Brown, R. V., North Texas State University.
Use of Kelthane to control mites in Drosophila.

A recent study (Brown, R. V., 1965, J. of Econ. Entomol. 58:156-157) indicated the value of Kelthane (1, 1-bis (p-chlorophenyl) 2,2,2-tri-chloroethanol) for control of the genetic mite Histiostoma laboratorium (Hughes, R., 1950, J. Wash. Acad. Sci. 40:177-183) formerly called Histiostoma genetica (Stolpe, S. G., 1938, Anat. Rec. 72:133-134). Some additional experience in the use of Kelthane has been acquired in eliminating mites in Drosophila stocks of two other laboratories. The method used was to wash and autoclave bottles to be used for media. Bottle interiors were rinsed in a Kelthane suspension of 400 ppm and allowed to drain and dry (or nearly). A cornmeal-agar media was added. Flies were transferred to these bottles. As soon as the next generation began emerging, they were transferred to similarly treated bottles with fresh media. The third generation flies were examined and were found to be free of mites.

Another procedure was tried and found to be of value. Bottles heavily contaminated with mites were treated as follows: (1) All adult flies were removed and discarded; (2) a 75 ppm solution was poured into the bottles and quickly poured out; (3) bottles were stoppered. Less than one percent of the flies that emerged had mites one week later. This procedure requires quickness in rinsing, and cultures that are vigorous with large numbers of developing flies, as the rinsing removes many of the pupae.

Pre-treatment of bottles before addition of media allowed for drying of bottles. This was more satisfactory than when bottles with media in them were treated and stoppered without drying, as higher concentrations of Kelthane were not so toxic in dry bottles.

One incidental observation that may be of interest to some workers was noted. The toxic concentrations of Kelthane did not appear to be equally toxic for all stocks. Stocks that appeared most susceptible were ec cv v f, f, and v. This is simply an observation and has not been investigated.

Ditman, W. F. Purdue University.
An improved method for determining visual depth preferences in large numbers of D. melanogaster.

Efficient techniques for determining visual preferences of D. melanogaster are often desirable for behavioral studies. Usually flies are allowed to crawl singly through T- or Y-tubes, the arms of which differ on some visual dimension such as brightness or hue. This method is time consuming if large numbers of flies are to be tested. Also the small size of the tubes precludes testing preferences for multi-dimensional visual stimuli such as form or depth.

To overcome the restrictions of the T- or Y-tube a large shaft was used. Fifty flies were released at the bottom and removed at the top. Inside, the shaft was painted flat black and was approximately 14" L x 7" W x 27" H. The final (upper) 8" of the shaft was tapered to 12" L x 6" W. Atop the shaft was a collection box 12" L x 6" W x 3" H inside. This collecting box was divided into two 6" L x 6" W compartments by means of a clear plastic partition 3" high. The top of the collection box was a pane of glass mounted to slide aside for removal of the flies with an aspirator. The visual stimuli were placed above the glass. All light entering the shaft entered through the glass.

When different stimuli are placed over the two 6" x 6" compartments in this two-choice situation flies attracted to the first stimulus collect in one compartment, while flies reacting to the second stimulus gather in the other compartment. Flies are released from the culture bottle by the removal of a small trap door above the bottle. The negative geotropism and positive phototropism of D. melanogaster encourage the flies to fly toward the two visual stimuli. Thirty seconds after the flies are released, the sliding trap doors beneath the two 6" x 6" collecting chambers are closed, effectively isolating the two groups of flies which have chosen between the two different stimuli. (See Fig. 1).

To create a depth stimulus, a 4' x 4' x 2' high inside box was centered above the collecting chambers. A mirror, 4' x 2', was placed in the center of the box in a vertical plane directly above the plastic partition of the collecting box. On either side of the mirror sheets of translucent white plexiglass were suspended. The plexiglass had 3" square pieces of black construction paper glued to the underside in a checkerboard pattern.

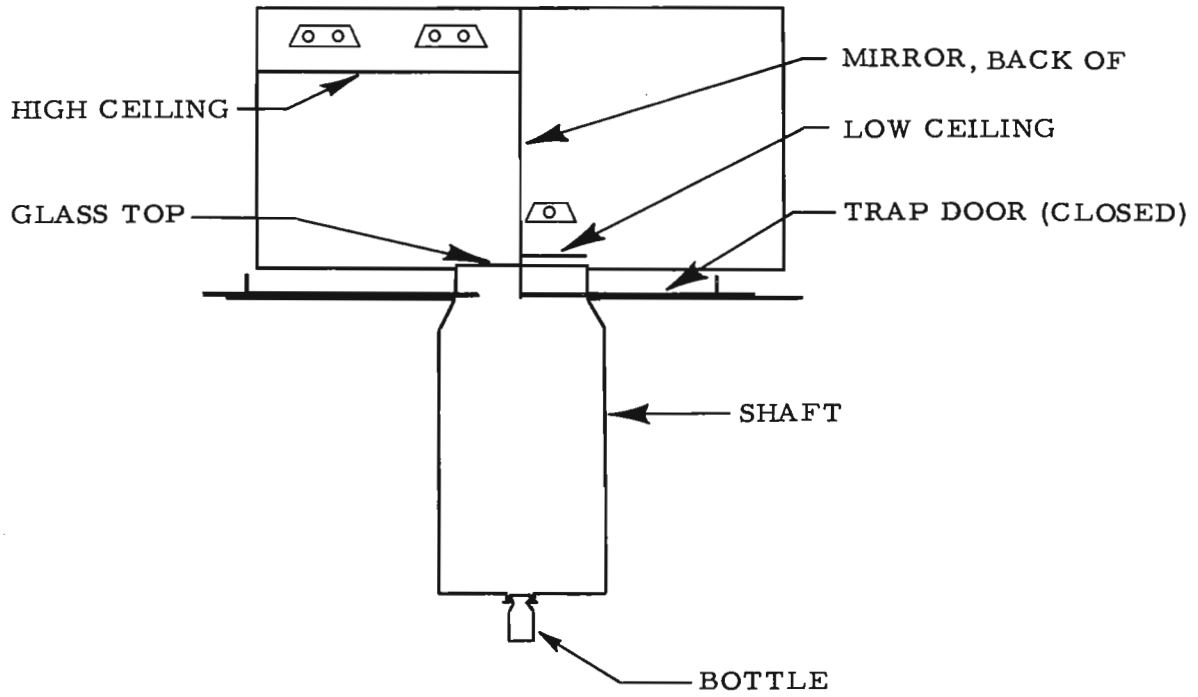

FIG. 1. BASIC VISUAL PREFERENCE APPARATUS WITH VISUAL DEPTH STIMULI

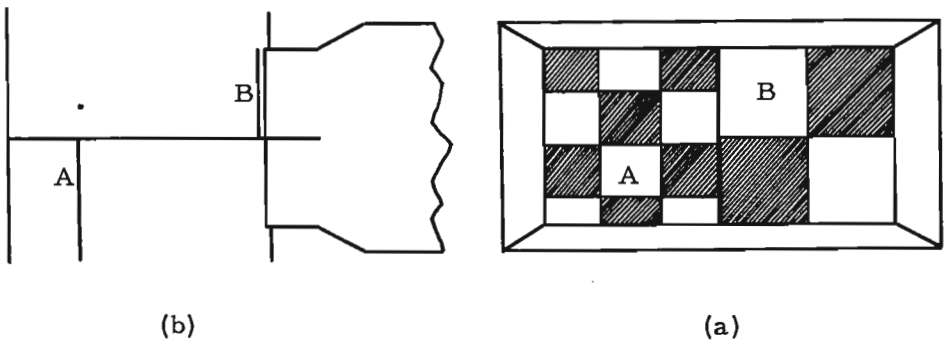

FIG. 2. a- VISUAL DEPTH STIMULI (A & B) AS SEEN FROM THE BOTTOM OF THE SHAFT. b- ACTUAL ARRANGEMENT OF A & B

Fluorescent lamps were mounted above the plexiglass. One plexiglass sheet was lowered until it rested on the glass pane of the collecting box, while the other piece of plexiglass was raised to a height of 14" above the glass pane. In this way an illusion of depth in the form of a high and low "ceiling" was created. Fig. 2 is an illustration of the appearance of the apparatus from inside looking up. The mirror is used to eliminate the cue of interposition. The edges of the plexiglass are not visible. With this arrangement only two depth-perception cues are available to the flies: motion parallax and textural differences of the stimulus' surfaces.

Doane, W. W. Yale University, New Haven, connecticut. Separation and quantitation of α-amylases with disc electrophoresis.

An improved method was developed to analyze α-amylases in Drosophila separated by acrylamide gel disc electrophoresis. The procedure provides both quantitative and qualitative estimates of enzyme activity from single flies or individual tissues. (A previous method, DIS 40:97, was abandoned.)

The technique of Ornstein & Davis (1962) is employed with minor modifications: 1) omit $K_4Fe(CN)6$ from small pore gel, 2) use 1/2 the amount of N,N,N',N'-tetramethylethylenediamine, 3) substitute 0.47 M tris-phosphate buffer, pH 6.9, for tris-HCl in large pore gels, and 4) add 1 µl. 10% 3-dimethylaminopropionitrile (DMAPN) to each sample gel to insure polymerization. Supernatant from a single fly, homogenized in 10 to 15 µl. distilled water and centrifuged in a capillary tube, is put directly on top of spacer gel, mixed with 0.1 ml. large pore gel and layered-over with another 0.1 ml. of this gel. Electrophoresis is done at 4°C. with a constant current of 4 ma per gel tube for 50-60 minutes.

After electrophoresis, gels are removed from their tubes and placed on a corrugated rack in a moist chamber. A glass plate (3 1/4" x 4"), coated with a starch-acrylamide film, is placed over the gels, weighted (125 gm.), and left to incubate at 25°C. for 30 minutes. Plates are made by mixing 2 ml. small pore gel as usual but altering the pH to 7.4 and including 1.5% Connaught hydrolyzed starch, previously boiled 5 min. in the water used in making "small-pore solution #2". Ten µl. of 10% DMAPN solution is added and the mixture spread between two glass plates, one of which is coated on its periphery with dried albumin. Cover-glass chips are set in the albumin to separate the plates so the film forms with uniform thickness. (Film thickness, sample size and incubation time must be suitably adjusted for densitometric analysis.) Films are polymerized over a fluorescent bulb 15-20 min. and immersed in tris-HCl buffer, pH7.4 (optimum for melanogaster, Doane, unpubl.), where they may be stored under refrigeration 1-2 weeks or used immediately. Prior to use, plates are rinsed with water, the outsides dried, and the two separated from one another, leaving the starch-acrylamide film on the side edged with albumin. The latter plate is placed directly on electrophoresed gels to incubate, care being taken to avoid any additional moisture.

Following incubation, the starch-acrylamide plate is set in I-KI reagent 1-2 min. to stop the reaction and to render unhydrolyzed portions of the film blue. Not only do amylase bands stand out distinctly (ranging from lighter shades of blue to nearly colorless), but the imprint of the individual gels may be discerned along with their origins and leading edges (the latter caused by tracking dye). The stained plate is rinsed with water, then 7% acetic acid, and re-covered with the other glass plate removed prior to incubation.

The stained starch film, sandwiched between two plates, may be stored several months without deterioration by placing it, with a little 7% acetic acid, in a container in the dark and under refrigeration. It serves a double purpose: 1) it is used as a photographic negative for contact prints of amylase banding patterns, and 2) it is scanned densitometrically (Joyce-Loebl Microdensitometer) to determine relative activity in different bands (see research note, this issue). Amylases in the electrophoresed gels remain unharmed and may be cut out, eluted, and further analyzed.