Grant, B.S., G. Bean and W.L. Harrison. College of William and Mary, Williamsburg, Virginia. A Drosophila eclosion fraction collector (DEFC).

Eclosion rhythm studies require an around-the-clock method for recording the time-of-day when flies emerge from the puparium. Periodic manual transfer from cultures proves far too inconvenient and exhaustive to personnel, particularly in long term experiments. An automated method employed by Pittendrigh and his associates at Princeton (see Zimmerman, et al., J. Insect Physiol., 14) results in the death of harvested adults. Since our experiments require living flies, a different method of collection had to be devised.

The system we have developed provides for the automatic hourly collection of newly emerged live flies without the necessity of handling individual pupae. Eggs are deposited on completely filled 1-1/4 X 1-3/4 inch plastic food cups. An open ended 1-1/4 X 4 inch clear plastic chimney is taped to the food cup to form a large vial. After numerous dark pupae appear on the inner surface of the chimney, it is removed from the food cup and attached to the DEFC (see e in Figure). (In order to avoid sampling only early developers additional clean chimneys should be subsequently placed on the food cups until the larvae are depleted.) Adults are continuously removed from the chimney once they have freed themselves from their pupae cases. This is

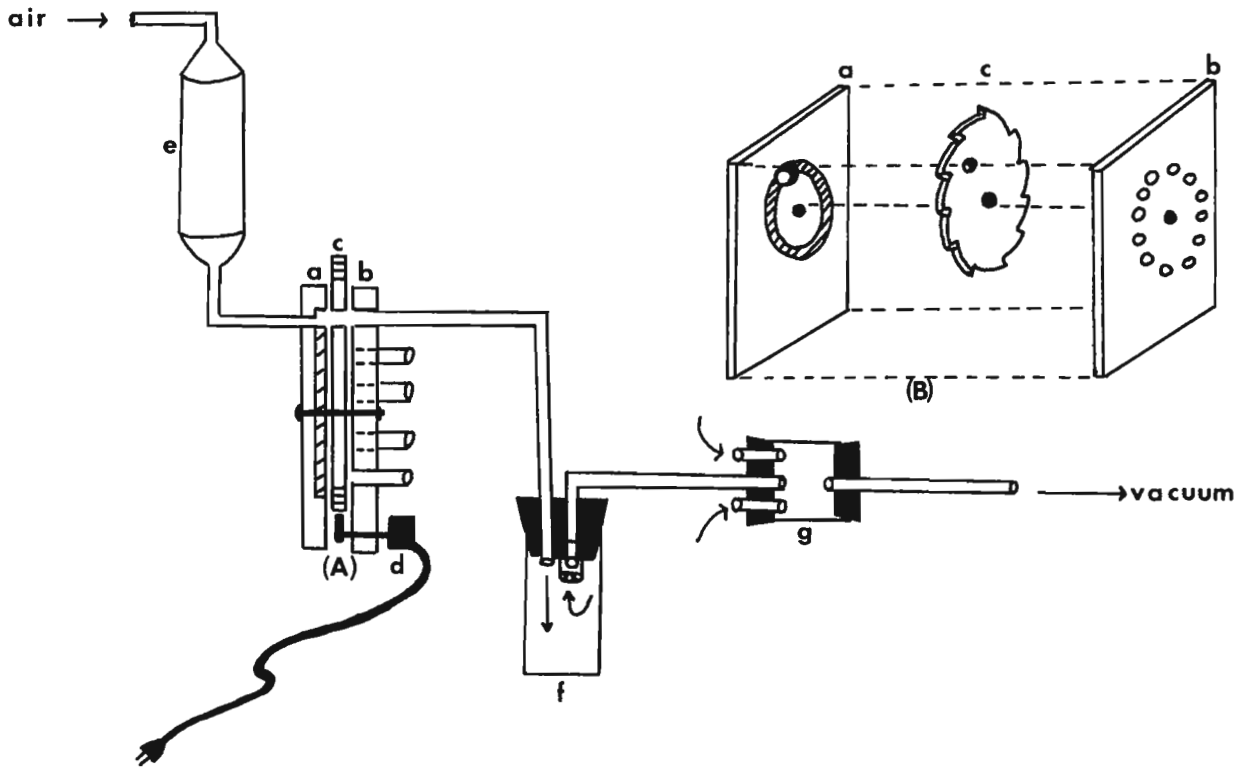
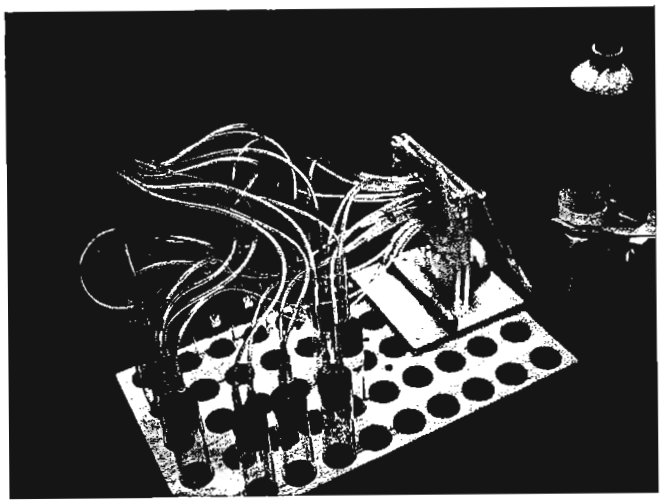


Figure 1. Drosophila eclosion fraction collector. (A) Cross-sectional diagram of sorter (B) Exploded view of sorter. Explanation in text.

accomplished by an air current generated from a carefully balanced combination of air being pumped into the upper end of the chimney and withdrawn at the lower end via a vacuum pump. This aspiration should be fairly gentle lest the flies be injured in transit, but the air stream within the chimney must be of sufficient force to effectively remove adults after eclosion. The flies are then drawn through an exhaust tube to the sorter.

The heart of the DEFC is the sorter. It is constructed from Lucite and consists of a 3-1/4 inch geared wheel (c) sandwiched between two 4 X 5-1/2 inch plates. Lightly greased paper gaskets actually separate these three pieces for the purpose of lubricating the wheel and to insure a reasonable air seal. The front plate (a) has a single 1/4 inch hole drilled through it which intersects a circular grooved track on its inner surface. The back plate (b)

has a ring of twelve 1/4 inch equally spaced holes, each of which is connected by tubing (1/8 inch inside diameter) to one of twelve collecting vials (f). The holes in plate b communicate with the track in plate a by way of a single hole through the geared wheel which permits only one hole in plate b to be open at any given time. Flies from the chimney enter the track in plate a through the single hole in that plate and then are drawn through the opening in the gear wheel to be shunted down one of the collection holes in plate b. There is only one route open at any given hour to a collection vial. The wheel is turned 1/12th revolution every hour by a lever extending from the axle of a small one-RPH electric motor (d). This turning time takes about seven minutes which allows a given vial to be open for the major portion of the hour. All twelve vials are connected to a single vacuum block, but appreciable pressure is present only in the particular vial open to traffic.



Our machines were designed to run unattended for twelve hours. At the end of this period, the collection vials are replaced with an empty set. The flies collected from the preceding block of time can then be counted and sexed for use in prescribed matings. The machine, of course, can be modified to run for longer periods of time by altering the size and numbers of teeth on the geared wheel and the corresponding number of collecting holes in plate b to accommodate the grace period of virginity for the species under investigation or for, perhaps, a specified photocycle in the laboratory.

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Paika, Inder J. University of Nebraska, Lincoln, Nebraska. Application of air drying technique to the preparation of chromosomes in testes of adult males.

Crozier, 1968 (Stain Technology, 43: 171-173) has described an air drying technique applied to the preparation of chromosomes of Drosophila larval ganglion cells.

His method has been extended to testicular material of adult flies of D. affinis. Adult males were injected with a small quantity of 0.1% colcemide in Bodenstein's solution and after 1½ to 2 hours the testes were dissected out in 1% sodium citrate solution and left there for 15 to 20 min., after which the testes were fixed in freshly prepared acetic-methanol (1:3) for about 30 mins. After fixation the material was put in a drop of 60% acetic acid on a clean warm slide for about 30 seconds. A very small drop of acetic-methanol (1:3) was then added to the dissociated tissue and the slide tilted to regulate spreading and allowed to dry in the air. Staining was done with lactic-acetic-orcein (2gm. synthetic orcein in 50 ml. glacial acetic acid and 50 ml. 85% lactic acid) at 45°C. for about one hour. The slide was then placed vertically in acetic-ethanol (1:3) until the coverslip dropped off. After de-hydration with 95% and absolute ethanol the preparation was mounted in euparal.

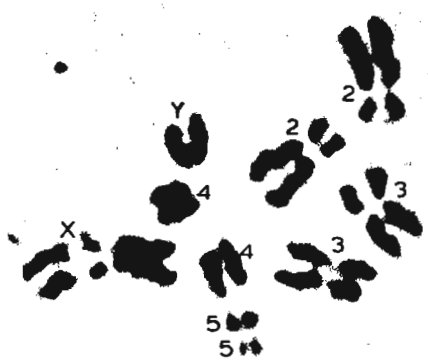

Fig.1. Phase contrast photomicrograph of primary spermatocyte of D.affinis (Chadron State Park, Nebraska) prepared by air drying technique applied to adult testes.