# CHAPTER 5

# Raising Large Quantities of *Drosophila* for Biochemical Experiments

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## I. Introduction

Many experiments using the techniques of biochemistry and molecular biology require large amounts of experimental material. *Drosophila* is a suitable organism for such efforts; it is relatively easy to grow adequate quantities of embryos, larvae, and adults. Mass cultures of *Drosophila* have been used in the preparation of a variety of cellular components, including RNA, proteins, DNA, and nuclei. In this chapter, we describe the system we use to rear and maintain a population of about 400,000 flies capable of producing 100 g of embryos a day.

METHODS IN CELL BIOLOGY, VOL. 44

## II. The *Drosophila* Colony

The minimal requirements for growth of the flies are simple and can be easily met. However, to obtain maximum yield of embryos, the following environmental conditions should be maintained: humidity of 70%, temperature of 25°C, and a constant 24-hr light–dark cycle. Constant conditions are critical if a strict schedule of inoculation, feeding, and collection is to be maintained. For added convenience, one may use a reverse light cycle to take advantage of the burst in egg-laying at dusk (a 50–100% increase in the number of embryos/hour for the first dark hours). Twenty-four-hour cycles of 12–14 hr light, 12–10 hr dark have been successfully used; the 12:12-hr cycle appears to be more readily entrained (Rensing and Hardeland, 1967). The maintenance of stringent environmental conditions requires some mechanical sophistication, such as a specially constructed room containing temperature and humidity servomechanisms (variation should be no more than ±0.3°C, ±2% humidity), even air flow distribution, and a light-regulating timing unit. Such a control chamber is initially expensive; however, close control will result in at least a doubling of productivity (in grams of embryos/hour/fly), which is highly significant in view of labor costs.

The following system was developed by D. S. Hogness, W. J. Peacock, and L. Prestidge at Stanford University Medical School Department of Biochemistry and has been used in our lab for over 20 years (Elgin and Miller, 1978). Embryos are harvested from feeding trays placed in population cages holding 50,000 adults. The adult flies are raised over a 22-day cycle, with cycles overlapping so that when one adult population has become less productive, another is ready to take its place. More closely overlapping cycles may also be used.

Day 1: Inoculation of containers with embryos (population A)
Day 5: Feed larvae (A) at third instar
Day 12: Transfer flies (A) to cages
Day 15: Inoculation of containers with embryos (population B)
Day 19: Feed larvae (B) at third instar
Day 22: Dispose of adults (A)
Day 26: Transfer flies (B) to cages

Egg laying generally peaks on the third or fourth day after transfer of the adults and declines linearly at about 5% per day thereafter. A population of 400,000 (eight cages) will produce 30–120 g embryos/day, depending on the age of the adults and strain used.

### A. Feeding of Flies—Harvesting of Embryos

The adult flies are fed yeast food (see recipes below). A single strip of yeast paste is spread on a grape plate, and the whole plate is placed inside the fly cage. Although the grape plate is probably fed on lightly, its predominant

purpose is to stimulate egg laying on its surface as a consequence of the attractive aroma and high surface humidity. The amount of yeast to be spread on the plate is determined by the amount of time the plate is to be left in the cage. For 6- to 12-hr collections, we find that a large strip of yeast about 3 cm wide and 0.5 cm deep down the center of the long axis of a grape plate is sufficient. If desirable, the yeast can be omitted for a short-time collection ($\leq$ 2 hr). However, the rate of egg production can be severely compromised if the flies remain unfed for longer periods of time. Also, if short collection times are used, it is advisable to avoid using grape plates directly from the refrigerator. Instead, allow the plates to warm up to room temperature to increase the yield of embryos.

At each collection one unties the curtain and shakes vigorously to get all flies into the cage; grape plates can then be inserted and/or removed. To remove grape plates, pick them up, turn vertically, and bang sharply on the bottom of the cage to knock off the adults. Bring the plate into the curtain tube and blow on the plate to remove any remaining adults. After the plate is removed, shake any adults in the curtain tube back into the cage and retie the curtain. (It should be noted that this technique creates a considerable amount of airborne allergen; people susceptible to hay fever, etc. will frequently become allergic to *Drosophila*. We recommend the use of 3M's 8500 Comfort Mask and gloves for all persons making transfers and collecting grape plates.)

The feeding and collection schedule used will depend on the stage desired. For collections to isolate nuclei, we use a schedule with three collection times a day: 9 a.m., 3 p.m., and 9 p.m. The overnight (9 p.m.–9 a.m.) collection is allowed to mature for 6 hr (9 a.m.–3 p.m.); it is then harvested and stored frozen at −80°C. The morning (9 a.m.–3 p.m.) and evening collections (3 p.m.–9 p.m.) are aged for 6 and 12 hr, respectively, to give more precisely staged embryos (6–12 hr and 12–18 hr). If very large amounts of fresh rather than frozen embryos are desired, one can cover and store the grape plates at 4°C until sufficient embryos have been recovered. It has been reported that embryos can be stored for up to 3 days at 4°C (Biggin and Tjian, 1988).

When the embryos are ready to harvest, they are washed off the plates using saline solution (7 g NaCl/liter) and collected in a Teflon-coated pan. A nylon paint brush and a squirt bottle containing the saline solution are useful in this procedure. Any remaining lumps of yeast and embryos are resuspended using the paint brush. For ease in completing this and the subsequent washing steps, the yeast food originally placed on the plate is usually left behind and the embryos are collected by brushing the area not covered by yeast. When all the plates have been cleaned, the saline solution containing embryos and contaminating adults is differentially filtered using a plexiglass filter holder (see Fig. 1). A screen of 630-mesh Nitex, held within the plexiglass lid that fits atop the upper cylinder, is used to trap the adults. The 116-mesh Nitex screen below (Nitex No. 3-160/53) is held in place between the two Plexiglass cylinders. The 116-mesh Nitex is cut to fit the cylinders and trimmed to weigh exactly 1 g to

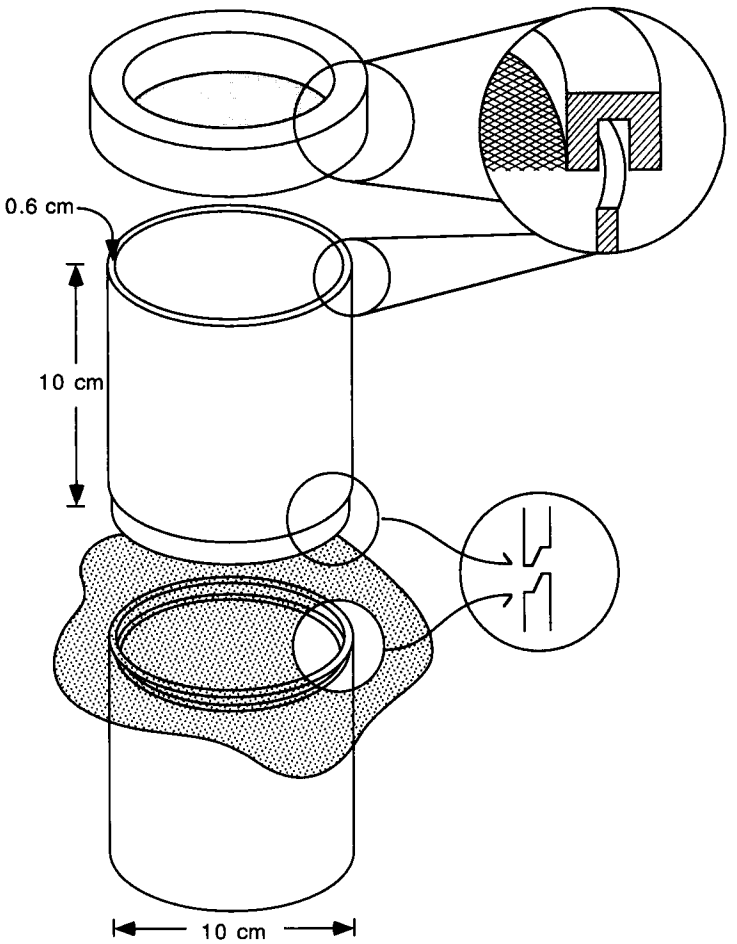


**Fig. 1** Plexiglass filter holder for washing embryos. The top piece is filled with Nitex H630 to trap adults and designed to fit down inside the top cylinder. The lower filter, Nitex H116, will trap embryos and is held in place by the interlocking cylinders.

assist in determining the weight of the collected embryos. The embryos are washed thoroughly with water at room temperature (or colder) and finally washed with saline solution. After the final wash, the cylinders are separated and the mesh is folded with the embryos inside. The mesh/embryo packet is then placed between several layers of paper towels, and the entire stack is gently pressed to remove wash solution. The packet is then weighed to determine the amount of embryos collected.

The washed embryos can be used immediately for experimental purposes or to continue the population; alternatively, they can be frozen at −80°C for future

experiments. If the embryos are to be frozen, they are removed from the Nitex, transferred to foil, wrapped tightly, and frozen in flat packages of no more than 40 g. The frozen embryos appear to be the equivalent of fresh embryos for the preparation of nuclei for several months; subsequently, some desiccation appears to occur and the yield of nuclei/gram embryos decreases. Note that the harvesting of embryos subjects them to anaerobic conditions; if the embryos are spread on a grape plate and allowed to recover, they will show induction of the heat–shock response at about 10% of maximum inducible levels. This can be avoided by freezing the harvested embryos immediately in liquid nitrogen without allowing any opportunity to recover from anaerobic conditions.

If it is important to know the accuracy of the timing in the collection the embryos, a 2-hr collection can be stained (0–2 hr embryos) with Feulgen (method of W. J. Peacock and L. Prestidge, personal communication). Embryos are dechorionated with 50% Clorox, washed in saline, and fixed for 1 hr in 3:1:5 methanol–glacial acetic acid–toluene (two changes). They are then rehydrated by successive equilibrium in 100, 95, 70, 50, 30, and 10% ethanol and water, for a minimum of 5 min in each. The last four solutions contain 0.1% Triton X-100. The DNA in the embryos is then hydrolyzed by immersing the embryos in 1 *N* HCl plus 0.1% Triton X-100 at 60°C for 8 min. Subsequently, the embryos are washed with water and stained in Feulgen stain plus 0.1% Triton X-100 for 40–60 min. Following staining, early syncytial embryos will be white, blastula embryos will be pink, and cellular embryos will be deep purple, corresponding to the DNA content. In a typical assessment of a 2-hr collection in which 2580 embryos were stained and counted, 2306, or 89.4%, were white (preblastula), 225, or 9.9%, were pink (blastula), and 19, or 0.7%, were purple (cellular).

[Feulgen stain preparation (W. J. Peacock, personal communication). Bring 200 ml distilled water to a boil and pour into a beaker containing 1 g basic Fuchsin. Stir for 5 min. Cool to 50°C and then filter (Whatman No. 1 paper). Add 30 ml 1 *N* HCl and 3 g $K_2S_2O_5$; stir ca. 1 min. Pour into capped, light-tight flask and let stand ca. 24 hr at room temperature. Add 2 g activated charcoal, stir a few minutes, and filter. Store in refrigerator in dark. The stain should be made fresh every 2 weeks.]

## B. Inoculation for Continuing Culture

Inoculation of growth chambers for the next generation of adult flies is carried out as follows. The embryos collected on the Nitex filter (approximately 23 g or scaled up proportionately) are added to a 250-ml graduated cylinder containing 20 ml of a 0.7% NaCl–0.1% Triton X-100 solution. The cylinder is filled to 100 ml, covered tightly, and inverted several times. The embryos are allowed to settle at 1 g for 10 min (to about the 50 ml point). Note that the *Drosophila* embryos are sticky and that labware, pipets, and any other items should be rinsed just before use with a small amount of NaCl–Triton X solution to keep the embryos from sticking. After settling, the top solution is removed with an

aspirator or poured off. The cylinder is now filled to 230 ml with 0.7% NaCl–13% sucrose solution and inverted to achieve mixing. Sucrose increases the viscosity of the solution so that it is easier to obtain an even suspension of the embryos. The suspension is poured into a large beaker. The embryos are maintained in even suspension by magnetic stirring; stir just fast enough to keep the embryos suspended in the solution. Two milliliters of embryo suspension is added to a larval container using a wide-mouthed transfer pipet and is distributed evenly over the surface. Finally, a small amount of dry Fleischmann's yeast is sprinkled over the surface and the containers are closed and placed in the environmentally controlled room. The embryo concentration in the solution can be determined by counting the embryos from 0.5-ml aliquots of a 10-fold dilution on black filter paper using a dissecting microscope. The concentration is adjusted to 3000 eggs/milliliter. After a few trial sessions, the counting step may be omitted and a simple weight of embryos per milliliter NaCl solution can be used.

On Day 5 (assuming the inoculation to be Day 1), the larvae are fed a few milliliters of the larval food solution. The larval food solution is placed with a squeeze bottle on the bottom of each container around the edge where the food meets the sidewall of the container. Under proper conditions, the adults emerge on Day 11 or 12, depending on the age of the embryos used for inoculation, and are transferred to the population cages. Generally, 2500–4000 flies (2.5 to 4.0 g depending on strain and rearing conditions) will hatch in each container. It should be noted that these procedures are designed to minimize the human labor input; the flies are crowded in both the larval and adult cages. However, increasing the number of embryos per tub any further results in decreased yield and causes developmental delays of the overcrowded animals.

When being cultured, *Drosophila* can have several competitors, generally mites or mold. To avoid these problems, several precautions are necessary. As an aid in slowing down the growth of mold, propionic acid and tegosept are added to most media. In addition, to help cut down the number of spores as well as guard against mites, all equipment (including adult cages) is washed in 50% Clorox. It is also possible to autoclave many of the media preparations if necessary.

### C. Transfer of Flies

The flies are transferred to the population cages as follows. The larval growth container in which the adults have emerged is held on its side (to prevent immobilized adults from becoming stuck in the food) and $CO_2$ is introduced with tubing through the Nitex screening in the lid. The adults are pooled together in a clean dry tub with a screened lid but without food. Adults are collected from several larval growth containers until approximately 50 g of flies (50,000 flies) have been accumulated. The anesthetized adults are then dispersed on the bottom of the population cage, and a grape plate containing a strip of yeast food is also placed in the cage.

## D. Cages

### 1. Larval Containers

The plastic containers used to grow the larvae have been of several types, but generally are about 1 liter in volume and of approximately equal height and diameter, with a larvae- and fly-tight lid (see Fig. 2). Critical parameters are (1) a configuration such that proper humidity is maintained; (2) sufficient surface area for larvae to crawl up the walls and pupate; and (3) a proper surface such that the food adheres firmly to the bottom of the container and the larvae will crawl up and pupate on the sides of the container. It is most convenient to use disposable plastic containers, minimizing labor costs. Louisiana Plastics 32SRL-N 32-ounce natural plastic containers with LR32P lids work well. The lids must be modified by cutting a 7- to 8-cm hole and filling the hole with a 64-mesh Nitex screen inset (Nitex high capacity No. 3-64/65). The holes are easily made

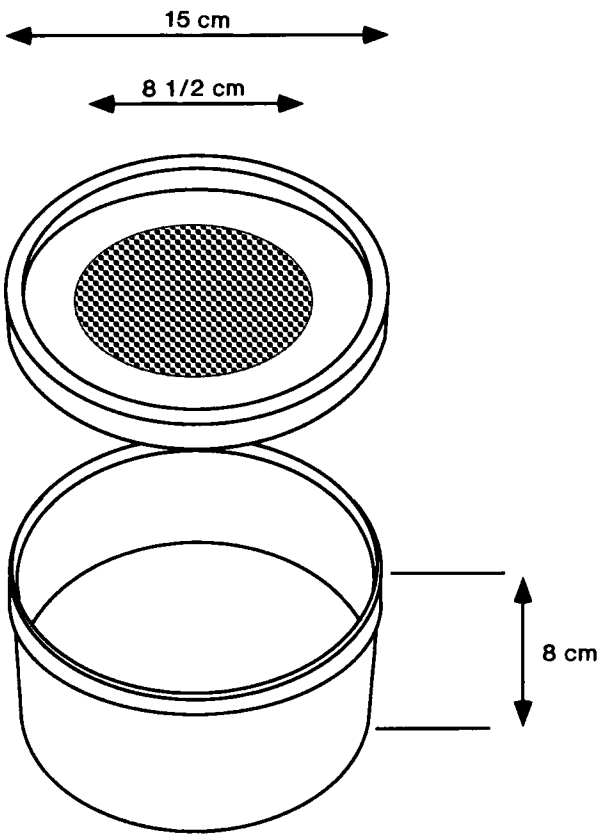


**Fig. 2** Plastic larval culture tubs. The hole in the lid is covered by H64 mesh Nitex.

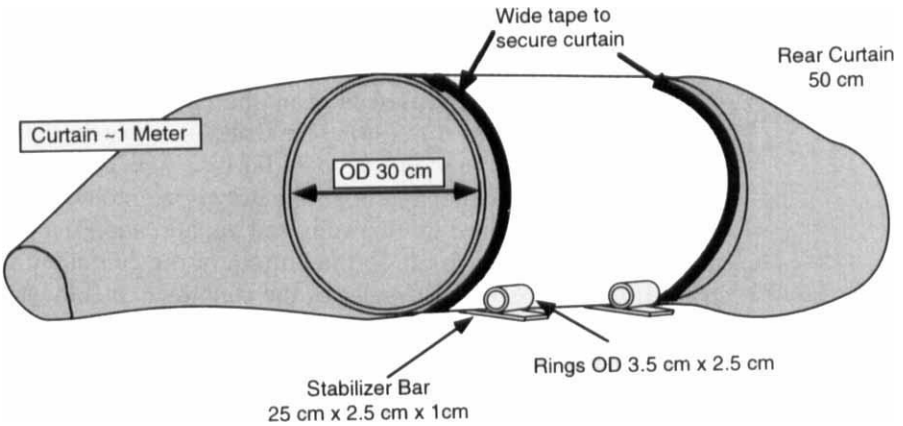


**Fig. 3** Population cages. The dacron curtains are held in place by wide masking tape and are loosely knotted to seal the cage.

by using a drill press and the Nitex can be "melted" into the top with an appropriate sealing iron (e.g., Super Sealer, Clamco Corp., Cleveland, Ohio). After each use, the lids are frozen or soaked in 50% Clorox, washed, and reused.

### 2. Population Cages

The population/embryo collection cages are made from 40-cm sections of 30-cm O.D. (12 inch) standard cast leucite or other acrylic tubing 0.6 cm (1/4 inch) thick (see Fig. 3). This seamless design makes for easy cleaning and low breakage. The cylinder is mounted horizontally on plastic supports. (Note that a flat sheet of plastic can be formed into a tube; this is frequently cheaper.) After curtain end pieces are slipped on the tube, the curtain edges are sealed securely with masking tape. The curtains are dacron, ca. 70 × 70 threads/inch (Dupont). The back curtain can be a single flat piece; the front curtain is a 750-cm to 1-m-long cylinder, closed with a knot.

## III. Media Recipes

1. *Cornmeal Media* (22 liters, for 80–100 larval containers, for eight cages with ca. 50,000 flies each)

| | |
|---|---|
| Water | 22,000 ml |
| Agar | 250 g |
| D(+)-glucose (Sigma No. G-8270) | 1313 g |
| Sucrose (table sugar grade) | 655 g |

| | |
|---|---|
| Cornmeal | 2273 g |
| Yeast 500 + vitamins | 398 g |
| Acid Mix A (see below) | 253 ml |
| Tegosept (see below) | 150 ml |

Add cold water to large kettle or other large pot appropriate for combining all ingredients. Slowly add dry ingredients to cold water, mixing to avoid lumping. Stir constantly, bring mixture to a boil, and cook until mixture is thickened and homogeneous. A motorized stirrer is convenient for mixing. Cool to 60°C. Add Acid Mix A and Tegosept; the final pH should be 4.2–4.3. Dispense into tubs; avoid getting cornmeal media on sides of tub. Allow each container to cool completely before use.

2. *Tegosept*

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|---|---|
| *p*-hydroxybenzoic acid methyl ester | 50 g |

The *p*-hydroxybenzoic acid methyl ester (Sigma No. H-5501) is dissolved in 500 ml of 95% ethanol.

3. *Acid Mix A*

| | |
|---|---|
| 100% propionic acid | 418 ml |
| 85% phosphoric acid | 41.5 ml |

Add the propionic and phosphoric acid to 500 ml water; adjust volume to 1 liter with water.

4. *Larval Food* (for 80–100 containers)

| | |
|---|---|
| Water (cold) | 604 ml |
| Propionic acid | 1.4 ml |
| Active dry yeast | 270 g |

Combine water and acid; slowly add yeast while stirring constantly. A consistency of maple syrup is desirable. Use immediately by squirting a ring of the yeast larval food around the tub where the food meets the sidewall. A hand-held plastic squirt bottle with a short tip is convenient for this procedure.

5. *Grape Plates* (5 liters, ca. 70 plates)

| | |
|---|---|
| Water | 11,000 ml |
| Grape juice, 48-oz cans | six cans |
| Agar | 450 g |
| D(+)-glucose (Sigma No. G-8270) | 1160 g |
| Sucrose (table sugar grade) | 580 g |
| Yeast 500 + vitamins | 360 g |
| Acid Mix A | 224 ml |
| 1.25 *N* NaOH | 440 ml |

Combine cold water and the six cans of grape juice in a large kettle. Slowly add dry ingredients to cold liquids, mixing to avoid lumping. Continue to stir

and bring mixture to a boil; simmer for 10 min, scraping the sides of the kettle occasionally. Cool to 60°C. Add Acid Mix A and NaOH solution. Dispense ca. 200 ml of solution into 8 × 10 × $\frac{5}{8}$-inch styrofoam meat-packing trays (e.g., Amoco Stock No. FT8SW; it is important to use the "webbed" foam plates for ease in handling). Allow trays to cool, cover with SaranWrap, and store in the refrigerator in small plastic trash bags.

6. *Yeast Food for Adults*

| | |
|---|---|
| Active dry yeast | ~300 g |
| Water (cold) | 450 ml |
| Propionic acid | 2.72 ml |

Combine water and acid; stir constantly while slowly adding yeast and mix thoroughly. Loosely cover the container and store in refrigerator. The container used should be large enough to allow some expansion of the live yeast culture. Note: A consistency of smooth peanut butter is desirable; add more yeast if necessary.

## IV. Materials Used

1. Disposable plastic containers and styrofoam meat packing trays are available from local packaging supply houses.
2. Nitex is a nylon monofilament bolting cloth. The number indicates the opening size in micrometers. Available from Tetko, Inc. P.O. Box 346 Lancaster, NY 14086.
3. Yeast 500 + vitamins is available from SPI Nutritionals 3300 Hyland Avenue Costa Mesa, CA 92626.
4. Active dry yeast (Fleischmann's) can be purchased in cases of 12 2-lb bags from any grocery supply company.
5. Agar. We have found that it is not necessary to use a high-quality grade of agar (e.g., Bacto agar). We obtain our agar in 25 kilo lots from Colony Imports 226 7th Street, Suite 103 Garden City, NY 11530.

The list of suppliers and equipment is given for the convenience of others working in the field; no warranty or endorsement is to be construed.

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