MEIOTIC DRIVE IN NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER. V. ON THE NATURE OF THE SD REGION[1,2]

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SECOND chromosomes were collected from nature which, when heterozygous with a normal chromosome II in males, are present in functional sperm much more often than the expected 50 percent. This phenomenon, named segregation-distortion, was found to depend on a locus named Segregation-distorter (symbolized *SD*), which is located near the centromere (and probably in the right arm) of chromosome II.

Studies on the formal genetics of *SD* have been reported by SANDLER, HIRAIZUMI and SANDLER (1959) and by SANDLER and HIRAIZUMI (1959, 1960). From the evidence presented there, the following propositions, relevant to the present discussion, appear to be true.

I. The phenomenon of segregation-distortion is operative only in heterozygous *SD* males—not in homozygous *SD* males nor in females.

II. The actual distorted ratios may be visualized as resulting from some sort of misreplication (formally equivalent to a chromosome break) of the SD^+-bearing chromosome, conditioned by *SD*, which somehow causes a large proportion of the SD^+-containing spermatocytes to behave abnormally so that functional SD^+-bearing sperm are not produced.

III. In order for *SD* to have this effect, the *SD* region must synapse with the SD^+ region of the homolog. Thus, when *SD* is heterozygous with a structurally rearranged homolog, as for example the Curly inversion, *In(2LR)Cy*, segregation is normal.

IV. At, or near, the tip of IIR there is a stabilizing modifier of *SD*, *St(SD)*. In the presence of this modifier, *SD* is *stable* and the segregation ratio is constant and high (often 20:1 or more) from male to male; in the absence of *St(SD)*, *SD* action becomes somewhat variable *(semistable)* and, rather frequently, changes spontaneously to a highly variable state *(unstable)*.

V. If *SD*, from certain *SD* lines, is passed from a male into a female (so balanced that crossing over in chromosome II does not occur), and *SD*-bearing male progeny are collected from such individual females, two types of male sibships are observed. From some females (called "unconditioned"), the sons produce the expected distorted ratios in their progeny; from others (termed "conditioned"), one half of the sons produce the expected distorted ratios whereas the other half produce a normal 1:1 segregation. In the next generation, however,

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all males distort irrespective of whether or not their father distorted. This phenomenon has been named conditional distortion.

VI. Normal, SD^+-bearing, second chromosomes often differ one from another in their sensitivity to the distorting action of *SD*. These differences seem localized in the SD^+ region.

The present study is concerned with the structure of the *SD* region. Data have been collected that support the conclusions that there are at least two separable elements that function in respect of the phenomenon of segregation-distortion. One of these is the *SD* locus itself which is responsible for the misreplication on the SD^+-bearing homolog. Closely linked and to the right of *SD* there is an Activator of *SD*, *Ac(SD)*, that is necessary in order for *SD* to operate. Both *SD* and *Ac(SD)* are located within a small chromosomal aberration that (a) causes a striking reduction in crossing over in the *SD* region and (b) directs the activated *SD* locus to cause a misreplication in the homolog of the chromosome carrying the aberration.

The experimental evidence supporting this interpretation of the *SD* region is presented here.

Crossing over in the SD *region:* Crossing over between *pr* and *cn* has been measured in heterozygous *SD* and homozygous SD^+ females. The markers, purple and cinnabar, span both the centromere and the *SD* locus, with *cn* (on the standard map) being five times as far to the right of the centromere as *pr* is to the left. Normal, SD^+-bearing second chromosomes of various sensitivities were made heterozygous with a chromosome marked by *pr cn* (which, itself, is rather insensitive) in females and crossed to *pr cn* males. In addition, *SD*-bearing chromosomes of three types were made heterozygous with *pr cn* in females and crossing over measured. These three types were: (1) *R(SD)-4*, which arose by recombination from an original *SD* chromosome and which carries no gross rearrangements, carries the mutant allele of *bw*, (brown) and, since it has been inherited from the male parent, would produce the standard semistable distribution of k values if tested in males (Proposition IV); (2) the same *R(SD)-4* inherited from female parents, thus resulting in a proportion of cases (i.e. one half of the *SD* offspring of conditioned females) which, if tested in a male, would have shown no distortion (Proposition V); and finally, (3) *R(SD)-4, St(SD)*, a standard *R(SD)-4* onto which has been placed the stabilizing modifier, *St(SD)*, resulting in a line which, when inherited through males, produces a standard stable distribution of k values (the parameter k is defined as the proportion of functional *SD*-bearing sperm produced by a heterozygote) in sons (Proposition IV). The results of these tests are presented in Table 1.

It can be seen that the control frequency of recombination (i.e. in homozygous SD^+-bearing females) is relatively constant irrespective of the sensitivity of the SD^+-bearing chromosome. Similarly the crossover values are constant in heterozygous *SD* individuals regardless of the "distorting state" of *SD*. Crossing over in heterozygous *SD* females, however, is uniformly very much lower than in homozygous SD^+ females. The reduction in recombination thus appears to depend on

TABLE 1

Crossing over between pr *and* cn *in crosses of females heterozygous for* pr cn *and various* SD *and* SD$^+$*-bearing second chromosomes, by* pr cn *males*

Constitution of parental females*	Phenotype of progeny				Percent recombination
	pr cn	+	*pr*	*cn*	
Tγ/ pr cn	4022	4424	52	45	1.14
Canton-S/ *pr cn*	1465	1530	17	17	1.12
Canton-S/ *pr cn*	4158	4561	53	44	1.10
Su(SD), bw/ pr cn	3707	3769	52	46	1.29
al/ pr cn	5462	6653	85	61	1.19
Mean					1.17
R(SD)-4/ pr cn	1581	1689	1	0	0.03
R(SD)-4/ pr cn	3831	3964	3	3	0.08
R(SD)-4/ pr cn	4116	4166	6	4	0.12
R(SD)-4, bw$^+$ St(SD)/ pr cn	5353	6552	7	8	0.13
Mean					0.09

* *Tγ* (Tokyo) is a very sensitive wild-type strain derived from a collection near Tokyo, Japan. Canton-S is a standard inbred strain of intermediate sensitivity; the two Canton-S experiments differ only in that the two sets were done at different times. *Su(SD), bw* is a second chromosome carrying the recessive allele of *bw* and a very insensitive *SD*$^+$ region (from the *bw^D* strain). The *al*-bearing second chromosome is also rather insensitive. The first two *R(SD)-4* experiments were done at different times, but in both cases the *SD*-bearing chromosome was inherited from the male parent. The third *R(SD)-4* experiment differed in that the *SD*-bearing chromosome was inherited from the female parent (so balanced, however, that no crossing over occurred in those females). All three tests involved the standard, semistable, *R(SD)-4*. In the final test, a *R(SD)-4* chromosome, made stable by the addition of the stabilizing modifier, *St(SD)*, was used.

the presence of the *SD* region and does not vary depending on the *k* value distribution characteristic of the chromosome when tested in males.

Further tests show that this depression in crossing over is almost—if not exclusively—confined to the right arm of chromosome II, and furthermore, the suppression effect rapidly decreases in magnitude with increasing distance of the measured region from *SD*. These two inferences come from the results of matings between females heterozygous for *R(SD)-4* and a chromosome marked by *b pr cn en* and males *b pr cn en*. The marker black is located to the left of *pr* while engrailed is located to the right of *cn*. The control in this case is *Tγ/b pr cn en* females by homozygous recessive males. The results are shown in Table 2.

Here it can be seen that the *b pr* region is not significantly different in the experimental and control sets, the *pr–cn* region shows the dramatic reduction in the heterozygous *SD* set as before, and the *cn–en* region shows a reduction in recombination in the experimental set to about one half that of the controls. Thus it is clear that the depression in crossing over is almost certainly a function of the *SD* region itself and that this depression is confined to a localized region in the right arm of chromosome II.

Characterization of recombinants: From crosses of *R(SD)-4/pr cn* females by homozygous *pr cn* males, 25 *pr* recombinants (symbolized *R(pr)* followed by an identifying number) and 25 *cn* recombinants (symbolized *R(cn)* followed by an identifying number) were selected for further study. These recombinants were backcrossed to the standard *cn bw* strain for a number of generations to insure uniformity of genetic background and then tested (1) for their ability to distort

TABLE 2

Crossing over between b *and* pr *(region I),* pr *and* cn *(region II) and* cn *and* en *(region III) in crosses of females heterozygous for* b pr cn en *and* Ty, *in one case, and,* R(SD)-4 *in the other, by* b pr cn en *males*

Phenotype of progeny	Constitution of female parent *Ty/ b pr cn en*	*R(SD)-4/ b pr cn en*
b pr cn en	3939	2522
+	5050	3256
b	158	93
pr cn en	150	75
b pr	72	4
cn en	75	3
b pr cn	281	73
en	209	61
b cn en	4	1
pr	9	0
b en	9	4
pr cn	8	1
b pr en	1	0
cn	0	0
b cn	0	0
pr en	0	1
Percent crossovers		
Region I	3.39	2.87
Region II	1.62	0.15
Region III	5.10	2.30

the standard, sensitive, *cn bw* second chromosome, and (2) for their sensitivity to *SD-5*—a stable *SD*-bearing chromosome. The results of these tests are given in Table 3, 4, 5, and 6. The recombinants in Table 3 are separated according to their sensitivity, as shown in Table 5. Similar groupings are made in Tables 4 and 6.

It is immediately evident from these results that *SD* is not a simple locus for the reason that, while none of the *pr* recombinants exhibit segregation-distortion, only about one half of the *cn* recombinants distort (Tables 3 and 4). There are two types of *pr* recombinants recovered—those that do not distort and are insensitive and those that also do not distort, but are of intermediate sensitvity. There are, on the other hand, three distinct types of *cn* recombinants—nondistorting, almost completely sensitive; slightly distorting and somewhat less sensitive; and those that distort and exhibit complete insensitivity. This last class while all clearly exhibit segregation-distortion, are nevertheless all "weaker" distorters than the original *R(SD)-4* that typically exhibits a mean *k* value of about 0.93. It may be that all of these lines have become unstable, since the semistable *R(SD)-4* does, on occasion, become unstable (Proposition IV), but it seems more probable that the *SD* region in these distorting *cn* recombinants has been changed as a consequence of the recombination. We shall refer to the distorting *cn* recombinants as *SD*, to the slightly distorting *cn* lines as SD^{sa} (*SD*-semiactive) and to the nondistorting *cn* recombinants as SD^{in} (*SD*-inactive).

Another line of evidence indicating that the *SD cn* recombinants carry a changed *SD* region is as follows. It has been shown (Proposition I) that an *SD*-bearing chromosome is completely insensitive to the distorting action of another *SD* chromosome. Although the data in Table 6 indicate that the *SD cn* recombinants (as defined in the preceding paragraph) are indeed insensitive, there were in fact a small number of highly sensitive cultures in some *SD cn* recombinant lines. These cultures were omitted from the tabulations in Table 6, but their presence definitely indicates a change from normal *SD* behavior in these *cn* recombinants.

The change in the nature of *SD* apparently requires a crossover in the immediate vicinity of *SD*. This is evidenced by the fact that ten *b* recombinants and ten *cn* recombinants from *b pr cn en/R(SD)-4* females were tested, and all could be shown to carry an unchanged *SD*.

A sample of each type of *pr* recombinant was made heterozygous with a normal

TABLE 3

The results of tests for segregation-distortion by pr *recombinants from* pr cn/R(SD)-4 *females. The testcross is:* R(pr)/ cn bw ♂ ♂ × cn bw ♀ ♀

Recombinant controls*	Phenotype of progeny *cn bw* 1802	+ 2188	*k* 0.55
1	627	769	0.55
2	634	769	0.55
3	614	700	0.53
6	603	730	0.55
11	706	830	0.54
50	659	799	0.55
52	725	886	0.55
54	966	1228	0.56
70	810	946	0.54
72	1028	1294	0.56
75	508	549	0.52
80	564	752	0.57
82	426	545	0.56
Mean			0.55
4	643	762	0.54
5	684	759	0.53
9	630	646	0.51
10	701	860	0.55
51	822	984	0.54
61	1174	1371	0.54
64	648	728	0.53
71	762	1048	0.58
73	1215	1415	0.54
74	645	671	0.51
81	220	175	0.44
83	293	363	0.55
Mean			0.53

* *cn bw*/ +♂♂ × *cn bw* ♀♀.

chromosome marked by *cn* in females, and crossing over between *pr* and *cn* was measured. Similar tests were made of females heterozygous for each type of *cn* recombinant and a normal chromosome marked by *pr*. The results of these tests are presented in Table 7. From these data, the following inferences can be drawn. (1) The element contributing the major component to the crossover suppression in the *SD* region is associated with the insensitivity of *SD*, because the insensitive *pr* recombinants and the insensitive *cn* recombinants (those that are *SD*) show a level of crossing over comparable to the original heterozygous *SD* lines, while crossing over in the more sensitive *pr* and *cn* recombinants is uniformly much higher. (2) It is not possible to separate SD^{in} from SD^{sa}, among the *cn* recombinants, on the basis of crossing over. (3) All of the recombinants have been changed by the crossover in the vicinity of *SD*, as is evidenced by the fact that crossing over is lower in all of the tested recombinants than in the controls. Thus all of the

TABLE 4

The results from tests for segregation-distortion by cn *recombinants from* pr cn/ R(SD)+ females. *The testcross is:* R(cn)/ cn bw ♂♂ × cn bw ♀♀

Recombinant controls*	Phenotype of progeny *cn bw* 1858	*cn* 2264	*k* 0.55
3	369	1810	0.83
8	373	1075	0.74
9	183	2105	0.92
15	321	1654	0.84
16	552	1459	0.73
19	433	2025	0.82
53	172	1209	0.88
57	305	2118	0.87
59	389	1585	0.80
62	589	1757	0.75
65	321	1568	0.83
Mean			0.82
1	744	1031	0.58
5	1571	2964	0.65
6	979	1294	0.57
12	1191	1633	0.58
56	631	910	0.59
Mean			0.59
2	822	937	0.53
4	1177	1268	0.52
7	1177	1278	0.52
10	1190	1356	0.53
13	826	893	0.52
14	1218	1327	0.52
17	1013	1192	0.54
18	1229	1484	0.55
20	1083	1258	0.54
Mean			0.53

* *cn/cn bw* ♂♂ × *cn bw* ♀♀.

TABLE 5

Tests for the sensitivity of pr *recombinants from* pr cn/ R(SD)-4 *females. The testcross is:* SD-5/R(pr) ♂♂ × pr cn ♀♀

Recombinant controls*	Phenotype of progeny *pr* 1477	+ 1833	*k* 0.55
1	656	855	0.57
2	496	569	0.53
3	667	695	0.51
6	568	742	0.57
11	486	835	0.63
50	437	424	0.49
52	343	579	0.63
54	501	611	0.55
70	536	692	0.56
72	689	826	0.55
75	681	799	0.54
80	771	930	0.55
82	380	441	0.54
Mean			0.56
4	260	1174	0.82
5	383	940	0.71
9	347	836	0.71
10	124	1005	0.89
51	301	1399	0.82
61	167	1195	0.88
64	300	803	0.73
71	756	2047	0.73
73	342	756	0.69
74	291	558	0.66
81	443	893	0.67
83	501	1348	0.73
Mean			0.75

* *pr*/+ ♂♂ × *pr cn* ♀♀.

recombination observed that has occurred between *pr* and *cn*, in heterozygotes for *SD*, occurs in the crossover suppressing element of the *SD* region.

A summary of the number and types of recovered recombinants is given in Table 8.

On the nature of the crossover reduction: If the crossover reduction in heterozygous *SD* females is due to a small chromosomal aberration in the vicinity of *SD*, then this crossover reduction should be a reflection of heterozygosity for the aberration. Accordingly, two experiments to test this point were performed. First, various *pr* recombinant females were made heterozygous with a number of *cn* recombinants, and crossing over between *pr* and *cn* was measured. These results are presented in Table 9. It can be seen that heterozygotes for *pr* and *cn* recombinants, each of which showed fairly high crossing over when heterozygous with a normal chromosome, exhibit a frequency of crossing over also in the intermediate range, but lower than either shows when heterozygous with a normal chromo-

TABLE 6

Test for the sensitivity of cn *recombinants from* pr cn/ R(SD)-4 *females. The testcross is:* SD-5/ R(cn) ♂♂ × pr cn ♀♀

Recombinant control*	Phenotype of progeny		k
	cn	+	
	1256	1366	0.52
3	366	357	0.49
8	223	262	0.54
9	301	338	0.53
15	512	497	0.49
16	262	316	0.55
19	278	326	0.54
53	264	256	0.49
57	259	270	0.51
59	193	240	0.55
62	604	610	0.50
65	401	403	0.50
Mean			0.52
1	98	1619	0.94
5	153	2551	0.94
6	484	2258	0.82
12	77	1621	0.95
56	191	1673	0.90
Mean			0.91
2	65	1584	0.96
4	108	1409	0.93
7	52	2255	0.98
10	100	1757	0.95
13	66	1756	0.96
14	46	1393	0.97
17	49	1862	0.97
18	46	2037	0.98
20	21	2459	0.99
Mean			0.97

* *cn*/+ ♂♂ × *cn bw* ♀♀.

some. When either a low crossover *pr* recombinant is tested against a high crossover *cn* or a low crossover *cn* is tested against a high crossover *pr* recombinant, the result is indistinguishable from the lower crossover valued recombinant when tested against a normal chromosome. When, however, a low crossover *pr* recombinant is tested against a low crossover *cn* recombinant, the result is a higher level of crossing over than either shows against a normal chromosome. Indeed, the level of crossing over here is higher than in heterozygotes for two high crossover recombinants. Thus it may be concluded that (1) the crossover suppression results from heterozygosity for some genetic element in the *SD* region, and (2) homozygosity for this element probably results in a higher than normal crossover rate.

Further evidence supporting conclusion (2) above is obtained from the following experiments. A chromosome carrying the markers, *b en*, and one carrying *b SD en* were made heterozygous with (1) a normal chromosome marked by *cn*,

(2) *R(cn)-3* which is *SD cn*, and (3) *R(cn)-17* which is SD^{in} *cn*. Crossing over between *b* and *cn* and between *cn* and *en* was measured. The results are given in Table 10. For the case of region I *(b—cn)*, heterozygous *SD* combinations show a reduction of about one crossover unit—undoubtedly reflecting the virtual lack of recombination between *pr* and *cn*. The heterozygote for *R(cn)-17* exhibits roughly the control value (or perhaps slightly lower) while the homozygous *SD* combination, *b SD en/R(cn)-3*, exhibits a higher than normal rate. For the case of region II *(cn–en)* the heterozygous *SD* combinations show the reduction to

TABLE 7

Crossing over between pr *and* cn *in female heterozygotes carrying (1)* pr *recombinants from* pr cn/ R(SD)-4 *females and a normal chromosome marked by* cn *and (2)* cn *recombinants, from this same source, and a normal chromosome marked by* pr. *The controls are* pr/cn. *The males, in all cases, are homozygous* pr cn

Constitution of parental female	Phenotype of progeny				Percent recombination
	pr	*cn*	*pr cn*	+	
pr/cn	4183	4120	41	57	1.17
R(pr)-4/ cn	2185	2255	19	17	0.80
R(pr)-5/ cn	4936	4812	32	29	0.62
R(pr)-7/ cn	5185	5110	28	35	0.61
R(pr)-9/ cn	5396	5367	37	27	0.59
R(pr)-61/ cn	5318	4867	27	30	0.56
R(pr)-64/ cn	4839	4791	37	39	0.78
Mean					0.66
R(pr)-1/ cn	5845	6243	11	16	0.22
R(pr)-3/ cn	4483	4518	8	6	0.16
R(pr)-11/ cn	5233	5280	8	9	0.16
R(pr)-50/ cn	5459	5804	6	4	0.09
R(pr)-52/ cn	5324	5382	11	8	0.18
R(pr)-54/ cn	3481	3505	4	6	0.14
Mean					0.16
pr/ R(cn)-5	4945	6817	50	25	0.63
pr/ R(cn)-12	5732	6708	35	28	0.50
pr/ R(cn)-56	1578	1653	7	15	0.68
Mean					0.60
pr/ R(cn)-2	2955	2987	23	22	0.75
pr/ R(cn)-4	6223	6459	46	49	0.74
pr/ R(cn)-7	6322	6648	51	43	0.72
pr/ R(cn)-13	3527	3629	18	16	0.47
pr/ R(cn)-14	4046	4498	30	35	0.76
pr/ R(cn)-17	6040	6081	33	33	0.54
pr/ R(cn)-18	6076	6346	46	44	0.72
Mean					0.67
pr/ R(cn)-3	5114	5414	4	2	0.06
pr/ R(cn)-9	3613	4277	1	2	0.04
pr/ R(cn)-53	3138	3091	4	6	0.16
pr/ R(cn)-57	2266	2598	2	5	0.14
pr/ R(cn)-59	2921	2900	3	7	0.17
pr/ R(cn)-62	2580	2930	4	3	0.13
Mean					0.12

about one half that of the controls as has been observed earlier, while the others show approximately the control value.

Thus the conclusions that the reduction in crossing over in heterozygous *SD* comes about as the result of heterozygosity for an element and that homozygosity for this element yields a higher than normal crossover rate are confirmed here. The fact that the element in question causes a reduction in crossing over when heterozygous, can be separated into partially functioning subunits (that is. all of

TABLE 8

A summary of the number and distinguishable types of recombinants obtained from R(SD)-4/ pr cn *females*

Type of recombinant	Number recovered	Mean distorting action	Mean sensitivity to *SD*	Mean crossover value between *pr* and *cn*
SD^{in} *cn*	9	0.53	0.97	0.67
SD^{sa} *cn*	5	0.59	0.91	0.60
SD cn	11	0.82	0.52	0.12
pr SD^{+}	13	0.55	0.56	0.16
pr SD^{+}	12	0.53	0.75	0.66

TABLE 9

Crossing over between pr *and* cn *in crosses of females carrying* pr *recombinants from* pr cn/ R(SD)-4 *females and* cn *recombinants, from this same source, by* pr cn *males. The control is* pr/cn

Constitution of parental female	Phenotype of progeny *pr*	*cn*	*pr cn*	+	Percent recombination
pr/cn	6349	6090	65	64	1.03
R(pr)-4/ R(cn)-2	6138	6175	31	32	0.51
R(pr)-4/ R(cn)-13	5131	5409	24	31	0.52
R(pr)-4/ R(cn)-56	5357	5228	38	21	0.55
R(pr)-5/ R(cn)-14	4677	5006	25	17	0.43
R(pr)-61/ R(cn)-14	3726	4355	19	19	0.47
Mean					0.50
R(pr)-4/ R(cn)-57	2749	2727	3	3	0.11
R(pr)-4/ R(cn)-62	2491	2568	3	4	0.14
R(pr)-5/ R(cn)-9	4647	4675	2	2	0.04
R(pr)-61/ R(cn)-9	4982	4962	8	7	0.15
Mean					0.11
R(pr)-54/ R(cn)-2	4301	4788	5	2	0.08
R(pr)-54/ R(cn)-13	3141	3568	2	6	0.12
R(pr)-54/ R(cn)-56	3313	3361	9	5	0.21
Mean					0.14
R(pr)-1/ R(cn)-3	5176	5442	43	39	0.77
R(pr)-50/ R(cn)-59	5450	5670	80	40	1.07
R(pr)-52/ R(cn)-9	5225	5352	58	39	0.91
R(pr)-54/ R(cn)-57	3462	3486	27	29	0.80
R(pr)-54/ R(cn)-62	5066	5491	56	31	0.82
Mean					0.87

the recombinants show some depression in crossing over), and that homozygosity for the element yields a higher than normal crossover rate suggests—but by no means proves—that the element in question is an insertion or duplication of genetic material in the *SD* region.

Reconstitution of SD: Since it is clear that *SD* is not a simple locus, it seemed possible that a functioning *SD* might be reconstituted in recombinants between *R(cn)* and *R(pr)*. A series of such re-recombinants were obtained by collecting wild-type chromosomes from *R(pr)/R(cn)* heterozygous females. Tests for segregation-distortion in such re-recombinants is given in Table 11. It can be seen that only in the one case that the *pr* recombinant was insensitive and the *cn* recombinant SD^{sa} was a functional *SD* recovered. It may be noted, however, that re-recombinants from SD^{sa} give uniformly higher *k* values than re-recombinants from SD^{in} which is additional evidence that SD^{sa} and SD^{in} are distinct.

In addition, about 30 *pr cn* re-recombinants were collected from these same crosses and tested for segregation-distortion. None of these were distorters.

A test of the crossover properties of a sample of wild-type re-recombinants is given in Table 12. It is evident that the reconstituted distorter chromosome exhibits the typical low crossover property, while the nondistorting re-recombinant from the same *pr* recombinant shows the usual intermediate level of recombination. In addition, re-recombinants from a high crossover *pr* recombinant and a high crossover *cn* recombinant show a level of crossing over slightly higher than that of either parent.

Interpretation of results: The following properties of the *SD* region have been elucidated by the experimental data presented here.

1. Crossing over in the immediate vicinity of *SD* is reduced by about a factor of ten.

2. This reduction in recombination is independent of the "state" of *SD* or of SD^{+}.

TABLE 10

Crossing over between b *and* cn *(region I) and between* cn *and* en *(region II) in crosses of females heterozygous for* b en *on one chromosome and* cn *on the other, by* b pr cn en *males.* R(cn)-3 *is* SD; R(cn)-17 *is* SD^{in} *(Table 4)*

Phenotype of progeny	Constitution of parental female					
	b en/cn	*b SD en/cn*	*b en/R(cn)-3*	*b SD en/R(cn)-3*	*b en/R(cn)-17*	*b SD en/R(cn)-17*
b en	2414	3978	2270	3017	2434	1731
cn	2964	5231	2699	4318	2853	2761
b cn	121	101	79	200	114	63
en	104	90	70	178	76	43
b	124	96	61	189	106	53
cn en	88	75	49	96	91	41
b cn en	3	3	0	2	4	3
+	4	4	3	5	2	2
Percent crossovers						
Region I	3.98	2.07	2.91	4.81	3.45	2.36
Region II	3.76	1.86	2.16	3.65	3.57	2.11

3. The crossover suppression diminishes rapidly with increasing distance of the measured region from *SD*.

4. The effect on crossing over is confined to the right arm of chromosome II.

5. The reduction in crossing over comes about as a consequence of heterozygosity for an element.

TABLE 11

Tests for segregation-distortion by wild-type re-recombinants from females heterozygous for nondistorting pr *recombinants and* cn *recombinants that are either* SD^{in} *or* SD^{sa}. *The cross is of males, heterozygous for the wild-type re-recombinant and* cn bw, *by* cn bw *females*

Recombinant	*cn bw*	+	*k*	Recombinant	*cn bw*	+	*k*
R(pr-4/cn-2)	432	503	0.54	*R(pr-61/cn-14)*	660	784	0.54
	529	561	0.51		918	1100	0.55
	781	875	0.53		456	491	0.52
	488	506	0.51		584	663	0.53
	684	773	0.53		685	700	0.51
	694	744	0.52		689	810	0.54
	546	604	0.53	Mean			0.52
	772	930	0.55				
	795	798	0.50	*R(pr-4/cn-56)*	545	674	0.55
	635	631	0.50		596	649	0.52
	904	913	0.50		743	867	0.54
	674	718	0.52		864	998	0.54
	669	716	0.52		518	671	0.56
					709	903	0.56
R(pr-4/cn-13)	477	517	0.52				
	688	732	0.52	*R(pr-5/cn-1)*	680	762	0.53
	662	685	0.51	*R(pr-5/cn-5)*	653	750	0.53
	667	734	0.52		543	612	0.53
	620	707	0.53		116	117	0.50
	747	827	0.53		256	331	0.56
	602	584	0.49				
	865	891	0.51	*R(pr-5/cn-12)*	414	514	0.55
	782	816	0.51		599	792	0.57
	* 604	650	0.52				
	743	742	0.50	*R(pr-61/cn-5)*	563	752	0.57
	855	964	0.53	*R(pr-61/cn-12)-2**	955	1497	0.61
	629	621	0.50	*-3**	1168	1475	0.56
				*-4**	912	1377	0.60
R(pr-5/cn-14)	773	771	0.50		259	447	0.63
	415	460	0.53	Mean			0.56
	818	803	0.50				
	759	919	0.55	*R(pr-52/cn-4)*	875	1029	0.54
				R(pr-54/cn-13)	1170	1208	0.51
				*	1086	1282	0.54
					1479	1529	0.51
					1380	1442	0.51
				Mean			0.52
				R(pr-54/cn-56)	260	1260	0.83

TABLE 12

Crossing over between pr *and* cn *in females, heterozygous for* pr cn *and wild-type recombinants from females carrying* pr *recombinants and* cn *recombinants from* pr cn/R(SD)-4 *females by* pr cn *males. The lines used are those marked by an asterisk in Table 2*

Recombinant	Phenotype of progeny				Percent recombination
	pr cn	+	*pr*	*cn*	
R(pr-54/cn-13)	5339	5701	35	28	0.57
R(pr-4/cn-13)	4856	5563	34	20	0.52
R(pr-54/cn-56)	5260	5807	7	6	0.12
R(pr-61/cn-12)-2	3916	3982	35	34	0.87
R(pr-61/cn-12)-3	4191	4864	44	38	0.90
R(pr-61/cn-12)-4	4191	4460	43	22	0.75

6. Homozygosity for this element results in a rate of recombination somewhat higher than normal.

7. The properties of the three types of *cn* recombinant and two kinds of *pr* recombinant that are recovered from crossing over in the *SD* region have been tabulated in Table 8. It should be noted that in no instance is crossing over normal, nor are any completely normal *SD*-bearing chromosomes recovered.

8. From heterozygotes for a nondistorting, insensitive *pr* recombinant and a semiactive, somewhat insensitive *cn* recombinant, an active, completely insensitive, low crossing over *SD*-bearing chromosome has been recovered.

Since there is nothing in these data to suggest otherwise, we assume that they are formally interpretable in terms of a linear sequence of elements. It is, of course, possible to construct any number of models based on other assumptions, but this does not seem profitable at this time. Thus we assume that closely linked, and to the right of *SD* itself, there is an Activator of *SD*, *Ac(SD)*, that is necessary in order that *SD* function. On this interpretation SD^{in} is *SD* alone while SD^{sa} is *SD Ac(SD)*. These two elements alone, however, do not account for the recombination results, nor can we understand why *SD Ac(SD)* is such a weakly distorting *SD* chromosome nor why the *SD cn* recombinants are weaker distorters than the original *SD* line. To account for these facts we may suppose the *SD* region to be an aberration (and, for the reasons given above, possibly an insertion or duplication of genetic material) in which *SD* and *Ac(SD)* reside. All of the exchanges in the *SD* region apparently occur within this aberration which acts cumulatively on crossing over so that a part of the aberration yields the intermediate level of crossing over while a majority or all of the aberration results in the low level of exchange. Supposing the aberration to be an insertion accounts for the fact that (1) it acts more or less additively in crossing over, (2) that it reduces crossing over by virtue of heterozygosity and (3) that homozygosity for the region results in a higher than normal rate of exchange. The aberration, however, must also have the effect of enhancing segregation-distortion and suppressing sensitivity. This point will be developed below.

A schematic representation of the postulated *SD* region and the exchanges that can take place within it is shown in Figure 1. Exchange "a" produces an insensi-

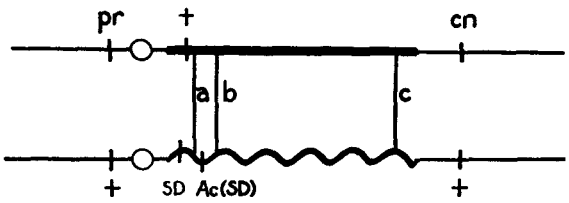

FIGURE 1.—A schematic representation of the *SD* region. The indicated crossovers are those that occur between the markers *pr* and *cn*. The heavy lines represent homologous sections in the *SD* region. The wavy line is the aberrant region responsible for the reduction in crossing over and for insensitivity of the original *SD*-bearing chromosome.

tive *pr* recombinant and an SD^{in} *cn* recombinant; exchange "b" produces insensitive *pr* and SD^{sa} *cn* recombinants; and exchange "c" yields a semisensitive *pr* and *SD cn* recombinant.

On the action of the aberration: From the foregoing, the effects of the aberration on crossing over are understandable. It is necessary, however, to postulate, in addition, effects on the phenomenon of segregation-distortion itself. In particular, the aberration evidently enhances the action of *SD* since the original *SD* line, containing the aberrant region intact, produces higher k values than the recombinant *SD cn* lines which have lost some of it, and these produce higher k values than the SD^{sa} recombinants which have lost most of the aberrant region. A second effect of the aberration is that it imparts insensitivity to chromosomes carrying it since the *pr* recombinants that show very reduced crossing over are insensitive to a standard *SD*, and those that exhibit an intermediate level of crossing over are more sensitive. Similarly, the original *SD* chromosome and the *cn* recombinants that show low crossing over are insensitive, and those that carry *SD Ac(SD)*, while much more sensitive than these, are less sensitive than those that carry only *SD*.

To explain these results we make three assumptions based on the prior formalism presented by SANDLER, HIRAIZUMI and SANDLER (1959) and summarized in Proposition II. (1) An activated *SD* chromosome will cause a distorting misreplication in either itself or its homolog—but not both in the same meiosis. (2) The misreplication always occurs in the region of the aberration (the heavy line in Figure 1). (3) The aberrant region (the wavy line in Figure 1) never misreplicates. These three assumptions account for all of the observations thus far in the following way. First, *SD* normally shows a high k value owing to the fact that *SD* carries the aberration and therefore always affects its homolog rather than itself. Second, *SD* is completely insensitive to the action of another *SD* because it carries the aberrant region which cannot be affected by *SD*. Third, the *SD cn* recombinants are weaker distorters than the original lines because when any section of the aberration is missing from an *SD Ac(SD)*-bearing chromosome, the k value goes down owing to some proportion of misreplication of the *SD*-bearing chromosome itself instead of the homolog. Fourth, the presence of the aberrant region, or any part of it, on the homolog in an *SD* heterozygote will imply a degree of insensitivity of the homolog in proportion to the amount of the aberrant section

that it carries owing to the immunity of the aberration to the action of *SD*. Finally, the fact that the SD^{sa} recombinant shows any abnormal segregation at all is accounted for by its carrying a small part of the abnormal region (Figure 1) which implies a slightly higher probability of the homolog misreplicating than *SD* acting on itself.

A test of this general hypothesis suggests itself. If a *pr* recombinant carrying most of the abnormal region (i.e., an insensitive, low crossover *pr* recombinants) is made heterozygous with an *SD Ac(SD)*-bearing *cn* recombinant in males, then a distorted segregation ratio *against* the *SD*-bearing chromosome should result. Accordingly, *R(cn)-1* which is SD^{sa} and *R(cn)-7* which is SD^{in} were made heterozygous with a number of *pr* recombinants in males, and the segregation ratios measured. These results are given in Table 13. It can be seen that when the *SD*-bearing chromosome does not carry *Ac(SD)* (i.e., *R(cn)-7*), there is no distorted segregation. Likewise, when the *pr* recombinant does not carry most of the aberration (i.e., semisensitive *pr* recombinants), there is no distorted segregation irrespective of the *cn* recombinant. When, however, *SD Ac(SD)* is heterozygous with a *pr* recombinant carrying most of the aberration, a distorted segregation ratio favoring the *pr* recombinant is evident. Not only are the mean *k* values different from 0.50, but in every test individual cultures gave high *k* values (greater than 0.80).

TABLE 13

The results from crosses of males, heterozygous for various R(pr)/R(cn) *combinations, by* cn bw *females. The* k *value given in the last column is the proportion of* R(pr) *progeny recovered*

Constitution of parental male	Phenotype of progeny		
	cn	+	*k*
pr-4/cn-1	1479	1467	0.50
pr-64/cn-1	3555	3474	0.49
Mean			0.50
pr-4/cn-7	1579	1789	0.53
pr-64/cn-7	1869	1892	0.50
Mean			0.52
pr-1/cn-1	2163	3880	0.64
pr-2/cn-1	1728	2468	0.59
pr-3/cn-1	1852	2922	0.61
pr-6/cn-1	2399	4471	0.65
pr-11/cn-1	1489	3448	0.70
pr-50/cn-1	1140	2201	0.66
pr-52/cn-1	2485	4092	0.62
Mean			0.64
pr-1/cn-7	2826	3391	0.55
pr-2/cn-7	2370	2552	0.52
pr-3/cn-7	2220	2619	0.54
pr-6/cn-7	2723	2844	0.51
pr-11/cn-7	2210	2719	0.55
pr-50/cn-7	1375	1538	0.53
pr-52/cn-7	1399	1537	0.52
Mean			0.53

The *pr* recombinants should be of two types: those carrying *Ac(SD)* and those lacking it (Figure 1). There do indeed appear to be differences among lines in these experiments in respect of the amount of distortion of the segregation ratios. These differences may be due to whether or not a *pr* recombinant carries *Ac(SD)*—and if it does it may result in either a higher or lower *k* value than if it does not. The differences may, on the other hand, be continuous reflecting the amount of the aberrant region that any *pr* recombinant happens to carry.

Finally, it ought to be noted that since no distortion is evident in any case in which the *SD*-bearing chromosome does not carry *Ac(SD)*, even though some *pr* recombinants should carry *Ac(SD)*, it would appear necessary to suppose that *Ac(SD)* must be in coupling with *SD* in order to activate it.

DISCUSSION

The results and interpretation presented in this report indicate that the *SD* region is extremely complex. The notion of an Activator of *SD*, necessary in order that *SD* act, coupled with the prior notion that *SD* causes something formally equivalent to a chromosome break in its homolog (SANDLER, HIRAIZUMI and SANDLER 1959), strongly suggests analogy with the "*Ac–Ds*-like" systems in maize (MCCLINTOCK 1951, 1956). Indeed, the possibility indicated above, that an extra *Ac(SD)* might inhibit *SD* action would be very suggestive of the action of *Ac* in maize. In the maize systems, however, no obvious analogy with the immune aspect of the aberration exists—this element seems unique to the *SD* system.

It should be emphasized, finally, that the notion that the aberration is an insertion or duplication and the crossover model presented are merely formalisms; the true structure of the *SD* region may cytologically be quite different from what is inferred genetically. For example, the question of how the aberration shares homology with the corresponding section on the normal homolog is unresolved because the exchanges in this region between *SD* and a normal chromosome might well be occurring in the heterochromatin in which the rules of homology and crossing over are extremely complex and nonspecific (for example LINDSLEY 1955).

Evidence has been presented previously (SANDLER, HIRAIZUMI and SANDLER 1959) indicating that synapsis in the *SD* region is necessary in order that segregation-distortion occur. In particular, if structurally abnormal SD^{+}-bearing second chromosomes are used as homologs for *SD*, distortion is suppressed or eliminated entirely. For example, *In(2LR)Cy/SD* males uniformly produce *k* values of approximately 0.50. This inversion has a break point in the centromere region, and thus presumably interferes with synapsis in the *SD* region.

From a female heterozygous for *In(2LR)Cy* and *cn bw*, a *Cy cn bw* chromosome was recovered, which proved, upon testing, to carry *In(2L)Cy* but no inversion on the right arm which is now marked by *cn bw*. Furthermore, crosses of *In(2L)Cy cn bw/Ty* females by *cn bw* males yielded 578 *Cy cn bw;* 480 *Cy cn;* 12 *Cy bw;* 12 *Cy;* 14 *cn bw;* 4 *cn;* 586 *bw;* and 689 + progeny. In this cross,

therefore, crossing over between the right break point of *In(2L)Cy* and *cn* (which must be roughly equivalent to crossing over between the centromere and *cn*) is 1.8 percent which is approximately that observed in the *pr cn* region. Thus it appears that synapsis in *In(2L)Cy* heterozygotes is normal in the *SD* region and therefore. under the hypothesis that synapsis is required for distortion, the *In(2L)Cy cn bw* chromosome ought to be sensitive.

Accordingly, *SD-5/ cn bw* males were crossed to *In(2L)Cy cn bw/cn bw* females. and in the F_1 *SD-5/cn bw* and *SD-5/ In(2L)Cy cn bw* males were collected and crossed to *cn bw* females. In the control cross (*SD-5/cn bw* males), 137 *cn bw* and 4.197 + progeny were recovered giving a control $k = 0.97$. In the experimental cross (*SD-5/In(2L)Cy* males), 188 *Cy cn bw* and 3,879 + progeny were produced giving an experimental $k = 0.95$. Thus it is clear that the *In(2L)Cy* chromosome is approximately as sensitive as the standard *cn bw* chromosome.

The fact that *In(2LR)Cy*, in which synapsis in the *SD* region is disturbed, is not sensitive, whereas *In(2L)Cy*, in which synapsis is normal near *SD*, is sensitive, provides additional evidence that synapsis in the *SD* region is related to the phenomenon of segregation-distortion. This argument, of course, is subject to the assumption that the crossover results in the female may be used as an indicator of the synaptic situation in the male.

It is now clear, however, that synapsis in the *SD* region is always abnormal owing to the presence of the crossover suppressing aberration in the *SD* region. These results may, therefore, be most simply interpreted as meaning that it is not just intimate synapsis that is prerequisite for distortion—but, rather, what is required is some special synaptic condition that is disturbed by a break point in the immediate vicinity of *SD*. Perhaps a part of the reduction in distortion that accompanies the loss of a section of the abnormal region is due to a breakdown of the optimum synaptic condition.

The results presented here explain the behavior of an exceptional *SD*-bearing recombinant reported earlier (SANDLER and HIRAIZUMI 1960). It had been shown that at the tip of IIR, closely linked to bw^+, there is a Stabilizer of *SD*, *St(SD)*, in the presence of which *SD* is stable and in the absence of which *SD* is less stable (i.e., exhibiting considerable variation in k values from male to male). Thus all recombinants between the locus of *cn* and the locus of *bw* in *SD/cn bw* heterozygotes lose *St(SD)* and are therefore less stable than the original *SD* line. However, one recombinant, *R(SD-5)-26*, appeared to result from a double crossover, one exchange between *SD* and *cn* and the other between *bw* and an inversion break point whose relative position is known. This recombinant should carry *St(SD)* but, in fact, showed a very low k value (mean $k = 0.76$). It is now clear that this recombinant, in which an exchange occurred between *SD* and *cn* lost a part of the aberration, since all recombination in the *SD* region occurs within the abnormal region, and this undoubtedly accounts for the low k value.

It has been noted previously (SANDLER and HIRAIZUMI 1959) that structurally normal, SD^+-bearing second chromosomes differ, one from the other, in their sensitivity to *SD* (i.e., in the characteristic k value distribution observed when

they are tested against some standard *SD* line). The data presented here on the substructure of the *SD* region provide evidence on the exact location of the differences among SD^+ lines of different sensitivities.

It should be noted in the first place that insensitivity of SD^+ chromosomes almost certainly comes about by a mechanism different from the insensitivity imparted by the aberration carried by SD-bearing chromosomes. The argument for this point will be presented in detail in a subsequent communication but in essence it is that insensitivity of SD^+ chromosomes comes about by an inactivation of the *SD* allele. The evidence here is that the action of an *SD* chromosome is impaired by heterozygosity with an insensitive SD^+ allele for some generations after the insensitive allele has been removed from the system ("translocal modification"; SANDLER and HIRAIZUMI 1959). The insensitivity of the aberration, on the other hand, apparently comes about by virtue of a nonreactivity of the aberration-bearing homolog of *SD* itself, since otherwise *SD* should not have acted on itself as in the experiments described above.

Furthermore, the sensitivities exhibited by the various classes of *pr* recombinants and *cn* recombinants almost certainly imply that differences in sensitivity of SD^+-bearing chromosomes are localized at the *SD* locus precisely. This is so for the following reasons. The insensitive region of SD^+-bearing chromosomes is known to be generally in the centromere region (SANDLER and HIRAIZUMI 1959). The sensitivity of this region from the *pr cn* second chromosome (that chromosome from which the *pr* and *cn* recombinants were derived) backcrossed to *cn bw* for many generations (to make the chromosome comparable to the recombinants) was tested in a cross of *SD/pr cn* males by *cn bw* females. The progeny included 1,567 *cn* and 6,090 + flies; thus the average k value was 0.80. This is very similar to the sensitivity of the semisensitive *pr* recombinants (Table 8). Thus those *pr* recombinants that contain just the SD^+ element from *pr cn* exhibit the sensitivity of *pr cn*. Much more convincing, however, is the fact that those *cn* recombinants that are SD^{in}, and therefore contain all of the *pr cn* centromere region except the SD^+ element, are completely sensitive. These observations certainly suggest strongly that the relative insensitivity of *pr cn* is located precisely at the SD^+ element.

Finally, it is perhaps worth noting that these data provide additional evidence that the *SD* system has existed in nature for a long time, for it must, indeed, have required a long time for the complexity of the *SD* region indicated by these studies to have evolved.

SUMMARY

The segregation-distorter locus *(SD)* is in the centromere region of chromosome II. It has been found that the *SD* region is genetically complex. The data collected may be interpreted in the following way. The *SD* locus itself causes a misreplication (formally equivalent to a break of some sort) either in its immediate vicinity or in a comparable region of its homolog. Closely linked and to the right of *SD* there is an Activator of *SD*, *Ac(SD)*, which must be present in coupling with *SD* in order that *SD* function. Both *SD* and *Ac(SD)* are located within

a small chromosomal aberration (which can be thought of formally as a duplication or insertion) that has the property of being immune to the action of the *SD Ac(SD)* complex. Thus the aberration results in (a) *SD* being immune to its own action and (b) the *SD* chromosome always acting on its homolog.

It has been possible to separate the elements in the *SD* region by crossing over and also to reconstitute *SD* from its constituent parts by further recombination. In addition, by separating appropriate parts of the *SD* region and making them mutually heterozygous in males, a system in which the *SD*-bearing chromosome acted on itself instead of its homolog was found.

The exact evidence bearing on this interpretation of the *SD* region is presented and the implications of this complexity discussed.

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