Schalet, A. University of Leiden, The Netherlands. Crossing over in the major heterochromatic region of the X chromosome in normal and inverted sequences.

This note briefly summarizes the results of a number of experiments designed for various purposes in which the major heterochromatic region of the X chromosome in Drosophila melanogaster was delimited by the su(f) and y^+ markers.

EXPERIMENT 1: ♀♀ 1J1 y^{J1} sc^{J1} v f mal^{Fl} 120 bb^+.Dp(sc^{V1} y^+)/1J1 y^{J1} sc^{J1} v f mal^1 su(f) bb^+ x ♂♂ Y/y ac In49 v f mal^1 su(f). Since all regularly produced ♂ offspring die except 1/2 of the crossovers between 120 and the duplication to the right of the centromere: a) crossing over between su(f) and y^+ is scored in two classes of ♀♀ but only 1 class of ♂♂. b) crossing over between 120 and su(f) is scored in only one class of ♂♂. Crossing over between mal and su(f) is scored only in ♀♀ (mal^{Fl}/mal^1 = mal^+ under the conditions of this experiment). The % crossing over given in the table takes these factors into account.

EXPERIMENTS 2 & 3: ♀♀ y^+ sc^8 bb su(f)mal^{Fl} f sc^8/y^{S1} sc^8 bb^+ mal^1 f sc^8 x ♂♂ Y/y ac In49 v f mal^1 su(f). In contrast to the other experiments flies were cultured on a potato based, rather than cornmeal, medium. During the collection of parental ♀♀ for parts of experiment 3, it was observed that the eye color of some mal^{Fl}/mal^1 ♀♀ were mutant or intermediate rather than wild type. The temperature during the critical periods of development was sufficiently low as to have expected only wild type eyes (Schalet, 1971). Indeed, more than 800 ♀♀ observed during the collection for 3d were all wild type. Failure of the complementation reaction was seen again among the ♀♀ offspring of this experiment in which the temperature during their development did not exceed 25. For this reason crossing over between mal and su(f) could not be accurately measured. These difficulties were not encountered in experiments 1 and 2 where the equality of mal and mal^+ ♀♀ within both non-crossover and crossover classes testifies to the reliability of the data with respect to this point.

EXPERIMENT 4: ♀♀ y^+ sc^8 bb su(f) w^a sc^8/y^{31d} sc^8 bb^+ Tu w^a sc^8 x ♂♂ Y/y w^a f^5 su(f).

EXPERIMENT 5: ♀♀ y^+ sc^8 bb su(f) w^a sc^8/y sc^4 Tu w^a sc^8 x ♂♂ Y/y w^a f^5 su(f). In these experiments the nearly white eye color of the w^a su(f) combination was used to mark the presence of the mutant su(f) allele. In experiment 4, three possible crossovers between su(f) and y^+ failed to breed: 1 ♀ could have been produced by maternal non-disjunction or a triploid; 2 apparent ♂♂ could have been 2X;3A intersexes. Two additional flies showing intersex characteristics were noted. The 11% value for the Tu to su(f) interval does not include data from ♂♂ carrying the y^{31d} marker. Although inclusion of such data would only raise the overall value to 11.6%, the 13.7% found among y^{31d} ♂♂ was considered to be spuriously high in part because of a consistent deficit of non-crossover y w^a Tu ♂♂. In experiment 5 the expected deficit of progeny marked with y was realized. Again the numbers of non-crossover ♂♂ marked with y was consistently smaller than the ♀♀ marked with y, and this led to a spuriously high 10.9% value for the Tu to su(f) interval among y ♂♂ as compared to the 8.8% among y ♀♀. On the contrary, for equal numbers of y^+ marked ♂♂ and ♀♀ the values were 6.9% and 7.0% respectively.

The slight bb allele in the y^+ sc^8 chromosomes of experiments 2-5 probably arose as a consequence of the exchange by which su(f) was inserted into the sc^8 chromosome. The bb phenotype was first noticed in tests of the original y^+ sc^8 su(f) mal^1 f v sc^8 chromosome from which the chromosomes used here were derived. Although all experiments involved mass matings of 5-12 parental ♀♀/culture, the following features of the data may be noted:

Crossover related bb mutants can be generated in a chromosome of normal sequence (c.f. Atwood 1969). In experiment 1a among the 43 tested chromosomes recombinant for the su(f) to y^+ interval there were two cases of independent origin in which ♀♀ showed a bb phenotype over a sc^4 sc^8 chromosome. In one of these cases, from a single set of cultures derived from five parental ♀♀, there were at least 6 offspring marked with su(f) and y^+ which showed similar bb phenotypes. All 6 offspring belong to the complementary crossover class were bb^+.

Premeiotic origin of at least some crossovers in the su(f) to y^+ interval. The example just cited suggests that a single gonial exchange was responsible for the observed crossovers. Supporting evidence for gonial exchange comes from the kind of distribution seen in experiment 2b. Here 9 of 19 crossovers were found among offspring derived from 12 parental ♀♀ in a single set of cultures out of the 13 sets scored in this experiment. Additional support comes from experiment 3b. In a special case, not included in the table, there were 30 y ac ♀♀ offspring in a single set of cultures derived from 12 parental ♀♀. The appearance of these ♀♀ suggest that at least one of the parental ♀♀ had lost the distal tip of the y^{S1} sc^8 chromosome due to a prior X Y exchange in the germ line of her father. Subsequently, an apparent

Spontaneous crossing over in heterochromatic and adjacent euchromatic regions of X chromosome in normal and inverted sequences

Experiment	Sequence	Days of Oviposition	Total Offspring	Crossovers su(f)-y$^+$	Crossovers mal-su(f)	Crossovers l(1)20-su(f)	Temperature range during Development of parental ♀♀
1a Nov. '69	normal	1-14	26,600 ♀♀	45 0.116	436 1.6	9 0.068	21-25; 17 (see text)
1b	normal	1-14	25,200 ♀♀	7 0.028	355 1.4	4 0.032	21-25
2a Feb. '71	sc^8/sc^8	1-12	7,400 ♂♂ & ♀♀	3 0.040	80 1.8		25
2b	sc^8/sc^8	1-12	17,500 ♂♂ & ♀♀	19 0.108	238 2.2		17 from just before puparium formation to at most 11 hrs. before eclosion; otherwise 25
3a June '71	sc^8/sc^8	1-6	15,800 ♂♂ & ♀♀	8 0.050			22-26; mostly 25
3b	sc^8/sc^8	1-8	20,000 ♂♂ & ♀♀	5 0.025			22-26
3c	sc^8/sc^8	1-6	10,000 ♂♂ & ♀♀	7 0.070			17 as larvae; otherwise 22-26
3d	sc^8/sc^8	1-6	29,400 ♂♂ & ♀♀	20 0.068			17 as larvae and pupae; otherwise 25
3e	sc^8/sc^8	1-4	7,100 ♂♂ & ♀♀	5 0.070			32-35 for 12 hrs. during first or second larval instar; otherwise 22-26
					Crossovers Tu-su(f)		
4 Jan. '72	sc^8/sc^8	1-8	38,800 ♂♂ & ♀♀	11 0.028 14 0.036	3296 11.0 (see text)		23-25
5 Jan. '72	sc^8/sc^4 sc^8	1-8	24,800 ♂♂ & ♀♀	6* 0.024	348 7.0 735 8.8 (see text)		23-25

* Subsequent tests have shown: 1 w^a Tu ♂ received from his mother an X chromosome carrying the markers y$^+$ su(f) Tu w^a and a segregating duplication with at least su(f)$^+$ present. 1 ♀ that was phenotypically y w failed to breed. 4 other flies were genuine crossovers between bb and su(f).

gonial exchange could have occurred to the left of su(f), since the phenotypes of the exceptional ♀♀ were: 2 y ac f^+ mal^+ and 28 y ac f mal. The 1 y ac f^+ mal^+ ♀ which was bred gave 11 y ac v mal ♂ offspring and 0 y ac f^+ mal^+ ♂♂.

Some crossovers in the su(f) to y^+ interval do not involve the bb locus. This is demonstrated by the 6 crossovers in experiment 5 in which the parental ♀♀ were heterozygous for the bb deficient sc^4 sc^8 chromosome. At least 1 of these crossovers appears to involve the region between bb and su(f) in the y^+ sc^8 bb su(f) w^a sc^8 chromosome. Whether the heterochromatic region to the left of the bb locus in this chromosome was involved in the other crossovers has not been tested yet.

Possible temperature influence on crossover frequency and changes in redundant sequences. Data presented in the table suggest the possibility that ♀♀ cultured at 17 during at least part of their pre-imaginal development may exhibit about twice the amount of crossing over in the su(f) to y^+ interval as ♀♀ cultured at the higher temperatures. The 0.116% crossing over in experiment 1a may be an example of this temperature enhancement, since parental ♀♀ were inadvertantly placed at 17 for 3 or 4 days prior to eclosion. Thus, the maintenance of stock cultures at around 17, in a manner which permits unrestricted crossing over to take place in the heterochromatic region, may serve to promote changes in redundancy not only within the bb locus, but also within repetitive sequences located on either side of the bb locus. The su(f) locus could be included among such structures. The crossover data discussed here has not taken into account the possibility that the wild type and mutant alleles of this locus represent different degrees of redundancy or complexity, and that some of the observed crossovers between su(f) and y^+ may have been exchanges within this locus. These conjectures concerning the organization of su(f) are based upon features previously enumerated (Schalet, Genen en Phaenen, 1970) which include suppression of lz^1 and intensification of lz^{37} by $su(f)^1$, and enhanced by the observations of Voss concerning the 1^{3DES} allele of su(f) and $su(1^{3DES})$ reported in DIS 46 and 47.

Comparisons of crossover frequencies in normal and inverted sequences. Crossover frequencies between su(f) and y^+ in normal and inverted sequences under comparable temperature conditions appear to be similar, although the data are too scanty to preclude a 1.5-fold increase. (The 0.4% crossing over at the bb locus in a sc^8 chromosome reported by Schalet (1969) remains an unexplained anomaly.) A perusal of the literature reveals that, almost invariably, crossing over in the segment proximal to f-B in the normal chromosome is increased at least 1.5-fold in the sc^8 (type) chromosome (see also experiment 4 for Tu to su(f) interval), while data based on the segment proximal to car fails to show a consistent comparable increase. Yet, Braver (1956, 1957) reported that the region comparable to the car to y^+ interval in the sc^8 chromosome, namely, the car to w interval in the rst^3 inversion, showed a 3 to 4-fold increase in crossing over as compared to the normal chromosome.

X-ray induced crossing over in inverted sequences. Along with the untreated series of experiments 2, 4 and 5, other ♀♀ were exposed to 3,000 R and crossovers, especially between su(f) and y^+, were scored. For experiments 2 and 4 induced crossovers (2-5.7%) were found for all days of oviposition, except that days 5-6 were not scored. When allowance is made for the difference in X-ray exposure, frequencies and curves were roughly comparable to Roberts' (1969) c(3)G experiment. Induced crossing over did not generate detectable bb locus deficiencies. In experiment 2, when the total exposure was divided into two equal fractions, one hour apart, crossing over was reduced for all days of oviposition and reductions ranged from 14% to 22% below the unfractionated series. In experiment 5, induced crossovers were found only among eggs laid during the first two days of oviposition at a low frequency of about 0.2%.

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Hanly, E.W. University of Utah, Salt Lake City, Utah. The effect of Actinomycin D on ommatidial bristle development.

In a recent publication (Hanly and Hemmert 1967) the fact that Actinomycin D inhibits the development of D. melanogaster ommatidial bristles in vitro was reported. It was noted that when the developing eye (24-30 hr following puparium formation of Oregon-R at 26°C) attached to the optic lobe of the brain is dissected and cultured in Schneider's medium containing low concentrations of Actinomycin D, ommatidial bris-