more frequently we use a sheet of facial tissue dipped in a fresh suspension of baker's yeast in water, about 100 grams of yeast to 600 cc. of water. Thus on a minimum of agar adult flies are provided adequate surface for feeding and later larvae are supplied adequate volume of food. With this procedure there is no difficulty encountered in reculturing as the food mass has formed a cake which does not easily shake loose by the time a new generation emerges.

For experimental cultures (but not for stocks) the following method is used. Corn-meal, Karo, agar medium enriched with brewer's yeast is made up and poured into suitable containers such as large covered glass dishes or aluminum pans. As Moldex is used, dishes of this medium may be kept in the refrigerator for a week or longer. A supply of cardboard parallelograms about an inch and a half long and cut on the bias is made up. A dish of medium is turned out onto a flat tray and cut into blocks about one inch long and a quarter inch thick. Cardboard strips and blocks of medium are of a width to fit into the size of vial used. Blocks of medium are placed on the cardboard strips and a drop of heavy yeast suspension added. For convenience in handling, glass vials are held together

in sets of seven with rubber bands. These vials are set out on the table, pairs of etherized flies dropped into them and then the vials are turned on their sides, cardboard strips with medium inserted, vials plugged and placed in the horizontal position in trays, and allowed to stand for the deposition of eggs and emergence of young larvae. After several days the vials are unplugged, parent flies released, and with forceps the cardboard strips are removed. Into each vial is poured a suspension of baker's yeast in water (see above), and a sheet or part of a sheet of facial tissue folded once and pushed down to the bottom of the vial. For a large vial 100 mm. x 15 mm., one fourth full of yeast suspension, a double sheet of facial tissue will absorb the liquid. The tissue should then be pulled back slightly from the bottom of the vial to ensure the complete soaking up of the liquid medium. The cardboard strip containing medium, eggs and larvae is then dropped in on the paper and the vial replugged and set in an upright position.

The larvae burrow very readily all through the paper base, provided too much yeast suspension has not been used. Experience will indicate how much paper and yeast suspension to use for vials of different sizes. Pupation takes place mostly in the paper. By this method the yield per vial is large, the flies are of uniform size, reach maximum size for the species, and owing to the abundant and easily available food supply larval competition is cut to a minimum. There is less spread in the emergence period than with other media. However, flies should be examined within two or three days of emergence as the paper-yeast suspension is not an adequate diet for adults. A small piece of fresh solid medium added after pupation will keep the adults over if they cannot be examined soon after emergence.

For some years I have used the method described above for all experimental cultures. For such large species as *Drosophila hydei*, *D. robusta*, and *D. funebris* yields of 300 flies per vial are not uncommon, and the average yield is over 150 flies. Furthermore, vials are more easily cleaned than where agar medium has been poured into them. Even where there is an abundant agar supply the author recommends this procedure for experimental cultures. In case agar supplies are depleted, thick corn-meal or other culture media which are difficult to pour might readily be cut into blocks for use as described above.