Berendes, H. D. Max Planck Institut f. Biologie, Abt. Beermann, Tübingen, Germany. Electron microscopical mapping of giant chromosomes.

Electron microscopical studies of selected regions of giant chromosomes which have been sectioned parallel to the chromosome axis permit the construction of chromosome maps from which the exact number of bands can be determined. The map made by light-microscopical investigation can be improved by the location of additional submicroscopic bands. Moreover, morphological changes in the submicroscopic structure of active regions (puffs and Balbiani rings) can be investigated at different phases of their active period.

The method for sectioning squashed salivary gland chromosomes developed by Sorsa and Sorsa (1) was modified to permit the selection of particular chromosome regions to be sectioned. Isolated glands are fixed in 6% glutaraldehyde, buffered 10% neutral formaldehyde, or Carnov's fixative for 10-20 min. and squashed on siliconized slides in 40% acetic acid or in the fixative. Directly after squashing, the coverglass is removed on dry ice and the preparations post-fixed in a methanol-10% neutral formaldehyde series (M:F, 1:0, 9:1, 6:4, 4:6, 1:9, 10 min. each step). Subsequently, the preparations are stained in filtered heamalum Mayer (Merck) for 10 min. and dehydrated in an ethanol series of which the last steps (80%-100%) are saturated with uranyl acetate. Capsules filled with Epon are placed on top of a well spread nucleus or group of nuclei selected light-microscopically and marked on the underside of the slide. After polymerization and removal of the capsules from the slide by dry ice, the capsules are placed in a holder (fig. 1) which is adapted to a Leitz Ortholux light-microscope. The chromosome region to be sectioned can now be selected (fig. 2), marked on the plastic and the pyramid can be made according to these marks.

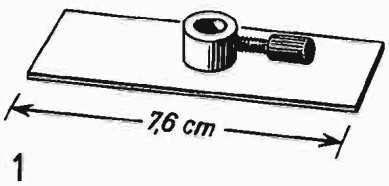

Fig. 1. Holder for capsules adapted to the Leitz Ortholux.

Fig. 2 Oil-immersion image of chromosomes on top of the capsule after trimming the pyramid. The X chromosome is indicated.

Fig. 3 Low power micrograph of the tip of a female X chromosome fixed with Carnoy's fixative. The white region is indicated. The bands 1A5,6; 1B3,4; 1E3,4 clearly demonstrate their compact singular nature.

This method was used to make a map of the banding pattern of section 1-4 of the female X chromosome of D. melanogaster (fig. 3). The pattern of bands is the same with all fixatives used. From 35 series of sections the maximum number of bands was determined and mapped. A comparison of this map with the revised map of Bridges (2) revealed characteristic differences. The electron microscopical map contains a maximum of 85 bands from X-1A to X-3F, whereas the map of Bridges contains 136 bands in this section. This discrepancy can partly be explained by the absence of double bands in the EM-map in 36 of the 38 band pairs indicated by Bridges (see Fig. 3). The remaining difference of 33 bands consists mainly of very thin bands on the map of Bridges.

It is evident that the reduction in the number of bands to about 60% of the number indicated by Bridges may have an important bearing not only on the correlation of known genetic factors with particular bands, but also on the calculation of the mean DNA content per band.

Lit: (1) Sorsa and Sorsa, Chromosoma 22 (1967); (2) Bridges, J. Hered. 29 (1938).

Hotchkiss, Sharon K. and J. K. Lim.
Wisconsin State University, Eau Claire, Wisconsin. Mutagenic specificity of Ethyl methanesulfonate affected by treatment method.

Preliminary experiments were conducted to determine if there is a difference in the mutagenic effects of Ethyl methanesulfonate (EMS) when fed and when injected into Drosophila. 0.025 M EMS in 1% sucrose solution was prepared by adding 0.24ml of EMS (Eastman Kodak) into 100ml of 1% sucrose solution and aerating for a few minutes with a hypodermic syringe. Two-day old males of the constitution $sc^8 \cdot Y \cdot B^S / y^2 w^i ct^6 f$ were injected with approximately 0.16 microliter of the EMS solution. Twenty-four hours after injection, the males were mated individually to two virgin females of the composition $ysc^{S1} ln49 sc^8$; dp bw; st p^p. Males of the stock used in the injection experiment were fed on the EMS solution for 24 hours and were mated individually to two virgin females of the stock used in the injection experiment. The matings in each experiment were kept for four days, after which males were discarded. Upon eclosion of F_1 flies, females were sib-mated for sex-linked recessive lethal detection and F_1 males were mated back to two virgin females of the composition $ysc^{S1} ln49 sc^8$; dp bw; st p^p for detection of translocations II-III, Y-II, Y-III, and Y-II-III.

If 25 or more wild type males were present in the sib-population, without any y w ct f male, a lethal was scored and retested for two generations. The presence of 40 or more flies in the expected classes was used as the criterion in screening for translocations (the actual number ranged from 41 to 156 with a mean of 73.7). The results are summarized in the following table:

Type of Treatment	Sex-linked recessive lethal			Translocation		
	Number tested	Number detected	Per cent	Number tested	Number detected	Per cent
Injection	508	29	5.7%	958	0	0%
Feeding	612	328	53.6%	760	19	2.5%

Of the 19 translocations detected, nine were T II-III and the remaining 10 were TY-III. None involving Y-II and Y-II-III were detected. A random sample of three from the nine T II-III was taken for salivary chromosome examination. All three samples showed reciprocal translocation between the second and third chromosomes, confirming the detection based on the genetic data.

The results indicate a quantitative difference in the effects of EMS when fed and when injected since the frequency of sex-linked recessive lethals induced by feeding was nearly ten times the frequency induced by injection. A qualitative difference, the production of translocations when fed but not when injected, is strongly suggested by these results, however, larger scale experiments will have to be conducted to eliminate the possibility that this, also is a quantitative difference only.