Spieth, H.T. University of California, Davis, California. Rearing techniques for the Hawaiian species Drosophila grimshawi and Drosophila crucigera.

The species of endemic Hawaiian Drosophila are notoriously difficult to rear under laboratory conditions. Modification of standard rearing procedures and food media (Wheeler and Clayton 1965) has enabled several laboratories to maintain stocks of a number of species but an inordinate amount of attention and time must be given the flies. Furthermore, pair matings have been difficult to achieve and typically only mass matings are successful. The following procedures have, however, been developed for rearing both D. grimshawi and D. crucigera in large numbers with relatively small time, energy and cost expenditures. Pair matings can be successfully achieved with adult mortality reduced to a minimum.

REARING UNITS. Square-shaped pint milk bottles with standard cotton or other type plugs are used as rearing containers.

FOOD STICKS. Cut rectangular strips (115 mm x 13 mm) from 3 mm (1/8") thick sheets of plexiglas. Cut plexiglas tubing, O.D. 22 mm (7/8"), I.D. 19 mm (3/4"), transversely into short sections, i.e. 19 mm (3/4") long. Cement three sections of tubing with Dupont household cement into a compact row crosswise in the central area of one side of a plexiglas stick. Such a food stick can be inserted into a large mouthed milk bottle, but I use small mouthed bottles with a 1 1/8" opening. It is necessary therefore after the cement is thoroughly dry to bevel the outer edges of the three pieces of tubing. This is easily accomplished by holding the assembled food stick at an angle against a power driven sanding belt. Small half moons of material are sanded away from both sides of each of the three tubular sections until the food stick can be easily inserted into the rearing bottle. The sanded material collects as a mass on the inner surfaces of the cylinders and can be removed after cooling.

FOOD SPONGES. Using a #12 cork borer, cut sponge plugs 3/4" thick from sheets of dry cellulose sponges. Thoroughly wash the sponge plugs, using a laboratory detergent, and thoroughly rinse; squeeze out the excess water and place the damp sponges into glass jars capped with aluminum foil; sterilize on dry cycle. The sterilized, slightly damp sponges fit snugly in the tubes of the food sticks and readily absorb the liquid media.

LIQUID MEDIUM. Banana-Malt-Liver (BML)

500 ml distilled water	10 gm Difco Bacto-Liver
80 gm banana peel	2.5 ml propionic acid
25 gm Fleischmann's Dry Diamalt	2.5 ml ethyl alcohol

PREPARATION OF BML. The bananas from which the peel is taken should be firm, i.e. slightly under-ripe with a yellow to greenish-yellow color. Place the peel, Diamalt, Bacto-liver, and approximately 400 cc water in a blender; blend at moderate speed until the peel is moderately disintegrated. The peel should not be completely pulverized. Place the mixture in a cooking vessel and use the remaining water to rinse the blender and add the liquid to the cooking vessel. Bring medium to a boil, simmer for 10 minutes, remove and pour into a large sterile pyrex bottle. Then add the propionic acid-ethyl alcohol mixture to the food, agitate, and store under refrigeration until used. The liquid medium should have a pH of approximately 4.0 and will keep well for several weeks.

SOLID MEDIUM. Diamalt, Cereal and Liver (DCL)

1000 ml distilled water

8 gm agar	20 gm Difco Bacto-Liver
45 gm cereal mixture *	160 gm banana peel
10 gm brewer's yeast **	5 ml propionic acid
45 gm Fleischmann's Dry Diamalt	5 ml ethyl alcohol

* Cereal mixture: 15 gm Gerber's High Protein Cereal
5 gm Kellogg's Concentrate
15 gm Kretschmer's Wheat Germ
10 gm Kellogg's Special K Cereal

Place all four dry ingredients in a blender and blend until thoroughly homogenized. It is convenient to prepare a large amount of cereal mixture beforehand and to store it in the refrigerator.

** I used Wheast instead of brewer's yeast. Wheast is a granular material consisting of inactive, dried Saccharomyces fragilis yeast which has been grown on fresh cottage cheese whey. It is available

from the Knudsen Creamery Co., P.O. Box 2335, Terminal Annex, Los Angeles, California 90054.

PREPARATION OF DCL. Place the agar and 500 cc water in a cooking vessel and heat until the agar is dissolved.

Place the banana peel and 400 cc water in a blender; blend until the peel is thoroughly homogenized. Add remaining solid ingredients and blend combined mixture until thoroughly homogenized. Add mixture to the agar-water in the cooking container, rinse the blender jar with remaining 100 cc water and add the liquid to the cooking vessel. Bring contents to a boil; simmer 10 minutes; remove from heat and allow to cool a few minutes. Add the alcohol-propionic mixture and stir thoroughly. Pour the hot medium into large shell vials or bottles, plug and store under refrigeration.

PREPARATION OF REARING BOTTLES. Fold a Tomac Kerchief or similar type material to form a pad approximately the size of the bottom of the rearing bottle. Insert the pad so arranged that it covers the bottom of the bottle. Use the pad without the addition of water.

Insert a sponge plug into each of the three cylindrical holders on a food stick and then thoroughly wet (i.e. soak the sponge) with the following liquids:

Sponge 1. distilled water

Sponge 2. 10% Karo solution (i.e. 10 cc light Karo syrup dissolved in 90 cc distilled water, boiled 10 minutes)

Sponge 3. BML liquid. Along with the amber colored liquid a sufficient amount of fine particles of the banana peel should be dispensed to form a thin coating of peel fragments on the end of the sponge plug.

I typically insert the sponge plugs into a number of food sticks, laying each food stick on its side with the ends of the sponge plugs facing upward. Using polythene wash bottles, one for each liquid, I squirt the liquids onto the sponge plugs until they are thoroughly wet.

Introduce 15 to 25 adult flies into the rearing bottle; drop in a prepared food stick and plug the bottle tightly with a cotton stopper.

Adults of D. crucigera and grimshawi cannot withstand prolonged exposure to temperatures exceeding 20°-21°C and are most comfortable at 18°-20° (65°-68°F). Under such conditions females will begin to lay eggs at 24 days but will not reach full productivity until 36 days. The flies are changed every 7 days at which time the food sticks of the 24+ day old flies are checked under a binocular scope for eggs, which can be identified by the appearance of long respiratory filaments on the surface of the BML sponge.

If eggs have been laid, withdraw the sponge with forceps and introduce into a vial or bottle containing solid food (DCL); plug the container with cotton. Lay the sponge on its side so that the egg filaments are exposed to the atmosphere. Return the vial containing the egg-bearing sponge to the rearing cabinet.

The mature larvae of both crucigera and grimshawi refuse to pupate in the solid food vials; rather, they engage in a period of wandering. In the field they leave their natural food sites and eventually burrow into the soil where pupation occurs. Natural conditions can be simulated by utilizing "sand jars".

Using a one quart or half-gallon mason jar with a screw-top lid, place a 3 to 4 inch layer of well-washed dry sand in the jar and wet well (but do not soak) with distilled water. After wetting the sand, unplug the vials containing the larvae and set the open vial upright in the sand layer. Discard the bell dome lid but retain the band. Place a 5 x 5" piece of fine bolting silk (grit gauze) on top of the jar and place the band over this; screw down tightly so that the cloth is smoothly stretched in place. I use #53 Nitex bolting silk sold by Trabler, Ernst and Tralber, 420 Saw Mill River Road, Elmsford, N.Y. If grit gauze is unavailable, muslin cloth can be substituted.

The mature larvae crawl out of the food vial over the surface of the sand, often up the sides of the jar; unless there exists a humidity gradient in the jar they will vigorously attempt to crawl out of the jar. They cannot penetrate the bolting silk or moderately heavy muslin but can and do penetrate almost any cotton plug. Eventually they burrow into the sand and pupate 1 - 3" under the surface. If the sand is too wet, they drown; if too dry, they desiccate.

The adult eventually emerges from the pupal case, pushes its way upward through the sand and then can be collected. The most effective method is to use an aspirator. I use one made

from a section of glass tubing, one end of which is covered with a piece of bolting silk and thrust into a section of rubber or tygon tubing. Adults should be collected on the day that they emerge and immediately placed on fresh food.

NOTES OF CAUTION. (1) The sponges in the food sticks must be thoroughly soaked, and especially the distilled water sponge. The adults must have liquid other than that in the food. The humidity in the bottle must be kept high, at least 90% relative humidity. If the rearing bottles are tightly plugged and if the humidity of the rearing room or chamber is not excessively low, the amount of liquid in the three sponges of a food stick will maintain the humidity in the bottles at an adequate level for 7 - 8 days. (2) The flies should not be crowded for they are pugnacious, especially the males. Fighting breaks their wings and increases mortality. (3) The sponge plugs can be repeatedly re-used if they are washed, rinsed and sterilized after removal from old food sticls. (4) The food sticks should be washed, rinsed, and dried after use but will not withstand sterilization. (5) The bananas should be washed in a mild detergent solution and then thoroughly rinsed before they are peeled.

Reference: 1. Wheeler, M.R. and F.E. Clayton 1965 DIS 40:98.

Travaglini, E.C. and D. Tartof. Institute for Cancer Research, Philadelphia, Pennsylvania. "Instant" Drosophila: A method for mass culturing large numbers of Drosophila.

In order to breed large numbers of Drosophila with minimum effort, we have modified Doane's procedure (DIS 45:189) for rearing larvae by substituting cellucotton (absorbent wadding, non-sterilized, obtainable at The Drug House, Philadelphia, Pennsylvania) for the plastic pad used for the substratum on which the larvae feed. Since the larvae ingest the cellucotton when it is soaked with a yeast-sucrose solution, they tend to tunnel through the cellucotton and therefore have a larger surface area to feed upon than the pad surface alone, and because of the easier access to food, it allows larger numbers of larvae to be raised in a box. Also, the cellucotton is disposable after being used.

The procedure is as follows: three layers of cellucotton are placed in the bottom of each polyethylene plastic box (5-1/2" x 7-1/2" x 3-1/2"; Freezette-flat, manufactured by Polly-flex) in whose detachable lid a ventilation hole (3" diam.) has been cut and covered by two layers of double-thick gauze secured with tape. To each box, 200 ml of the following medium is added (40 g of fresh brewer's yeast, 20 g of sucrose, and 140 ml of acid mix A). Acid mix A (0.4% propionic acid and 0.06% phosphoric acid) (Lewis, E.B., DIS 34:117) can be made up in ten liter quantities and stored at room temperature until used. Then, on the surface of each pad, a filter paper (2" diam., Whatman #1) containing 0.5 - 0.7 ml of fertilized eggs (proferably 18-hr embryos) is placed. Care should be taken not to drown or dry out these embryos before they hatch; the filter paper should be damp but not wet! If flies are desired, the boxes are incubated at 25°C in a properly ventilated area for six days and then after pupation, the cellucotton pads are transferred directly to population cages where the flies will hatch; after hatching, the cellucotton pads are discarded. Each box will yield approximately 5-7 g of adult flies.

If larvae are to be harvested, 100 ml of H_2O should be added to the cellucotton in each box just as the larvae begin to climb and another 300 ml H_2O 1 hour before the actual harvest takes place; the water will cause all the larvae to climb out of the cellucotton onto the sides of the box. The larvae are harvested by scraping them from the sides of the box with a spatula. Each box yields approximately 12 g of uniformly sized larvae.

This method has also been adapted to breeding flies in stock bottles over a period of two weeks at 25°C. In order to control the pH of the nutrient medium over this period of time, the yeast-sucrose medium had to be modified. The procedure is as follows: a wad of cellucotton (2" x 8", 2 layers thick) is pushed into the bottom of a bottle and this is wetted by the addition of a mixture of 6 g yeast, 3 g sucrose and 50 ml acid mix A, then 10 pairs of mature adults are put into a bottle. Each bottle yields approximately 1 g of flies.

(Work supported by USPHS grants CA-01613, CA-06927 and RR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.)