Table 1. Extraction of eye pigments using different procedures.

Mutant	Absorbance at 480 nm: 40 heads in 4 ml of AEA (after 24 h)	40 heads homogenized in 4 ml of AEA	40 heads split and placed in 4 ml of AEA (after 24 h)
scarlet	0.368	0.548	0.544
brown	0.006	0.042	0.002
sepia	0.018	0.050	0.023

Table 2. Extraction, purification and quantitation of the brown pigment ("xanthommatins") from **D.melanogaster**. w.t.= water treatment. v.o.l. = volume of the organic layer.

Strain*	Absorbance at 492 nm of organic layer throughout the purification procedure of dihydroxanthommatin: Before w.t. (v.o.l.=2.3ml)	After 1st w.t. (v.o.l.=1.3ml)	After 2nd w.t. (v.o.l.=1.0ml)
bw (150 heads)	0.249	0.328	0.402[a]
v (150 heads)	0.037	--	0.011
v (150 whole flies)	0.092	--	0.045
cn se (150 heads)	0.013	--	--
Hn^{r3} v (250 heads)	0.052	0.015	--

* bw = brown, v = vermilion, cn se = cinnabar sepia; Hn^{r3} v = Henna-recessive[3] vermilion.

[a]Considering the differences in volume between the taken and discarded aliquots of the organic layer after each treatment, the amount of dihydroxanthommatin of the bw mutant in the final solution was 70% that of the initial extract.

Quantitation of the brown pigment ("xanthommatins"): This is carried out following Butenandt et al.'s (1960) procedure for the isolation of dihydroxanthommatin, which has been modified to suit quantitative purposes. Fly heads (around 150, 75 from males and 75 from females, nine-day-old adults) are homogenized in 1.5 ml 2 M HCl. Sodium metabisulfite (10 mg) and n-butanol (2 ml) are added and the mixture is tumbled for 30 min. After centrifugation at 4000 g for 5 min, the organic layer is separated. An aliquot of 1.7 ml is mixed with 10 mg of sodium metabisulfite and 1.5 ml of water. The mixture is tumbled and centrifugated again. After repeating the procedure with 1.2 ml of the washed organic layer, its absorbance is measured at 492 nm. Values can be corrected in order to obtain the amount of dihydroxanthommatin in the initial extract, considering the differences in volume between the aliquot taken and the aliquot discarded of the organic layer after each treatment.

The following mutants were used to test the above procedure: brown, vermilion (no "xanthommatins"), cinnabar sepia (no "xanthommatins", no "drosopterins" and high levels of sepiapterin) and vermilion Henna-recessive[3] (no "xanthommatins", small amounts of "drosopterins" and high amounts of sepiapterin and acetyldihydrohomopterin, a minor pigment of the eyes). As shown in Table 2, fly heads are preferred to whole flies because interferences from body pigments can be avoided. After the second treatment of the butanolic layer with water, interferences from head pigments other than dihydroxanthommatin are considerably reduced. At the same time, dihydroxanthommatin is concentrated up to 160% of the initial concentration, making the quantitation more precise. We have found that the absorbance of dihydroxanthommatin versus the number of heads is linear at least up to 0.900.

References: Butenandt, A., E. Biekert, H. Kubler & B. Linzen 1960, Hoppe Seyler's Z. Physiol. Chem. 319:238-256; Clancy, C.W. 1942, Genetics 27:417-440; Ephrussi, B. & J.L. Herold 1944, Genetics 29:148-175.

Wallace, Bruce. VPI & SU, Blacksburg, Virgina USNA. A $100 incubator.

The material required for the construction of the incubator box includes two 4' x 8' sheets of 1/4" plywood finished on one side, 32 sq ft of 3/4" polystyrene insulating panels, 60 feet of 1" x 1" (actually 3/4" x 3/4") wood strips, hinges, and latches.

The plywood sheets are cut according to the pattern shown in Figure 1 (I had mine custom cut at the lumber yard). Wooden strips are glued and nailed carefully around the outside edge (rough side) of one sheet of each component (door, top, end, bottom, and back). The polystyrene sheets are cut to fit within the edging strips; scrap pieces can be used as well as larger ones because they won't be seen. The second panel of each component is glued and nailed (finished side out) over the strips and polystyrene insulation. (The pattern allows for saw cuts; notice, however, that the larger door components (D_2) can be lengthened, if necessary, so that the total length of the two door components is $35\frac{1}{2}$").

Figure 2 shows that, when assembled, the top rests on the ends, and that the ends overlap the bottom. The back overlaps the ends and bottom; the top rests on the back. Both glue and nails are used in assembling the different components. The incubator can then be stained and varnished.

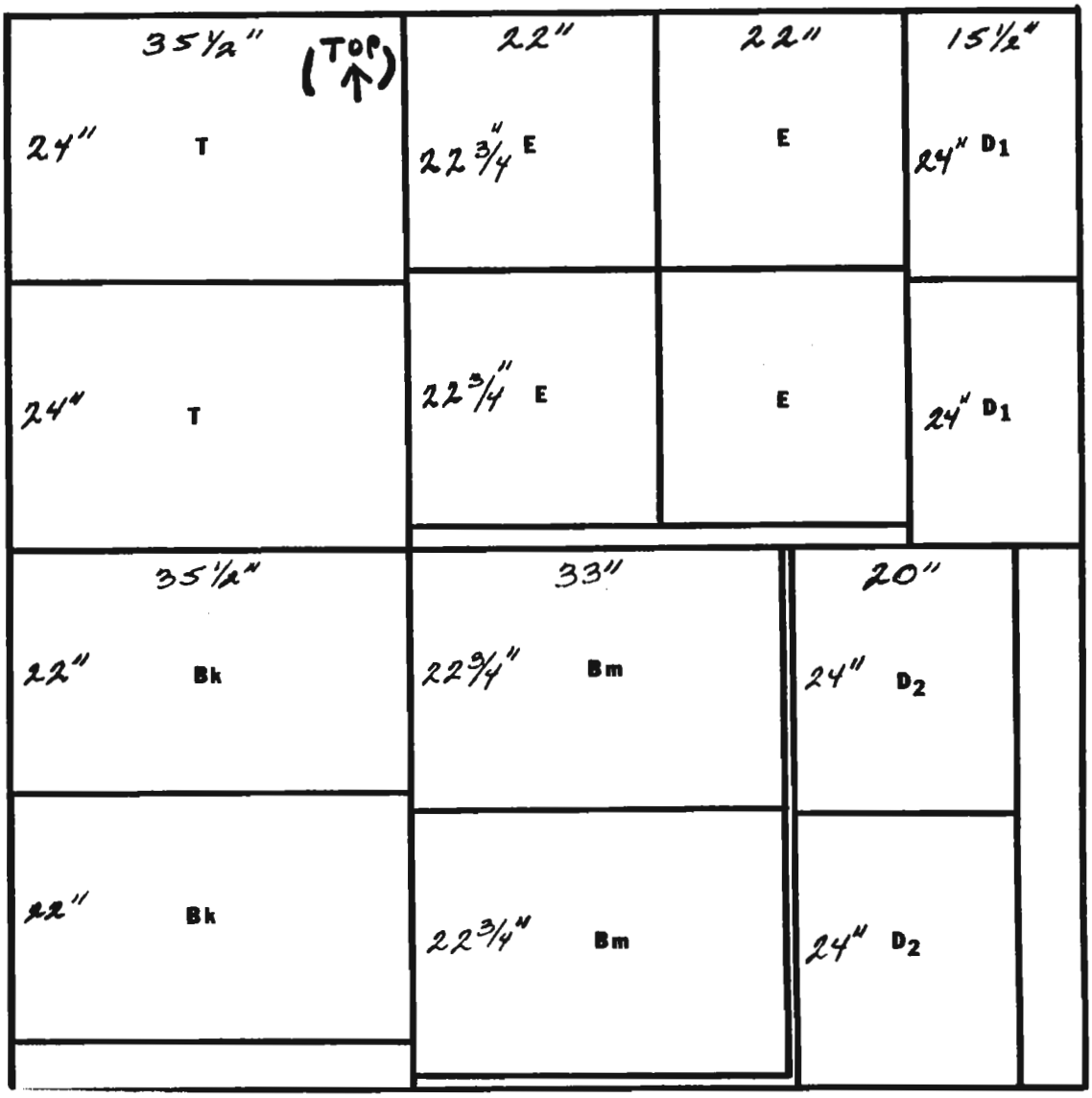

Figure 1. Cutting pattern for two 4' x 8' sheets of 1/4" plywood. T = top; E = end; D_1 and D_2 = door; Bk = back; and Bm = bottom. Two pieces are needed for each component because polystyrene paneling is sandwiched between for insulation. If, because of saw cuts, pieces D_1 are not 15½" wide, the width of pieces D_2 can be increased so that the total width of the two completed doors is 35½".

Details concerning the interior can probably vary--even be improved--according to different tastes. I had a spare shelf from a Fisher electric oven, 22½" deep and 28" long. That shelf, shown in Figure 2, determined the position of the divider that separates the heating element (two 40 watt bulbs) from the bottle compartment.

The divider is made of two pieces of 1/4" plywood 20-3/4" x 22-3/4" (not included in the two 4' x 8' sheets). Matching circular holes about 3" in diameter are cut in the upper front; matching rectangular holes about 3" x 12" are cut in the lower rear. A piece of hardware cloth covering the latter opening is sandwiched between the two pieces of plywood which are glued and held together by strategically located stove bolts. A small circulating fan is mounted on the circular hole; it blows warmed air from the heating compartment into the bottle compartment while cooler air returns through the lower, rectangular hole.

I bolted the Fisher oven shelf (made of expanded metal) to the divider before it, with its attached shelf, was guided into channels at the top and bottom of the incubator. The channels were formed by fastening strips of 1/2" quarter round molding in parallel, leaving a half-inch gap for holding the divider. The other end of the shelf was then bolted to the end of the incubator through pre-bored holes. I then closed the front of the heating chamber with a piece of plywood about 4-3/4" x 20-3/4" in size.

The two 40 watt bulbs that heat the incubator are mounted in ceramic sockets on the floor of the incubator within the heating chamber (Figure 2). I have arranged matters so that the fan is "on" only when the bulbs are "on" (the fan must be wired in parallel with the bulbs; it does not run if wired in series).

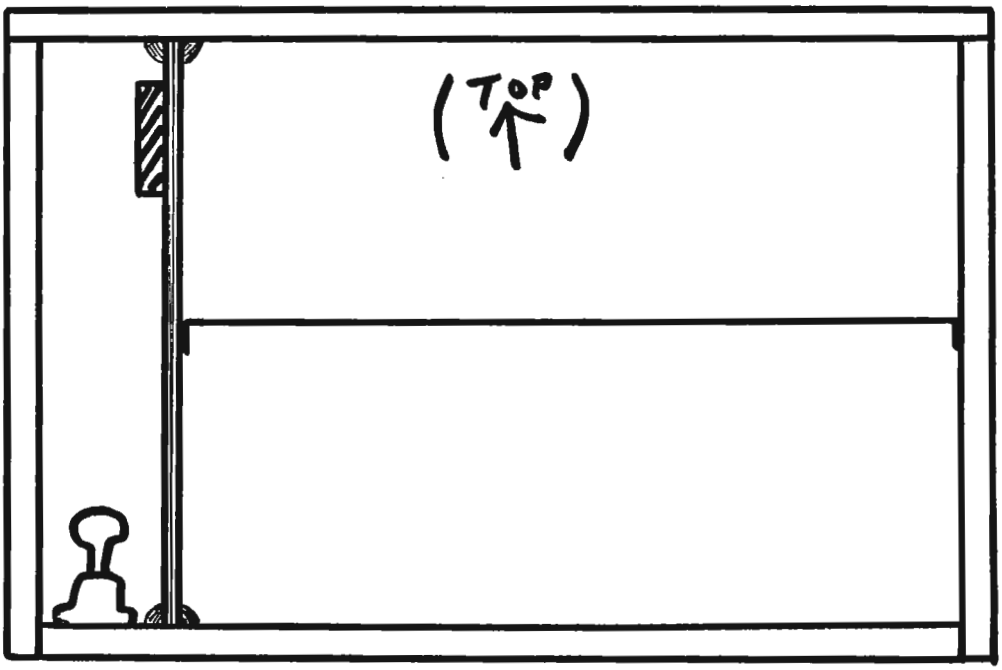

Figure 2. Scheme showing the assembly of the ends, bottom and top. The back overlaps the bottom and the ends; the top rests on the back and the two ends. The doors are mounted flush with the top and the ends; they overlap the bottom. Strips of felt may be needed on the front edges of the top and bottom to give the door a proper seal. The circulating fan is shown attached to the divider whose position, in my case, was determined by the length of the Fisher oven shelf.

The thermostat which controls the temperature of the incubator is mounted outside on the end opposite the heating chamber. The sensing bulb is passed through the end, low and in the rear; it is mounted on the back of the bottle compartment not far from the rectangular return opening in the divider. A one-inch hole was bored in the rear corner of the top at the end opposite the heating chamber. A thermometer, mounted in a rubber stopper, is placed in this hole. The thermometer bulb is protected from breakage by a small strip of hardware cloth that is fastened diagonally across that corner of the incubator, near the top. The thermometer extending above the incubator has been protected by attaching a jar lid (through which a circular hole 1" in diameter was punched) around the thermometer, and screwing the glass jar into the lid. My incubator is mounted on four 4" lengths of a square oak table leg that otherwise had been consigned to the scrap heap.

Aside from items that are available at most hardware stores (plywood, polystyrene panels, wire, light bulbs and ceramic sockets, wire, cabinet, hinges, latches, and Mortite caulking for plugging the holes that were drilled in the incubator walls for the thermometer, thermostat, and wiring, two items were purchased elsewhere: 3" square fan, D33,588 from Edmund Scientific Co., 101 East Gloucester Pike, Barrington, NJ 08007 ($20.00 including shipping costs); 800 watt thermostat, FT-7, from A.M. Leonard Inc., 6665 Spiken Road, Piqua, OH 45356 ($24.00, including shipping).

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Incident light microscopy of SSP chromosomes.

For cytological mapping of polytene chromosomes, transmission light and electron microscopy have been used almost exclusively so far, because surface structure studies of native chromosomes have not been able to yield any detailed information concerning the banding patterns. This is the case even in scanning electron microscopic analyses (Iino & Nagura 1980). Using the surface spread polytene (SSP) chromosome preparation technique, however, where chromosomes are spread laterally and longitudinally, more structural details are depicted in transmission light (Kalisch 1982) and electron microscopy (Kalisch & Whitmore 1983) than can be seen in well-extended squash preparations. Through the spreading process the SSP chromosomes are flattened enough so that individual bands become distinguishable as surface structures due to their supercoiled DNA (in comparison with the uncoiled DNA of the interbands). We have shown previously with scanning electron microscopy that the surface pattern of SSP chromosomes is identical with the one which can be seen using transmission electron microscopy (Kalisch & Jacob 1983). In this preliminary note, we show that even incident light microscopy together with differential interference contrast (DIC) can be used for a detailed pattern analysis of SSP chromosomes in Drosophila.