Atherton, D. and J. Gall. Yale University New Haven, Connecticut. Salivary gland squashes for in situ nucleic acid hybridization studies.

In situ nucleic acid hybridization studies require chromosome preparations that can withstand air-drying, alkali denaturation, incubation at elevated temperatures, and conventional autoradiography, while still maintaining adequate morphological detail. We have found Drosophila polytene chromosomes among the most challenging objects so far tested. The following procedure provides permanently stained autoradiographs of in situ hybrids in which banding patterns are nearly as good as in the best temporary mounts.

Select a late third instar larva, which has crawled up the wall of the bottle and has stopped moving. Those with extruded spiracles and somewhat hardened cuticle often provide the best glands. Animals should be from well-fed, uncrowded bottles. D. melanogaster larvae should be raised at a low temperature (18°C).

Dissect out the salivary glands in Ringer's solution, including the duct but without the fat bodies. Transfer the glands on a needle to a small dish of freshly prepared ethanol-acetic fixative (3:1) and cover the dish. The glands will turn white immediately.

While the glands are fixing (2-5 min.) prepare a siliconized coverslip by removing it from ethanol and wiping it to remove all dust. On the coverslip place a small drop of 45% acetic acid (7-8µl). The volume is critical.

Remove the glands from the 3:1 with forceps and watch them under the dissecting microscope while the fixative evaporates. Just before the 3:1 is completely evaporated place the glands in the drop of 45% acetic acid and allow them to swell. Remove any pieces of dust from the coverslip. We prefer strong incident illumination against a black background.

Gently lower a subbed slide toward the preparation until it just touches the drop. Pick up the coverslip with the slide. Survey the preparation under low or medium powers of the phase contrast microscope (16X-40X). At this point there should be many intact cells as well as free, but intact nuclei.

Place the slide, coverslip up, between two pieces of bibulous paper. While holding one end of the slide, tap the area over the coverslip about ten times with the eraser end of a pencil. Tap very hard for melanogaster, less hard for some other species with larger nuclei, such as virilis and hydei. Do not make any special effort to prevent lateral motion of the coverslip. Under no circumstances should sustained pressure be applied to the coverslip area.

Check under the microscope. At this point cytoplasm should be flowing and the chromosomes should have broken out of the nuclear envelope. Tap again in the same manner as before, and recheck under the microscope. Continue until the chromosome arms are in the desired configuration.

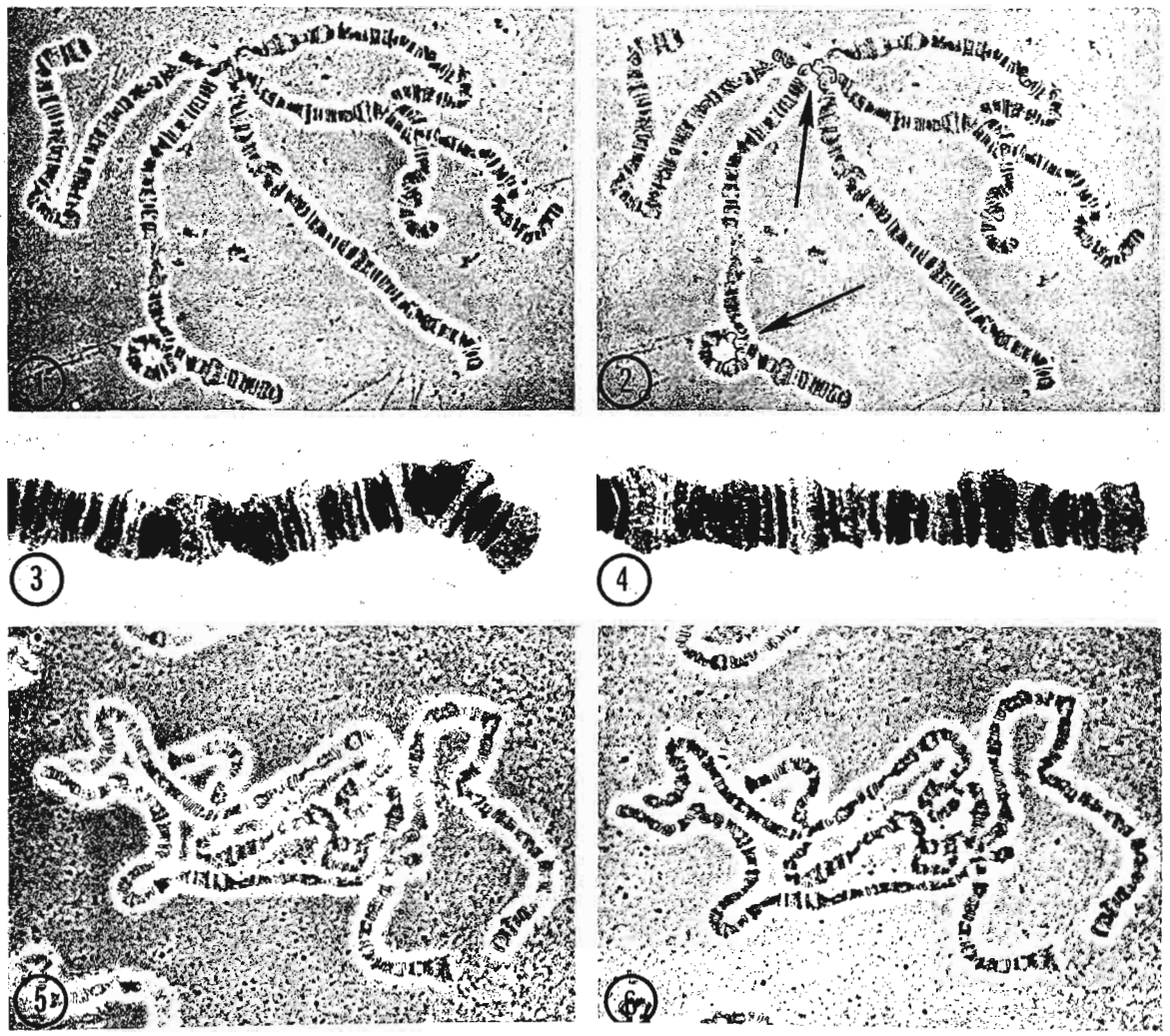
Note that the first taps should break the nuclear envelope, make the cytoplasm flow, and cause the chromosome arms to extend freely into the liquid. Subsequent taps eliminate the excess liquid and cement the chromosome arms into the desired position on the slide. Do not "squash" the preparation as is usually done for salivary gland material. Preparations which are adequately squashed for conventional cytology will not be flat enough for in situ hybridization, while those squashed flat enough by finger pressure will usually be badly distorted.

Flatten the preparation by placing the slide on a warming plate at 40-45°C. About once a minute check the preparation by observing under the 25X phase objective for a) cytoplasmic flow, b) the presence of heavy, refractile bands, and c) the condition of the chromocenter. A preparation should be left on the warming plate until the cytoplasm has stopped flowing, the heavier bands have been pressed until they are no longer refractile, and the chromocenter has not been pressed into a gray blob. These criteria apply specifically to observations made with the Zeiss 16X, 25X, or 40X Neofluar objectives. If during the monitoring stage the chromocenter begins to lose its morphology, remove the slide whether or not the cytoplasm is flowing and some bands are still refractile. Dust between the slide and coverslip will permit air to enter as the solution evaporates, thereby ruining the preparation.

Place the slide, coverslip down, on a flat block of dry ice in a covered container, and leave for 10-20 minutes. With a sharp razor blade in hand and an open Coplin jar of 100% ethanol standing by, quickly lift the slide from the dry ice, place the razor blade under the edge of the coverslip, flip off the coverslip and plunge the slide into the ethanol. Leave the slide in the ethanol for at least 12 hours in order to harden the tissue; it may be left for several days if desired. Remove the slide, stand it vertically, and allow it to dry. Check the slide under the phase contrast to see how good the preparation is. The best slides will have little cytoplasm, well spread chromosome arms, and all bands grey or black, even though viewed in air without a coverslip or mounting medium. Adequate preparations will have

some refractile bands. In these the alkali denaturation step during the in situ hybridization procedure will cause some swelling.

For in situ hybrids proceed as described in Gall and Pardue (Methods in Enzymology, XXI,



Figs. 1 and 2. Chromosomes of D. melanogaster before (1) and after (2) treatment with 0.07N NaOH for 2 minutes to denature the DNA. This preparation demonstrates the ideal degree of flattening, and shows only minimal damage after the alkali treatment (arrows in (2) indicate "ballooning" in the chromocenter and at regions of overlaps). Photographed as a dry preparation without mounting medium or coverslip. Because of the great difference in refractive index between the specimen and air, only the very flattest preparations will show gray or black bands. Zeiss 25X Neofluar phase, X310.

Figs. 3 and 4. Two examples of chromosome 2L from a preparation which had been air dried, denatured with 0.07N NaOH, stained with Giemsa, and permanently mounted in Permount. Fig. 3 is from the nucleus shown in (1) and (2). Zeiss 100X planapochromat, bright field, X1,200.

Figs. 5 and 6. Chromosomes before (5) and after (6) 0.07N NaOH to demonstrate the appearance of an adequate, but not ideal squash. Note the overall high refractility with many bands brighter than background. As in (1) and (2) the contrast is slightly lower after alkali, presumably due to extraction of material. Photography exactly as in (1) and (2). X330.

470-480, 1971). However, omit step 9 of that paper (acid treatment to remove histones). Acid treatment reduces the amount of hybrid formed and interferes with good staining.

The procedure outlined here can, of course, be used for routine stained preparations without hybridization. In this case, the flatness of the chromosomes is less critical. Giemsa stain provides excellent contrast of bands and demonstrates nucleolar DNA clearly. It may be preceded by RNase or a brief acid hydrolysis to reduce cytoplasmic staining (1N HCl, 60°C, 5 min.).

Materials needed: Siliconized coverslips: Place coverslips (18 mm square, #1 thickness) in a 1% Siliclad solution for a few moments, rinse in distilled water, and allow to dry on paper toweling. Store the coverslips dry and do not worry about water marks. Just before needed, place a few coverslips in 95% ethanol. Subbed slides: Clean new slides in detergent, rinse well with tap water, and place for a few moments in a solution of gelatin and chrome alum (0.1% gelatin in 0.01% chromium potassium sulfate). Dry slides vertically in a slide carrier, and store in a dust-free box. Solutions: Immediately before use, prepare "3:1" fixative, consisting of 3 parts absolute ethanol + 1 part glacial acetic acid. Keep stock solutions of 45% acetic acid, and any convenient insect Ringer's solution.

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PERSONAL AND LABORATORY NEWS

J.L. Ménsua, formerly Assistant Professor in the Department of Genetics, University of Barcelona, has accepted a charge of Professor in the Autonomous University of Barcelona (Bellaterra), where he is head of a new Laboratory of Genetics.

Heinz Tobler has left the University of Zürich and is now Full Professor and Director of the Institute of Zoology, University of Fribourg, 1700 Fribourg, Switzerland. He heads a group working on problems of determination and differentiation of imaginal discs.

Ross MacIntyre will be on sabbatic leave at the Department of Genetics, University of California at Davis, Davis, California from February through July, 1973 (from Cornell University, Ithaca, New York).

The Drosophila Species Collection at the University of Texas at Austin still exists, and stocks are available upon request from either M.R. Wheeler or R.H. Richardson. Although support is insufficient for long-term survival, efforts are still being made to obtain additional support necessary to maintain the collection. With tightening of research funds, the financial support for the facility was no longer possible as a component of a research project. With the enthusiastic and invaluable world-wide support of numerous Drosophila workers, about 1/3 of the N.I.H. approved support level has been shifted to the N.I.H. Research Resources Branch. Until August 31, 1973, sufficient additional interim support for temporary maintenance of the facility is being supplied from local and piecemeal support. In the meantime, N.S.F. has reviewed a proposal to share support with N.I.H., but there were no funds for new museum support and N.S.F. has not been able to allocate funds to this facility. Unless additional funds become available by next August the facility will be lost, but optimism still prevails.

Ravi Dutt Narda, 32, former Associate Professor of Genetics, Department of Genetics, Punjab Agricultural University, Ludhiana, India, died on August 23, 1971. Dr. Narda did his M.Sc. in 1963 and Ph.D. in 1966 from Punjab University, Chandigarh, India, under the supervision of Dr. G.P. Sharma. He joined the Department of Genetics, Punjab Agricultural University, in 1966 as Assistant Professor and was promoted to Associate Professor in 1970. His research work included behavioural genetics, speciation and mutation studies in Drosophila.