Figure 2 shows the structure of a cage. The ceiling was wrapped with cheese-cloth to provide ventilation. The food was provided daily on trays (20 x 13.5 x 2 cm). New trays were placed through the openings on the bottom of the cage, pushing the old ones into an anaesthetizing box in order to collect flies from the medium surface. The anaesthetized flies were put back into the cage through the cheese-cloth sleeve of the side opening.

All equipment mentioned above was made of transparent plexiglass.

The embryo harvesting trays were placed and removed in the same way. Each harvesting tray (Fig. 3) consisted of a flat piece of silon sponge, a piece of polyester material and some fine nylon gauze. The sponge piece (20 x 13.5 x 1.5 cm) was wetted with a solution containing 1.5% acetic acid, 2.5% ethanol and covered with the polyester material whose colour resembled the food medium colour. A thick suspension of Baker's yeast was layered and the nylon gauze was placed over it. In this way the desired rough medium surface which is the preferable place of egg deposition was obtained (Spencer 1950; Elgin & Miller 1978). In addition the nylon gauze prevented flies from sticking to the yeast suspension. The embryos were collected within two hours during the oviposition activity of the flies.

Egg harvesting was carried out by washing the nylon gauze in water and filtering the washing water through a sintered glass funnel S_1.

The method described above allowed us to collect 10-15 g of synchronous Drosophila embryos for a laying period of two hours, employing a population of 50 to 70000 flies.

References: Comings, D.E., D.C. Harris, T.A. Okada & G. Holmquist 1977, Exp. Cell Res. 105: 349-365; Elgin, S.C.R. & D.W. Miller 1978, in: The Genetics and Biology of Drosophila, v2b, pp.112-120 (Ashburner et al., eds.), Academic Press, NY; Mitchell, H.K. & A.M. Mitchell 1964, DIS 38: 135-137; Spencer, W.R. 1950, in: Biology of Drosophila, pp.535-590 (M.Demerec, ed.), NY - L.

Real, M.D., J. Ferré and J.L. Ménsua. University of Valencia, Spain. Methods for the quantitative estimation of the red and brown pigments of **Drosophila melanogaster.**

Quantitative estimation of the red (drosopterins) and brown (xanthommatins) eye pigments of **Drosophila melanogaster** in eye color mutants has normally been carried out following Clancy's "double extraction" procedure (1942). This method is based in the extraction of the red pigment in AEA (30% ethyl alcohol acidified with HCl to pH 2.0) by placing the heads in this solvent for 24 hr. Then, the brown pigment is extracted by transferring the heads into a solution of AMA (absolute methyl alcohol containing 1% by volume of dry HCl).

Ephrussi & Herold (1944) systematically studied Clancy's procedure and found it inappropriate for the analysis of the brown pigment in mutants having also drosopterins. They reported that the "double extraction" had two main problems: the AEA seems to render insoluble a part of the brown pigment, and second, some brown pigment is extracted by the AEA.

We have revised the procedures reported in the literature for selective extraction of the eye pigments. In mutants containing both eye pigments, an accurate quantitative estimation can be made using the following methods:

Quantitation of the red pigment ("drosopterins"): Fly heads (around 40, 20 from males and 20 from females, nine-day-old adults) split longitudinally into halves, are placed in AEA (3 ml) for 24 hr (Ephrussi & Herold 1944). Then, the extract is filtered through an inert filter (glass fiber) and the absorbance measured at 480 nm. The absorbance is linear versus the number of heads at least up to values around 0.900.

In order to test the above procedure, the following mutants were chosen: scarlet (it does not have "xanthommatins"), sepia (it has no "drosopterins" and it is the mutant known to accumulate the highest amount of the yellow pigment sepiapterin) and brown (it only has "xanthommatins"). Table 1 shows that the extraction of the red pigment is higher when the heads are split longitudinally or when homogenized with AEA. Interferences due to the yellow and brown pigments were higher when heads are homogenized than when split into halves (Table 1). Only in the case of the sepia mutant was the yellow pigment contribution important. In all the other eye color mutants, the "drosopterins"/sepiapterin ratio is high enough to minimize sepiapterin contribution to "drosopterins" quantitative estimation.

The stability of the red pigment in AEA has been compared with its stability in 0.1 M NaOH and 0.1 M HCl. After 72 hr, the loss of absorbance at 480 nm of a homogenized extract of scarlet heads is: 2% in AEA, 9% in HCl and 90% in NaOH.

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.