from the standard pasteur pipette. The eggs are kept in suspension and from sticking to the gauze by means of a small magnetic stirrer, revolving at about 74 revs./minute. The figures for flow rate and stirring are those which give optimum egg-hatching rates with minimum culture infection. The eggs undergo 15 minutes of this treatment and then the egg chamber is removed from the apparatus. The egg chamber must be washed through with sterile water before plating so as to remove any C.D.B.A.C. Prolonged exposure to this detergent was found to kill eggs. Some strains appear more sensitive than others to the length of time spent in C.D.B.A.C. The egg chamber is then dismantled at (F) in a sterile hood and the eggs plated on agar using the procedure of Sang (1956).

Reference: Sang, J.H. (1956). J. Exp. Biol. 33:45-72.

Lewis, E.B. and L. Craymer. California Institute of Technology, Pasadena, California. Quinacrine fluorescence of Drosophila chromosomes.

We describe below a modification we have developed for Drosophila of the quinacrine-fluorescent staining methods developed by Caspersson and his colleagues (Expt. Cell Res. 58: 141-151, 1969) for plant and, later, human chromosomes. Our findings are in general agreement with those of Vosa (Chromosoma 31: 446-451, 1970) who reports selective staining with this dye of the long arm of the Y chromosome and of the fourth chromosome in Drosophila melanogaster. In addition we have observed a bright fluorescing band in region 81F (3R) of the salivary gland chromosomes. Also in larval ganglion metaphases a weakly fluorescent spot is visible in the basal heterochromatic region of X, 2L, 2R and 3L, while 3R has two such spots. The Y has at least three strongly fluorescing spots in Y-long and at least one in Y-short (Y^{closed} has five spots visible); the fourth chromosomes appear at metaphase as two very bright fluorescent bodies. Adult muscle or brain tissues show in resting nuclei a large, usually single, fluorescent body, which may represent a chromocentral fusion of the fluorescent spots found in the basal part of each chromosome arm. The XY male has a somewhat brighter body, evidently due to fusion of the Y chromosome as well. It may prove possible therefore to "sex" somatic resting nuclei of adult tissues.

The resting nuclei of imaginal disc cells also have a single large fluorescing body in XX or XO tissues but tend to have two such bodies, of approximately equal fluorescent intensity, in XY or XXY resting nuclei. We interpret this to mean that in these rapidly dividing tissues the Y often does not fuse with the chromocenter. We have also extended this observation by studying males carrying an extra Y-long arm attached to X in addition to a normal Y. Such males often show three fluorescing bodies in the resting nuclei of their imaginal wing discs instead of two, suggesting that extra Y's do not tend to fuse with one another in imaginal disc tissue.

A. Procedure for staining Drosophila salivary gland chromosomes or resting nuclei of many larval or adult (except brain) tissues.

1. Dissect larvae in 45% acetic acid. Place tissue in a small drop of 45% acetic acid on a siliconed coverslip. Lower a slide which has been "subbed"* over the drop; after it touches the drop, invert the slide; blot to remove excess mounting fluid; tap the coverslip sharply over the tissue area with a blunt instrument to disperse cells; cover with absorbent tissue and squash with strong pressure.

2. Immerse slide in liquid nitrogen until bubbling stops (or freeze on a block of dry ice).

3. Pry off coverslip with a razor blade. Dip slide in 95% alcohol for about a minute and then into absolute alcohol for a minute. Remove and dry by waving in the air.

4. Flood area over tissue with a few drops of an 0.5% to 2% solution of quinacrine hydrochloride in 45% acetic acid and stain for one or two minutes. (Batches of Gurr's "atebrin" or Sigma's quinacrine have proved satisfactory.)

5. Drain off staining solution and quickly dip slide into a jar of 95% ethanol followed by one or two transfers through absolute ethanol. The total time in the alcohols should be 20 seconds or less at 25°C to avoid excessive destaining. Insufficient rinsing may result in excessive background fluorescence in the final preparation.

6. Remove slide from the absolute ethanol and quickly dry by waving the slide vigorously in air. Heating at this stage tends to destain the preparation.

7. To mount, place a drop of sucrose solution (0.5 to 1.0 molar in double distilled water) in the center of a coverslip. Invert the slide over this coverslip until it touches

the drop. Quickly reinvert and blot firmly to remove excess mounting fluid. Seal with clear nail polish.

B. Procedure for staining adult brain tissue.

1. The same procedure as that described for the larvae is used except that the percentage of acetic acid in both the dissecting fluid and the quinacrine staining solution is reduced from 45% to 10%.

C. Procedure for staining larval ganglia for metaphase chromosome studies (modified from DIS 34: 118-119).

1. Dissect larvae in a solution of 1.0% Na Citrate in distilled water. Place the dorsal ganglia in a drop of this solution for 10 minutes on a slide. Warm the slide on a hot plate at 40°C for one minute (this hastens separation of sister chromatids). Pass the ganglia into a pre-fixative composed of equal parts of 45% acetic acid and 95% ethanol and leave for 30 seconds. Then remove tissue and place in a drop of 45% acetic acid on a siliconed coverslip. Continue with procedure described in part A, par. 1 above.

*3 gm gelatin. 600 ml distilled water. Heat to dissolve gelatin. Cool. Add chrom. alum - $KCr(SO_4) \cdot 12 H_2$) - 300 mg. Dip slides, drain and allow to dry in dust-free container.

TEACHING NOTE

Potter, J.H. University of Maryland, College Park, Maryland. A demonstration of compensation for an inherited biochemcial defect in D. melanogaster.

A simple demonstration of compensation for an inherited biochemical defect can be carried out by beginning students using D. melanogaster. In essence, students supply kynurenine to larvae of vermillion mutants which cannot convert tryptophan to kynurenine, one of the steps in the synthesis of omnochrome pigments. Since students frequently do not distinguish vermillion from wild type flies, they use the white-eyed, double mutant, vermillion brown. Vermillion brown larvae fed kynurenine develop brown eyes. To emphasize the specificity of the block, students also feed kynurenine to the double mutant, cinnabar brown. Cinnabar brown mutants develop white eyes whether or not they receive kynurenine.

Experimental procedure: Students set up two cultures each of vermillion brown and cinnabar brown mutants in 80 x 25 mm. shell vials containing 5 ml of Carolina Instant Drosophila Medium. As soon as larvae appear the parents are removed and the medium in one vial of each genotype is injected with 0.2 ml of a kynurenine-antibiotic solution. The medium in the other two vials is injected with 0.2 ml of plain antibiotic solution. The injections are made with a 2 1/2 ml syringe without a needle inserted in a hole made in the medium with an applicator stick. Injections are repeated every two days until pupae appear. The adults are scored in the usual way. The kynurenine treated, vermillion brown, flies are mated after scoring and their progeny scored for eye color to demonstrate that the genotype has not been changed by the kynurenine treatment.

The kynurenine antibiotic solution is similar to that used by Parsons and Green (1959) for culturing eye discs: 0.05% streptomycin, 0.033% penicillin and 1.00% D.L. kynurenine can be obtained from Sigma Chemical Co., St. Louis, Missouri, at $14.00/gram.

References: Parsons, P.A. and M.M. Green, 1959, Proc. Nat. Acad. Sci., Wash. 45: 993.

MATERIALS REQUESTED OR AVAILABLE

H.R. Feijen, University of Malawi, Genetics Section, P.O. Box 5200, Limbe, Malawi, would be grateful to obtain reprints on speciation in Drosophila and reprints on systematics of Drosophila.