

Fig. 3. Counting head:

A Cross-sectional view.

B Longitudinal view.

(1) transmitter.

(2) air flow through suction pipe.

(3) receiver.

v_{air} , according to: $d_{min} = v_{air} \times t_{int}$; with t_{int} = duration of the interval between 2 impulses. (for $f = 100,000/s$ and $\pi = 10 \mu s$ we have $t_{int} = 5 \mu s$ per interval).

The maximum number of flies which theoretically can be counted within a given time, n_{max} , may be determined according to the following equation:

$$n_{max} = \frac{v_{air}}{l_{fl} + d_{min}} ; \text{ where } v_{air} \text{ represents the air speed at the sensor, } l_{fl} \text{ the mean fly length and } d_{min} \text{ the minimum distance between 2 flies being sucked through the channel.}$$

With a suction power of 500l/h and the diameter of the suction tube being 2 mm the mean air speed at the sensor is about 45 m/s. Taking a fly length of 2.5 mm and a minimum distance of $45,000 \text{ mm/s} \times 5 \mu s = 0.225 \text{ mm}$, theoretically about 15,000 flies may be reliably counted.

With flies as well as with poppy seeds (diameter: <1mm), we found counting deviations of less than 0.5%. Even very small flies--which are found especially in population experiments--were counted with the same accuracy.

Losses of flies being killed during the counting procedure are less than 0.5%.

When the apparatus is used daily, problems with counting accuracy may arise from the dirt being deposited in the suction tube. Therefore, we established a signal that shows optically when the transmission through the suction pipe drops below a critical value. At the same time the counting process is interrupted. This is effected by DC-coupling the impulses caused by the flies and leading them to an extreme low-frequency pass combined with an integrator (Fig. 2).

More detailed information can be obtained on request from the first-named author.

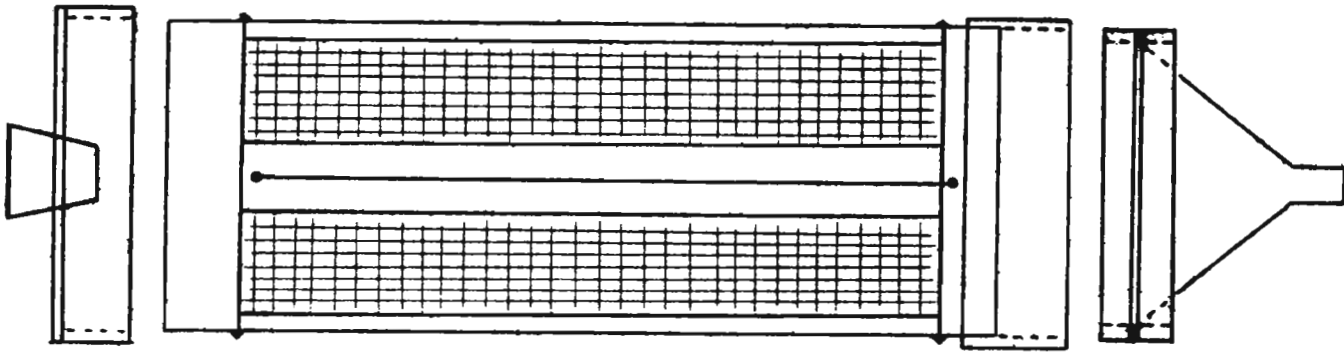
By varying the diameter of the suction tube and if necessary the suction power this counting device may be applied to other biological objects.

References: Cuperus et al. 1969, DIS 44:134-135; Cuperus et al. 1970, DIS 45:176.

Wallace, B. Virginia Polytechnic Institute, Blacksburg, Virginia USNA.
A modular mating chamber for *Drosophila*.

Although density has been one of many variables studied by those interested in the mating behaviors of *Drosophila*, it appears that no one has designed a mating chamber in which the density of flies can be altered by incremental changes in the size of the chamber as well as by altering the number of flies in a chamber of constant size. The chamber described here is of modular construction; its size can be varied by combining two, three, or even more of the basic units. The single unit has been used successfully in studying the kinetics of the mating behavior of a sepia strain of *D. melanogaster* for densities ranging from 5 males plus 10 females to 320 flies of each sex over a half-hour period. Because the results of replicated tests were highly consistent, this mating chamber seems suitable for studying a number of aspects of mating behavior.

The basic unit of the mating chamber is a 12" length of rigid clear plastic tubing $4\frac{1}{2}$ " O.D. ($4\frac{1}{4}$ " I.D.; i.e., $1/8$ " wall). Attached to one end is a $1\frac{1}{2}$ " sleeve of the same type of



clear plastic tubing cut from a piece of 4-3/4" O.D. (4 1/2" I.D.); this sleeve overlaps the main tube one-half inch. Because of the imprecision in the manufacture of plastic tubing, it may be necessary to cut the sleeve in order for it to fit over the main unit; if so, the gap must be filled by cementing in an appropriately sized piece from a 1 1/2" ring sacrificed just for this purpose.

Vanes of nylon window screening (1 1/4" strips) are provided as resting surfaces for the flies. These are supported by pairs of crosswires (cut from straightened coat hangers) that are inserted through small holes drilled in the walls of the chamber. One pair is located just behind the sleeve; the other just more than 1" from the opposite end of the tube (so the wires do not interfere with the sleeve of the next module, or with the terminal cap). The wires are fixed in place by brushing clear fingernail polish into and over the holes in the walls of the tube.

The nylon vanes are held taut by attaching one end to 1 1/4" pajama elastic; the elastic and the nylon strip can then be glued to cloth-mounted picture hooks using Elmer's glue to moisten (and supplement) the glue that is already on the mounting cloth.

Moisture is provided for the flies by a dampened tubular wick that extends (with light weight spiral springs at either end) from the intersecting wires at one end of the chamber to those at the opposite end. The spring tension keeps the wick from sagging against any of the four vanes. The wick is put in place and removed with the help of a small hook shaped from a piece of coat hanger wire.

The chamber is capped at one end by a 1" ring of 4-3/4" O.D. plastic (extended in diameter as needed) to which a circular plate of clear plastic is cemented. A hole bored into the plastic provides a port through which flies may be introduced. The hole should accommodate a 25 mm x 95 mm shell vial; it is closed by a rubber stopper.

A funnel is provided at the opposite end for the removal of flies. A 1" ring of 4 1/2" O.D. plastic (which, of course, can fit into the terminal sleeve of the mating chamber) holds the funnel. A 1/2" ring of 4 1/2" O.D. plastic from which an appropriately sized segment has been removed is cemented snugly within the 1" ring. A 1/2 pint Fairgrove funnel (#174, Aluminum Housewares Co., Inc., Maryland Heights, Missouri 63043) with its rim unrolled and flattened fits within the 1" ring and rests on the 1/2" inner ring. A second 1/2" ring is then cemented within the 1" ring, on top the flattened rim of the funnel. A rubber stopper fits over the tip of the funnel; nylon curtain material is fastened over the hole in the stopper by means of Divro plastic rubber.

The various components of the mating chamber, when properly assembled, fit together snugly. The pieces are held firmly in place, however, by the use of 3/16" wooden pins that are fashioned from doweling; each pin protrudes about one inch from a "head" consisting of a one-half or three-quarter inch segment of 1" doweling. The large heads make handling of the pins convenient. Holes, 3/16" in diameter, are drilled on opposite sides of each component. In the mating chambers I built, one hole was drilled on either side of a terminal cap; one of these (the black hole) was identified with a Magic-marker pen. This cap then served as the model for drilling holes into each mating chamber. Care was taken to pin the first hole before drilling the second one on the opposite side; for each pair, the Magic-marker was used to identify the black hole. The mating chambers were used in turn as models for drilling holes in other terminal caps and in the sleeves at the ends of the mating chambers; holes in the sleeves served as models for drilling holes in the funnel assembly. In each case, the

pieces were held together as firmly as possible as the new hole was drilled; the first one was then pinned before drilling the second; and the appropriate one in each case was marked with black ink. As a result, the pieces are freely interchangeable. When needed, a fly-tight seal can be made by smearing Elmer's glue on the "male" end of the basic unit (or funnel) and allowing it to stand overnight on brown paper toweling. When the excess paper is trimmed off the following day, a thin paper gasket remains on the plastic wall at the end of the tube. Such gaskets have been sufficient to seal small spaces caused by inaccuracies in the construction of the present chambers.

When flies are to be removed from the mating chamber, CO₂ is led into the unit through the opening in the terminal plate; the tube from the CO₂ tank pierces a cork that fits into the port hole of the terminal plate. The nylon-covered stopper on the funnel allows displaced air to escape without building up pressure within the chamber. *The use of ether in conjunction with mating chambers as large as these would seem ill-advised.*

Because the flies seem to prefer the funnel as a resting place, future tests are planned in which a clear plastic plate, rather than a funnel, will be inserted into the sleeve at that end of the unit.

TEACHING NOTE

Perez-Chiesa, Y. University of Puerto Rico, Río Piedras PR-USA. Incidence of *Drosophila melanogaster* flies with melanotic tumors for demonstrating conditionality, penetrance and variable expression.

A sex-linked, temperature-sensitive melanotic tumor mutation in *Drosophila melanogaster*, *tu(1)Szt^{ts}* (Rizki & Rizki 1980) is excellent for demonstrating conditionality, penetrance and variable expression with changes in temperature. It also allows for learning the chi-square contingency test and for discussing dosage compensation in *Drosophila*, as well as other

aspects of insect physiology. As reported by Rizki & Rizki (1980) *tu(1)Szt^{ts}* larvae develop melanotic tumors at 26°C, whereas 18°C inhibits tumor formation. However, penetrance may vary in melanotic tumor strains depending also on genetic background, crowding conditions and food media used (Sparrow 1978).

Experimental Procedure: Students are given two stocks of *D. melanogaster*: wild type, non-tumor forming strain and *tu(1)Szt^{ts}*. They set up two cultures of each stock and place them in incubators: one culture of each at 18°C, the others at 26°C. Three days later the parents are removed and their progeny is allowed to continue development at the same temperature at which they started. After eclosion students classify the flies in terms of sex and mutant phenotype: presence of melanotic tumors. The tumors are usually found in the abdomen and less frequently elsewhere. The students are asked to determine whether there are significant differences in the incidence of flies with tumors between the sexes and between the stocks used at each temperature. We have done the experiment at 22°C vs 29°C; there will be tumor formation at 22°C but the incidence of flies with tumors is still significantly different from that of flies grown at 29°C. Cultures can be coded to avoid bias.

References: Rizki, T.M. & R.M. Rizki 1980; Wilhelm Roux's Archives 189:197-206; Sparrow, J.C. 1978, IN The Genetics and Biology of *Drosophila* (Ashburner & Wright eds), V2b, Academic Press, London p277-313.

