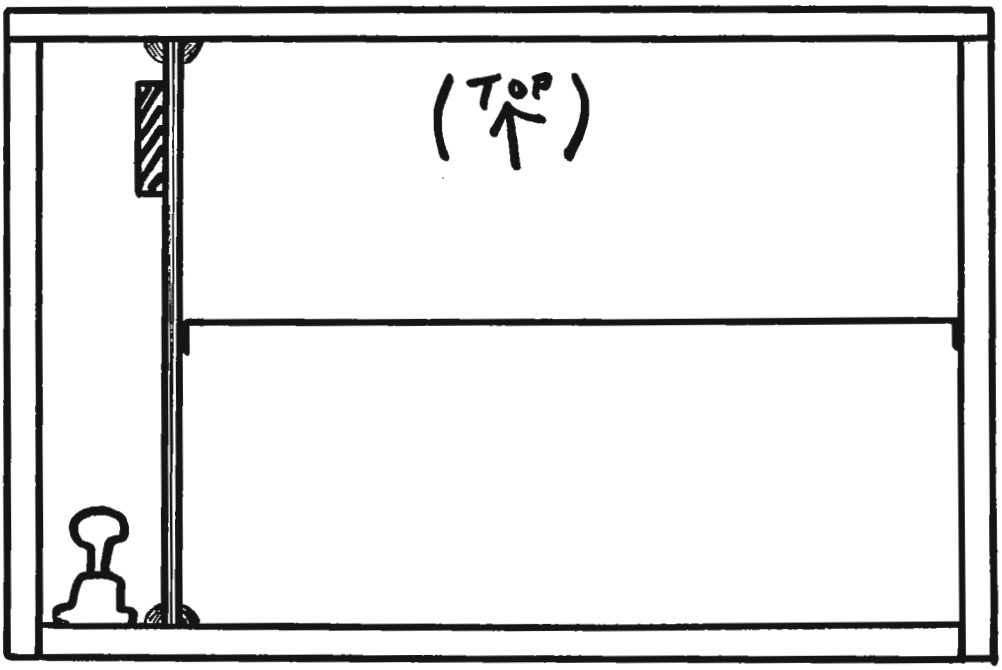

Figure 2. Scheme showing the assembly of the ends, bottom and top. The back overlaps the bottom and the ends; the top rests on the back and the two ends. The doors are mounted flush with the top and the ends; they overlap the bottom. Strips of felt may be needed on the front edges of the top and bottom to give the door a proper seal. The circulating fan is shown attached to the divider whose position, in my case, was determined by the length of the Fisher oven shelf.

The thermostat which controls the temperature of the incubator is mounted outside on the end opposite the heating chamber. The sensing bulb is passed through the end, low and in the rear; it is mounted on the back of the bottle compartment not far from the rectangular return opening in the divider. A one-inch hole was bored in the rear corner of the top at the end opposite the heating chamber. A thermometer, mounted in a rubber stopper, is placed in this hole. The thermometer bulb is protected from breakage by a small strip of hardware cloth that is fastened diagonally across that corner of the incubator, near the top. The thermometer extending above the incubator has been protected by attaching a jar lid (through which a circular hole 1" in diameter was punched) around the thermometer, and screwing the glass jar into the lid. My incubator is mounted on four 4" lengths of a square oak table leg that otherwise had been consigned to the scrap heap.

Aside from items that are available at most hardware stores (plywood, polystyrene panels, wire, light bulbs and ceramic sockets, wire, cabinet, hinges, latches, and Mortite caulking for plugging the holes that were drilled in the incubator walls for the thermometer, thermostat, and wiring, two items were purchased elsewhere: 3" square fan, D33,588 from Edmund Scientific Co., 101 East Gloucester Pike, Barrington, NJ 08007 ($20.00 including shipping costs); 800 watt thermostat, FT-7, from A.M. Leonard Inc., 6665 Spiken Road, Piqua, OH 45356 ($24.00, including shipping).

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Incident light microscopy of SSP chromosomes.

For cytological mapping of polytene chromosomes, transmission light and electron microscopy have been used almost exclusively so far, because surface structure studies of native chromosomes have not been able to yield any detailed information concerning the banding patterns. This is the case even in scanning electron microscopic analyses (Iino & Nagura 1980). Using the surface spread polytene (SSP) chromosome preparation technique, however, where chromosomes are spread laterally and longitudinally, more structural details are depicted in transmission light (Kalisch 1982) and electron microscopy (Kalisch & Whitmore 1983) than can be seen in well-extended squash preparations. Through the spreading process the SSP chromosomes are flattened enough so that individual bands become distinguishable as surface structures due to their supercoiled DNA (in comparison with the uncoiled DNA of the interbands). We have shown previously with scanning electron microscopy that the surface pattern of SSP chromosomes is identical with the one which can be seen using transmission electron microscopy (Kalisch & Jacob 1983). In this preliminary note, we show that even incident light microscopy together with differential interference contrast (DIC) can be used for a detailed pattern analysis of SSP chromosomes in Drosophila.

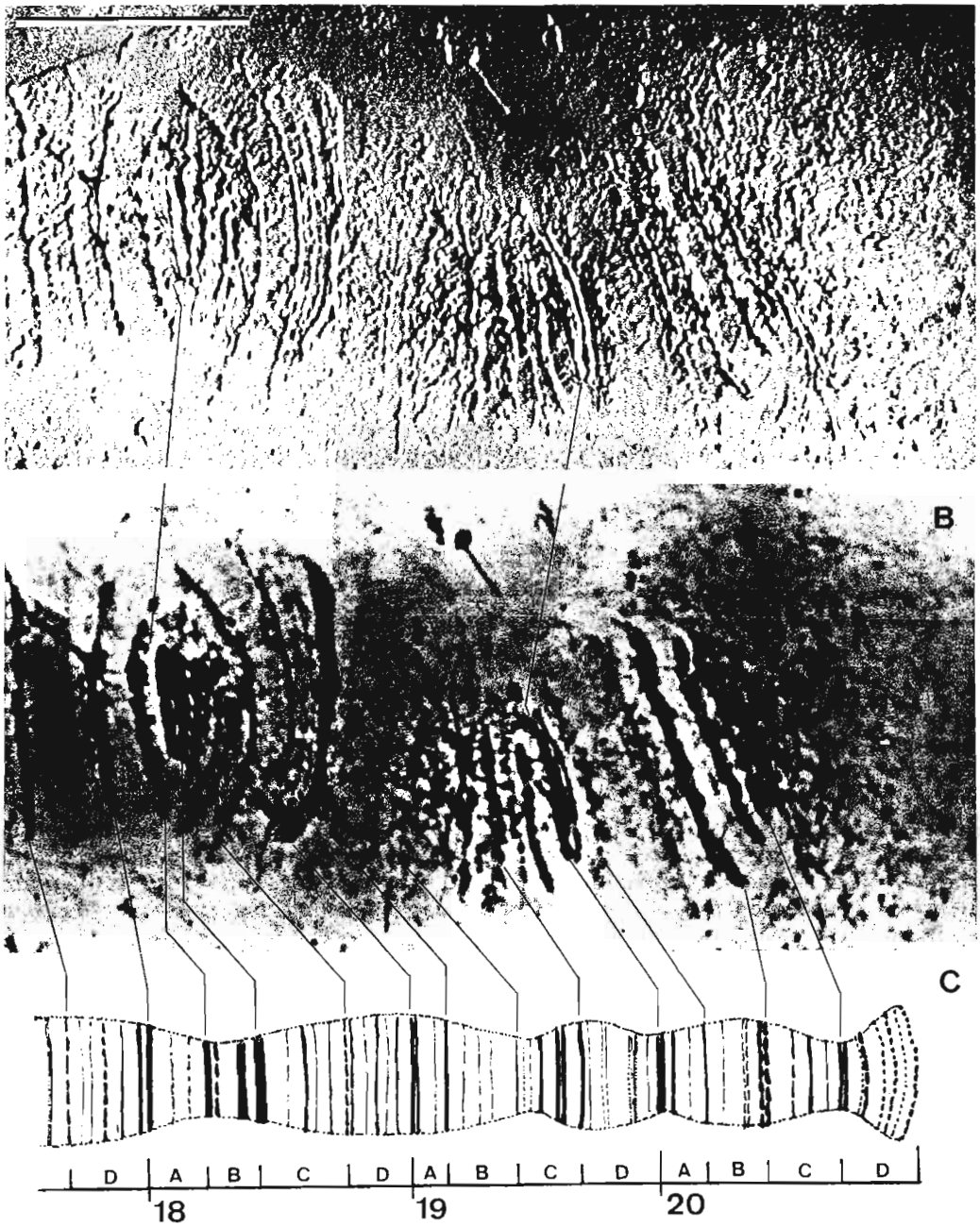

Figure 1. Region 17D-20D of a SSP chromosome-X from a late third instar larva of a **D.hydei** wildtype strain (Sao Paolo). **(A)** Incident light, differential interference contrast (Epiplan 80/0.95 Pol objective). **(B)** Transmission light, phase contrast (Planapo 63/1.4 Ph3 oil objective), green filter (11nm FWHM). **(C)** Chromosome map of Berendes (1963) based on transmission light microscopic analyses of many squash preparations. Micrographs were taken with ZEISS Universal Microscope on Agfapan 25 ASA film. Methodological details of SSP chromosome preparation, pattern analysis and incident light microscopy have already been published elsewhere (Kalisch & Whitmore 1983; Kalisch et al. 1985a and b). Bar = 20 µm.

In Figure 1 the same specimen is depicted with incident (A) and transmission light (B). In both cases patterns are identical, with the exception that in sections with a lower degree of spreading, some additional structures (faint chromosome bands and tight double bands) can be seen with incident light (compare structures labeled in A and B). Additional structures seen in (A) also coincide with the pattern seen by transmission electron microscopy (unpubl. data).

For incident light microscopy the chromosome preparation was sputtered with a 4nm gold layer (on top of the chromosomes for better reflection of the incident light). Even better results should be expected if microscope slides with a thicker gold or silver layer were to be used and the SSP chromosomes, in contrast, prepared on top of it. The phase contrast depiction in (B) is unstained. A subsequent staining with Orcein did not show an improvement in the finer details but rather only an overstaining of the prominent band groups.

The chromosome map in (C) shows the pattern known so far from many transmission light microscopical studies on well-extended squash preparations. The pattern given by this map can not be depicted,

however, from an individual squash preparation due to the thickness of polytene structures on the one hand and the juxtaposition of the individual bands on the other. A homologous chromosome-X region with a higher degree of longitudinal spreading has already been published (Kalisch 1982). Note that in comparison with the chromosome map (C), more structural details are to be seen in the depictions of both papers than in squash preparations (for example: 207) and that the telomeric section (20D) of chromosome-X is always flared in SSP chromosomes. Additionally, in Fig. 1 of this paper, a structural disorder is shown in 19C. This comes from ectopic pairing which is often found between 19C and 20A (unpubl. data).

The resolution of incident light microscopy can be improved further through the use of the ZEISS Laser Scanning Microscope, as could be shown recently with SSP chromosomes of Chironomus (Kalisch et al. 1985b). In that paper we demonstrated the methodological advantages and disadvantages of the technique in different aspects of polytene chromosome mapping.

The intention of this note is to demonstrate the possibility of also using incident light microscopy for the relatively low polytene chromosomes of Drosophila and to emphasize the following advantages of this technique: Use of unstained SSP chromosomes; surface depictions, which show the distribution of total chromosome DNA in individual bands; additional information about pattern details compared with light microscopic techniques used so far.

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References: Berendes, H.D. 1963, Chromosoma (Berl.) 29:118-206; Iino, A. & T. Naguro 1980, Cytobios 27:157-165; Kalisch , W.-E. 1982, DIS 58:85-87; Kalisch, W.-E. & H.J. Jacob 1983, Cytobios 36:39-43; Kalisch, W.-E. & T. Whitmore 1983, Cytobios 37:37-43; Kalisch, W.-E., T. Whitmore & H. Reiling 1985a, Cytobios 141:47-62; Kalisch, W.-E., T. Whitmore & A. Siegel 1985b, J. Microsc., in press.

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The Great Clone Mill Tramp Disaster

Three brothers who pooled one of the biggest ass-raising clone mills in North America on 5,000 acres in southwestern Montana were killed where they lay this morning by tramping clones. Here's the story:

Inside the clone mill, the gene vats were cooking. Sterile workers clocked in and out and tramps loitered in the crawl space, under the warming boilers, eating whatever fell through the safety net and the floor cracks, drinking seepage that killed them by the dozens and changed some into a broad mix of beastly forms.

It was mall of horror and a school of blood from which a few escaped to roam abroad as huge beavers, buttfish, goose-necked businessmen and oreodonts, preying on everything that wasn't them, sometimes cooking fat bits in brute lard brought to a slow boil.

The mill owners, I.P. Freely, Seymour Butts and Wazee Moose, like the Stooges, were sleeping together on the desert floor one evening when some goose-necked business types, cloned accidentally when a mill worker spat unchewed goose fat, passed by in company with a rabid brute and a numbskull. They asked the mill owners for the time, but the three merely wheezed and whimpered and turned over all at once. This angered the unnatural clones, and their greasy circuits screamed "Kill!"

At the clone mill, more tramps had come to drink the seepage. Thousands were metamorphosed, to parade abroad and launch satellites of the parent company, Hour of the Beast Ltd.

Services for the mill owners will be held at Lamanno Panno Fallo, a sunnyside stooge mortuary. Survivors are Clone Freely, Clone Butts and Clone Moose, all sons; Yellow Bleacher, founder of Mixmeat Pies, a close friend; a mutant shrew and a gaggle of beakwomen; and Minnie and Michael Rat, the prexy and his spouse, a Detroit squirrel.