The Australian Drosophila fauna is dominated by members of the subgenus Scaptodrosophila. Most of the latter species cannot be collected by fruit baiting and have to date proved impossible to culture, but species of the coracina group (Bock and Parsons, 1978) are attracted to fruit baits and have been cultured successfully by the method mentioned above. When ready for pupation, larvae of the coracina group species crawl to the top of their vial and "skip", landing either on the surface of the sand into which they immediately burrow, or on the side of the sand jar, in which case the process is repeated.

If a culture of one of the above group of species is maintained in a stoppered vial when the larvae are ready to pupate, most larvae crawl to the top of the vial, attempt to squeeze past or burrow into the stopper, and die; pupal integuments are formed in some cases, but the pupae are generally inviable. A small yield of adults of the next generation may be obtained from the minority of larvae remaining to pupate in the food medium itself. However, if the sand jar technique is used, a very substantial yield of adults of the next generation can be obtained.

Except for D. coracina itself which is Japanese, the species of the coracina group are Australian. D. coracina has been regarded as difficult to culture (Toda, pers. comm.), but newly-collected specimens arriving from Japan went readily into culture using the sand jar technique. D. coracina breeds in tree sap (Toda, 1977). The breeding sites of the Australian species are not yet known, although it seems likely from experience with collection methods that some at least may breed in rain forest fruits or fungi. Separate larval feeding and pupation sites are clearly suggested by the behavior of the final instar larvae at the point of pupation, and it is not inconceivable that the technique described above may be extended to species of other groups in which the larvae normally pupate away from the source of food.

The recipe for the larval medium that we use is 36 gm agar, 72 gm dried yeast, 108 gm raw sugar, and 24 ml of 10% nipagin in 75% ethanol. All ingredients are boiled for 5 min in 1000 ml water, then a further 1000 ml cold water with 10 ml priopionic acid is added before the medium is dispensed. This medium is more suitable than one with live yeast.

We have cultured the following species with the sand jar technique: D. coracina, D. lativittata, D. enigma, D. nitidithorax, D. specensis, and a new species of this group recently discovered on Lord Howe Island. In some cases adding a piece of mushroom appears to facilitate oviposition, a not unexpected finding given that the last two species at least are additionally and preferentially attracted to rotted mushroom baits in the wild when given a choice of baits.

Culture temperature is important, since 18-20°C appears more suitable than 25°C, a common D. melanogaster culture temperature. This is predictable since many Australian rain forest species are difficult to culture at temperatures as high as 25°C, as is also true of many Hawaiian endemic species (Carson et al. 1970).

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Cobel-Geard, S.R. and H. Gay. University of Michigan, Ann Arbor. A new simplified method for the preparation of neuroblast mitotic chromosomes from D. melanogaster.

A number of procedures have been devised for spreading metaphase stage chromosomes of neural ganglia from Drosophila melanogaster third instar larvae. We wish to report a technique which has been used in our laboratory with great success to obtain large numbers of neuroblast slide preparations. These are highly suitable for in situ hybridization experiments because they contain well-spread metaphase stages with longer, less condensed chromosome arms. Our procedure is an adaptation of a technique described by Holmquist (1975) and based on an ear-

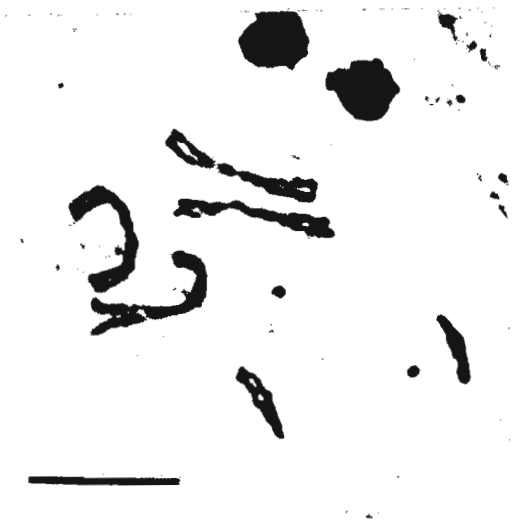

Fig. 1. Mitotic chromosomes of D. melanogaster for third instar neuroblast cells. X2160 Bar = 10 μ

lier method reported by Lewis and Riles (1960). The modifications we have made substantially simplify the above procedures, requiring less individual manipulation of fragile neural ganglia and eliminating the need for a slide warmer. The resulting slides can be stored as air-dried preparations and are suitable for repeated cytochemical treatments.

The procedure for preparing 20 slides is as follows:

1) Third instar larvae should be selected from well-fed and uncrowded bottles.

2) Twenty neural ganglia (dorsal and ventral maintained as a unit) are dissected out in Becker's solution and placed in a watch glass or other small shallow dish containing 5 ml of Schneider's embryonic cell culture medium (Schneider 1964) at room temperature. The ganglia can easily be picked up in a droplet of fluid without crushing the tissue using very fine forceps with the droplet adhering between unclosed tips.

3) After 20 ganglia have been placed in the medium, add 0.04 ml of a 100 μg/ml refrigerated stock solution of colcemid (final concentration 0.7 μg/ml) and incubate at room temperature for 45 minutes.

4) During the incubation period place 20 dust-free slides (acid washed and subbed with a gelatin solution -- 0.5 g gelatin/500 cc H_2O + 0.05 g chrome alum, $KCr(SO_4)_2$) on an ethanol-swabbed flat countertop. Subbed slides should be stored with dessicant in the refrigerator during hot and humid months to prevent peeling of the subbing solution. Other equipment and solutions should be assembled at this time: 3 pasteur pipettes and bulbs, 1% sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 50% acetic acid, and 18 mm diameter coverslips soaking in 95% EtOH.

5) After the incubation the ganglia are transferred, one per slide, to single drops of 1% sodium citrate by again taking care to avoid damaging the tissue.

6) The neuroblast cells of the first ganglia are hypotonically swollen for 10 minutes, at which time the majority of the hypotonic solution is removed by slow suction with a pasteur pipette. Immediately a new pipette is used to add a drop of 50% acetic acid before the tissue dries out (a second person performing this task is helpful, but not necessary). This step should be carried out rapidly by moving down the line of slides in an assembly line fashion.

7) After the first specimen has been fixed for 5 minutes, an ethanol-rinsed coverslip that has been wiped clean with lint-free lens paper is placed over the drop. To spread the tissue, the coverslip is gently tapped with a small blunt tool, like the wooden handle end of a dissecting probe. Care should be taken to avoid slippage.

8) To remove excess moisture, the slide is then placed between layers of tissue paper and pressure is exerted with a rolling movement of the index finger.

9) The slide is then placed coverslip side down onto a flat surface of dry ice. Continue down the line until all slides are on the dry ice block.

10) After allowing the specimens to freeze for approximately 30 minutes, the coverslips are quickly flipped off with a scalpel blade, and the slides immersed in 100% EtOH and stored overnight at -4°C.

These neuroblast slide preparations are then air dried before using for in situ hybridization. To facilitate microscope scanning for metaphase stages, we stain our slides with 3% Giemsa in 0.01 M sodium phosphate buffer for 20 minutes.

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