Kircher, Henry W., Kenneth G. Goodnight, Robert W. Jensen. University of Arizona, Tucson, Arizona. A medium for Drosophila that are difficult to rear in the laboratory.

Many species of Drosophila spend their larval stage in leaves that are rotting on the forest floor in the Hawaiian Islands (1). Most of these species cannot be maintained in the laboratory on normal banana media or even the enriched medium recently devised by Wheeler and Clayton (2). We had some success rearing these flies last summer in Hawaii with the following medium. It's presented here as a starting point for further nutritional experiments with species of Drosophila that are difficult to rear in the laboratory.

Ingredients:

1. Brewer's yeast, U.S.P., is stirred overnight three times with several volumes of 2:1 v/v chloroform-methanol to remove the lipid fraction (Note 1). Between extractions the yeast is filtered thoroughly with a vacuum on a Buchner funnel. The final product is dried in air and finally in an oven at 50°C until no trace of solvent remains.
2. Turtox brand agar, General Biological Supply House, Chicago, Ill. (Note 2).
3. Fructose, propionic acid.
4. Enzymatic hydrolysate of soy protein, Nutritional Biochemicals Co., Cleveland, Ohio (Note 3).
5. B-Vitamins, thymine and choline chloride.
6. β-Sitosterol, cholesterol and ergosterol.

Preparation of One Liter of Medium:

1. The extracted yeast (35g.) is ground thoroughly in a mortar with 0.5 g. of the sterol. A single sterol or a mixture of the three above can be used.
2. The following vitamins, thymine and choline chloride are dissolved (riboflavin dispersed) in 100 ml. water. The mixture is stirred magnetically before a 10 ml. aliquot is added to 1 liter of medium. It is conveniently kept in the refrigerator in a small flask containing a magnetic stirring bar.

For 10 liters of medium:

Thiamine	20 mg.
Nicotinic acid	120 mg.
Riboflavin	100 mg.
Calcium pantothenate	160 mg.
Biotin	4 mg.
Pyridoxine	25 mg.
Folic acid	30 mg.
Thymine	20 mg.
Choline chloride	600 mg.

3. One liter of water (Note 4) add 15 g. agar is brought to a boil. To the hot solution is added 10 g. fructose, 20 g. soy protein hydrolysate, 10 ml. of the vitamin suspension, 5 ml. propionic acid and 35 g. of the yeast-sterol mixture.
4. After the yeast has been thoroughly wetted and dispersed, the medium is autoclaved (20 min., 15 psig), and when cool, is poured into autoclaved stoppered shell vials. It can be used for axenic or xenic rearing of Drosophila.

Note. 1. The leaf-breeding Hawaiian Drosophila do not use yeasts in nature. The yeast sterols (mainly ergosterol) may inhibit the utilization of the necessary sterol (cholesterol or β-sitosterol). Other yeast lipids may also be disadvantageous to the flies.

Note 2. Erk and Sang (3) have reported that Difco Bacto-agar is toxic to certain species of Drosophila.

Note 3. Hagen (4) has shown that the oriental fruit fly, when grown axenically, does much better when a partially hydrolyzed protein is furnished the young larvae. We have also observed this in our work with D. pachea. With this species, enzymatically hydrolyzed casein was toxic.

Note 4. The replacement of water with a hot aqueous extract of the plant or the lipid extract of the plant in which the Drosophila larvae are found may be beneficial here to supply feeding or ovipositional stimulants.

References

1. W. B. Heed, University of Arizona, unpublished work.
2. M. R. Wheeler and F. Clayton DIS, 40: 98 (1965).
3. F. C. Erk and J. R. Sang, J. Insect Physiol., 12, 43 (1966).
4. K. S. Hagen, Nature, 209, 423 (1966).