A MODIFIED MODEL OF SEGREGATION DISTORTION IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Elements of the Segregation Distorter (*SD*) system of *Drosophila melanogaster*, *Sd* and *Rsp*, were analyzed and the following points were established: (1) The model of multiple alleles at the Rsp^s locus proposed by MARTIN and HIRAIZUMI (1979) is supported by our observations. (2) A modifier of *SD*, tentatively symbolized *M(SD)*, was found close to *cn* (*2R*-57.5). (3) *Sd* heterozygous males were found to show, under certain genotypic condition, almost complete sterility.——Based upon these observations, the following modified model of segregation distortion is proposed: (1) The *M(SD)* locus produces a multimeric repressor protein that binds to the *Rsp* locus as a necessary condition for normal spermiogenesis. *M(SD)* homozygotes produce a repressor *M(SD)/M(SD)*; whereas, a homozygote for its normal allele *M+(SD)* produces a *M+(SD)/M+(SD)* repressor. *M(SD)/M+(SD)* heterzygotes produce a *M(SD)/M+(SD)* repressor. (2) The *Sd* locus produces a certain product that, like an inducer in the lactose system of *E. coli*, tends to bind with the repressor complexed with the *Rsp* locus. This binding disrupts the repressor-*Rsp* complex, causing *Rsp* locus to be turned on. The product of *Rsp* transcription, in turn, results in sperm dysfunction. (3) Rsp^i, an allele of *Rsp*, has a strong complexing affinity with the repressor such that the Rsp^i-repressor complex is "resistant" to the inducing activity of *Sd* product. Rsp^s, on the other hand, has a weaker complexing affinity than that of Rsp^i, and the degree of affinity varies among different Rsp^s alleles.——A possible extension of the above model is discussed.

MORE than 20 years ago, Segregation Distorter (*SD*) was discovered in a natural population of *Drsophila melanogaster* in Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959). Heterozygous SD/SD^+ males backcrossed to normal SD^+/SD^+ females produce progeny containing the *SD* element in an excess over the expected 50%—usually 80% or more. This distorted transmission frequency is due to dysfunction of sperm containing the normal SD^+ element (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DEMARCO 1967).

Many important discoveries about this system have since been made by a number of investigators (reviewed by HARTL and HIRAIZUMI 1976), yet the mechanism of segregation distortion is not yet wholly understood. The *SD* system has been thought to consist of two distinct major elements (HARTL 1974). One element, *Sd*, is located to the left of, but very close to, the *pr* locus (*2L*-54.4) on the

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left arm of chromosome *2*; the other element, *Rsp*, is located within the centromeric heterochromatin of the right arm of chromosome *2*, proximal to the *stw* locus (*2R*-55.1) (GANETZKY 1977). There are two allelic alternatives of *Rsp*—sensitive (Rsp^s) and insensitive (Rsp^i); a chromosome carrying Rsp^i is not distorted by the *SD* chromosome.

According to the model proposed by HARTL (1973), these two elments interact as follows: it is assumed that as a necessary condition for sperm maturation, the *Rsp* locus must be complexed with the product of the *Sd* locus, which is further assumed to be a multimeric regulatory protein. Three kinds of regulatory multimers may be distinguished: Sd^+/Sd^+, which is assumed to be capable of interaction with both Rsp^s and Rsp^i; Sd^+/Sd heteromultimers, which complex preferentially with Rsp^i; and *Sd/Sd* homomultimers, which can complex with neither Rsp^s nor Rsp^i. The majority of the regulatory protein in the Sd^+/Sd male is assumed to be Sd^+/Sd heteromultimers. In 1977, GANETZKY proposed a model of segregation distortion similar to that of HARTL, but with the following modification: rather than the binding of an *Sd* product at the *Rsp* locus being a necessary condition for normal spermiogenesis, it is this binding that causes sperm dysfunction. It is assumed that the *Sd* product complexes more readily with Rsp^s than with Rsp^i and that the amount of *Sd* product is limited with respect to the number of binding sites available. No function is ascribed to the Sd^+ locus. In order to explain reduced male fertility of some genotypes, GANETZKY further assumes that when no Rsp^s locus is available, the *Sd* product can bind to an Rsp^i locus.

Although the two models above differ somewhat from each other, both predict that homozygous $Sd\ Rsp^s/Sd\ Rsp^s$ males should show greatly reduced fertility, if not complete sterility. MARTIN and HIRAIZUMI (1979) critically examined this prediction and found that *Sd* homozygosity *per se* did not cause male sterility. This strongly suggests that either some important element, or some specific interaction among elements in this system, has not yet been incorporated into the model of segregation distortion.

Three marked chromosome *2* lines relevant to the present study, *al dp b pr sp*, *cn bw* and $lt\ stw^s$, and one *SD* line, *R(SD)cn-14*, were employed by MARTIN and HIRAIZUMI (information for those lines are given in MATERIALS), and they generated various recombinants between *pr* and *cn* (*2R*-57.5) loci in the *R(SD)cn-14/ al dp b pr sp* females. By using those recombinant chromosome lines, they found instances where the *Sd* heterozygous males, even in the absence of Rsp^i, could cause segregation distortion. In order to explain those observations, MARTIN and HIRAIZUMI (1979) proposed a model of multiple alleles at the Rsp^s locus such that the Rsp^s allele (hereafter, Rsp^{s-3}) located on the *al dp b pr sp* chromosome had a somewhat higher probability of complexing with the repressor protein than did the Rsp^s allele (hereafter, Rsp^{s-2}) located on the *cn bw* chromosome. On the other hand, the Rsp^s allele (hereafter, Rsp^{s-1}) located on the $lt\ stw^s$ (*lt*: *2L*-55.0) chromosome had a much smaller probability of complexing with the protein than did Rsp^{s-2}. (It should be noted here that a similar result to that mentioned above, *i.e.*, segregation distortion without Rsp^i, was observed by HARTL

(personal communication some six years ago, although he made no further studies on this subject.)

The *lt stw*^{s} chromosome is "super-sensitive" to segregation distortion; hence no recombinants that may be generated in the *R(SD)cn-14/lt stw*^{s} female should include any suppressors of segregation distortion. This certainly will help us interpret the data without even slight complications due to any possible partial suppressors in the genome, as was the case in the previous study (MARTIN and HIRAIZUMI 1979).

In the present study, a new set of recombinants between the *lt* and *stw*^{s} loci were generated from *R(SD)cn-14/lt stw*^{s} females, and the same tests as described in the previous report (MARTIN and HIRAIZUMI 1979) were carried out. The original purpose of the present study was to confirm the model of multiple alleles at the *Rsp*^{s} locus proposed by MARTIN and HIRAIZUMI (1979) in an almost completely "sensitive" genetic background without partial suppressors. The results, however, provided some important additional information, based on which we are now able to propose a modified model of segregation distortion, as will be presented below.

MATERIALS

The chromosome *2* lines employed in the present study are listed below.

Original lines: (1) *cn bw*: A standard chromosome *2* line marked with 2 recessive eye color mutants, *cn* (cinnabar eye color, *2R*-57.5) and *bw* (brown eye color, *2R*-104.5). (2) *al dp b pr sp*: A chromosome *2* line carrying 5 recessive mutants, *al* (aristaless, *2L*-0.01), *dp* (dumpy wing, *2L*-13.0), *b* (black body color, *2L*-48.0), *pr* (purple eye color, *2L*-54.4) and *sp* (speck, *2R*-107.0). (3) *lt stw*^{s}: A chromosome *2* line carrying 2 mutants, *lt* (light eye color, *2L*-55.0) and *stw*^{s} (an allele of *stw*, straw body color, *2R*-55.1). This chromosome line is associated with a recessive lethal gene. (4) *In(2LR)SM5*: A multiply inverted chromosome *2* balancer. This chromosome carries the mutant markers *al*^{2}, *Cy* (Curly wings, *2L*-6.1), *lt*^{v}, *cn*^{2} and *sp*^{2}. In this report, this chromosome will be referred to as *SM5*. (5) *R(SD)cn-14*: A chromosome *2* line showing a moderate degree of segregation distortion when made heterozygous with the *cn bw* chromosome in the male. This chromosome carries the marker *cn*, and so far no structural abnormality has been found. (For the origin of this chromosome, see HIRAIZUMI and NAKAZIMA 1967.) For simplicity, this chromosome will be abbreviated as *cn-14* throughout this paper. This chromosome carries a recessive lethal gene (or genes) that seems to be independent of the *SD* system. (6) *SD-72*: A chromosome *2* line isolated in 1956 from a natural population in Madison, Wisconsin. This chromosome shows a strong degree of segregation distortion against the *cn bw* chromosome. This chromosome carries one para- and one pericentric inversion.

All of the chromosome *2* lines listed above have been kept in this laboratory by backcrossing, through heterozygous males, to the standard *cn bw* females for at least 20 generations. Therefore, all the genetic backgrounds associated with these lines were, except for the second chromosome, derive from the *cn bw* stock. For the convenience of the reader, the *k* values ($k =$ the frequency of *SD* chromosomes recovered in backcross progeny) for the chromosome lines listed above are given in Table 1 (data from MARTIN and HIRAIZUMI 1979).

As can be seen in Table 1, the two "normal" chromosome lines (*lt stw*^{s} and *al dp b pr sp*), when made heterozygous in males with the standard *cn bw* chromosome, show *k* values that are slightly larger than the theoretical 0.5. After adjusting for differential viabilities between the *cn bw* and wild phenotypes, however, those *k* values can be considered essentially equal to 0.5. It can also be seen in Table 1 that the *lt stw*^{s} chromosome, when compared with the standard *cn bw* chromosome, is "super-sensitive" to the action of Segregation Distorter. The *al dp b pr sp* chromosome line, on the other hand, has a slightly reduced sensitivity when compared to the standard *cn bw* chromosome, although the reduction is not so large.

TABLE 1

A list of k *values for several combinations of basic chromosome 2 lines*

Genotype of male parents with respect to:		
Visible markers	*Sd* and *Rsp* loci	$\bar{k}$
cn bw/al dp b pr sp	$Sd+\ Rsp^{s-2}/Sd+\ Rsp^{s-3}$	0.505 (17)
cn bw/lt stw^3	$Sd+\ Rsp^{s-2}/Sd+\ Rsp^{s-1}$	0.507 (55)
al dp b pr sp/cn-14	$Sd+\ Rsp^{s-3}/Sd\ Rsp^{i}$	0.856 (24)
cn bw/cn-14	$Sd+\ Rsp^{s-2}/Sd\ Rsp^{i}$	0.922 (20)
lt stw^3*/cn-14*	$Sd+\ Rsp^{s-1}/Sd\ Rsp^{i}$	1.000 (21)
cn bw/SD-72	$Sd+\ Rsp^{s-2}/Sd\ Rsp^{i}$	1.000 (20)

The genotype of female parents was, in all cases, *cn bw* homozygous. The k values were computed for the right-hand chromosomes shown. The number of replications is shown in parentheses (data from MARTIN and HIRAIZUMI 1979).

Recombinant chromosome lines: Many independent recombinant chromosome lines were generated in *cn-14/lt stw*^{3} females by crossing over between the *lt* and *stw*^{3} loci. Exact progeny counts were not made, but approximately 10,000 progeny from the *cn-14/lt stw*^{3} females were examined (a total of 30 matings with 4 transfers every 6 to 7 days, each brood producing 80 to 90 progeny), among which 10 *lt stw*+ and 10 *lt*+ *stw*^{3} male progeny were recovered (1 *lt stw*+ line was lost accidentally before tests could be made). Female recombinant progeny were not scored, but assuming a 1:1 sex ratio, the above results provide a rough estimate of the recombination frequency, 0.0040 ($20 \times 2/10{,}000$), between the *lt* and *stw*^{3} loci. Recombinant lines were also generated in the same genotype of females, this time by isolating recombinants between *stw*^{3} and *cn* loci. One *lt stw*^{3} *cn* and 2 *lt*+ *stw*+ *cn*+ recombinant males were isolated. No progeny count was made for this mating; therefore, an estimate of the recombination frequency between the *stw*^{3} and *cn* loci is not available.

METHODS

The crosses employed in this study take the following form: 2 *cn bw* females × 1 x/y male, where x and y are the second chromosomes carrying either one, both, or none of the *Sd* and *Rsp*^{i} elements. SANDLER and HIRAIZUMI (1961) showed that the k value was somewhat dependent upon the age of the parental *SD* heterozygous male. In order to minimize such an aging effect, the age of the male was restricted to 1.0 to 1.5 days at the time when the mating was initiated, and the parents were kept in a vial for only 3 days, then discarded. Under these experimental conditions, the number of progeny produced per male is not an accurate measure of the number of functional sperm produced, since the inseminated females were discarded before they used up all of the sperm they received. Although these conditions may not be sensitive enough to allow detection of any slight changes in male fertility (the number of functional sperm), any large reduction in the number of functional sperm per male would be observed.

RESULTS

Characterization of recombinant chromosomes: A method quite similar to that employed by HARTL (1974) and by MARTIN and HIRAIZUMI (1979) was used to characterize each of the recombinant chromosomes for their *Sd* and *Rsp*^{i} elements. For convenience in some of the experimental procedures, the *lt*+ *stw*^{3} *cn*+ and the *lt*+ *stw*+ *cn*+ recombinant chromosomes [hereafter, $R(+\ stw^3\ +)$ and $R(+\ +\ +)$, respectively] were marked with the *bw* mutant, and these marked recombinant

chromosomes will be abbreviated as $R(+\ stw^3\ +)\ bw$ and $R(+\ +\ +)\ bw$, respectively. The *lt stw*$^+$ *cn* and the *lt stw*^{3} *cn* recombinant chromosomes will be referred to as $R(lt + cn)$ and $R(lt\ stw^3\ cn)$, respectively. The following two crosses were examined: (a) *cn bw* ♀ × *R/cn bw* ♂, where *R* is one of the recombinant chromosomes described above. (b) *cn bw* ♀ × *R/SD-72* ♂, where *R* is one of the recombinant chromosome lines, $R(lt + cn)$ or $R(lt\ stw^3\ cn)$. The results are summarized in Table 2 for the $R(+\ stw^3\ +)$, $R(+\ stw^3\ +)\ bw$, $R(+\ +\ +)$ and $R(+\ +\ +)\ bw$, and in Table 3 for the $R(lt + cn)$ and $R(lt\ stw^3\ cn)$ recombinant chromosome lines.

In Table 2, there are two clearly distinct groups, designated A and B; *i.e.*, one including lines that distort the *cn bw* chromosome (Group B), and the other including lines that are distorted by the *cn bw* chromosome (Group A). The "suicide" distortion in Group A can be easily understood, based on the model of multiple alleles at the *Rsp*^{s} locus proposed by MARTIN and HIRAIZUMI (1979). The recombinant lines in Group A can then be categorized as *Sd Rsp*$^{s-1}$ and the lines in Group B as *Sd Rsp*^{i}.

One other point emerging in Table 2 is that the *k* values of lines in Group B, although all of them appeared to be distinctly larger than 0.5, were generally much smaller than the *k* value of 0.922 for the original *cn-14* chromosome (see Table 1). Since the *lt stw*^{3} chromosome does not contain any suppressor of segregation distortion, the reduction in *k* values must be due to loss of an enhancer element located in the original *cn-14* chromosome outside of the *Sd-Rsp* region. Specualtion about the nature of this "enhancer" will be presented later.

TABLE 2

A list of k *values for various* R(+ stw^3 +), R(+ + +), R(+ stw^3 +) bw *and* R(+ + +) bw *recombinant chromosomes*

Group	Line number	Mating 1	Mating 2
	2	0.345 (35)	0.316 (20)
	3	0.389 (24)	0.290 (20)
A	9	0.198 (30)	0.348 (20)
	10	0.332 (26)	0.357 (13)
	Unweighted average	0.316	0.328
	1	0.640 (19)	0.683 (20)
	4	0.649 (20)	0.683 (17)
	5	0.602 (19)	0.732 (20)
B	6	0.589 (20)	0.645 (20)
	7	0.690 (30)	0.629 (19)
	8	0.689 (20)	0.710 (19)
	101	0.729 (33)	0.663 (30)
	102	0.708 (33)	0.609 (30)
	Unweighted average	0.662	0.669

Mating 1: *cn bw* females × *cn bw/R*(+ *stw*^{3} +) or *R*(+ + +) males. Mating 2: *cn bw* females × *cn bw/R*(+ *stw*^{3} +) *bw* or *R*(+ + +) *bw* males. The *k* values were computed for the right-hand chromosomes shown above in the male genotypes, and the number of replications is shown in parentheses. Genotypes of line numbers 101 and 102 were *R*(+ + +) for Mating 1 and *R*(+ + +) *bw* for Mating 2. The average number of progeny per replication was about 60.

TABLE 3

A list of k *values for various* R(lt + cn) *and* R(lt stw^3 cn) *recombinant chromosomes*

Group	Line number	Mating 1	Mating 2
	2	0.544 (30)	0.513 (13)
A	6	0.532 (30)	0.482 (13)
	Unweighted average	0.538	0.498
	1	0.511 (30)	1.000 (17)
	3	0.545 (30)	1.000 (15)
	4	0.518 (30)	1.000 (20)
B	5	0.526 (30)	1.000 (13)
	7	0.430 (30)	1.000 (16)
	8	0.494 (26)	1.000 (13)
	10	0.478 (30)	1.000 (17)
	101	0.484 (25)	1.000 (10)
	Unweighted average	0.498	1.000

Mating 1: *cn bw* females × *cn bw/R(lt + cn)* or *R(lt stw^3 cn)* males. Mating 2: *cn bw* females × *R(lt + cn)/SD-72* or *R(lt stw^3 cn)/SD-72* males. The *k* values were computed for the right-hand chromosomes shown in the male genotypes above, and the number of replications is shown in parentheses. The genotype of line number 101 was *R(lt stw^3 cn)*. The average number of progeny per replication was about 60.

Let us now look at Table 3 for the *R(lt + cn)* and *R(lt stw^3 cn)* recombinant chromosome lines. In Mating 1, each of the 10 lines shows a *k* value essentially equal to 0.5; but in Mating 2, there are two clearly distinct groups, designated A and B. The recombinant lines in Group A are not distorted by *SD-72*, whereas those lines in Group B are distorted. The lines in Group A will then be categorized as carrying *Sd$^+$ Rsp^i* and those in Group B as *Sd$^+$ Rsp^{s-1}*. It should be noted that during the process of generating recombinant chromosomes between the *lt* and *stw^3* loci, about ⅓ of the exchange events took place between *lt* and *Rsp*, both of which are located in the proximal heterochromatin around the centromere. This was somewhat a surprising observation since it is widely accepted that crossing over in heterochromatin is absent, or nearly so. This matter will be discussed later.

Suicide segregation distortion not involving the Rsp^i *allele:* An example of suicide segregation distortion (SANDLER and HIRAIZUMI 1960; HARTL 1974) in males heterozygous for *Sd* but free of *Rsp^i* was presented in Group A of Table 2. The male genotype was *cn bw, Sd$^+$ Rsp^{s-2}/R(+ stw^3 +), Sd Rsp^{s-1}* or *cn bw, Sd$^+$ Rsp^{s-2}/R(+ stw^3 +) bw, Sd Rsp^{s-1}* and the average *k* values for *Sd*-bearing chromosomes were 0.316 and 0.328, respectively. The suicide distortion was examined for several additional combinations, and the results are summarized in Table 4.

As mentioned earlier, MARTIN and HIRAIZUMI (1979) suggested that the ability to bind with the repressor protein is highest for *Rsp^{s-3}*, next for *Rsp^{s-2}* and the lowest for *Rsp^{s-1}*. The results in Table 4 are consistent with this suggestion; the magnitude of suicide distortion was predictable from this hypothesis. Similar to the case in the *al dp b pr sp, Sd$^+$ Rsp^{s-3}/R(+ +) bw; Sd Rsp^{s-3}* genotype (MARTIN and HIRAIZUMI 1979), the *lt stw^3, Sd$^+$ Rsp^{s-1}/R(+ stw^3 +) bw, Sd Rsp^{s-1}* males

TABLE 4

Suicide distortion in males of several genotypes without Rsp^1 *alleles*

Genotype number*	$(\bar{k})$**	$\bar{k}$†	Number of males tested	Sterile males (%)	Mean number of progeny per male
1	1.000	0.475	62	11 (17.74)	40.00
2	0.922	0.316	115	0 (0.00)	70.93
3	0.922	0.328	73	0 (0.00)	44.11
4	0.856	0.071	28	0 (0.00)	58.14
5‡	0.492	0.023	70	0 (0.00)	66.60

* (1) *lt* stw^3, *Sd*+ $Rsp^{s-1}/R(+\ stw^3\ +)$ *bw*, *Sd* Rsp^{s-1}; (2) *cn bw*, *Sd*+ $Rsp^{s-2}/R(+\ stw^3\ +)$, *Sd* Rsp^{s-1}; (3) *cn bw*, *Sd*+ $Rsp^{s-2}/R(+\ stw^3\ +)$ *bw*, *Sd* Rsp^{s-1}; (4) *al dp b pr sp*, *Sd*+ $Rsp^{s-3}/R(+\ stw^3\ +)$ *bw*, *Sd* Rsp^{s-1}; (5) *SM5*, *Sd*+ $Rsp^i/R(+\ stw^3\ +)$, *Sd* Rsp^{s-1}.

** The *k* value for *cn-14* in the *x/cn-14* male, where *x* is the left-hand chromosome shown for each genotype.

† *k* value was computed for the right-hand chromosome shown in each male genotype.

‡ HARTL (1975) showed that the *Cy* balancer chromosome was associated with the Rsp^i allele.

gave an average *k* value of about 0.5, as might be expected since this genotype was homozygous for Rsp^{s-1}.

Segregation distortion in males heterozygous for two complementary recombinant chromosomes: The *R(+* stw^3 *+), Sd* Rsp^{s-1} or *R(+* stw^3 *+), Sd* Rsp^i chromosome lines were made heterozygous with the *R(lt + cn), Sd*+ Rsp^{s-1}; *R(lt + cn), Sd*+ Rsp^i; or the *R(lt* stw^3 *cn), Sd*+ Rsp^{s-1} recombinant chromosomes and the *k* values were examined. Results are shown in Table 5.

As can be seen in Table 5, the *k* value for *Sd*-bearing chromosome was, as before, 0.5 when the male was homozygous for either Rsp^{s-1} or Rsp^i. However, one point should be mentioned. Male fertility in genotypes 1 and 2 was drastically

TABLE 5

Fertility and k *values for males heterozygous for two complementary recombinant chromosome lines*

Genotype number*	Number of males		Number of progeny**		$\bar{k}$†	*m*	*n*
	Tested	Sterile (%)	Left	Right			
1	101	73 (72.28)	0.69	0.52	0.431	7	3
2	63	39 (61.90)	0.89	0.83	0.481	1	3
3	62	1 (1.61)	52.10	0.08	0.002	2	3
4	23	0 (0.00)	0.04	64.17	0.999	2	1
5	50	0 (0.00)	0.04	46.98	0.999	1	3
6	18	0 (0.00)	29.22	30.56	0.511	2	1

In this table, *m* is the number of *R(lt + cn)* or *R(lt* stw^3 *cn)* recombinant chromosome lines examined and *n* the number of *R(+* stw^3 *+)* recombinant chromosome lines tested.

* (1) *R(lt + cn), Sd*+ $Rsp^{s-1}/R(+\ stw^3\ +)$, *Sd* Rsp^{s-1}; (2) *R(lt* stw^3 *cn), Sd*+ $Rsp^{s-1}/R(+\ stw^3\ +)$, *Sd* Rsp^{s-1}; (3) *R(lt + cn), Sd*+ $Rsp^i/R(+\ stw^3\ +)$, *Sd* Rsp^{s-1}; (4) *R(lt + cn), Sd*+ $Rsp^{s-1}/R(+\ stw^3\ +)$, *Sd* Rsp^i; (5) *R(lt* stw^3 *cn), Sd*+ $Rsp^{s-1}/R(+\ stw^3\ +)$, *Sd* Rsp^i; (6) *R(lt + cn), Sd*+ $Rsp^i/R(+\ stw^3\ +)$, *Sd* Rsp^i.

** Number of progeny recovered containing the chromosome shown in the left or in the right-hand side of each male genotype.

† *k* value was calculated for the right-hand chromosome shown in each male genotype.

reduced—males of these genotypes were almost completely sterile. Although the homozygous *stw*^{s} genotype seems to cause a certain degree of male sterility, the male sterility observed here is not due to such an effect since genotype 1 is phenotypically wild type. It should be noted that genotype 1 in Table 4 (*i.e.*, *lt stw*^{s}, *Sd*$^{+}$ *Rsp*$^{s-1}$/*R*(+ *stw*^{s} +) *bw*, *Sd Rsp*$^{s-1}$) showed a *k* value of about 0.5 and a somewhat reduced but nevertheless fair degree of male fertility. The only difference between genotype 1 in Table 4 and genotype 1 (or 2) in Table 5 was that the *Sd*$^{+}$ *Rsp*$^{s-1}$ chromosome in the former genotype was the original *lt stw*^{s} chromosome, whereas it was the *R*(*lt* + *cn*) or *R*(*lt stw*^{s} *cn*) recombinant chromosomes in the latter.

As mentioned above, the *cn-14* chomosome seems to contain a modifier that enhances the activity of *SD*. Let us tentatively call this element *M(SD)*; *M(SD)* for enhancer of *SD* and *M*$^{+}$ *(SD)* for its normal allele. Since all of the *R*(+ *stw*^{s} +), or *R*(+ + +) recombinant chromosomes lost *M(SD)*, all, or at least the majority of the complementary recombinants [*R*(*lt* + *cn*) or *R*(*lt stw*^{s} *cn*)] should contain *M(SD)*. Thus, it appears that in the *Sd Rsp*$^{s-1}$/*Sd*$^{+}$ *Rsp*$^{s-1}$ male, when the *M(SD)* element is also present in the genome, *M(SD)* enhances the activity of *SD*, and both of the homologues are distorted (rendered dysfunctional) in very high frequencies. In heterozygous *Rsp*^{i}/*Rsp*$^{s-1}$ males the *k* values for *Sd* deviated extremely from the theoretical 0.5; they were very high (almost 1) or very low (almost 0) depending upon whether *Sd* is in coupling or repulsion phase with *Rsp*^{i}. These extreme deviations from 0.5 are expected from the model of multiple alleles at the *Rsp* locus proposed by MARTIN and HIRAIZUMI (1979). Male fertility for these genotypes, as well as for the *Rsp*^{i}/*Rsp*^{i} homozygote, was fair. The increased fertility for these genotypes was due to an increased number of progeny recovered in the *Rsp*^{i} class (increased number of functional sperms containing *Rsp*^{i}).

Male fertility: As was mentioned earlier, the scheme in the present experiment does not permit estimation of the number of functional sperm transferred to the female; yet, it allows us to make qualitative comparisons when fertility of the male (number of functional sperm) is drastically reduced. It was shown in the previous section that a modifier, *M(SD)*, is also involved in male fertility. The number of progeny produced per mating, for each segregating class separately, and the frequency of completely sterile males for those genotypes presented in Tables 4 and 5, together with those for several other genotypes, are summarized in Table 6. In order to make the summary presentation and understanding somewhat easier, the genotypes of the males were presented with respect only to the *Sd*, *Rsp* and *M(Sd)* loci, omitting the visible mutant markers.

There seem to be three groups in Table 6 with respect to male fertility. The *Sd*$^{+}$ homozygous males, regardless of the composition with respect to the *Rsp* and *M(SD)* loci, showed almost complete fertility, and the average number of progeny produced under the present experimental condition was in the range of 70 to 80 per mating. Segregation frequencies in these genotypes were all very close to 0.5. The second group consists of a single genotype, *Sd*$^{+}$ *Rsp*$^{s-1}$ *M(SD)*/*Sd Rsp*$^{s-1}$ *M*$^{+}$ *(SD)*, and in this genotype, about 60 to 70% of the males were completely sterile. The average number of progeny per mating was only 1 to 2 when sterile

TABLE 6

A list of male fertility (per cent of sterile males and number of progeny recovered) for various combinations of chromosome lines

Genotype of male	Number of males tested	Sterile males (%)	Mean number of progeny			Phenotype of male
			Left	Right	Total	
Sd+ Rsp^{s-1}M(SD)/Sd Rsp^{s-1}M+(SD)	63	61.90	0.89	0.83	1.72	*stw^3*
Sd+ Rsp^{s-1}M(SD)/Sd Rsp^{s-1}M+(SD)	101	72.28	0.69	0.52	1.21	+
Sd+ Rsp^{s-1}M+(SD)/Sd Rsp^{s-1}M+(SD)	62	17.74	20.89	19.11	40.00	*stw^3*
Sd+ Rsp^{i}M(SD)/Sd Rsp^{s-1}M+ (SD)	62	1.61	52.10	0.08	52.18	+
Sd+ Rsp^{s-1}M(SD)/Sd Rsp^{i}M+(SD)	50	0.00	0.04	46.98	47.02	*stw^3*
Sd+ Rsp^{s-1}M(SD)/Sd Rsp^{i}M+(SD)	52	0.00	0.15	50.13	50.29	+
Sd+ Rsp^{s-1}M(SD)/Sd Rsp^{i}M+(SD)	23	0.00	0.04	64.17	64.22	+
Sd+ Rsp^{s-1}M+(SD)/Sd Rsp^{i}M+(SD)	37	21.62	9.19	23.65	32.84	*stw^3*
Sd+ Rsp^{i}M(SD)/Sd Rsp^{i}M+(SD)	18	0.00	29.22	30.56	59.78	+
Sd+ Rsp^{s-1}M+(SD)/Sd+ Rsp^{s-1}M(SD)	80	0.00	41.51	35.50	77.01	*lt*
Sd+ Rsp^{s-1}M+(SD)/Sd+ Rsp^{i}M(SD)	22	0.00	40.55	40.50	81.05	*lt*
Sd+ Rsp^{s-2}M+(SD)/Sd+ Rsp^{s-1}M(SD)	231	1.30	34.71	34.77	69.48	*cn*
Sd+ Rsp^{s-2}M+(SD)/Sd+ Rsp^{i}M(SD)	60	3.33	33.98	39.40	73.38	*cn*
Sd+ Rsp^{s-3}M+(SD)/Sd+ Rsp^{s-1}M(SD)	55	0.00	35.11	36.20	71.31	+
Sd+ Rsp^{s-3}M+(SD)/Sd+ Rsp^{i}M(SD)	22	0.00	35.41	38.00	73.41	+

males were included in calculation; it was 5 to 6 even when the sterile males were excluded. Although the number of progeny was very small, the segregation frequency for this genotype was almost the normal 1:1. The third group consists of the remaining genotypes, which are all *Sd/Sd+* heterozygotes, but which have several different combinations of *Rsp* and *M(SD)* alleles. The genotypes in this group showed 0 to 22% male sterility and the average number of progeny produced per mating was about 50, which is somewhere between the first two groups. The segregation frequencies of *Sd* varied, depending upon the genotype for the *Rsp* and *M(SD)* alleles, from virtually 0 to 1.

One important observation that had not been noted in the past is that the *Sd/Sd+* heterozygote can, depending upon the genotype with respect to the *Rsp* and *M(SD)* alleles, be almost complete male sterile.

A modified model of segregation distortion: It has been suggested repeatedly in the previous sections that there is a modifier, *M(SD)*, of *SD* that was originally located in the *cn-14* chromosome. In order to understand how this element *M(SD)* enhances *SD* activity and how it interacts with *Sd* and *Rsp*, several trials were made to construct a model to explain the data thus far obtained. We have reached the following modified model of segregation distortion, which is operationally equivalent to the classic model of the lactose system of *E. coli*.

(1) The *M(SD)* locus produces a multimeric repressor protein that is bound to the *Rsp* locus, and this is the necessary condition for normal spermiogenesis. No restrictions are made on the amount of the repressor. An *M(SD)* homozygote produces the *M(SD)/M(SD)* repressor, whereas an *M+(SD)* homozygote produces the *M+(SD)/M+(SD)* repressor. An *M(SD)/M+(SD)* heterozygote is assumed to produce the *M(SD)/M+(SD)* repressor.

(2) The *Sd* locus produces products that behave like the inducer in the lactose system of *E. coli*. This *Sd* product binds with the repressor that is complexed with the *Rsp* locus. When this binding occurs, the repressor can no longer remain complexed with the *Rsp* locus, and it is released from *Rsp* locus. This allows the *Rsp* locus to initiate transcription and is the cause of sperm dysfunction. No restrictions are made at this time on the amount of *Sd* product. Rather, we simply assign a parameter q to represent the probability that the *M(SD)* product becomes unbound from the *Rsp* locus in the presence of *Sd* product. At this moment, no functions are ascribed to Sd^+.

(3) Rsp^i is an allele of *Rsp* that has a "stronger" binding affinity with the repressor, such that the complex of Rsp^i and repressor is "resistant" to the inducing activity of the *Sd* product. Rsp^s, on the other hand, has a repressor-binding affinity that is "weaker" than that of Rsp^i, and the degree of that affinity varies among different Rsp^s alleles.

It should be clarified at this point that the "stronger" or "weaker" affinities here are defined with respect to response to the inducer activity of the *Sd* product. Therefore, when a male does not carry *Sd*, Rsp^i and Rsp^s may not be distinguishable; they will behave in much the same way. It is assumed that the *M(SD)* and $M^+(SD)$ alleles produce repressors with somewhat different structures, and this difference may cause different affinities for *Rsp* and different responses to the inducer.

According to the model proposed above, all Sd^+/Sd^+ homozygotes should show, regardless of the genotype with respect to the *Rsp* and *M(SD)* loci, normal spermiogenesis since there is no inducer produced in the genome. This model provides a straightforward explanation for the segregation distortion in *Sd* Rsp^i/Sd^+ Rsp^s, and the suicide distortion in *Sd* Rsp^s/Sd^+ Rsp^i. Let us define, for simplicity, $q^i[M(SD)/M^+(SD)]$ or $q^s[M(SD)/M^+(SD)]$, as the value of q for Rsp^i, or Rsp^s, when the male has the $M(SD)/M^+(SD)$ genotype. The value of q for other genotypes, $M^+(SD)/M^+(SD)$ and $M(SD)/M(SD)$, can be defined in the similar way. In the Sd^+ Rsp^i $M(SD)/Sd$ Rsp^{s-1} $M^+(SD)$ and Sd^+ Rsp^{s-1} $M(SD)/Sd$ Rsp^i $M^+(SD)$ males shown in Table 6, for example, the value of q^i $[M(SD)/M^+(SD)]$ can be assumed to be relatively small, since the average number of progeny in the Rsp^i class is more-or-less comparable to that of the non-*Sd* males. The value of $q^{s-1}[M(SD)/M^+(SD)]$ is, on the other hand, almost one since the average number of progeny in the Rsp^{s-1} class is practically zero when compared with that of non-*Sd* males. In *Sd* Rsp^{s-1} $M(SD)/Sd^+$ Rsp^{s-1} $M^+(SD)$ males, both of the *Rsp* alleles have the same probability, $q^{s-1}[M(SD)/M^+(SD)]$ and, since this value is almost one as shown above, the male will be almost sterile, as observed. Since the present model involves, besides *Sd* and *Rsp*, one more element, *M(SD)*, the flexibility of the model increases greatly to explain the observed data, although the quantitative measures of interactions among the three elements have not yet been established and await future studies. Some observations can be made, however, In Sd^+ Rsp^{s-1} $M^+(SD)/Sd$ Rsp^{s-1} $M^+(SD)$ males, the total number of progeny produced per male, about 40, appears to be smaller than that produced by non-*Sd* males, but it is definitely larger than that of Sd^+ Rsp^{s-1} $M(SD)/$

Sd Rsp^{s-1} $M^+(SD)$ males. Although it is not possible to estimate the value of q^i $[M^+(SD)/M^+(SD)]$ accurately from the present data, it is clear that the value of $q^{s-1}[M^+(SD)/M^+(SD)]$ is definitely smaller than one. This indicates that the repressor produced by $M^+(SD)$ homozygotes, when complexed with Rsp^{s-1}, is more resistant to the inducing effect of the *Sd product than is the repressor* produced by $M(SD)/M^+(SD)$ heterozygotes.

DISCUSSION

The main observations made in the present study can be summarized as follows: (1) The model of multiple alleles at the Rsp^s locus proposed by MARTIN and HIRAIZUMI (1979) was found to be consistent with the present observations. (2) There is a modifier, $M(SD)$, of segregation distortion, which was originally located in the *cn-14* chromosome. (3) *Sd* heterozygosity can cause, under certain genotypic conditions, almost complete male sterility.

The suicide distortion, as shown in Table 4, can best be explained in terms of multiple alleles at the Rsp^s locus. One may still suspect, however, that the apparent suicide distortion is in fact due to some kind of zygotic mortality of progeny genotypes. In order to make this point clear, egg-adult survival rates were examined. Virgin females from the Tokyo wild-type strain were mated individually to $R(+\ stw^3\ +)$ *bw*, *Sd* Rsp^{s-1}/*SM5* males. After ensuring that each Tokyo female was fertilized, three females were placed in a vial containing the same medium as that used for breeding experiments. Females were allowed to lay eggs for 12 hours. The number of eggs and the number of emerging adult flies were scored to estimate the egg-adult survival rate. Control matings, Tokyo ♀ × *cn bw* ♂, and Tokyo ♀ × *cn bw/SM5* ♂, were also conducted. Results are shown in Table 7.

Table 7 clearly shows there is no difference between control and experimental matings in the egg-adult survival rate of progeny.

The precise position of $M(SD)$ has not yet been mapped, but the fact that all of the *Sd* Rsp^i recombinants carried $M^+(SD)$ rather than $M(SD)$ indicates that $M(SD)$ is located outside of the *Sd-Rsp* region, and suggests that it is to the right of *Rsp* somewhat close to the *cn* locus. GANETZKY (1977) reported a strong enhancer, $E(SD)$, in or near the proximal heterochromatin of the left arm of chromosome 2. Obviously his $E(SD)$ is different from $M(SD)$, at least in its position. It is, of course, possible that an enhancer like $E(SD)$ may also be present in our

TABLE 7

Egg to adult stage survival rates in progeny of three matings shown

Parental genotypes	No. of eggs examined	No. of adults emerged			Percent survival (No. adults/No. eggs)
		+	*SM5*	Total	
Tokyo females × *cn bw* males	437	362	—	362	82.84
Tokyo females × *cn bw/SM5* males	246	106	108	214	86.99
Tokyo females × $R(+\ stw^3\ +)$ *bw*, *Sd* Rsp^{s-1}/*SM5* males	312	21	251	272	87.18

cn-14 chromosome, but we simply claim that there is a (additional) modifier *M(SD)* located at a position different from that of *E(SD)*.

As mentioned above, GANETZKY (1977) located *Rsp* in the proximal heterochromatin of the right arm of chromosome *2*. We found, however, a fairly high frequency of recombination between *lt* and *Rsp* (about 0.0013). If GANETZKY is correct, we have to assume the occurrence of a very high frequency of recombination in the proximal heterochromatin since the *lt* locus is located in the proximal heterochromatin int he left arm of chromosome *2*.

A control mating, *lt stw^s/al dp b pr sp* ♀ × *lt stw^s* ♂, produced only one *lt stw$^+$* recombinant out of 4,835 progeny scored. This gives an estimate of recombination frequency of about 0.0002 between *lt* and *stw^s*. A comparison of this frequency with that in the *cn-14/lt stw^s* female mentioned earlier (about 0.0040) suggests that the *cn-14* chromosome increases the crossover frequency in the *lt-stw^s* region, including the heterochromatic region. The relation to possible *SD* activity in females is an interesting subject to be studied in the future.

The mechanism of how *M(SD)* enhances segregation distortion is a matter of mere speculation at this time, but the three elements in the system, *Sd*, *Rsp* and *M(SD)*, have led the authors to compare the *SD* system with the lactose system in *E. coli*, except that in the *SD* system the inducer is coded by one element, *Sd*, which is intrinsic to the genome and does not come from outside the genome. As the reader might recognize, the present model is not particularly new. It is a combination of the previous two models proposed by HARTL and by GANETZKY; the present model assumes that complex formation at the *Rsp* locus is a necessary condition for normal spermiogenesis, which is exactly what HARTL proposed. The present model proposes that the binding of *Sd* product (inducer) to the repressor complexed at the *Rsp* locus is the cause of sperm dysfunction; this is essentially the same as GANETZKY's proposal, although he postulated the binding of *Sd* product to the *Rsp* locus. The major difference between the present and the previous models is that, in the present model, the repressor that complexes with *Rsp* is not the product of *Sd*, but of the modifier *M(SD)* locus. It was shown in the previous section that the *q* value (the probability that the repressor complexed with *Rsp* locus is unbound in the presence of *Sd* product) varied, depending upon the genotype at the *M(SD)* locus. In the present study, because of the limited number of genotypes employed, only two alleles at that locus, *M(SD)* and *M$^+$(SD)*, were assumed. It is likely that this locus, like *Rsp*, may also consist of multiple alleles, each with a characteristic mode of interaction with the *Rsp* alleles and the *Sd* product.

When *Sd* is absent from a genome, spermiogenesis will proceed normally, since there is no inducer present and the *Rsp* locus will remain turned off regardless of the allelic constitution at the *Rsp* and *M(SD)* loci. When *Sd* is present, there will appear varying degrees of segregation distortion and male sterility depending upon the allelic constitution at the *Rsp* and *M(SD)* loci. Depending upon the genotype, *Sd* heterozygous males can show strong segregation distortion, with considerable to very much reduced fertility; alternatively, it can show a 1:1 segregation ratio with near normal to almost zero fertility. Presumably the same or a

similar situation exists for *Sd* homozygotes. The present model certainly does not contradict the near-normal male fertility of *Sd* homozygotes reported by MARTIN and HIRAIZUMI (1979). The genotype they employed was *Sd* Rsp^{s-s} $M^{+}(SD)$/ *Sd* Rsp^{s-s} $M^{+}(SD)$ and, according to the present model, it may be that the value of q^{s-s} $[M^{+}(SD)/M^{+}(SD)]$ is relatively small. Although a considerable degree of sperm dysfunction may occur in this genotype, since $q^{s-s}[M^{+}(SD)/M^{+}(SD)]$ may not be completely zero, q is small enough to allow the male to produce a nearly normal number of progeny under the present experimental conditions. Recall that the value of $q^{s-1}[M(SD)/M^{+}(SD)]$ was practically one, but q^{s-1} $[M^{+}(SD)/M^{+}(SD)]$ was definitely smaller than one. It should also be recalled that the *Sd* Rsp^{s-1} $M^{+}(SD)/Sd^{+}$ Rsp^{s-1} $M(SD)$ males were almost completely sterile. This suggests that *Sd* Rsp^{s-1} $M^{+}(SD)$/*Sd* Rsp^{s-1} $M(SD)$ males are also sterile, although we have not made the actual observations yet. *Sd* homozygous males can thus be completely sterile or nearly normally fertile.

So far, we have treated, although not so specified, the inducer as a monomer. Actually it may be a multimer, although the data at hand do not allow us to distinguish between these two possibilities.

Throughout the previous discussions we have assumed that the binding of the regressor to the *Rsp* locus is stable and that spermiogenesis proceeds normally when the inducer is absent. Although this may generally be the case, it may not invariably be so. The q value has been defined only in the presence of *Sd*, but this concept may be extended to include cases where *Sd* is not present. We have not assigned any function to hte Sd^{+} locus, but it may, in fact, code for a product that interacts with the repressor-*Rsp* complex in a way similar to the *Sd* product, but with much reduced efficiency. Sd^{+} may also consist of multiple alleles. If all or some of these speculations are true, it could be that considerable sperm dysfunction may take place even in the absence of *Sd*, as in the case reported by HAUSCHTECK-JUNGEN and HARTL (1978), and that segregation distortion, even in the absence of *Sd*, may occur with certain specific combinations of alleles at the *Sd*, *Rsp* and *M(SD)* loci. In fact, the suicide distortion in the *Mr* system of *D. melanogaster* (HIRAIZUMI 1971, 1977, 1979; MATTHEWS *et al.* 1978) could be one example of such a combination.

A final word should be given to the significance of the present model, especially in its extended form, in population genetics and evolution. The present model suggests that the q value, which relates to successful completion of spermiogenesis, is dependent upon a specific combination of alleles at the *Rsp*, *M(SD)* and (presumably) *Sd* loci. Undoubtedly, a combination of alleles giving a q value of zero will have a selective advantage, and it will become fixed in a population or species. However, there could be many such "optimum" combinations occurring in natural populations, creating a large amount of genetic variation regulating the success of spermiogenesis, which, in turn, regulates the "Mendelian" segregation ratio in populations.

Certainly, many studies are still needed to better understand the mechanism of segregation distortion and its evolutionary implications—evolution of the regu-

latory mechanism of the Mendelian segregation ratio. The story of segregation distortion is not yet over—rather, it is just getting started.

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LITERATURE CITED

GANETZKY, B., 1977 On the components of segregation distortion in *Drosophila melanogaster*. Genetics **86**: 321–355.

HARTL, D. L., 1973 Complementation analysis of male fertility among the segregation distorter chromosomes of *Drosophila melanogaster*. Genetics **73**: 613–629. ——, 1974 Genetic dissection of segregation distortion. I. Suicide combinations of *SD* genes. Genetics **76**: 477–486. ——, 1975 Genetic dissection of segregation distortion. II. Mechanism of suppression of distortion by certain inversions. Genetics **80**: 539–547.

HARTL, D. L. and Y. HIRAIZUMI, 1976 Segregation distortion. pp. 615–666. In: *The Genetics and Biology of Drosophila*, Vol. 1b. Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

HARTL, D. L., Y. HIRAIZUMI and J. F. CROW, 1967 Evidence for sperm dysfunction as the mechanism of Segregation Distortion in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **58**: 2240–2245.

HAUSCHTECK-JUNGEN, E. and D. L. HARTL, 1978 DNA distribution in spermatid nuclei of normal and segregation distorter males of *Drosophila melanogaster*. Genetics **89**: 15–35.

HIRAIZUMI, Y., 1971 Spontaneous recombination in *Drosophila melanogaster* males. Proc. Natl. Acad. Sci. U.S. **68**: 268–270. ——, 1977 The relationship among transmission frequency, male recombination and progeny production in *Drosophila melanogaster*. Genetics **87**: 83–93. ——, 1979 A model of the negative correlation between male recombination and transmission frequency in *Drosophila melanogaster*. Genetics **93**: 449–459.

HIRAIZUMI, Y. and K. NAKAZIMA, 1967 Deviant sex ratio associated with segregation distortion in *Drosophila melanogaster*. Genetics **55**: 681–697.

MARTIN, D. W. and Y. HIRAIZUMI, 1979 On the models of segregation distortion in *Drosophila melanogaster*. Genetics **93**: 423–435.

MATTHEWS, K. A., B. E. SLATKO, D. W. MARTIN and Y. HIRAIZUMI, 1978 A consideration of the negative correlation between transmission ratio and recombination frequency in a male recombination system of *Drosophila melanogaster*. Japan. J. Genetics **53**: 13–25.

NICOLETTI, B., G. TRIPPA and A. DEMARCO, 1967 Reduced fertility in *SD* males and its bearing on segregation distortion in *Drosophila melanogaster*. Atti. Acad. Naz. Lincei. **43**: 383–392.

SANDLER, L. and Y. HIRAIZUMI, 1960 Meiotic drive in natural populations of *Drosophila melanogaster*. V. On the nature of the *SD* region. Genetics **45**: 1671–1689. ——, 1961 Meiotic drive in natural populations of *Drosophila melanogaster*. VIII. A heritable aging effect on the phenomenon of Segregation-Distortion. Can. J. Genet. Cytol. **3**: 34–46.

SANDLER, L., Y. HIRAIZUMI and I. SANDLER, 1959 Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion. Genetics **44**: 233–250.

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