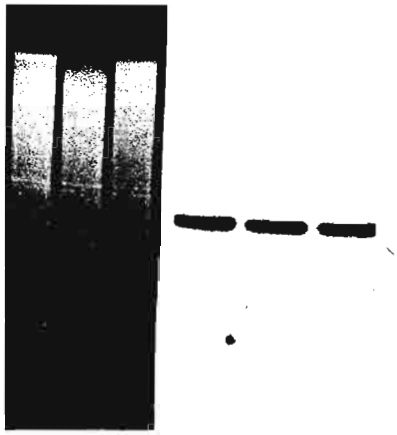

a b c a b c

We have found the method to be an easy adjunct to genetic experiments, since instead of discarding the appropriate genotypes at the time of scoring, they are retained at -70°C for use in recombinant DNA experiments. It should also be noted that storage of adults in this way provides an excellent source of undegraded total and polyadenylated RNA from heads as assayed by Northern blots (M.Healy, unpubl.). Since nearly two-thirds of the polysomal RNA complexity of the entire life cycle is present in adult heads (Levy & Manning 1981), adult heads may be a far more useful source of nucleic acids than has previously been imagined.

References: Oliver,D.V. & J.P.Phillips 1970, DIS 45:58; Spradling, A.C. 1981, Cell 27:193-201; Levy, L.S. & J.E. Manning 1981, Developmental Biology 85:141-149.

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Egg sterilization without dechorionation.

We have adapted a method used for sterilizing mosquito eggs for use with fruit fly eggs. Eggs laid over a 3-5 hr period are loosened with a brush from agar flooded with water. A yeast paste made from sterile killed baker's yeast is used on the agar as an oviposition stimulant. Eggs are collected in a small fine meshed sieve and washed to remove visibly adhering particles. Eggs are then transferred to a sterile 50 ml container with 20 ml of 80% alcohol. Following a suggestion by H.Gordon a vacuum is applied (about 5-10 Hg is adequate) for about a minute. Presumably the alcohol is a wetting agent which is effectively pulled into the interstices of the chorion under vacuum. After 45-60 min in alcohol, using a sterile sieve, the eggs are transferred to a 50 cc sterile snapcap jar with 20 cc aqueous 0.3% Hyamine 10X which has been autoclaved for about 5 min. This compound is available from Sigma as methylbenzethonium chloride. An alcohol sterilized cap is snapped on and the eggs are shaken for 30-40 min. Finally the eggs are placed in sterile water and pipetted into sterile media. In a typical experiment where approximately 10 eggs were transferred to each media vial 85% of the vials remained free of contamination.

Success of this method seems to depend on using only 200-300 eggs per container, using "clean" parents (we do not add live yeast to our cultures) and using "fresh" eggs where there are no hatched larvae. Overnight eggs are less successful.

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Method of preparation of Drosophila for scanning electron microscope studies.

Modification of the method of Hodgkin & Bryant (1978) for preparation of Drosophila for SEM studies has resulted in a simple procedure which yields excellent results with minimal distortion of the specimen.

Adult flies were first etherized and submerged in either 70% ethanol or 70% acetone; less damage was observed in the specimens dehydrated with ethanol. In addition, soaking in 70% ethanol for two to three days appeared to reduce damage. The flies were then dehydrated as follows: 70% ethanol for 15 min, 95% for 15 min, and three changes of 100% for 15 min each. Specimens were then placed in sample holders constructed as described by Postek et al. (1980) and slowly critical point fluid. The use of the sample holders necessitates longer diffusion time, but reduces damage due to excessive handling. Whole flies were mounted directly on metal stubs with carbon paint which eliminated charging. Appendages and heads were attached to glass coverslips with double-stick cellophane tape which gives a smooth background; the coverslips were then mounted on metal stubs. Specimens were sputter coated with approximately 200 A of gold-palladium. Specimens were examined with a JEOL 35C scanning electron microscope using an accelerating voltage of 10 kV and a load current of approximately 100 μA. Polaroid 4x5 Land Film Type 55/Positive-Negative produced the best quality prints.

Fig. 1. D.simulans tarsal claw. Acetone dehydration, attached with double-stick tape. Bar = 10 µm.

Fig. 2. D.simulans. Ethanol dehydration, attached with carbon paint.

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References: Hodgkin,N.M. & P.J.Bryant 1978, IN The Genetics and Biology of Drosophila, V2c (Ashburner & Wright, eds), Academic Press, New York, p337-358; Postek et al. 1980, Scanning Electron Microscopy, A Students Handbook, Ladd Research Industries, Inc, p.143-144.

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A new fungicide for Drosophila medium.

Heavy and persistent fungal infections which were resistant to the propdionic acid and Nipagin (Tegosept) which are routinely added as fungicides to the yeast-sucrose-agar medium we use (based on Carpenter 1950) led us to look for a more effective fungicide. Benzimidazole compounds are often used as fungicides in agriculture and horticulture. We now routinely add methyl benzimidazole carbamate as the fungicide for our Drosophila medium. This compound is insoluble in water and only sparingly soluble in ethanol. We make a solution of 200 µg/ml of MBZ in ethanol. (It is effective even though this may not all dissolve unless left for an extended period.) 10 ml of this solution is added to 500 ml of Carpenter's medium after autoclaving. This addition has no discernible effect on fertility, fecundity, development rate or viability of any of our stocks of D.melanogaster or D.hydei. We have not tested for any mutagenic effect and care should be taken if studying non-disjunction as these compounds inhibit microtubular function and effect non-disjunction in fungi. We have now used this fungicide for two years and had no problems with fungal infections during that time. Yeasts appear to grow on this medium. We obtain methyl benzimidazole carbamate from BASF (UK) Ltd., Lady Lane, Hadleight, Ipswich, Suffolk, IP7 6BQ, U.K.

Reference: Carpenter, J.M. 1950, DIS 24:96-97.