Sawicki, J.A. and W.W. Doane. University of California School of Medicine, San Francisco and Arizona State University, Tempe, Arizona. Preparation of "small" polytene chromosomes for cytological analysis.

The small size of polytene chromosomes in larval tissues such as the midgut and fat body of D. melanogaster precludes their cytological analysis for banding and puffing patterns by standard light microscopic techniques. To overcome this difficulty, we used two different approaches to study midgut polytene chromosomes of late third instar larvae: (1) Scanning Electron Microscopy (SEM), and (2) light microscopy coupled with genetically increased levels of polyteny.

SEM Approach. The SEM procedure we adopted is a modification of the procedure described by Mitchell and Lipps (1975). Banding patterns and "puffs" displayed by midgut chromosomes prepared by the following procedure could be satisfactorily compared with those displayed by salivary gland chromosomes which were prepared by either SEM or standard light microscopic methods.

1. Dissect tissue (e.g. three midguts) in Drosophila Ringer's solution (Ephrussi and Beadle 1936).
2. Transfer tissue to 50 µl of 45% acetic acid and rinse 3 times with the acid.
3. Replace acid with 20 µl of a 0.2% solution of orcein (natural) in a 1:1 mixture of lactic acid (85%):glacial acetic acid (v/v). Let tissue stand at room temperature for 1-3 hours in staining mixture.
4. Add 50 µl of 45% acetic acid.
5. Suck mixture containing tissue in and out of a small, siliconized glass needle such as a dispopipette flame-drawn to a fine tip.
6. Allow mixture to settle for a few minutes and remove surface layer containing cytoplasmic material with a siliconized dispopipette.
7. Add a small amount of 45% acetic acid to the nuclei remaining and quickly transfer to a very small volume (about 1-2 µl) containing 20-30 nuclei to a siliconized slide. Cover immediately with a "subbed" coverslip (10 x 10 mm) on which 5 µl of the 0.2% lacto-aceto-orcein stain has been dispensed in a circle of small drops. For subsequent identification, mark the side of the coverslip not in contact with the sample by a small dab of Kodak 910 cement colored blue with Azure B Bromide.
8. Spread chromosomes by gently tapping coverslip with a pencil eraser and then pressing with thumb.
9. Study the spread chromosomes with a phase contrast microscope and make an outline diagram of the relative positions of the chromosomes on the coverslip. Chip one corner of the coverslip for reference purposes and photograph particularly good spreads. To prevent distortion of the chromosome preparation due to drying out, limit the time for this entire step to less than one hour.
10. Remove coverslip by supercooling it in liquid nitrogen and flipping it off with a razor blade.
11. Transfer coverslip to 100% ethanol immediately, before ice crystals can form.
12. Let coverslip stand in 100% ethanol for about 20 minutes to dehydrate preparation.
13. Transfer coverslip to amyl acetate and leave it there for 1-3 hours.
14. Critical point dry preparation.
15. Examine coverslip with a light microscope to ensure chromosome spreads are still there. Place a small dab of DAG 154® (colloidal graphite in iso-propanol) next to each spread on the sample side; this non-conducting substance acts as a marker in the SEM.
16. Coat with gold/palladium as soon as possible after critical point drying so that samples do not absorb moisture.
17. View in SEM (45°>).

Genetic Approach. To increase polyteny of midgut chromosomes, two stocks, each carrying a different allele of the giant locus (gt 1-0.9), were used to generate third instar larvae heterozygous for the alleles gt^1 and gt^{X11} (Kaufman 1972). One stock, gt^1 w^a, is homozygous viable for the gt^1 allele. The other stock, y^{sc} gt^{X11}/FM6, is homozygous lethal for the gt^{X11} allele, which is maintained over the FM6 balancer chromosome.

Under suitable rearing conditions, the polytene chromosomes of the anterior midgut and the posterior midgut from late third instar larvae of the genotype gt^1/gt^{X11} proved to be large enough to permit accurate banding and puffing designations (Sawicki and Doane, in preparation). gt^{X11}/FM6 newly emerged females were mated to newly emerged gt^1/Y males. After an initial egg-laying period of two days, the flies were transferred daily to fresh culture bottles for 10

days and reared at 18°C. Larvae that appeared 9-10 days after egg-laying were discarded. The genotype of these larvae was either $gt^1/FM6$, FM6/FM6, or FM6/Y. The desired gt^1/gt^{X11} third instar larvae climb the walls of the food bottle 15-17 days after egg-laying.

A rich food medium was used for rearing flies and larvae which was either sugar-based or starch-based. The sugar medium contained 10% (w/v) anhydrous dextrose (J.T. Baker Chemical Co.), while the starch food contained 5% (w/v) cornstarch (ICN Pharmaceuticals, Inc.). Both media also contained 15% (w/v) Brewer's yeast, 1.2% (w/v) agar, and 1 part buffered propionic acid (Lewis 1960) plus 5,000 Units each of penicillin-streptomycin (Grand Islands) for every 100 parts of food medium.

Chromosome preparations from the anterior and posterior midgut regions of single gt^1/gt^{X11} third instar larvae were prepared for examination with the light microscope and compared with salivary gland preparations. Larvae were dissected in Drosophila Ringer's solution and the tissue was fixed immediately for three minutes in 3:1 ethanol:glacial acetic acid. After transferring the tissue to 45% glacial acetic acid for one minute, midgut preparations were stained for 2-1/2 minutes in 2% lacto-aceto-orcein while the staining time for salivary glands was 1-1/2 minutes. Chromosomes were spread in the usual manner.

While either of the above approaches was satisfactory for banding and puffing analysis of midgut polytene chromosomes, the genetic approach proved the least time consuming. It may be assumed that the combined application of both approaches to "small" polytene chromosomes would improve the resolution of banding patterns beyond that observed by using either approach alone.

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References: Ephrussi, B. and G.W. Beadle 1936, Am. Nat. 70:218; Kaufman, T.C. 1972, Genetics 71:s28; Lewis, E.B. 1960, DIS 34:117; Mitchell, H.K. and L.S. Lipps 1975, Biochem. Genet. 13:585.

Shearn, A. Johns Hopkins University, Baltimore, Maryland. Reintroduction of y^+ onto a TM3 chromosome.

Some time ago, I planned an experiment which depended on using the y^+ marker which is supposed to be on TM3 balancer chromosomes. However, none of the TM3 chromosomes tested had y^+. Therefore, I set out to reintroduce it onto a TM3 which carried Sb and Ser. Females with the genotype $y;Dp(1;3)sc^{J4},y^+$ / y;TM, ri pP sep bx^{34e} Sb e^s Ser were treated with 4000r of γ-irradiation and mated to $y;+/_{\neg}$ + males. Progeny which were y^+, Sb and Ser were selected and tested further. In this way we recovered several TM3 chromosomes containing y^+. They are reasonably stable because the unirradiated recombination frequency of y^+ with TM3 is 0.000. A stock with such a y^+ containing TM3 chromosome is available for distribution (see stocklist, DIS 54).