CYTOLOGICAL AND GENETIC OBSERVATIONS OF MEIOTIC DRIVE IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

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Many exceptions to Mendel's law of independent segregation have been noted. If the inequality of recovery of two homologous chromosomes is due to a meiotic event, these homologues are said to exhibit meiotic drive.

This study was designed 1) to ascertain the effect of irradiation at the onset of meiosis on the meiotic mutant *Segregation-distorter* in order to determine the meiotic stage during which the mechanism of *SD* is sensitive, and 2) to observe the meiotic drive systems of *SD* and the abbreviated sc^4sc^8 X together ("Double-Drive") both cytologically and genetically at 18° and 28°C. Analysis of the interaction of these systems and correlation of the nonrandom polar segregation of sc^4sc^8 with its genetic recovery tested the hypothesis that the mechanism of both drive systems involves a predetermined functional-nonfunctional polarity which is independent of the drive systems which respond to it.

Irradiation treatments were administered to very late third instar male *SD* larvae. To observe the effects of

sperm storage, part of the experimental and control groups were aged after eclosion as virgins. The first four daily broods from unstored irradiated males exhibited depressed k values. The percentage of aberrations and the depression of the k values per brood appear to be positively correlated. Using the proportion of aberration products as an index, stored males appear to transfer few sperm which were in meiotic or premeiotic stages at the time of treatment.

Depressed k values were exhibited by both irradiated and control groups which were stored. Lack of mating activity and possibly resultant prolongation of Meiosis I appear to "unstabilize" and depress the drive of SD.

The sex ratios (♀♀/total) of cn bw progeny in experimental and control groups were in excess of 60%. A modification of Hiraizumi's SD-X chromosome homology hypothesis is presented to account for the lack of a reciprocal decrease in the sex ratios of SD progeny.

In the study of "Double-Drive," the nonrandom segregation of sc^4sc^8 or the nondisjunctional nullo product away from the bacterial pole correlated with genetic recovery, supporting the hypothesis that a predetermined functional-nonfunctional polarity exists in the primary spermatocyte. At 18°C, sc^4sc^8 expresses increased drive when in the presence of SD. Both drives are slightly reduced when together at 28°C. Thus, the data indicate that the drive systems of SD and sc^4sc^8 are not competitive in their interaction, and that the mechanism of SD is not independent of that to which sc^4sc^8 responds.

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By

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To my mother and father

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I. INTRODUCTION

Many exceptions to Mendel's law of independent segregation have been noted. These abnormal progeny ratios may be due to 1) aberrant meiotic segregation, 2) gametic selection, or 3) post-fertilization selection. If the inequality of recovery of two homologous chromosomes is due to a meiotic event, these homologues are said to exhibit meiotic drive (Novitski and Sandler, 1956).

One of the meiotic drive phenomena occurring in Drosophila melanogaster, Segregation-distorter (SD), has been intensively studied. SD is known to exhibit the following properties: 1) A male heterozygous for SD and a structurally normal SD chromosome produces almost all SD progeny, with rare exceptions. 2) Females heterozygous for SD produce normal segregation ratios; their heterozygous sons exhibit abnormal k values (with certain exceptions). (The parameter k is defined as the proportion of functional SD-bearing sperm.) 3) When the SD chromosome is heterozygous with a structurally rearranged homologue having one breakpoint near the centromere, such as In(2LR)Cy, segregation-distortion is inhibited. 4) An SD chromosome appears to be insensitive to the action of another SD chromosome. 5) The complex SD locus lies in the centromeric region of chromosome II, between purple and cinnabar. To the right of SD is

Activator of SD,Ac(SD), which is necessary for SD to exhibit distortion. 6) There is a stabilizing modifier of SD,St(SD), at, or near, the tip of IIR. (Sandler, Hiraizumi, and Sandler, 1959; Sandler and Hiraizumi, 1960a, b, 1961a; Dennell and Judd, 1968).

Sandler, Hiraizumi, and Sandler (1959) presented a model for SD action which assumed that SD caused a break at SD^+. The lethality of the union of SD^+ sister chromatids would cause an excessive recovery of SD-bearing chromosomes. Their model was supported by preliminary cytological observations of dicentrics at Metaphase II and bridges at Anaphase II in about half of the division figures examined (Crow, Thomas, and Sandler, 1962).

Peacock and Erickson (1965) did an extensive cytological investigation of meiosis in the SD male. Completely normal spermatogenesis was found. They found that all products of meiosis in the male are cytologically evident and are presumably transferred to the female. A Drosophila melanogaster male can transfer 3,000 to 4,000 sperm to the vagina during a single mating (Kaufmann and Demerec, 1942). The capacity of the female storage organs is about 700 sperm (Kaplan, Tinderholt, and Gugler 1962; Lefevere and Jonsson, 1962). Peacock and Erickson reported that, when the number of sperm transferred by SD males was less than the storage capacity of the female the ratio of stored sperm to progeny was 2:1. This ratio approached 1:1 when the number of sperm transferred exceeded the storage capacity of the

female or when the insemination was of the order of 60 sperm or less. These same relationships were exhibited by control, wild-type males.

In order to explain abnormal ratios recovered from certain translocation males T (1:4) B^s, Novitski and I. Sandler (1956a, b) proposed that some of the sperm produced by a Drosophila melanogaster male were regularly nonfunctional, and that particular chromosomes had certain propensities for being included in functional sperm.

The cytological observations and the sperm count versus progeny count ratios reported by Peacock and Erickson support this functionality hypothesis, and indicate that functional and nonfunctional sperm are a regular aspect of spermiogenesis in Drosophila. Peacock and Erickson postulated that the mechanism of Segregation-distorter was non-random orientation of the SD chromosome to the functional pole at Meiosis I.

The propensity of SD to orient towards the proposed functional pole of Meiosis I appears to vary. SD-bearing chromosomes with different strengths (meiotic drives) have been found in many natural populations, and exhibit k values from about .7 - 1.0 (Mange, 1961; Greenberg, 1962; Hiraizumi and Nakazima, 1965).

At least some SD chromosomes are temperature sensitive. From treatment of four different SD chromosomes with heat and cold, Mange (1968) found that temperature sensitivity was dependent on the stage of meiosis which a

sperm is undergoing and independent of the age of the male being treated. The most sensitive period occurred around early meiosis. The SD chromosomes which she reported to be greatly sensitive to heat treatment (30°C.) were recombinants. Both recombinants and natural SD chromosomes exhibited varying sensitivity to cold (19° C.).

Other meiotic drive systems in Drosophila melanogaster have been shown to be temperature sensitive at early meiosis, with the effect of temperature treatment being a more normal segregation ratio. The RD effect (high recovery rate of the X chromosome) is greatly depressed at 18°C, with the sex ratio (♀♀/total) being most reduced 7-8 days after treatment (Erickson and Hanks, 1961). Segregation ratios of both X^D: IV and X^P: Y from A-type Bar of Stone translocation males were very much altered by 18°C treatment of prepupae (Zimmering and Perlman, 1962).

Gershenson (1933) suggested that the unequal recovery of XO and XXY progeny resulting from primary non-disjunction in the Drosophila male was due to chromosomal loss because of lack of synapsis. In XY and XYY males with chromosomes which differed in amount and distribution of heterochromatin, chromosome loss appeared to occur only where homology was drastically reduced, supporting Gershenson's hypothesis that lack of pairing is related to apparent loss of chromosomes (Sandler and Braver, 1954).

Yet, males with sex chromosomes with greatly reduced homology exhibit more normal recovery of the Y chromosome

at 18°C than at 26°C (Zimmering, 1963). Since a modified univalent Y shows a similar increase in recovery at 18°C, the increase in recovery of the Y from XY males observed at cold temperatures does not appear to be due to increased pairing between X and Y. Using $\underline{sc^4sc^8}$/Y/Y males, Zimmering and Green (1965) found that the frequency of XY sperm recovered increased from 23% at 26°C to 43% at 18°C. Since, in such males, the $\underline{sc^4sc^8}$X is usually a univalent while the Y chromosomes form a bivalent, the normalizing effect on segregation again does not appear to be due to increased pairing. However, this was not demonstrated cytologically.

Peacock (1965) found that, while failure of the $\underline{sc^4sc^8}$X and Y chromosomes to synapse was common, these subsequent univalents usually travelled to the same pole at Anaphase I. The nondisjunction frequency was directly related to the frequency of synaptic failure. The occurrence of meiotic loss of unpaired chromosomes was negligible. Peacock postulated that the $\underline{sc^4sc^8}$ meiotic drive system was another case of nonrandom segregation of both disjunctional and nondisjunctional reciprocal products into functional and nonfunctional sperm.

The experiments in this study were undertaken to 1) ascertain the effect of irradiation at the onset of meiosis on Segregation-distorter and 2) observe two meiotic drive systems together, the $\underline{sc^4sc^8}$X and SD ("Double-Drive"), both cytologically and genetically, at 18° and 28°C.

Effect of Irradiation

The effects of heat and cold shock on SD and other chromosomes which exhibit abnormal segregation implicate early meiosis as the time of operation of these drive systems. Temperature treatments, however, are administered over relatively long periods of time. As a result, it is impossible to determine precisely the meiotic stage (or stages) which is critical to the drive systems. The functionality hypothesis would require the sensitivity to treatment to be exhibited prior to Anaphase I.

Irradiation treatments can be administered over periods of very brief duration. By irradiation of very late third instar larvae, primary spermatocytes will be the most advanced meiotic stage undergoing treatment (Cooper, 1950). Sensitivity of SD at this stage would support the hypothesis that functional and nonfunctional sperm are determined a Meiosis I and that the operation of the Segregation-distorter chromosome involves nonrandom orientation to the functional pole.

"Double-Drive"

The proposed functional and nonfunctional poles at Meiosis I provide a good working hypothesis to explain the mechanism of all meiotic drive phenomena. The operation of the meiotic drive systems of SD and sc^4sc^8 has been attributed to the nonrandom segregation of the chromosome or chromosomes involved to the functional or nonfunctional pole at Anaphase I

(Peacock, 1965; Peacock and Erickson, 1965). Analysis of the interaction of these systems together would test the hypothesis that the operation of both systems involves a predetermined functional-nonfunctional polarity which is independent of the drive system or systems being studied, or, if functionality is not predetermined, would provide information concerning the time of determination of the functional pole.

Genetic Tests

If the polarity of the primary spermatocyte is determined prior to Anaphase I, the two systems would not be competitive. If both phenomena utilize functional-non-functional polarity, the drives exhibited by SD and the $\underline{sc^4sc^8}$ systems in "Double-Drive" should be similar to those exhibited by these chromosomes when alone. In addition to the factors mentioned above, there are possible segregational interactions between the chromosomes involved. This would be reflected in further deviation from randomness in the association of chromosomes in recovered sperm.

If the driving chromosomes themselves determine the functional pole, $\underline{sc^4sc^8}$ and SD would be in a competitive situation if the mechanisms of both systems involved functionality. When these systems are present in the same role, there are eight possible gametic products:

Disjunctional products	Nondisjunctional products
$\underline{SD}\ \underline{sc^4sc^8}$	$\underline{SD}\ \underline{sc^4sc^8}$ Y
$\underline{SD}$ Y	$\underline{SD}$ nullo
$\underline{SD^+}\ \underline{sc^4sc^8}$	$\underline{SD^+}\ \underline{sc^4sc^8}$ Y
$\underline{SD^+}$ Y	$\underline{SD^+}$ nullo

If $\underline{SD}$ is stronger than $\underline{sc^4sc^8}$ in polar determination, the drive of $\underline{SD}$ in the "Double-Drive" situation would approximate the drive it exhibits alone, whereas the drive of the disjunctional and nondisjunctional products of the $\underline{sc^4sc^8}$ system would be reduced due to the loss of $\underline{sc^4sc^8}$ products which resulted in nonfunctional gametes. The reverse would be true if $\underline{SD}$ is the weaker competitor. If both systems are equal in polar determination strength, a high proportion of $\underline{SD}\ \underline{sc^4sc^8}$ and $\underline{SD}$ nullo products would be recovered, due to the competition between $\underline{SD^+}$ Y and $\underline{SD}\ \underline{sc^4sc^8}$ and between $\underline{SD}$ nullo and $\underline{SD^+}\ \underline{sc^4sc^8}$ Y.

Cytological Tests

The abbreviated $\underline{sc^4sc^8}$ chromosome can be distinguished from a normal X and from its Y homologue, thus allowing cytological observation of its segregational behavior at Anaphase I. This behavior was scored in the $\underline{sc^4sc^8}$ X system, and with the $\underline{sc^4sc^8}$ X system in the presence of $\underline{SD}$ ("Double-Drive") at 18° and 28°C. The granular inclusions reported by Peacock and Erickson (1964) were present in the stocks used in this study. These granules have been shown to be Bacteria or Rickettsiae, and their polar association was concluded to

be an independent response to the cytoplasmic gradient in meiotic cells to which "driving" chromosomes respond (Yanders, Brewen, Peacock, and Goodchild, 1968).

The presence and position of these inclusions in relation to the segregation of the chromosomes were noted. A positive correlation between non-random segregation to the pole lacking granular inclusions and subsequent genetic recovery would support the conclusion of Yanders et al (1968) that the polar association of these inclusions reflects a cytoplasmic gradient in meiotic cells associated with the determination of functionality of the gamete.

With the polar association of the granules, the segregational behavior of $\underline{sc^4sc^8}$ alone and in the presence of SD can be examined. If the granules are indicators of polarity, a difference in the behavior of $\underline{sc^4sc^8}$ in the two systems should be supported by genetic recovery ratios of the reciprocal products.

Evidence will be presented which supports the hypothesis that the mechanisms of the SD and $\underline{sc^4sc^8}$ meiotic drive systems involve nonrandom segregation to pre-determined functional and nonfunctional poles at Anaphase I. The data also indicate an interaction between the two systems and apparent repulsion between the SD-bearing chromosome and the sex chromosomes which is influenced by temperature.

II. MATERIALS AND METHODS

The stocks of Drosophila melanogaster used are listed below. Nutrient medium, modified after Carpenter (1950), was used for all experiments. All virgin females used were aged four to five days before mating.

Irradiation of SD

Very late third instar SD-72 male larvae were irradiated with 0 (Control) or 450r of X-rays with a General Electric Maximar-250-III, operating at 250kv, 15ma, with a .5mm copper, 1.0mm aluminum filter, giving an average dose of 110r/minute. The larvae used were from half-hour egg collections. For treatment, control and experimental larvae were suspended in 0.7% saline solution (Beadle and Ephrussi, 1936), .5mm in depth, in an open plastic petri dish.

In experiment I, 20 control and 50 experimental males were transferred without etherization to two virgin cn bw females per day for seven days from the day of eclosion. In experiment II, 10 control and 25 experimental males were transferred as described above for seven days, from the day of eclosion. Ten control and 15 irradiated males were stored for seven days as virgins, then mated as above for seven days. All progeny resulting from these matings were collected and scored for sex and eye color. Male progeny

with aberrant colors were tested for meiotic drive by matings with cn bw virgin females.

Cytological and Genetic Studies of Meiotic Drive

The sc^4sc^8 X chromosome used was a crossover product derived from an $In(1)sc^4$, $In(1)sc^8$ female. The distal breakpoints of these two inversions are similar. The proximal breakpoints, in the basal heterochromatin, are such that the crossover chromosome $Ins(1)sc^4sc^8$, having the distal region of $In(1)sc^4$ and the proximal region of $In(1)sc^8$, is deficient for a large portion of basal heterochromatin, (Cooper, 1959).

For the study of the $Ins(1)sc^4sc^8,y,y^+$ Y meiotic drive system, alone and in the presence of SD, the sc^4sc^8 stock, stock A, was infected with granules from cn bw females known to exhibit cytoplasmic granules. The granules are maternally inherited. Crosses between $sc^4sc^8,y/y^+Y$ ♂♂ and sc^4sc^8, y/Basc ♀♀ (with granules) or between ++/y+Y: SD, cn bw/SD^+++ ♂♂ (Stock B) and sc^4sc^8, y/Basc ♀♀ (with granules) yield two types of F_1 males which are distinguishable cytologically: sc^4sc^8, y/y^+Y and $Basc/y^+$ Y.

1. sc^4sc^8 without SD

$$\underset{\text{Basc}}{sc^4sc^8,y};\ \underset{\text{cn bw}}{\text{cn bw}}\ ♀♀ \times \underset{y^+\ Y}{sc^4sc^8,y};\ \underset{\text{cn bw}}{\text{cn bw}}\ ♂♂$$

$$\underset{\text{Basc}}{sc^4sc^8,y};\ \underset{\text{cn bw}}{\text{cn bw}}\quad \underset{\text{Basc}}{sc^4sc^8,y};\ \underset{\text{cn bw}}{\text{cn bw}}\quad \underset{y^+\ Y}{sc^4sc^8,y};\ \underset{\text{cn bw}}{\text{cn bw}}\quad \underset{y^+\ Y}{\text{Basc}};\ \underset{\text{cn bw}}{\text{cn bw}}$$

Cytologically distinguishable males

2. $\underline{sc^4sc^8}$ with $\underline{SD}$ ("Double-Drive")

$$\frac{sc^4sc^8,y;\ cn\ bw}{Basc\quad cn\ bw}\ ♀♀\quad x\quad \frac{+\ ;\ SD}{y^+Y\quad cn\ bw}\ ♂♂$$

$$\frac{sc^4sc^8,y;\ cn\ bw}{+\quad cn\ bw}\quad \frac{Basc;\ cn\ bw}{+\quad cn\ bw}\quad \frac{sc^4sc^8,y;\ cn\ bw}{y^+\ Y\quad cn\ bw}\quad \frac{Basc;\ cn\ bw}{y^+\ Y\quad cn\ bw}$$

$$\frac{sc^4sc^8,y;\ cn\ bw}{+\quad SD}\quad \frac{Basc;\ cn\ bw}{+\quad SD}\quad \frac{sc^4sc^8,y;\ cn\ bw}{y^+\ Y\quad SD}\quad \frac{Basc;\ cn\ bw}{y^+\ Y\quad cn\ bw}$$

Cytologically distinguishable males

The sib males with Basc X served as controls. These crosses were cultured at 18° and 28°C. Testes from F_1 prepupae and very early pupae were dissected in 0.7% saline, stained in acetoorcein, then squashed for observation under phase contrast. The prepupal and early pupal stages were selected because granule populations diminish greatly after these stages.

All primary spermatocytes with suitable Anaphase I divisions were scored. These cells were divided into three categories of granule populations: 1) polarized, where the granular mass is concentrated at one pole, 2) non-polarized, where the granular mass is distributed throughout the cell, situated between the poles at the equator, or evenly distributed at both poles, and 3) those cells with few or no granules. Disjunctional and nondisjunctional divisions were scored. In the case of polarized cells, the chromosomes travelling to the granular and non-granular poles were noted.

The sibs of those males dissected were mated to y; cn bw females for genetic tests of the sc^4sc^8 drives, nondisjunction rates and k (SD/total) values of the F_2 progeny. These crosses were cultured at 22.5°C.

Genetic Tests of Male Sibs of Dissected Males

1. sc^4sc^8 without SD

$$\frac{y;\ cn\ bw}{y\ \ cn\ bw}\ ♀♀ \quad x \quad \frac{sc^4sc^8, y;\ cn\ bw}{y^+\ Y\ \ \ cn\ bw}\ ♂♂$$

$\frac{sc^4sc^8, y;\ cn\ bw}{y\ \ \ cn\ bw}$	$\frac{y\ ;\ cn\ bw}{y^+Y\ \ cn\ bw}$	/	$\frac{nullo;\ cn\ bw}{y\ \ \ cn\ bw}$	$\frac{sc^4sc^8, y;\ cn\ bw}{\frac{y^+\ Y}{y}\ \ \ cn\ bw}$
from disjunctional gametes			from nondisjunctional gametes	

2. sc^4sc^8 with SD

$$\frac{y;\ cn\ bw}{y\ \ cn\ bw}\ ♀♀ \quad x \quad \frac{sc^4sc^8, y;\ cn\ bw}{y^+\ Y\ \ \ SD}\ ♂♂$$

$\frac{sc^4sc^8, y;\ cn\ bw}{y\ \ \ cn\ bw}$	$\frac{y\ ;\ cn\ bw}{y^+Y\ \ cn\ bw}$	$\frac{sc^4sc^8;\ cn\ bw}{y\ \ \ SD}$	$\frac{y\ ;\ cn\ bw}{y^+Y\ \ SD}$	/	$\frac{nullo;\ cn\ bw}{y\ \ \ SD}$	$\frac{sc^4sc^8, y;\ cn\ bw}{\frac{y^+\ Y}{y}\ \ \ SD}$
from disjunctional gametes					from nondisjunctional gametes	

Stocks

SD Segregation-distorter, SD-72, second chromosome (Sandler, Hiraizumi, and I, Sandler, 1959).

cn bw cinnabar and brown, second chromosome, exhibits granules (Madison).

y ; cn bw yellow, cinnabar, and brown (derived by Grant Brewen y^{b-32} (Oregon) and cn bw, Madison).

Stock B: $\frac{+}{+}$; $\frac{\text{cn bw}}{\text{cn bw}}$ ♀♀ x $\frac{+}{y^{+}\ Y}$; $\frac{\text{SD-72}}{\text{cn bw}}$ ♂♂ (derived by Yanders).

Males used for "Double-Drive" cross.

"Stock A with granules":

$\frac{\text{Basc}}{sc^{4}sc^{8},y}$; $\frac{\text{cn bw}}{\text{cn bw}}$ ♀♀ x $\frac{sc^{4}sc^{8},y}{y^{+}\ Y}$; $\frac{\text{cn bw}}{\text{cn bw}}$ ♂♂

Females used for "Double-Drive" cross.

"Stock A with granules" derived from:

1. Yanders' "Stock A":

$\frac{sc^{4}sc^{8},y;}{\text{Clb}}$ $\frac{\text{cn bw}}{\text{cn bw}}$ ♀♀ x $\frac{sc^{4}sc^{8},y;}{Y}$ $\frac{\text{cn bw}}{\text{cn bw}}$ ♂♂

2. Basc (Philadelphia).

3. $\frac{+}{y^{+}\ Y}$; cn bw ♂♂ from "Stock B".

4. cn bw with granules, (Madison).

Derivation of "Stock A with Granules"

1. $\frac{Basc}{Basc}$; $\frac{+\ +}{+\ +}$ ♀♀ x $\frac{+}{Y}$; $\frac{cn\ bw}{cn\ bw}$ ♂♂

2. $\frac{Basc}{Y}$; $\frac{cn\ bw}{+\ +}$ x $\frac{+}{+}$; $\frac{cn\ bw}{cn\ bw}$ ♀♀

(granules)

3. $\frac{Basc}{+}$; $\frac{cn\ bw}{cn\ bw}$ x $\frac{sc^4sc^8,y}{Y}$; $\frac{cn\ bw}{cn\ bw}$ ♂♂

(granules)

$\frac{Basc}{sc^4sc^8,y}$; $\frac{cn\ bw}{cn\ bw}$ ♀♀

(for "Stock A" with granules)

4. $\frac{sc^4sc^8,y}{ClB}$; $\frac{cn\ bw}{cn\ bw}$ ♀♀ x $\frac{+}{Yy^+}$; $\frac{cn\ bw}{cn\ bw}$

$\frac{sc^4sc^8,y}{Yy^+}$; $\frac{cn\ bw}{cn\ bw}$ ♂♂

(for "Stock A" with granules)

III. RESULTS

Effects of Irradiation on SD

Tables 1 through 6 and Figures 1 through 4 summarize the results of irradiating SD male larvae at or near the onset of meiosis. In experiment 1, the k value (SD/total) of the progeny of irradiated unstored males was depressed below that of the control group for the first six days of mating, with the lowest k values resulting from days 2 and 3. The brood of day 7 exhibited a k higher than any of the control group. In experiment 2, the k values of the unstored, irradiated males were depressed in all seven daily broods, with day 4 showing the greatest depression. Due to the large numbers of progeny, a test of significance is not necessary.

Certain aberrations appeared in progeny of irradiated larvae which did not occur in control groups. These aberrations provide an indication of which sperm batches were most affected by irradiation. The aberrations detectable are cinnabar and brown eyed flies. These aberrant progeny are probably the result of radiation-induced chromatid breaks between the cinnabar and brown loci. Breaks in analogous regions of non-sister chromatids and subsequent chromatid exchange would produce such aberrants. The SD Ac(SD) cn^+ chromatid would thereby carry the bw allele but no stabilizer

Table 1. SD-72 third instar larvae irradiated (450 r), unstored, control k = 95.8, 50 males, Experiment 1.

Paternal Age in Days	k(SD/total)	Percent Aberrations	Total Progeny	Total Aberations bw	Total Aberations cn
1	92.96	.47	428	2	0
2	85.72	.63	1265	4	4
3	85.62	.24	3701	8	1
4	91.79	.11	5534	6	0
5	92.31	.08	6155	4	1
6	90.32	.07	6120	3	0
7	97.25	.02	6120	1	0

Table 2. 20 control males, unstored, Experiment 1. No aberrations observed.

Paternal Age In Days	k	Total Progeny
1	95.71	632
2	95.69	1974
3	95.83	3302
4	95.71	2571
5	95.87	2699
6	96.01	2878
7	95.81	3005

Table 3. SD-72 third instar larvae irradiated (450 r), unstored, control k = 99.5, 25 males, Experiment 2.

Paternal Age in Days	k(SD/total)	Percent Aberrations	Total Progeny	Total Aberrations bw	Total Aberrations cn
1	97.84	.37	1067	4	0
2	97.95	.42	2405	10	0
3	96.44	.22	2249	5	0
4	95.98	.49	2436	10	0
5	96.82	.09	3430	3	0
6	97.02	.00	3695	0	0
7	97.09	.025	3954	1	0

Table 4. 10 control males, unstored, Experiment 2. No aberrations observed.

Paternal Age In Days	k	Total Progeny
1	99.43	1051
2	99.20	1496
3	99.44	1070
4	99.46	1303
5	99.36	776
6	99.64	822
7	99.42	517

Table 5. 15 SD-72 third instar larvae, irradiated (450 r), stored 7 days, Replicate 2.

Paternal Age In Days	k	Percent Aberrations	Total Progeny	Total Aberrations bw	Total Aberrations cn
7	97.86	.089	3360	3	0
8	97.11	.000	2389	0	0
9	98.41	.000	2008	0	0
10	98.68	.000	2039	0	0
11	97.66	.000	1880	0	0
12	97.09	.000	1753	0	0
13	98.61	.000	2083	0	0

Table 6. 10 SD-72 third instar larvae, control group, stored 7 days, Replicate 2.

Paternal Age in Days	k	Percent Aberrations	Total Progeny	Total Aberrations
7	99.23	none	2691	none
8	98.22		2139	
9	96.77		1918	
10	96.48		1703	
11	99.33		1649	
12	97.99		1541	
13	98.45		1160	

locus, St(SD). As such, it would exhibit a moderate, unstable k. The $SD^{+}AC(SD)^{+}cn$ chromatid would bear the normal allele for brown, bw^{+}, and the SD stabilizer, St(SD), and exhibit no drive.

All cn and bw male progeny were tested for drive by mating them with cn bw virgin females. The mean k for brown males was .772. The k for cinnabar males was .521.

Irradiated males mated from day of eclosion show the greatest number of aberrations in broods from days one through four in both replicates 1 and 2. The percentage of aberrations and the depression of k values per brood appear to be positively correlated. Due to the small numbers of aberrations in the data concerned, a nonparametric test of correlation is not applicable.

Aberrations appeared only in the first brood of the stored, irradiated males. These stored males exhibited k values similar to that of the stored control group. However, lower k values were exhibited by the stored control group than those of the unstored males. Lack of mating activity appears to increase the percentage of SD^{+} sperm transferred to the female. If aberrations are an indication of which sperm batches were in meiotic or premeiotic stages at the time of treatment, the stored males do not appear to transfer most of these sperm.

The over-all sex ratio ($\frac{♀♀}{\text{total}}$) of cn bw progeny from experiment 1 was .619. This represents a total of 2484 cn bw progeny. Experiment 2 cn bw progeny (682) had an

over-all sex ratio of .625. This supports the hypothesis of Hiraizumi and Nakajimi (1967) that SD has some homology with the X chromosome which reduces the probability of both travelling to the same pole.

Where the SD^+ cn bw chromosome reached the functional pole, it was accompanied by the X chromosome over 60 percent of the time. Thus, when SD cn^+ bw^+ travelled to the hypothesized non-functional pole at Anaphase I, it was accompanied by the Y chromosome more than 60 percent of the time. This implies a degree of repulsion between the SD-bearing chromosome II and the X chromosome comparable to the repulsion exhibited between homologues.

Cytological Observations of sc^4sc^8 and "Double-Drive"

The results of the cytological studies are given in Tables 7 and 8. The presence and position of the granular inclusions varied. In about half of the primary spermatocytes of all classes observed, the granules were polarized, the great majority of granules being situated in a mass or near one pole of the cell. The Basc control males exhibited polarization to about the same extent as the experimental groups. The granular masses of nonpolarized cells in both types of males were usually distributed on one side of the cell along the equator.

The position of the granular inclusions was undoubtedly disturbed by the squash procedure. In most cases where the granules were scored as polarized, the granular mass was

Figure 1. $\underline{k}$ values per daily brood. Irradiated and control males, Experiment 1.

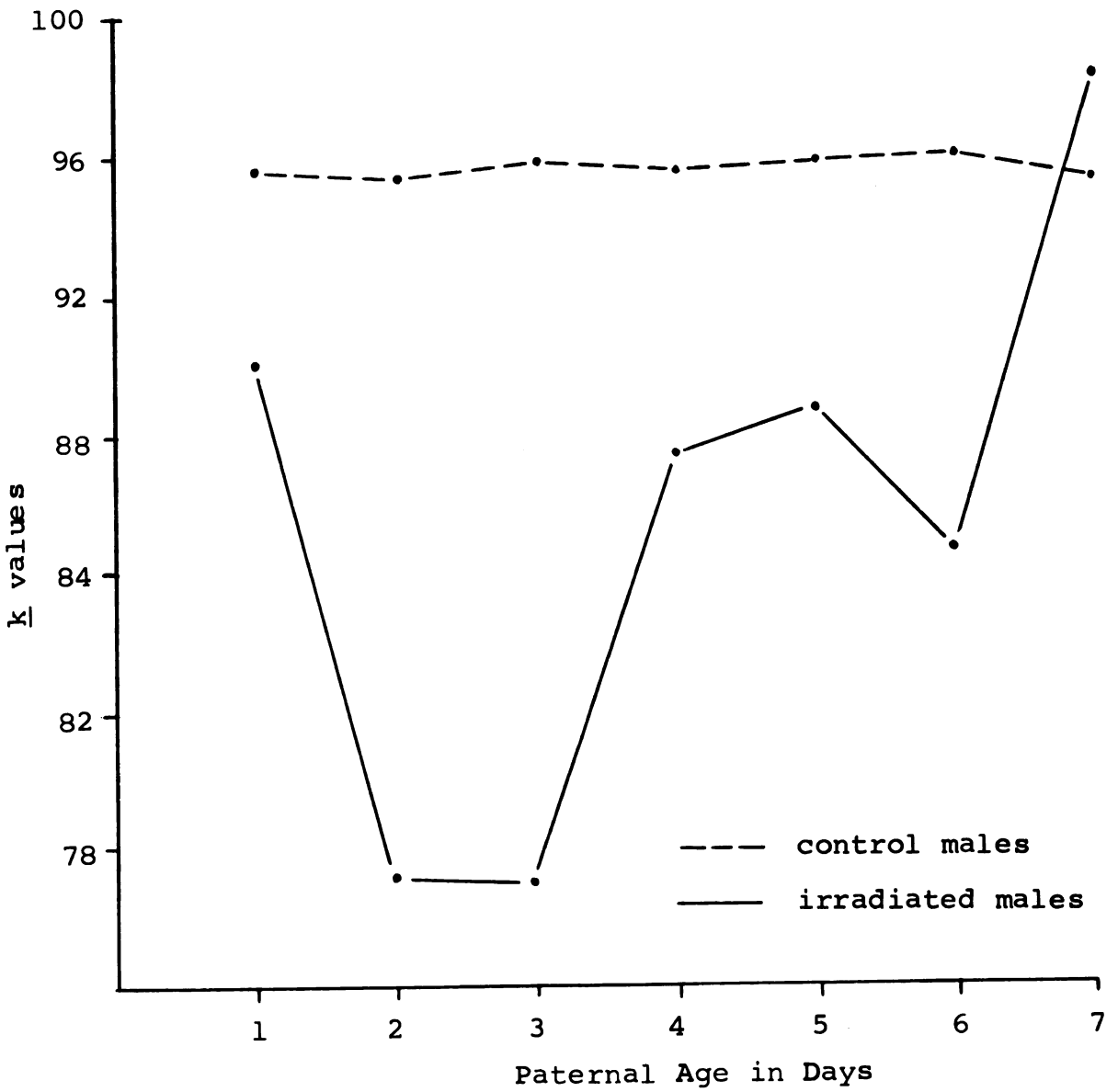
100
96
92
88
84
82
78
k values
control males
irradiated males
1
2
3
4
5
6
7
Paternal Age in Days

Figure 2. $\underline{k}$ values per daily brood. Irradiated and control males. Experiment 2.

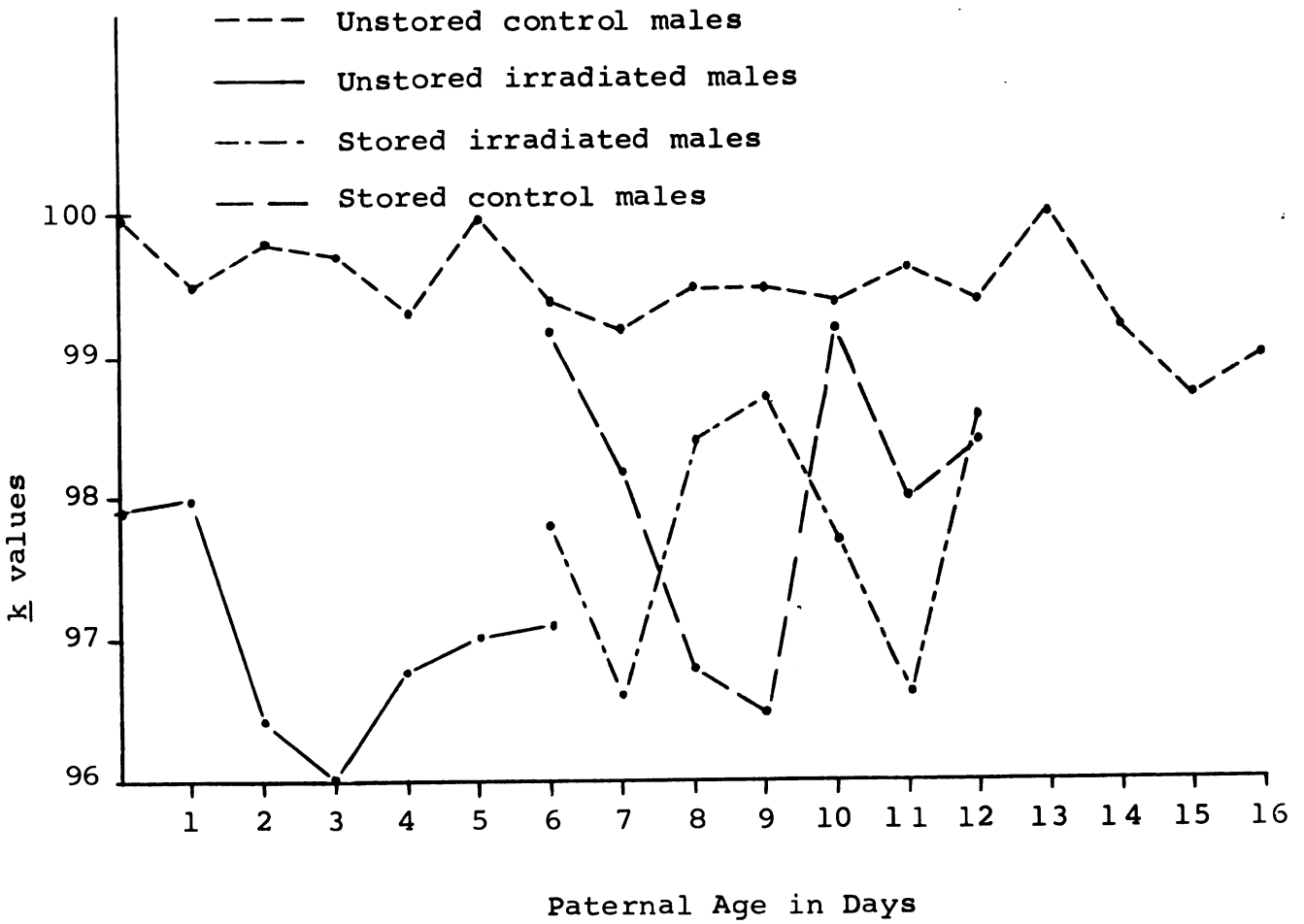
Unstored control males
Unstored irradiated males
Stored irradiated males
Stored control males
100
99
98
97
96
k values
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Paternal Age in Days

Figure 3. Percent aberrations per daily brood. Irradiated males, Experiments 1 and 2.

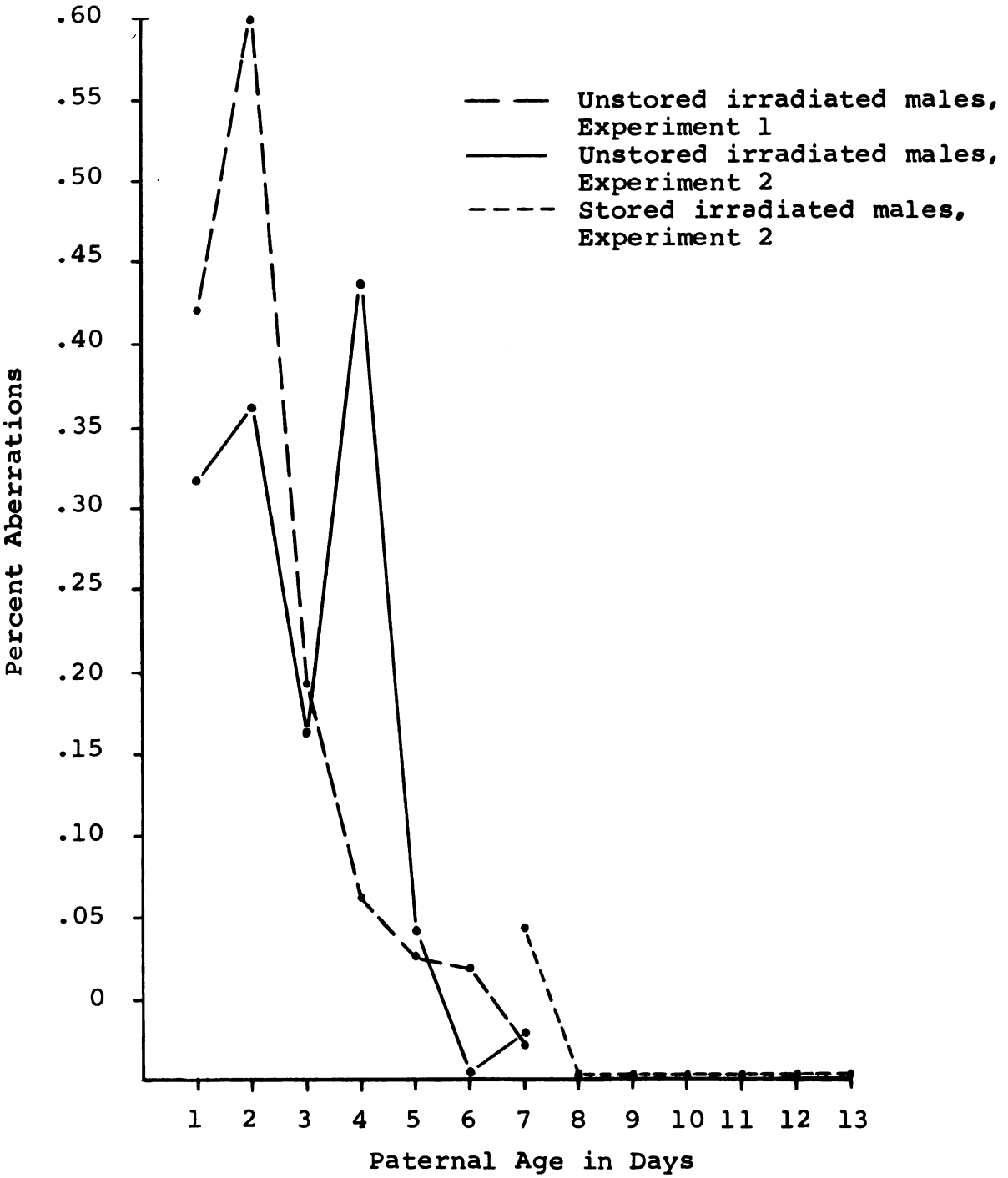

Figure 3. Percent aberrations per daily brood. Irradiated males, Experiments 1 and 2.

Table 7. Cytological Observations.

		Polarized Cells						Non Polarized Few or no Granules						Totals
		Disjunctional				N.D.*		Disj.**		N.D.*	Disj.**		N.D.*	
Temp.	o	4-8 to granules	4-8 away	Basc to	Basc away	XY to	XY away	4-8	Basc	XY	4-8	Basc	XY	
28°	D.D.***	33	51			69	37	43		33	19		25	310
	Basc			61	66				61			34		222
28°	4-8	19	34			25	14	38		17	31		25	203
	Basc			47	66				56			43		212
18°	D.D.***	31	43			23	15	29		9	20		12	182
	Basc			41	48				51			13		153
18°	4-8	34	42			9	5	21		19	17		2	149
	Basc			44	39				43			22		158
														1589

*Nondisjunctional
**Disjunctional
***"Double-Drive"

Table 8. Summary of cytological observations.

		Percent Polarity	Observ. n.d. rate	Polarized n.d.	Percent XY to gran.	Percent sc^4sc^8 to non-gran. pole	Percent M-5 to non-gran. pole
28°C.	Double-Drive	61.3	.529	.558	65.1	60.7	
	Basc sib	57.2	.000				52.0
	$\frac{sc^4sc^8}{Basc}$	45.3	.339	.424	64.1	64.2	
	sib	53.3	.000				48.7
18°C.	Double-Drive	61.5	.324	.339	60.5	58.1	
	Basc sib	58.2	.000				53.9
	$\frac{sc^4sc^8}{Basc}$	60.4	.235	.156	64.3	55.3	
	sib	52.5	.000				45.8

not located at the pole indicated by the spindle axis, but was positioned to one side of that pole. Usually, this difference between spindle axis and granular axis was slight. However, once the granular mass approached the equatorial region, it had to be considered to be unpolarized. Thus, these nonpolarized primary spermatocytes may have actually exhibited polarization prior to disturbance of the rigid spindle and granular position by squashing.

The observed nondisjunction rate was higher when $\underline{sc^4sc^8}$ was in the presence of SD at both 28° and 18°C. No nondisjunctional events were observed in the Basc class. At 18°C, the rates of nondisjunction in both sc^4sc^8 and "Double-Drive" primary spermatocytes was lower than those observed at 28°C.

In Basc-y Y cells, the sex chromosomes tended to undergo anaphase movement at the same time as the autosomes. In $\underline{sc^4sc^8}$ and "Double-Drive", however, the movement of the X and Y to the poles was generally precocious with respect to that of the autosomes. This undoubtedly is due to lower frequency of synaptic association because of the reduced X-Y homology with the $\underline{sc^4sc^8}$ gross heterochramatic deficiency.

The anaphase behavior with respect to the position of the granules in disjunctional or nondisjunctional polarized cells did not appear to be random. In the 28°C "Double-Drive" disjunctional class, $\underline{sc^4sc^8}$ travelled to the non-granular pole with a frequency of 61%. At 18°C, this frequency was reduced to 58%. At 28°C, the $\underline{sc^4sc^8}$ chromosome

oriented to the non-granular pole with a frequency of 64% of the time. At 18°C, this frequency was reduced to 55%. The nullo pole of nondisjunctional cells was usually that pole without the granular mass. In 28°C "Double-Drive" nondisjunctional cells, the pole to which $\underline{sc^4sc^8}$ X and Y travelled was the granular pole in 65% of such events. At 18°C, this frequency was 61%. The corresponding frequencies for $\underline{sc^4sc^8}$ were 64% at 28°C, and 64% at 18°C. The behavior of the Basc X appeared to be random with respect to its orientation to the granular pole.

Genetic Tests of sc^4sc^8 and "Double-Drive"

Tables 9, 10 and 11 summarize the results of the progeny tests of the sibs of males sacrificed for cytological study. The k values exhibited by "Double-Drive" males increased from 91.3% at 28°C to 98.9% at 18°C. The corresponding over-all rates of nondisjunction decreased from .512 at 28°C to .305 at 18°C.

When the $\underline{sc^4sc^8}$ drive system operated in the absence of SD the over-all rates of nondisjunction were lower than with SD. At 28°C and 18°C, the nondisjunction frequencies were .323 and .149 respectively. These results are in accord with those of Zimmering (1963), with the frequency of non-disjunction being reduced by almost half at 18°C (Zimmering cultured his $\underline{sc^4sc^8}$ males at 18° and 26°C.)

The presence of SD ostensibly increases the efficiency of the $\underline{sc^4sc^8}$ meiotic drive system at both high and low

Table 9. Summary of progeny tests - "Double-Drive."

Temp.	SD Yy^+ ♂	SD y ♀	cnbw Yy^+ ♂	cnbw y ♀	SD y ♂	SD y^+ ♀	cnbw y ♂	cnbw y^+ ♀	K	SD n.d.	cnbw n.d.	over-all n.d.	Total
28°	451	1453	145	61	2028	19	4	166	91.3	.518	.452	.512	4327
18°	1101	1792	17	3	1141	117	3	20	98.9	.303	.467	.305	4194

Table 10. "Double-Drive" segregation frequencies.

Segregation I	Segregation II	Frequency 28°C.	Frequency 18°C.
$\frac{sc^4sc^8}{Y}$	SD	.427	.336
	cnbw	.004	.034
$\frac{sc^4sc^8}{Y}$	cnbw	.001	.014
	SD	.263	.104
nullo	SD	.272	.469
$\frac{sc^4sc^8}{}$-Y	cnbw	.005	.038
nullo	cnbw	.001	.001
$\frac{sc^4sc^8}{}$-Y	SD	.028	.004

Table 11. Summary of progeny tests $\frac{sc^4sc^8}{}$,y.

Temp.	cnbw Yy+♂	cnbw y ♀	cnbw y ♂	cnbw y+ ♀	rate of n.d.*	x/x+y	o/o+xy	Total
28°	490	1722	1020	36	.323	.778	.966	3268
18°	399	487	99	54	.149	.550	.647	1039

temperatures. Yet, in the "Double-Drive" situation, the meiotic drive of SD increases at 18° whereas that of $\underline{sc^4sc^8}$ decreases. Without $\underline{sc^4sc^8}$, this SD(+/y+Y; $\frac{SD\text{-}72 \quad + \; +}{SD^+ \; cnbw}$) has a k value of 99.6 at 28°C and 99.1 at 18°C. Therefore, the presence of the $\underline{sc^4sc^8}$ system at 28°C appears to effectively reduce the drive of SD while increasing the drive of $\underline{sc^4sc^8}$. At 18°C, the interaction of the systems increases $\underline{sc^4sc^8}$ drive while the k of SD is apparently unaffected.

The relative frequencies of the reciprocal products of disjunctional and nondisjunctional events at 28°C were:

	X/X+Y	0/0+XY
"Double-Drive"	.718	.917
$\underline{sc^4sc^8}$	.778	.966

Thus, the drive of the disjunctional $\underline{sc^4sc^8}$ actually decreased in the presence of SD, as did the "drive" of the nondisjunctional nullo product. Therefore, at 28°C, the drive of both $\underline{sc^4sc^8}$ and SD are lowered. At 18°C, the results differ from those at 28°C.

	X/X+Y	0/0+XY
"Double-Drive"	.616	.893
$\underline{sc^4sc^8}$	.550	.647

The drives of both disjunctional and nondisjunctional products are increased when the $\underline{sc^4sc^8}$ system is in the presence of SD at 18°C. Therefore, at 18°C, the drive of SD is apparently unaffected by the presence of $\underline{sc^4sc^8}$, while that of $\underline{sc^4sc^8}$ is heightened.

Table 10 shows the frequency of segregation of disjunctional and nondisjunctional $\underline{sc^4sc^8}$ chromosomes with SD or cnbw. Considering disjunctional products, the association of sc^4sc^8 and SD decreases from .427 at 28°C to .336 at 18°C, while the reciprocal association of y^+Y with SD^+ cnbw increased from .004 to .034. Accordingly, the segregation of $\underline{sc^4sc^8}$ with SD^+ cnbw increased from .001 at 28°C to .014 at 18°C. From genetic evidence, it appears that the $\underline{sc^4sc^8}$ X and SD arrive at the functional pole (F pole) together with greater frequency at 28°C than at 18°C.

In the case of nondisjunctional products, the frequency with which the SD segregates to the nullo pole increases at 18°C (.272 at 28°C vs. .469 at 18°C), as does the frequency of $\underline{sc^4sc^8}$, Y - cnbw association. The segregation frequency of cnbw to the nullo pole remains the same (.001) at 18°C and 28°C. That of $\underline{sc^4sc^8}$, Y and SD decreases from .028 to .004.

The segregation of the nondisjunctional product and SD at high and low temperatures present a different picture than SD segregation with disjunctional products. Both nullo-SD and $\underline{sc^4sc^8}X,Y$ cnbw segregation frequencies increase at 18°C. Apparently, the probability of SD reaching the nullo F pole and of cnbw arriving with XY at the F pole increases at 18°C. The repulsion between SD and the $\underline{sc^4sc^8}X\text{-}y^+Y$ chromosomes increases as the temperature is lowered. The postulated homology between the SD complex and X (Hiraizumi, 1967) may be supported by these data. The abbreviated

$\underline{sc^4sc^8}$ X has less homology with the Y than a normal X (Cooper, 1959). However, the $\underline{sc^4sc^8}$ X and $\underline{y}^+$Y together may bear enough homology to the SD complex to cause such repulsion.

Correlation of Cytological Studies and Genetic Evidence

Tables 12 and 13 compare cytological observations with resultant progeny data. The frequency of nondisjunction in all primary spermatocytes and that exhibited in the F_2 are in agreement. A 3 x 2 X^2 test (Table 14) of rate of disjunction versus the presence and location of the granules, gives a X^2 value of 2.72. Therefore, the probability is about 30% that this distribution would occur in populations with homogeneous means.

These data support Yanders *et al* (1968) in their conclusion that meiotically driven chromosomes and the bacterial cellular inclusions are responding to the same cellular polarity, and that this polarity is associated with the determination of functional and non-functional gametes at Meiosis I (Peacock and Erickson, 1965). These bacterial inclusions appear to serve as an index of the normal polarity of a spermatocyte.

Yanders *et al*, derived the following parameter, *p*, as an estimate of the probability that the bacteria will be located at the nonfunctional pole of the anaphase I spindle:

$$s = pt + (1 - p)(1- t)$$

Where *s* is the observed frequency of occurrence of the bacteria with the Y chromosome in disjunctional anaphases,

Table 12. Correlation of cytological and genetic observations.

	Cytological n.d.*	Genetic n.d.*	n.d.* in Polarized cells	sc^4sc^8 to non granular pole	sc^4sc^8 in disjunctional gametes	XY to granules	nullo in n.d.* gametes
28°							
DD**	.559	.518	.632	.607	.718	.651	.917
4-8	.339	.323	.424	.642	.778	.641	.966
18°							
DD**	.324	.303	.309	.581	.616	.605	.893
4-8	.235	.147	.156	.553	.550	.643	.647

*n.d. = nondisjunction
**DD = "Double-Drive"

Table 13. Values for the parameter p for disjunctional and nondisjunctional products.

	Cytological Observations		Genetic Observations			
	Disjunctional	N.D.*	Disjunctional	N.D.*		
	X/X + Y (s) to non-granular pole	o/o + XY (s) to non-granular pole	X/X + Y (t)	o/o + XY (t)	Disjunctional p	Nondisjunctional p
28° D.D.**	.607	.651	.718	.917	.745	.681
28° 4-8	.642	.641	.778	.966	.755	.651
18° D.D.**	.581	.605	.616	.893	.849	.634
18° 4-8	.553	.643	.550	.647	1.03	.986

*N.D. = Nondisjunctional
**D.D. = "Double-Drive"

Table 14. Test for homogeneity of means.

3 x 2 X^2 Test, "Double-Drive" at 28°C.

$$X^2 = \frac{\Sigma\,(T/n^2) - \frac{G^2}{\Sigma n}}{\bar{y}\,(1 - \bar{\bar{y}})}$$

	Polar. cells	Non-polar cells	Agranular cells	Total	
Disjunc. cells	84	43	19	146	(G)
Nondisj. cells	106	33	25	164	
Sample size	190	76	44	310	(Σn)

Item	Computation					
Mean	.792	.566	.432	.47096	$\bar{\bar{y}}$	Σ means = 1.790
T^2	7056	1849	361	21316	G^2	$\Sigma T^2/n$ = 69.45
T^2/n	37.14	24.33	7.98	68.76	$G^2/\Sigma n$	

$1 - \bar{\bar{y}}$	.52904
$\bar{\bar{y}}\,(1 - \bar{\bar{y}})$	.253442
$\Sigma(T^2/n - G^2/\Sigma n)$	.69
X^2	2.72

or with the X and Y chromosomes in nondisjunctional anaphases (see Table 13), t is the coefficient of meiotic drive for X or nullo gametes from disjunctional or nondisjunctional gamete classes respectively. The calculated p values are given in Table 13.

The mean value of p for disjunctional and nondisjunctional products of $\underline{sc^4sc^8}$ males at 28°C is .703. Yanders et al (1968) reported a similar $\bar{p}$ value (.741) for $\underline{sc^4sc^8}$ at 25-26°C. At 28°C, the p values for disjunctional and nondisjunctional events in "Double-Drive" and $\underline{sc^4sc^8}$ are comparable. At 18°C, $\underline{sc^4sc^8}$ gave p values in close agreement. In "Double-Drive" at 28°C, however, the probability that the polarized bacteria are located at the nonfunctional pole is .849 in disjunctional gametes, and .634 in nondisjunctional gametes. In all cases, the probability of the bacteria being at the nonfunctional pole is greater in disjunctional gametes than in nondisjunctional gametes. The p value for $\underline{sc^4sc^8}$ at 18°C are in close agreement (difference = .017), as are the p values for "Double-Drive" at 28°C (difference = .064). The differences between nondisjunctional and disjunctional p values for "Double-Drive" at 18°C and $\underline{sc^4sc^8}$ at 28°C are .215 and .104 respectively.

The probability that the nonfunctional pole will exhibit granules appears to be influenced by environmental conditions such as temperature, and the extent and direction of this influence is dependent upon the chromosomes in the

drive system (or systems). The extent of the influence of temperature may differ in disjunctional and nondisjunctional gametes.

VI. DISCUSSION

The Effect of Irradiation on SD

The depression of the k values in the sperm batches of irradiated males indicates that the mechanism of SD is disturbed by irradiation. The period of sensitivity occurs prior to Anaphase I, probably during the period of synapsis at zygotene of Prophase I. The percentage of aberrations observed per sperm batch is inversely correlated with the k values exhibited. Since the probable origin of these aberrations is irradiation-induced chromatid breakage and subsequent rejoining of non-sister chromatids, the chromatids involved would most likely be in synaptic association. The sperm batches containing the greatest proportion of chromatids affected by these events also exhibited the lowest k values, suggesting that the SD mechanism was most sensitive to irradiation at the time of synaptic pairing.

This interpretation is supported by the work of Sandler, Hiraizumi, and Sandler (1959) who found that inhibition of pairing at the SD Ac(SD) region by inversions in the SD^{+} homologue greatly depressed the drive of SD. Irradiation at synapsis may reduce the effectiveness of SD by disturbing the intimate association of the chromatids.

Mange (1968) found the k values of SD males most reduced 7 to 11 days following treatment of temperature-

sensitive SD males for a 24 hour period at 30° or 19°C. The irradiation-induced reduction of k values in these experiments was most pronounced 7 to 10 days after treatment. Therefore, the period during which the SD mechanism is sensitive to temperature appears to coincide with the irradiation-sensitive stage. The k value depression observed after irradiation of SD-72 is comparable to that reported by Mange after cold shock of SD-72 for 24 hours at 19°C. (SD-72 was reported to be insensitive to heat treatment.)

Storage of both treated and control males affected the k of SD. Only the first sperm batch of treated males carried aberration products. The sperm in later batches were most probably not yet primary spermatocytes at the time of irradiation. The erratic k values of both the control and experimental groups can be attributed to prolonged storage of the adult male without mating. Storage apparently "unstabilizes" SD. Why storage should reduce the stability of SD is not clear, but sexual abstinence could retard the rate of spermiogenesis, and prolongation of the primary spermatocyte stage could cause disorientation at Anaphase I.

The sex ratios (♀♀/total) of cn bw progeny of SD/cn bw males were in excess of 60% in all groups. Hiraizumi (1967) reported such an increase in the proportion of females in cn bw, with a reciprocal decrease in the proportion of females in SD progeny. Because of this, he postulated a homology between the X chromosome and the SD Ac(SD) St(SD) complex, and this type of homology would account for the current

results. This would imply that a degree of repulsion exists between the SD bearing II and the X resulting in their tendency to segregate at Anaphase I to opposite poles.

However, a reciprocal decrease in the proportion of SD females was not observed in these data. The SD progeny and the homozygous cn bw stock exhibit similar sex ratios of about 50%. Since SD has been maintained by back crossing it to cn bw every generation, the SD bearing chromosome II should be essentially the only difference between the two stocks.

The absence of a reciprocal depression of the sex ratio in the two classes is difficult to reconcile with Hiraizumi's original homology hypothesis, which implied an "effective pairing" before Anaphase I. To account for these data, a modification concerning the chromosomal sequence of Anaphase I segregation is proposed: 1) If SD precedes the sex chromosomes to the functional or nonfunctional pole at Anaphase I, the probabilities of the X or Y reaching that pole are equal. That is, the arrival of the X at that pole is effectively a random and independent event. 2) If the sex chromosomes undergo Anaphase I segregation prior to the autosomes, and the X chromosome travels to the F-pole, the probability of SD orienting to the F-pole is reduced. The reciprocal Y; SD gamete is nonfunctional.

Effectively, this is unilateral repulsion. The chromosome does not "recognize" sufficient homology with the SD chromosome to disorient its movement at Anaphase I. The

SD complex on chromosome II, however, is somewhat repelled by an X-chromosome-bearing pole. A reciprocal decrease in the SD sex ratio would therefore not occur. The SD^{+} cn bw chromosome would have an increased potential of reaching the F-pole if the X preceded it. The reciprocal Y; SD gamete is lost. If the X segregated precociously to the nonfunctional pole, the strong drive of the SD chromosome to orient towards the F-pole would be unaltered, or slightly increased. If the drive of SD is somewhat increased by repulsion from an X-bearing nonfunctional pole, the subsequent increase in the proportion of functional Y; SD gametes would compensate for the loss of Y; SD nonfunctional gametes resulting from the repulsion of SD toward an X-bearing functional pole.

Relationships of Cytological and Genetic Data

Comparison of the rates of nondisjunction observed in primary spermatocytes and recovered in progeny of sib males indicates that the cytological and genetic data are measurements of the same meiotic event. Both methods indicated 1) an increase in the frequency of nondisjunction in $sc^{4}sc^{8}$ when in the presence of SD in "Double-Drive", at 18° and 28°C, 2) a decrease in nondisjunctional events in both $sc^{4}sc^{8}$ and "Double-Drive" at 18°C and 3) comparable rates of nondisjunction in both $sc^{4}sc^{8}$ and "Double-Drive" at 18° and 28°C.

Yanders et al (1968) concluded that the polarization of the bacteria at Meiosis I was an independent response to

a cytoplasmic gradient which regularly exists in the primary spermatocyte. This gradient renders the meiotic poles unequal, supporting the cytological model for meiotic drive proposed by Peacock and Erickson (1965). Their functionality hypothesis requires a predetermined polarity of the primary spermatocyte, with only one pole yielding functional sperm. On the basis of the p values calculated (p = the probability that the bacteria will be located at the nonfunctional pole at Anaphase I, Yanders et al, 1968), the observed frequency of association of one or the other reciprocal class of disjunctional or nondisjunctional events in $\underline{sc^4sc^8}$ is a reasonable estimate of the genetic recovery of that class.

The probability that the pole exhibiting polarized bacteria will be nonfunctional is slightly lower in nondisjunctional cells. In the cases of $\underline{sc^4sc^8}$ at 18°C and "Double-Drive" at 28°C, the p values for disjunctional and nondisjunctional cells differ by only .017 and .064 respectively. In $\underline{sc^4sc^8}$ at 28°C and in "Double-Drive" at 18°C, the differences are somewhat greater, .104 and .215. The p value differences do not correlate with the characteristic differential in recovery of nondisjunctional reciprocal products reported by Zimmering (1963) and Peacock (1965), and observed in both $\underline{sc^4sc^8}$ and "Double-Drive" at both temperatures. Although the propensity of the bacteria to be located at the nonfunctional pole at Anaphase I is significant in every category, there appears to be a variance with

temperature, chromosomal constitution, and segregation behavior at Anaphase I.

The 3 x 2 X^2 test for homogeneity of means indicated that polar, non-polar, and agranular cells did not differ significantly in frequencies of nondisjunction. Therefore, the polarized cells scored for the association of bacteria with the reciprocal products of disjunctional and nondisjunctional events can be considered to be representative of the population in general.

In addition to the cytological evidence that a polarity exists in the primary spermatocyte prior to Anaphase I movement, the genetic tests of $\underline{sc^4sc^8}$ and "Double-Drive" support the hypothesis that functionality is determined prior to chromosomal segregation. If the functional pole were determined by the driving chromosome itself, $\underline{sc^4sc^8}$ and $\underline{SD}$ would be in a competitive situation. Yet, at 18°C, the $\underline{SD}$ $\underline{k}$ value in "Double-Drive" is characteristic of the drive of this $\underline{SD}$ alone (.989), and the drive of $\underline{sc^4sc^8}$ is increased in the presence of $\underline{SD}$. At 28°C, the $\underline{k}$ of $\underline{SD}$ in "Double-Drive" was decreased (.917), as was the drive of $\underline{sc^4sc^8}$. These results indicate that the $\underline{SD}$ and $\underline{sc^4sc^8}$ systems were both responding to a gradient in the spermatocyte associated with functionality.

Hartl, Hiraizumi, and Crow (1967) proposed that the mechanism of $\underline{SD}$ was the production of dysfunctional $\underline{SD}^+$ sperms. Such a mechanism would be independent of any polarity gradient in the primary spermatocyte. This model is formally

equivalent to the chromosome breakage hypothesis concerning SD (Crow et al, 1962) but does not require cytological evidence of actual breakage events.

If SD action is independent of the cytoplasmic gradient to which $\underline{sc^4sc^8}$ responds, the drive of $\underline{sc^4sc^8}$ should be unaffected by that of SD. The occurrence of $\underline{sc^4sc^8}$ with SD or SD^+ would be randomly determined, those sperm bearing $\underline{sc^4sc^8}$;SD^+ being rendered dysfunctional. The present data indicate that the drives of these two systems are indeed related, with $\underline{sc^4sc^8}$ exhibiting an increased drive when the k of SD is higher (at 18°C) and a diminished drive when SD's k is lower (at 28°C). This is not compatible with the dysfunctionality hypothesis.

Sandler, Lindsley, Nicoletti, and Trippa (1968) consider SD to be in a class of meiotic mutants which disturb normal meiotic behavior of chromosomes. In their germinal cycle model, SD's action would most likely be exerted after centromeric association and the onset of pairing. The SD locus must pair with the SD^+ locus on its homologue in order to effect segregation-distortion (Sandler, Hiraizumi, and Sandler, 1959) and subsequent chromosomal disjunction is normal (Hiraizumi and Hartl, 1968).

The aberrant segregation of the $\underline{sc^4sc^8}$ chromosome is probably due to its gross heterochromatic deletions. Deleted X chromosomes characteristically exhibit greatly reduced pairing with the Y and nondisjunction (Lindsley and Sandler, 1958). It is possible, however, that a locus

(or loci) for normal genetic control of sex chromosome disjunction and Anaphase I segregation is included in the segment deleted from $\underline{sc^4sc^8}$.

While both $\underline{SD}$ and $\underline{sc^4sc^8}$ exhibit aberrant segregation, they do so for different reasons. In the case of $\underline{sc^4sc^8}$, the deletion of proximal heterochromatin produces a chromosome which is much smaller than the normal X, and considerably smaller than the Y. It has been shown that when the homologues differ in size, the smaller of the two is recovered more frequently (Novitski, 1956a). On the other hand, there is no difference in size between $\underline{SD}$ and its $\underline{SD^+}$ homologue, and the $\underline{SD}$ locus itself, presumably a single site in the heterochromatin, is responsible for the aberrant segregation. This is a qualitative difference in homologues, while that of $\underline{sc^4sc^8}$ is quantitative. Nevertheless, both $\underline{SD}$ and $\underline{sc^4sc^8}$ exhibit a pronounced meiotic drive, even though the mechanisms initiating the drives are dissimilar.

The model which best fits these results is the functional-nonfunctional pole hypothesis rather than the dysfunction hypothesis. If the chromosomes themselves determined polar functionality, as is required by the dysfunction model, the two driving systems would compete and one or both drives would be diminished. The present data do not indicate such competition. The cytologically observed drive of $\underline{sc^4sc^8}$ away from the granular pole at Anaphase I and its comparable genetic recovery support the existence of a predetermined functional-nonfunctional polarity

in the primary spermatocyte. Although the SD-bearing chromosome II is not cytologically distinguishable from its SD^+ homologue, the concomitant elevation and depression of the SD k value and sc^4sc^8 evident at 18°C and 28°C indicates that the mechanism of SD is not independent of the polarity gradient to which sc^4sc^8 responds.

V. SUMMARY

The research was designed 1) to ascertain the effect of irradiation at the onset of meiosis on Segregation-distorter in order to determine the meiotic stage during which the mechanism of SD is sensitive, and 2) to observe the meiotic drive systems of SD and the $\underline{sc^4sc^8}$ X together ("Double-Drive") both cytologically and genetically at 18° and 28°C. Analysis of the interaction of these systems and correlation of the nonrandom polar segregation of $\underline{sc^4sc^8}$ with its genetic recovery tested the hypothesis that the mechanism of both drive systems involves a predetermined functional-nonfunctional polarity which is independent of the drive systems which respond to it.

In the first study, irradiation treatments were administered to very late third instar male SD larvae. To observe the effects of sperm storage, part of the experimental and control groups were aged after eclosion as virgins. The first four daily broods from unstored irradiated males exhibited depressed k values. The percentage of aberrations and the depression of the k values per brood appear to be positively correlated. Using the proportion of aberration products as an index, stored males appear to transfer few sperm which were in meiotic or premeiotic stages at the time of treatment.

Depressed k values were exhibited by both irradiated and control groups which were stored. Lack of mating activity and possibly resultant prolongation of Meiosis I appear to "unstabilize" and depress the drive of SD.

The sex ratios (♀♀/total) of cn bw progeny in experimental and control groups were in excess of 60%. A modification of Hiraizumi's SD-X chromosome homology hypothesis is presented to account for the lack of a reciprocal decrease in the sex ratios of SD progeny.

In the study of "Double-Drive", the nonrandom segregation of $\underline{sc^4sc^8}$ or the nondisjunctional nullo product away from the bacterial pole correlated with genetic recovery, supporting the hypothesis that a predetermined functional-nonfunctional polarity exists in the primary spermatocyte. At 18°C, $\underline{sc^4sc^8}$ expresses increased drive when in the presence of SD. Both drives are slightly reduced when together at 28°C. Thus, the data indicate that the drive systems of SD and $\underline{sc^4sc^8}$ are not competitive in their interaction, and that the mechanism of SD is not independent of that to which $\underline{sc^4sc^8}$ responds.

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APPENDIX

APPENDIX

Basc

constitution: $In(1)sc^{S1}L_{sc}{}^{8}R_{+S}$, $sc^{S1}{}_{sc}{}^{8}w^{a}$ B.
synthesis: Muller
synonym: M-5: Muller-5
references: Spencer and Stern, 1948, Genetics 33: 43-74
properties: Male and homozygous female viable and fertile; X/0 male poorly viable, variegated for y, ac, and probably l(1)J1. Suppresses crossing over in X, but less so than Binsc because In(1)S = In(1)6A1-3;10F10-11A1 less effective than In(1)d1-49 =In(1)4D7-E1;11F2-4. Routinely used in detection of sex-linked recessive lethals.

cn: cinnabar

location: 2-57.5
origin: Spontaneous
discoverer: Clausen, 20i8.
references: 1924, J. Exptl. Zool. 38: 423-36
phenotype: Eye color bright red, like v or st. Ocelli colorless. Eye color darkens with age, but ocelli remain colorless. Larval Malpighian tubes pale yellow (Beadle, 1937, Genetics 22: 587-611). Nonautonomous in development of pigment of transplanted eye disks (Beadle and Ephrussi, 1936, Genetics 21: 230), cn blocks conversion of kynurenine to 3-hydroxyhynurenine, which has been identified as the cn^{+} hormone (Butenandt, Weidel, and Schlossberger, 1949, Z. Naturforsch. 4b: 242-44). RK1.
cytology: Proximal to 44C, based on its inclusion in Dp(2;3)P32 = Dp(2;3)41A;42D-E;44C-D;89D7-E1 (E. B. Lewis).

bw: brown

location: 2-104.5
discoverer: Waaler, 19j15.
references: 1921, Hereditas 2: 391-94.
Sturtevant and Beadle, 1939, An Introduction to Genetics, Saunders, p. 64 (fig.).
phenotype: Eye color light brownish wine on emergence, darkening to garnet. Red pigments lacking; ommochromes at 87 percent normal level (Nolte, 1954,

J. Genet. 52: 111-26). Adult testes and vasa colorless. Larval Malpighian tubules pale yellow (Beadle, 1937, Genetics 22: 587-611). Produces white eyes in combination with v, cn, or st. Eye color autonomous when transplanted into wild-type host (Beadle and Ephrussi, 1936, Genetics 21: 230). RK1.

cytology: Placed between 59D4 and 59E1 by Bridges [1937, Cytologia (Tokyo), Fujii Jub. Vol. 2: 745-55], on the basis of its exclusion from the inner inversion of $In(2LR)bw^{V1}$ = In(2LR)21C8-D1;60D1-2 + In(2LR)40F;59D4-E1 and its inclusion in $In(2R)bw^{V\ De2}$ = In(2R)41A-B;59D6-E1. Based on the study of bw rearrangements, Slatis (1955, Genetics 40: 5-23) tentatively places bw in 59D9, 10, or 11.

other information: Separable into at least two subunits by recombination with bw and bw^{75} about 0.001 units to the left of bw^{59} and bw^{81} (Divelbiss, 1961, Genetics 46: 861).

y: yellow

location: 1-0.0.

origin: Spontaneous.

discoverer: E. M. Wallace, 11a

references: Morgan and Bridges, 1916, Carnegie Inst. Wash. Publ. No. 237: 27.

phenotype: Body color yellow; hairs and bristles brown with yellow tips. Wing veins and hairs yellow. Tyrosinase formed in adults (Horowitz). For the most part, y is autonomous in mosaics; tissue, however, over limited distances there is some nonautonomy [Hannah, 1953, J. Exptl. Zool. 123: 523-60 (fig.)]. Larval setae and mouth parts yellow to brown, hence distinguishable from the dark brown of wild type (Brehme, 1937, Proc. Soc. Exptl. Biol. Med. 37: 578-80; 1941, Proc. Natl. Acad. Sci. U.S. 27: 254-61). RK1

cytology: Placed in region 1A5-8 on basis of its being carried by the $X^{D}3^{P}$ element of $T(1;3)sc^{260-20}$ = T(1;3)1A8-B1;61A1-2 and by $Dp(1;f)sc^{260-27}$ = Df(1;f)1A8-B1;19F, but not being lost from Df(1)260-5 = Df(1)1A4-5 (Sutton, 1943, Genetics 28: 210-17).

$In(1)sc^{4L}sc^{8R}$: Inversion(1) scute-4 Left scute-8 Right

cytology: $In(1)1B3-4;19F-20C1^{L}1B2-3;20B-D1^{R}$; duplicated for 1B3, mitotic chromosomes deficient for the proximal third of hD, all of hC and hB, and the distal majority of hA (Cooper, 1959, Chromosoma 10: 525-88). About 0.6 the length of a normal X at metaphase.

origin: Recombinant containing left end of $In(1)sc^4$ and right end of $In(1)sc^8$.
discoverer: Gershenson.
references: 1933, J. Genet. 28: 297-313
1933, Biol. Zh. (Moscow) 2: 145-59, 419-24.
genetics: Duplicated for the sc locus, carrying both sc^4 and sc^8; deficient for the bb locus and the nucleolus organizer [i.e., $Df(1)bb^G$]. Shown by Ritossa and Spiegelmann (1965, Proc. Natl. Acad. Sci. U.S. 53: 737-45) to be deficient for all the DNA that is complementary to ribosomal RNA present in a haploid chromosome set. In the male, $In(1)sc^{4L}sc^{8R}$ frequently fails to pair with the Y and when it does the unpaired X and Y usually proceed to the same pole (Peacock, 1965, Genetics 51: 573-83). Furthermore, reciprocal meiotic products are not recovered with equal frequency, which Peacock interpreted as the result of non-random orientation of the first meiotic division with respect to the postulated functional pole of the primary spermatocyte. Irregularities in meiotic behavior of $In(1)sc^{4L}sc^{8R}$ in the male are affected by the Y chromosome present (Peacock, 1965) and the temperature at which meiosis occurs (Zimmering, 1963, Genetics 48: 133-38). $In(1)sc^{4L}sc^{8R}/Y/Y$ male gives quite regular segregation of the two Y's and low recovery of the X.