ON THE COMPONENTS OF SEGREGATION DISTORTION IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The segregation distorter (*SD*) complex is a naturally occurring meiotic drive system with the property that males heterozygous for an *SD*-bearing chromosome *2* and an *SD*+-bearing homolog transmit the *SD*-bearing chromosome almost exclusively. This distorted segregation is the consequence of an induced dysfunction of those sperm that receive the *SD*+ homolog. From previous studies, two loci have been implicated in this phenomenon: the *Sd* locus which is required to produce distortion, and the Responder (*Rsp*) locus that is the site at which *Sd* acts. There are two allelic alternatives of *Rsp*—sensitive (Rsp^{sens}) and insensitive (Rsp^{ins}); a chromosome carrying Rsp^{ins} is not distorted by *SD*. In the present study, the function and location of each of these elements was examined by a genetic and cytological characterization of X-ray-induced mutations at each locus. The results indicate the following: (1) the *Rsp* locus is located in the proximal heterochromatin of *2R*; (2) a deletion for the *Rsp* locus renders a chromosome insensitive to distortion; (3) the *Sd* locus is located to the left of *pr* (*2*-54.5), in the region from 37D2-D7 to 38A6-B2 of the salivary chromosome map; (4) an *SD* chromosome deleted for *Sd* loses its ability to distort; (5) there is another important component of the *SD* system, *E(SD)*, in or near the proximal heterochromatin of *2L*, that behaves as a strong enhancer of distortion. The results of these studies allow a reinterpretation of results from earlier analyses of the *SD* system and serve to limit the possible mechanisms to account for segregation distortion.

NORMALLY, an individual heterozygous for two allelic alternatives produces two kinds of gametes in equal frequencies. Those cases in which some aspect of transmission has been altered, such that one gametic type is recovered in excess of the other, have been termed meiotic drive (ZIMMERING, SANDLER and NICOLETTI 1970). Cases of meiotic drive have been reported in a variety of organisms including higher plants, mosquitoes, Drosophila, mice, and possibly humans (ZIMMERING, SANDLER and NICOLETTI 1970). One of the best characterized of these cases is the segregation distorter (*SD*) system in *Drosophila melanogaster*. Because an excellent recent review of segregation distortion has been written by HARTL and HIRAIZUMI (1976), only those features of the system which bear on the experiments to be described in this report will be discussed here.

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SD, a locus on chromosome *2*, was first discovered by Y. HIRAIZUMI in a natural population in Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959); subsequently *SD*-bearing chromosomes-*2* have been recovered from many other natural populations throughout the world (HARTL and HIRAIZUMI 1976). The characteristic feature of *SD* is that males heterozygous for an *SD*-bearing second chromosome and most normal (SD^+-bearing) homologs transmit a vast excess of the *SD*-bearing chromosome. In many cases, the proportion of *SD*-bearing progeny among the total offspring produced by such males (k) exceeds 99 percent.

Zygotic mortality as an explanation of these results was eliminated by the fact that the segregation ratio from heterozygous females is normal and, directly, by measuring egg hatch. Thus it was suggested that distortion resulted from the nonfunction of SD^+-bearing gametes (SANDLER, HIRAIZUMI and SANDLER 1959). It has since been directly shown by several workers (NICOLETTI, TRIPPA and DE MARCO 1967; HARTL, HIRAIZUMI and CROW 1967) that in situations where the number of functional sperm is the limiting factor, the fecundity of heterozygous *SD* males is reduced, relative to controls, proportionally to the amount of distortion. For example, males carrying an *SD*-bearing chromosome with a k value close to 1.00 were only half as fertile as control males. These data imply that gametes which received the SD^+ homolog are, in fact, rendered dysfunctional owing to the action of *SD*.

Although meiosis in heterozygous *SD* males is normal at the level of light microscopy (PEACOCK and ERICKSON 1965), anomalies in spermiogenesis consistent with the dysfunctional sperm hypothesis are observed in electron microscopic studies (NICOLETTI 1968; TOKUYASU, PEACOCK and HARDY 1976). For *SD* chromosomes that have k values close to 1.00, up to 32 of the 64 spermatid nuclei developing in the syncytial spermatid bundle appear abnormal in that the chromatin in these nuclei is incompletely condensed, often leading to subsequent problems in spermatid maturation. The abnormal nuclei are presumably those that contain the homolog of *SD*, because *SD* males heterozygous for a homolog that is insensitive to distortion show no aberrant sperm development (PEACOCK, TOKUYASU and HARDY 1972).

Given that sperm dysfunction is the basis for segregation distortion, there are two distinct mechanisms which can be suggested to account for the gametic lethality. On the one hand, it could be that in *SD* males the presence of *SD* in the secondary spermatocyte is necessary for maturation of the resulting spermatid. Alternatively, *SD* could interact with its homolog and cause it to become a gametic lethal. SANDLER and CARPENTER (1972) showed that the latter was correct by demonstrating that nullo-*2* sperm were recoverable from a heterozygous *SD* male, whereas diplo-*2* sperm (carrying both *SD* and SD^+) were not recovered.

The demonstration that *SD* in some way acts upon its homolog implies that there is a site on the homolog at which this interaction occurs. Recombinational dissections of the *SD* system have shown that this is indeed the case (SANDLER and HIRAIZUMI 1960b; HIRAIZUMI and NAKAZIMA 1967; HARTL 1974). The conclusion from such studies is that there are two major, recombinationally separable, loci involved in segregation distortion. One locus is called *Sd*, and its presence is

necessary for distortion to occur; closely linked and to the right of *Sd* is a second locus called Responder (*Rsp*). The responder locus exists in two forms—sensitive (Rsp^{sens}) usually carried by the homolog of *SD*, and insensitive (Rsp^{ins}) carried by all highly distorting *SD* chromosomes recovered from the wild. The presence of Rsp^{sens} is necessary for a chromosome to be sensitive to distortion. *Rsp* thus behaves as the site at which the *Sd* locus exerts its effect. HARTL (1974) maps the entire complex to the base of the second chromosome between the markers *Tft* (*2*-53.2) and *cn*(*2*-57.5).

In addition to these major loci, a number of modifiers are known throughout the genome which alter the degree of distortion to varying extents (SANDLER 1962; KATAOKA 1967; HARTL 1970; MIKLOS 1972b; TRIPPA and LOVERRE 1975).

Except in the formal terms just discussed, the mechanism of action of *SD* is unknown. Based on complementation for male fertility among a collection of different *SD* chromosomes, HARTL (1973) proposed a molecular model for distortion involving the binding of regulatory proteins at the *Rsp* locus. In their electron microscopic analysis, TOKUYASU, PEACOCK and HARDY (1976) noticed that the first anomalies seen in sperm development in *SD*-bearing males coincide closely in time with the transition from lysine-rich to arginine-rich histones—a transition that occurs as a normal step in Drosophila spermiogenesis (DAS, KAUFMANN and GAY 1964a,b). This observation, together with the localization (PARDUE, *et al.* 1972) of the histone structural genes by *in situ* hybridization to the base of *2L* (the region containing the *SD* complex), raises the possibility that the mechanism of distortion may involve the histone transition (KETTANEH and HARTL 1976).

In this connection, it becomes important to determine the cytological location of *Sd* and *Rsp*, since, if one or the other were to correspond to the histone genes, it would lend considerable weight to this idea. In addition, in order to construct any model for the mechanism of distortion, or to test predictions of such models, it is important to know the behavior of null alleles in the system. For example, no available data bear on the question of whether a normal allele of *Sd* exists, as opposed to the wild types being the absence of *Sd* (SANDLER and CARPENTER 1972). To these ends, induced deletions for *Sd* and for *Rsp* were collected and characterized genetically and cytologically.

A NOTE ON TERMINOLOGY

The nomenclature for the elements of the *SD* system, reviewed by HARTL and HIRAIZUMI (1976), has a long and confusing history. To clarify the nomenclature and to identify synonyms, a brief description of the name changes is given here.

The locus responsible for distortion was originally termed *SD* (SANDLER and HIRAIZUMI 1960b). Later, HARTL (1969) introduced the symbol *Sd* to refer to this locus, reserving *SD* to refer to the entire distorting chromosome. The distinction is a useful one and will be followed here. *Sd*+ is used by HARTL (1969) to indicate the allelic alternative of *Sd*. The same will be done here, but it should be pointed out that there is no evidence that *Sd*+ is something other than the absence of *Sd*.

The history of the locus concerned with sensitivity is more tangled. Originally, insensitivity was attributed to a chromosome aberration present on the *SD* chromosome, which is immune to the action of *Sd* (SANDLER and HIRAIZUMI 1960b). LEWIS (1962) showed that the aberration

was a pericentric inversion present on the *SD-72* chromosome used in the studies of SANDLER and HIRAIZUMI (1960b), but not present on other *SD* chromosomes. Thus, rather than the inversion itself being responsible for insensitivity, insensitivity was attributed to a locus within the inversion. This locus has been given a variety of designations.

HIRAIZUMI and NAKAZIMA (1967) repeated the earlier recombinational analysis of SANDLER and HIRAIZUMI (1960b), but used an *SD-5*-type chromosome that lacks the pericentric inversion. These authors called the locus which conferred insensitivity on the *SD* chromosome *Activator of SD [Ac(SD)]*. This is confusing because the term *Activator* was used first by SANDLER and HIRAIZUMI (1960b) to refer to a proposed locus, separable from the pericentric inversion, needed to activate the *Sd* locus; *Ac(SD)* was not, in their view, concerned with sensitivity. Nonetheless, *Ac(SD)* has been used in subsequent literature to refer to the insensitivity locus.

SANDLER and CARPENTER (1972) reinterpreted the earlier data of SANDLER and HIRAIZUMI (1960b), and reached conclusions consistent with the model of HIRAIZUMI and NAKAZIMA (1967). However, in their analysis *Ac(SD)* is replaced by the name *Receptor* to indicate the site of action of *Sd*. Finally, the locus underwent yet another name change (HARTL 1973) to become known as *Responder* (*Rsp*). The two alleles were referred to as *Rsp* (insensitive to *Sd*) and *Rsp+* (sensitive to *Sd*).

Here we will follow Hartl's nomenclature and use *Rsp* to indicate the site at which *Sd* acts. However, to indicate more simply the segregational properties of the two alleles, we will refer to them as Rsp^{ins} (Responder-insensitive), for the allele not distorted by *Sd*, and Rsp^{sens} (Responder-sensitive), for the one that is. *Rsp* will be used to refer to the locus itself.

A new component of the *SD* system is reported in this paper. It behaves as a strong positive enhancer of distortion and is therefore given the name *E(SD)*. This locus is different from the *Ac* of SANDLER and HIRAIZUMI (1960b) in both its cytological location and effects on distortion, and the two should not be confused. As with *Sd*, the allelic form of *E(SD)* will be called *E(SD)+*, although again it is not clear that *E(SD)+* is anything other than the absence of *E(SD)*.

MATERIALS AND PROCEDURES

Chromosomes: The following chromosomes were used in this study. For a complete description of the markers, see LINDSLEY and GRELL (1968).

1. *SD chromosomes* (= *Sd* Rsp^{ins}): *SD-72*, recovered from natural populations in Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959), $k > .99$, carries a pericentric inversion, *In(2LR)39–40; 42A* and a paracentric inversion in *2R*, *In(2R)NS* = *In(2R)52A2-B1; 56F9–13* (LEWIS 1962). *SD-5*, recovered in Madison (SANDLER, HIRAIZUMI and SANDLER 1959), $k > .99$, carries two nonoverlapping paracentric inversions in *2R*, *In(2R)45C-F; 49A* and *In(2R)NS* (LEWIS 1962). SD^{R-1} recovered in Rome, Italy, (SANDLER *et al.* 1968), has no structural rearrangements (NICOLETTI and TRIPPA 1967); $k = .97$, kindly supplied by DRS. B. NICOLETTI and G. TRIPPA.

2. "*Suicide chromosome*" (= *Sd* Rsp^{sens}): *R(cn)-10*, a recombinant derivative from *SD-36*, where *SD-36* is a chromosome identical to *SD-5*. This chromosome shows self-distortion when segregation is from an insensitive (*Sd+* Rsp^{ins}) homolog (HARTL 1975). See below for further discussion of this "suicide behavior." Constructed and kindly supplied by D. HARTL.

3. *Tester chromosomes* (= *Sd+* Rsp^{sens}): (1) *cn bw*, structurally normal, standard tester chromosome in *SD* studies, highly sensitive to distortion and (2) pr^{A2} *cn bw*, carries a mutation of *pr* newly induced by X-rays in the standard *cn bw* chromosome; the sensitivity remains unaltered.

4. *Heterochromatic deletions*: $Df(2R)M\text{-}S2^{10}$, originally isolated by SCHULTZ, deleted for most of the centric heterochromatin on *2R* (MORGAN *et al.* 1939), used in complementation analysis in these studies. Also used was a collection of heterochromatic deficiencies in *2L* and *2R* of varying extent. These were isolated as detachments from compound second chromosomes and generously supplied by A. HILLIKER. For a complete description of the isolation and characterization of these chromosomes see HILLIKER and HOLM (1975).

5. *Euchromatic deletions*: A set of deletions that covers the entire euchromatic portion of the base of *2L* has been isolated and cytologically characterized (WRIGHT, HODGETTS and SHERALD 1976). They were graciously supplied by R. HODGETTS. The deletions and their cytological breakpoints are shown in Figure 3; they were used in complementation tests.

Irradiation of chromosomes: Males 3–5 days old were irradiated with 5000 rad from a cobalt-60 source. Irradiated males were mated to appropriate females and discarded on the fourth day in order to sample only postmeiotically treated cells.

Tests for distortion: The ability of a chromosome to distort was measured by individually crossing 10–20 males heterozygous for the chromosome being tested and the *cn bw* chromosome to *cn bw* females. The segregation ratios are presented as *k* values, which are the proportion of *SD*-bearing progeny among the total offspring. When the chromosome being tested was itself marked with *cn* and *bw*, it was made heterozygous with a *cn bw* chromosome also carrying *pr*. These males were mated to *pr cn* mothers and *k* scored as the proportion of *pr*+ progeny.

In many instances, the chromosome tested for distortion was recovered as a product of irradiation and was, therefore, likely to differ in viability from the *cn bw* chromosome. Because such viability differences will affect the estimate of *k*, the reciprocal cross with the female parent heterozygous was performed to evaluate deviations owing to viability differences between the homologs. With this estimate, the *k* values can be corrected.

Tests for sensitivity: The sensitivity of chromosomes to *SD* was measured by crossing 10–20 single males heterozygous for the chromosome being tested and an *SD-5*, *SD-72*, or SD^{R-1} chromosome to *cn bw* females. As before, *k* values were calculated as the proportion of *SD*-bearing progeny; a value close to 0.50 indicates a chromosome insensitive to distortion. In several cases, a chromosome to be tested for sensitivity did not carry either *cn* or *bw*. To test these, an *SD* derivative carrying an X-ray-induced *bw* mutation was used. These derivatives have the same distortion ability as the unirradiated *SD* chromosomes ($k > .99$).

As in the test for distortion, the *k* value measured in the male cross was affected, in several cases, by viability differences between the *SD* chromosomes and its homolog; in these cases, corrections were made as described above.

Tests for induction of self-distortion ("suicide behavior"): This test is based on the observation by SANDLER and HIRAIZUMI (1960b) and by HARTL (1974, 1975) that recombinant *SD* chromosomes that carry *Sd*, but a sensitive responder (*Sd* Rsp^{sens}), will be recovered significantly less than 50 percent when heterozygous in a male with a homolog carrying *Sd*+, but Rsp^{ins} (*Sd* Rsp^{sens}/*Sd*+ Rsp^{ins}). Both chromosomes are transmitted with equal frequency from an *Sd* Rsp^{sens}/*Sd*+ Rsp^{sens} male.

The special behavior of the *Sd* Rsp^{sens} chromosome is used as an important diagnostic test for the presence of Rsp^{ins} (HARTL 1975). A chromosome may be insensitive to distortion in two different ways: namely, because it carries Rsp^{ins}, or because it carries a suppressor of *Sd*. If the chromosome carries Rsp^{ins}, it should induce the suicide behavior described above, with *Sd* acting at the Rsp^{sens} locus in coupling with itself. On the other hand, if a chromosome is insensitive because it carries a suppressor of *Sd*, then in males heterozygous for this chromosome and the *Sd* Rsp^{sens} chromosome, *Sd* does not operate and both chromosomes are recovered equally frequently.

The test is to mate males heterozygous for the *Sd* Rsp^{sens} chromosome marked by *cn* and the chromosome being tested to *cn bw* females. The degree of self-distortion by the *Sd* Rsp^{sens} chromosome is measured by the proportion of *cn*-bearing offspring among the progeny. In this case, a *k* value significantly less than 50 percent indicates suicide behavior.

THE *Rsp* LOCUS

Isolation of responder mutants: To devise a screen for mutations at the responder locus, it is necessary to ask whether Rsp^{sens} is the functional allele and insensitivity the absence of function, or *vice versa*? If the former obtains, it should be possible to recover a mutation to insensitivity in a sensitive chromosome,

whereas in the latter case, the opposite should be true. Because many wild-type stocks exhibit at least some degree of sensitivity to distortion (SANDLER, HIRAIZUMI and SANDLER 1959), it seems likely that Rsp^{sens} is the functional allele. Therefore, an attempt was made to recover X-ray-induced insensitive mutations on the sensitive *cn bw* chromosome.

Irradiated *cn bw* chromosomes were made heterozygous in males with *SD-72*; these males were individually testcrossed with homozygous *cn bw* females, and each cross checked for a reduced *k* value. Because *cn cw* progeny are white-eyed and *SD-72* red-eyed, it was possible to screen these crosses without counting progeny by simply noting the presence of white-eyed flies. In this manner, 5,160 irradiated *cn bw* chromosomes were screened; among these, five were recovered that exhibit insensitivity to *SD*; these were designated Rsp^{ins}.

Characterization of Rsp^{ins} *mutants:* Since numerous modifiers of *SD* that affect the degree of distortion are known, it is important to know whether the insensitive *cn bw* chromosomes recovered display all of the properties expected of a Rsp^{ins} allele, rather than those of one of these modifiers.

The first question is whether all five insensitive *cn bw* chromosomes are mutant at the same site and, if so, is this site at the base of chromosome *2*? It was found that along with the mutation to insensitivity, three of the Rsp^{ins} *cn bw* chromosomes (Rsp^{ins}*-1*, *-11*, and *-31*) had acquired a recessive lethal. Complementation tests among these indicated that all three shared the same lethal mutation. Because the Rsp^{ins} *cn bw* chromosomes were of independent origin, and because the lethal is not present in the unirradiated *cn bw* stock, this result argues that the lethal and the insensitivity are the result of a single X-ray-induced event and, therefore, that these three are all mutant at the same site.

The remaining two Rsp^{ins} *cn bw* chromosomes (Rsp^{ins}*-16*, and *-32*) were viable as homozygotes and in all heterozygous combinations with each other and with the three lethal-bearing Rsp^{ins} *cn bw* chromosomes. Thus, complementation analysis is not informative. However, if the mutation that confers insensitivity on the viable-Rsp^{ins} *cn bw* chromosomes were at a different site from that on the lethal-Rsp^{ins} *cn bw* chromosomes, it should be possible, in viable/lethal heterozygotes, to recover recombinants sensitive to distortion. Accordingly, females of the genotype lethal-Rsp^{ins}(*-1*, *-11*, *-31*) *cn bw*/viable-Rsp^{ins}(*-16*, *-32*) *cn bw* were mated to *SD/cn bw* males. Individual second chromosomes produced by the females were recovered in *SD*-bearing male progeny, and the sensitivity of these chromosomes was tested by mating to *cn bw* females. Among 789 chromosomes tested, none proved to be sensitive. This result indicates that if the lethal- and viable-Rsp^{ins} mutations represent two different sites, they can be no more than 0.38 map units apart (the upper limit of the 95% confidence interval based on a Poisson distribution, STEVENS 1942).

This experiment cannot definitely rule out the possibility of insensitivity resulting from a mutation at either of several sites which happen to be closely linked. Nonetheless, the failure to recover a sensitive recombinant, together with the indistinguishable behavior of lethal-insensitive and viable-insensitive

chromosomes in the tests described below, suggests that all five insensitive chromosomes are mutant at the same site.

Assuming that the five insensitive *cn bw* chromosomes define a single site, we now ask if that site maps in the interval between *pr* and *cn* at the base of chromosome *2*, the region where the Rsp^{ins} carried by *SD* chromosomes is known to reside (SANDLER and HIRAIZUMI 1960b; HARTL 1974). That this is the case is shown by the following experiments.

Insensitivity was mapped with respect to the marker *cn* by making the Rsp^{ins}*-1 cn bw* chromosome heterozygous with a wild-type chromosome in females and recovering *cn*-bearing chromosomes that were tested for sensitivity to distortion by *SD-72*. Of 115 *cn*-bearing chromosomes tested, only one was sensitive, having resulted from a crossover between Rsp^{ins} and *cn*. This places the *Rsp* locus 0.87 map units from *cn*, but does not indicate whether it is to the left or right.

To examine this, *pr cn* recombinants were recovered from *pr*/Rsp^{ins}*-1 cn bw* females. Each recombinant was tested for the presence of the lethal carried by the Rsp^{ins} chromosome, and for sensitivity to distortion. The results were as follows: of 122 *pr cn* recombinations, 32 carried the lethal and 90 were lethal free; without exception, the lethal was inseparable from insensitivity. These data indicate that Rsp^{ins}*-1* resides at a position to the right of *pr* and $32/122 = 0.26$ of the distance from *pr to cn*. Since the map distance from *pr* to *cn* is about 1.2 units, Rsp^{ins} maps 0.31 units to the right of *pr* and 0.89 to the left of *cn*. The latter result agrees well with the previous localization of Rsp^{ins}*-1* at 0.87 units from *cn*.

This mapping places the mutationally induced insensitive mutation in the same region as the naturally occurring Rsp^{ins} alleles, and is consistent with the interpretation that these mutations affect the *Rsp* locus.

In addition to map location, HARTL (1975) has suggested three criteria for defining a chromosome that carries *Sd*+, but is Rsp^{ins}. These are: (1) the Rsp^{ins} chromosome should not be a distorter; (2) it should be insensitive to distortion by an *SD* chromosome (*Sd* Rsp^{ins}) and, most importantly, (3) it should produce a suicide combination, *i.e.*, a recovery of the *Sd* Rsp^{sens} chromosome significantly less than 50 percent (HARTL 1974), from males heterozygous for this chromosome. Each of these requirements was examined for each of the five induced insensitive *cn bw* chromosomes.

To test for distortion by the insensitive *cn bw* chromosomes, males of the genotype Rsp^{ins} *cn bw*/*pr cn bw* were crossed to *pr cn* females. As a control, *cn bw*/*pr cn bw* males were mated to the same females. The results of these crosses are given in Table 1; they demonstrate that the Rsp^{ins} chromosomes do not themselves distort a sensitive chromosome.

The requirement for insensitivity to distortion was tested by crossing *SD* Rsp^{ins}*-i cn bw* males by *cn bw* females. Each Rsp^{ins} chromosomes was tested with three different *SD* chromosomes—*SD-72*, *SD-5*, and SD^{R-1}. In each of these cases, the reciprocal cross, *SD*/Rsp^{ins}*-i cn bw* females by *cn bw* males, was also performed; since *SD* is known to operate only in males, the expected *k* value in the female crosses is 0.50 and any deviations from this reflect relative viability dif-

TABLE 1

Test for the ability of Rsp^{ins} cn bw *chromosomes to distort a sensitive homolog. The cross is*: Rsp^{ins}-i cn bw/pr^{A2} cn bw ♂♂ × pr cn ♀♀

Chromosome tested	Phenotype of progeny *cn*	*pr cn*	*k*
Rsp^{ins}-1	686	660	0.51
Rsp^{ins}-11	819	889	0.48
Rsp^{ins}-16	721	775	0.48
Rsp^{ins}-31	878	813	0.52
Rsp^{ins}-32	1160	1155	0.50
cn bw	1410	1417	0.50

ferences. The *k* values from the male cross have been corrected for these viability differences.

The results of these crosses are shown in Table 2. It can be seen that the *SD* chromosomes strongly distort the unirradiated *cn bw*, but the *Rsp^{ins} cn bw* chromosomes exhibit *k* values close to 0.50. The one exception to this is *Rsp^{ins}-11*, which consistently has a *k* value somewhat closer to 0.60 than to 0.50. However, even in this case, there is no question but that the chromosome has become markedly less sensitive.

The third criterion for the demonstration of a *Rsp^{ins}* allele, the production of suicide behavior, is tested by crossing *Sd Rsp^{sens}/Sd+ Rsp^{ins}-i cn bw* males to *cn bw* females (Table 3). In each case, the recovery of the *Sd*-bearing chromosome is markedly less than 50 percent. The degree of self-distortion is similar to that observed by HARTL (1974; 1975) in tests of naturally occurring *Rsp^{ins}* alleles. For comparison, the effect of one of these [the *Rsp^{ins}* allele carried by *In(2L+2R) Cy bw* (HARTL 1975)] is also shown.

That the depressed recovery of the *Sd Rsp^{sens}* chromosome is the consequence of being heterozygous with a *Rsp^{ins}* chromosome in a male, rather than of some viability difference, for example, is demonstrated by the results of two control crosses. First, the recovery of the *Sd Rsp^{sens}* chromosome from a male also carrying the original, unirradiated, *cn bw* chromosome (*Sd Rsp^{sens}/Sd+ Rsp^{sens}cn bw*), is, if anything, slightly greater than of its homolog (k=0.57). Secondly, when the *Sd Rsp^{sens}* chromosome is segregating from an insensitive homolog [*Sd Rsp^{sens}/In(2L+2R)Cy, Cy Sd+ Rsp^{ins} bw*] in a female, where *Sd* does not operate, the two chromosomes are recovered equally frequently (k=0.48).

One final point is that self-distortion of the *Sd Rsp^{sens}* chromosome caused by *Rsp^{ins}-1* is higher than that produced by the other *Rsp^{ins}* chromosomes. The reason for this is not clear. HARTL (1975) suggested that modifiers which alter the ability of a *Rsp^{ins}* chromosome to induce self-distortion are segregating in various stocks. Whether or not such modifiers are present here is not known. The important point, however, is that in every case the insensitive *cn bw* chromosomes cause suicide behavior of the *Sd Rsp^{sens}* chromosome, indicating that they all carry *Rsp^{ins}*.

TABLE 2

Test for the sensitivity of Rsp^{ins} cn bw *chromosomes. The cross is: (A)* SD-i/Rsp^{ins}-j cn bw ♂ ♂ × cn bw ♀ ♀ *and (B) the reciprocal cross*

Genotype of parent	Type of cross	Phenotype of progeny* +	Phenotype of progeny* *cn bw*	*k*†
SD-72/Rsp^{ins}-1 cn bw	A	1629	1328	0.52
	B	1221	1063	
SD-72/Rsp^{ins}-11 cn bw	A	844	491	0.61
	B	1212	1058	
SD-72/Rsp^{ins}-16 cn bw	A	1712	1397	0.52
	B	1294	1144	
SD-72/Rsp^{ins}-31 cn bw	A	1290	1029	0.52
	B	1243	1074	
SD-72/Rsp^{ins}-32 cn bw	A	792	595	0.51
	B	1462	1131	
SD-72/cn bw	A	3525	0	1.00
	B	1436	1318	
SD-5/Rsp^{ins}-1 cn bw	A	1740	1715	0.48
	B	1543	1423	
SD-5/Rsp^{ins}-11 cn bw	A	1259	915	0.58
	B	1491	1498	
SD-5/Rsp^{ins}-16 cn bw	A	1675	1724	0.51
	B	1291	1394	
SD-5/Rsp^{ins}-31 cn bw	A	940	938	0.49
	B	1351	1309	
SD-5/Rsp^{ins}-32 cn bw	A	1545	1485	0.49
	B	1346	1259	
SD-5/cn bw	A	2181	8	0.99
	B	1465	1358	
SD^{R-1}/Rsp^{ins}-1 cn bw	A	1661	1612	0.48
	B	1172	1069	
SD^{R-1}/Rsp^{ins}-11 cn bw	A	1246	864	0.55
	B	902	774	
SD^{R-1}/Rsp^{ins}-16 cn bw	A	1554	1374	0.54
	B	1076	1098	
SD^{R-1}/Rsp^{ins}-31 cn bw	A	1299	1162	0.50
	B	843	735	
SD^{R-1}/Rsp^{ins}-32 cn bw	A	1488	1000	0.52
	B	996	734	
SD^{R-1}/cn bw	A	1573	48	0.96
	B	708	603	

* When *SD-5* or *SD-72* is heterozygous in the female parent, recombination between *cn* and *bw* is negligible because of the presence of inversions on *2R*. *SD^{R-1}* is inversion free, and recombination between *cn* and *bw* does occur (avg. map distance = 35). In this case, because *cn* and *Rsp* are tightly linked, the *cn bw*+ recombinants are added to the *cn bw* class and the *cn*+ *bw* recombinants to the + class in the Table above.

† The *k* values in the A crosses are corrected for viability differences, measured in the B crosses, as follows: the observed number of *cn bw* progeny in the A cross is multiplied by the ratio (+/*cn bw*) from the B cross to give a corrected number of *cn bw* progeny. *k* values are then computed in the usual way using the corrected number of *cn bw* progeny.

TABLE 3

Test for the ability of Rsp^{ins} cn bw *chromosomes to induce self-distortion of* R(cn)-10 (=Sd Rsp^{sens}). *The cross is: (A)* Rsp^{ins}-i cn bw/R (cn)-10 ♂ ♂ × cn bw ♀ ♀ *and (B) the reciprocal cross*

Chromosome tested	Type of cross	Phenotype of progeny *cn*	Phenotype of progeny *cn bw* (or *Cy bw*)	*k*
Rsp^{ins}-1	A	370	2248	0.14
Rsp^{ins}-11	A	805	1792	0.31
Rsp^{ins}-16	A	702	1539	0.31
Rsp^{ins}-31	A	764	1675	0.31
Rsp^{ins}-32	A	765	1499	0.34
cn bw	A	960	711	0.57
In (2L+2R)Cy bw	A	478	1217	0.28
	B	842	762	0.48

The evidence presented above, then, shows that both the map position and the three-fold operational test for *Rsp^{ins}* are fulfilled by the behavior of the five X-ray-induced insensitive *cn bw* chromosomes which argues that all five carry a new mutation at the same site and that this site is the *Rsp* locus. Having established that all five insensitive *cn bw* chromosomes are mutant at the *Rsp* site, we now indicate their allelic status by writing their numeral designation as a superscript (*i.e.*, *Rsp^{ins-1}*, *Rsp^{ins-11}*, etc.); henceforth they will be so represented.

Mapping the Rsp *site*: It now remains to localize the *Rsp* locus cytologically. The fact that three of the five *Rsp^{ins} cn bw* chromosomes carried a newly induced recessive lethal genetically inseparable from insensitivity suggested that both phenotypes might be the consequence of a deletion at the base of chromosome *2*, thus offering a means of localizing *Rsp*. However, an examination of the polytene chromosomes revealed no detectable chromosome aberrations. This indicated that any deletion present must be either too small to be seen or located in the heterochromatin. An alternative means of finding the cytological location of *Rsp*, based on the proximity of the lethal and *Rsp^{ins}*, was to use deletions in this region with known breakpoints to determine the location of the lethal.

Since the genetic mapping placed *Rsp* closer to *pr* than to *cn*, several deletions covering the base of *2L* proximal to *pr* were tested; none overlapped the lethal. A deficiency at the base of the right arm of chromosome *2*, however, proved lethal in combination with all three lethal-*Rsp^{ins}* mutants. This deficiency, *Df(2R)M-S2^{10}*, is special in that salivary analysis reveals no euchromatic portion of the chromosome is missing, but *2R* in mitotic metaphase chromosomes is only three-fourths its normal length (HILLIKER and HOLM 1975). Therefore, *Df(2R)M-S2^{10}* appears to be a deletion for most of the centric heterochromatin of *2R*. Consequently, several other chromosomes containing deletions in this region were obtained from A. HILLIKER, who isolated them as detachments of compound autosomes (for a complete description of these deletions and their isolation, see HILLIKER and HOLM 1975). Complementation analysis of the lethal-*Rsp^{ins}* chromosomes using these heterochromatic deletions, as well as

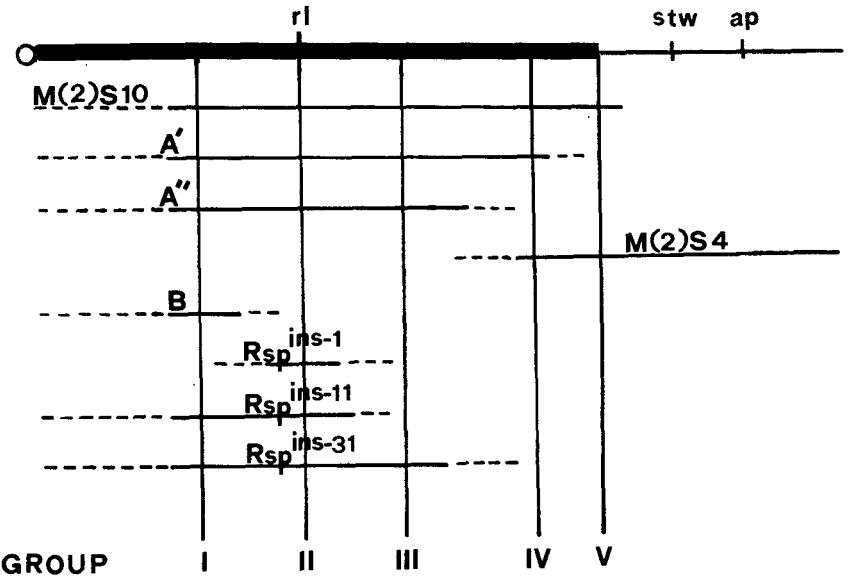

FIGURE 1.—Complementation map of *Rsp^{ins} cn bw* chromosomes with deletions and EMS-induced lethal mutations in *2R* heterochromatin (after HILLIKER 1976, his Figure 1). The centromere is indicated by the open circle and the heterochromatin by the heavy black line. The extent of the various deficiencies is indicated by the horizontal solid lines; the dashed lines represent the regions where the breakpoint cannot be specified precisely. The vertical lines indicate the relative position of the EMS-induced lethal loci designated by the Roman numerals below. An overlap between two deficiencies, or between a deficiency and a lethal locus signifies that this heterozygous combination is lethal.

several EMS-induced lethal mutations in the region also recovered by HILLIKER, yields the complementation map shown in Figure 1.

There are several features about this map that deserve notice. First, the lethal lesion for all three lethal-*Rsp^{ins}* chromosomes is contained in the heterochromatin. Second, *Rsp^{ins-11}* and *Rsp^{ins-31}* are themselves deletions because they overlap several loci. It cannot be established that *Rsp^{ins-1}* is a deletion because it lacks only the *rl+* locus. However, because the number of mutant sites in the heterochromatin is small relative to the amount of DNA present, the distance between sites is likely large; thus *Rsp^{ins-1}* may be a deletion for a sizeable amount of material, even though it encompasses only one known locus. Similarly, it is possible, although difficult to prove, that *Rsp^{ins-16}* and *Rsp^{ins-32}* are also deletions in the region, but which do not include a vital locus.

A related point is that the lethality of the lethal-*Rsp^{ins}* chromosomes is probably due not to mutation at the *Rsp* locus itself, but to the deletion of adjacent vital genes. The *Rsp^{ins}* chromosomes that are viable as homozygotes suggest that alterations of the *Rsp^{sens}* function do not produce any detectable effects on the somatic phenotype. A third feature of the map is that the exact complementation patterns for the three *Rsp^{ins}* chromosomes are different, indicating that they are, in fact, of independent origin, but, as expected, they all overlap in a common

region—the interval between the Group I and the Group III lethal loci. These data suggest that this interval contains the *Rsp* locus.

The fact that Rsp^{ins-11} and Rsp^{ins-91} are deletions, and that Rsp^{ins-1} is also likely to be a deletion, leads to the reasonable conclusion that Rsp^{ins} is a site at which *SD* acts to cause distortion of its homolog, and the absence or inactivation of this site is sufficient to render a chromosome immune to distortion. To test this hypothesis, the various chromosomes shown in Figure 1 were tested for their sensitivity to distortion by *SD*. The prediction was that those deletions which include the region containing *Rsp* would be insensitive to distortion.

The sensitivity of the various deletions was measured by crossing *SD/Df(2R)-i* males to *cn bw* females. The results of these crosses, shown in Table 4, present an ambiguous picture. The results for some of the deletions are in agreement with the hypothesis, while the outcome for others is not. For example, four of the deletions tested, *Df(2R)-14C*, *-2J*, *-10G*, and *-6C*, all members of the same complementation group (A″ in Figure 1), are insensitive to distortion by *SD-5*

TABLE 4

Segregation ratios from males (column 2) and females (column 3) carrying the indicated heterochromatic 2R *deficiency*

Genotype	Segregation ratios from ♂♂ × *cn bw* ♀♀	Segregation ratios from ♀♀ × *cn bw* ♂♂
SD-5/Df(2R)M-S2^{10}	0.72 (1294)	0.47 (1547)
SD-5/Df(2R)M-S2^{4}	0.98 (855)	0.50 (514)
SD-5/Df(2R)A′	1.00 (1216)	0.45 (1555)
SD-5/Df(2R)B	0.97 (1338)	0.50 (1854)
SD-5/Df(2R)2J	0.51 (3317)	0.51 (1589)
SD-5/Df(2R)10G	0.60 (3582)	0.44 (582)
SD-5/Df(2R)6C	0.53 (2137)	0.52 (1629)
SD-5/Df(2R)14C	0.48 (2830)	0.50 (1652)
SD-72/Df(2R)2J	0.52 (2346)	
SD-72/Df(2R)10G	0.60 (2381)	
SD-72/Df(2R)6C	0.53 (2121)	
SD-72/Df(2R)14C	0.54 (2067)	
R(cn)-10/Df(2R)M-S2^{10}	0.60 (1984)	
R(cn)-10/Df(2R)M-S2^{4}	0.67 (2174)	
R(cn)-10/Df(2R)A′	0.62 (2026)	
R(cn)-10/Df(2R)B	0.55 (2617)	
R(cn)-10/Df(2R)2J	0.33 (1812)	0.52 (1598)
R(cn)-10/Df(2R)10G	0.46 (1459)	0.55 (2039)
R(cn)-10/Df(2R)6C	0.46 (1334)	0.53 (780)
R(cn)-10/Df(2R)14C	0.14 (1052)	0.52 (1570)
Df(2R)2J/cn bw	0.52 (2081)	0.50 (1791)
Df(2R)10G/cn bw	0.49 (2116)	0.49 (2256)
Df(2R)6C/cn bw	0.48 (1872)	0.48 (1746)
Df(2R)14C/cn bw	0.52 (1897)	0.50 (1914)

In each case, the k value is given as the proportion of offspring bearing the chromosome written first. The number in parentheses is the total number of progeny.

and *SD-72*. To demonstrate that this insensitivity was the consequence of these chromosomes bearing Rsp^{ins}, as opposed to carrying a modifier of *SD*, *Sd* Rsp^{sens}/*Df(2R)-i* males were mated to *cn bw* females. As discussed before, the *Sd* Rsp^{sens} chromosome is expected to distort itself if its homolog carries Rsp^{ins} but not otherwise. As shown, *Df(2R)14C* and *Df(2R)2J* induce the same degree of suicide behavior as did the Rsp^{ins} *cn bw* chromosomes. This effect is less marked in the case of *Df(2R)10G* and *Df(2R)6C*, the *k* values corrected for viability differences being 0.41 and 0.43, respectively. The reason for the difference in strength is not clear, but here too the result suggests the presence of Rsp^{ins}. Finally, none of these four deletions is itself a distorter as indicated by the normal segregation ratios from *Df(2R)-i/cn bw* males (Table 4).

The insensitivity was shown to be inseparable from the deletions by backcrossing to the sensitive *cn bw* stock and allowing three generations of free recombination between these chromosomes. The deletion was then reisolated and retested for sensitivity to distortion. In no case did this affect the insensitivity of the deletions. Thus, by all of the operational criteria used before to define a Rsp^{ins} allele, these four deletions would be classified as Rsp^{ins} mutants, in agreement with the hypothesis presented above.

These results, however, are tempered by the behavior of other deletions in Table 4, which do not fit easily with the simple hypothesis. For example, *Df(2R)MS-2*^{10}, which spans the entire region, is partially sensitive to distortion, and *Df(2R)A'*, which should also delete the *Rsp* locus, is completely sensitive to distortion. In addition, HILLIKER (personal communication) has tested several other deletions covering this region, also isolated as detachments from compound autosomes, and found them to be sensitive to distortion. Why these deletions should remain sensitive to distortion while similar deletions are associated with insensitivity is not clear. Several possible explanations for this apparent contradiction will be considered in the DISCUSSION. Suffice it to say here that, although the matter remains unsettled, the majority of the data strongly favor the view that *Rsp* is located in the heterochromatin of *2R* and that a deficiency for it leads to insensitivity.

THE *Sd* LOCUS

Isolation of X-ray induced SD *revertants*: The same kinds of considerations discussed in respect of the isolation of Rsp^{ins} chromosomes apply to the isolation of *SD* revertants. Thus, if distortion results from the absence of some Sd^+ product, or from the loss of an important site, deletion of Sd^+ should turn a normal chromosome into a distorter. In addition, it should not be possible to revert an *SD* chromosome by X rays. Conversely, if *Sd* is a neomorph, for example, an X-ray-induced deletion of the *Sd* locus would produce an *SD* revertant, whereas such a deletion in a normal chromosome would not affect segregation.

To examine these relations, X-ray-induced *pr* deletions in *SD-72* and *cn bw* chromosomes were constructed and characterized on the assumption that some of these would include the *Sd* locus owing to the genetic proximity of *Sd* and *pr* (SANDLER and HIRAIZUMI 1960b). To isolate these deletions, *SD-72/cn bw* males

and *cn bw* males were irradiated and crossed to *pr cn* females and their progeny screened for any *pr*-eyed offspring. Six *pr* mutations were recovered in the *SD-72* chromosome and 26 in the *cn bw* chromosome. They were classified as deletions based on their lethality in combination with known deletions for *pr*. Because a deletion for *Sd* might be male-sterile, the *pr* deletions were recovered in both males and females to avoid selecting against a particular class of deletions.

The *pr*-bearing *SD-72* and *cn bw* chromosomes were tested for their ability to distort a sensitive *cn bw* chromosome, and the *pr*-bearing *cn bw* chromosomes were also tested for their sensitivity to distortion by an unirradiated *SD-72* chromosome. These results are presented in Table 5; they indicate that none of the *pr* deletions affected the segregational properties of the chromosomes on which they were induced. The *SD-72* chromosomes remain complete distorters, while the *cn bw* chromosomes remain sensitive to distortion and did not themselves become distorters.

It appears from these results that either *Sd* is not as close to *pr* physically as it is genetically, or that the *pr* deletions are limited in size. To distinguish between these two possibilities, the *pr SD-72* chromosomes and some of the *pr cn bw* chromosomes were examined cytologically. The largest deletion among the *pr SD-72* chromosomes extended from 38B to 39A-C (Figure 4c). All the remaining *pr SD-72* chromosomes proved to carry only small deletions of several bands around *pr*, located at 38A8-38B6 on BRIDGES' (1942) revised salivary map (WRIGHT, HODGETTS and SHERALD 1976). These small deletions were all contained within the region 38A5,6 to 38C1,2. The *pr cn bw* chromosomes, on the other hand, in-

TABLE 5

Segregation ratios from males of the indicated genotypes crossed by cn bw *females*

pr deficiency tested*	*k* values† when homolog is: *cn bw*	*SD-72*
A1	0.48 (2385)	1.00 (1099)
A2	0.50 (2827)	1.00 (1118)
A3	0.46 (7201)	1.00 (1382)
A4	0.48 (1594)	1.00 (1262)
A5	0.50 (1894)	1.00 (1317)
A6	0.49 (723)	1.00 (1325)
A7	0.48 (3164)	1.00 (1436)
A8	0.42 (3673)	1.00 (1287)
A9	0.49 (2222)	1.00 (817)
B5	1.00 (1226)	
B10	1.00 (2750)	
B11	1.00 (1942)	
B12	1.00 (2829)	
B14	1.00 (2615)	

The number in parentheses is the total number of progeny.

* Lines A1–A9 are *pr* deficiencies induced in the *cn bw* chromosome and lines B5–B14 are *pr* deficiencies induced in the *SD-72* chromosome.

† *k* values in the second column are given as the proportion of progeny carrying the *pr* deletion; in the last column as the proportion of *SD-72* progeny.

cluded some very large deficiencies extending almost two numbered sections. The reason for this nonrandom recovery of small deletions in the *SD-72* chromosome is not known.

It has been argued (SANDLER and CARPENTER 1972) that *Sd* resides between 37B2 and 39–40. The collection of *pr* deletions in the *cn bw* chromosome spans the region 37B to 39C, yet none of these deletions shows any ability to distort. From this it may be concluded either that *Sd* does not result from a deletion of *Sd*+ or that *Sd* does not reside within the region deleted. As will be shown below, the former alternative is probably correct.

Assuming this to be true, the absence of a revertant among the *pr SD-72* chromosomes must be because the *pr* deletions induced in this chromosome do not include the *Sd* locus. The *pr* deletions in *SD-72* span the region 38B to 39A-C. Therefore, *Sd* must reside outside these boundaries.

In a subsequent effort to induce deletions of the *Sd* locus, irradiated *SD* chromosomes were directly screened for alterations in their ability to distort. Males carrying *SD-5* were irradiated and the irradiated *SD-5* chromosome recovered in sons heterozygous with *cn bw*. These sons were individually mated to *cn bw* females and screened for a reduction in *k* value, evidenced by the production of white-eyed progeny. Among 4,000 chromosomes so screened, eight were recovered that had very reduced *k* values; these are designated SD^{Rev} chromosomes.

Characterization of SD *revertants*: It is necessary to consider the types of X-ray-induced events that could reduce the ability of an *SD* chromosome to distort. Among these are: deletion or inactivation of *Sd* itself; deletion or inactivation of an essential enhancer of distortion; induction of a suppressor of distortion; mutation of Rsp^{ins} to Rsp^{sens} (this would convert *Sd* Rsp^{ins} to *Sd* Rsp^{sens}, which does not distort a sensitive homolog); and perhaps some chromosome rearrangements, several of which have been reported to eliminate distortion entirely or even reverse the direction of distortion (NOVITSKI and EHRLICH 1970).

Because the *SD-5* chromosome already carries one or more recessive lethals, it is difficult in this case to ask whether any of the *SD* revertants had acquired a new lethal by performing complementation tests among the various *SD* revertants, as was done for the Rsp^{ins} mutations. It was decided, therefore, to first characterize the eight *SD* revertants with respect to the properties of distortion, to determine whether any distinctions could be made among them, and whether any of these behaved as if it carried a deletion or inactivation of the *Sd* site.

The first test was to measure the *k* value for each *SD* revertant to determine if the ability to distort had been partially reduced or if it had been eliminated entirely. The results of this test and the reciprocal cross are shown in Table 6. The segregation ratio in the reciprocal cross measures viability differences between the two chromosomes and thus allows computation of corrected *k* values. These corrected *k* values reveal that the eight revertants fall into at least two classes: SD^{Rev}*-2*, *-7*, *-16*, *-19* and *-37* all with *k* values close to 0.50, and SD^{Rev}*-1*, *-3*, and *-36* with *k* values around 0.70.

This distinction depends upon the observation of a segregation ratio significantly less than 0.50 for SD^{Rev}*-1*, *-3*, and *-36* in the reciprocal cross and the in-

TABLE 6

Test for the ability of SD^{Rev} *chromosomes to distort a sensitive homolog. The cross is: (A)* SD^{Rev}-i/cn bw ♂ ♂ × cn bw ♀ ♀ *and (B) the reciprocal cross*

Chromosome tested	Type of cross	Phenotype of progeny		k^*
		+	*cn bw*	
SD^{Rev}-*1*	A	1813	1389	0.73
	B	779	1584	
SD^{Rev}-*2*	A	745	716	0.48
	B	698	613	
SD^{Rev}-*3*	A	1202	1371	0.64
	B	839	1712	
SD^{Rev}-*7*	A	1463	1474	0.51
	B	1411	1486	
SD^{Rev}-*16*	A	623	571	0.50
	B	738	688	
SD^{Rev}-*19*	A	751	680	0.52
	B	623	630	
SD^{Rev}-*36*	A	2920	1741	0.71
	B	659	982	
SD^{Rev}-*37*	A	525	695	0.48
	B	511	633	
SD-5	A	2181	8	0.99
	B	1466	1359	

* k values in A crosses corrected for viability differences as explained in Table 2.

terpretation that this reflects a reduced viability of these reverted-*SD* chromosomes. If this assumption is incorrect—if, for example, the reduced k values signified instead some type of distorted segregation in the female—the corrected k values for these three revertants would be spuriously high.

In order to test the validity of this assumption, we may consider the expected results for the segregation of the *SD* revertants from an insensitive homolog. Because, in this case, distortion in the male should be eliminated, the observed k values in the male cross and the reciprocal cross should be comparable to one another, and, further, the corrected k values for the male cross should be close to 0.50. For this reason, the k values were measured in crosses of SD^{Rev}/Rsp^{ins-1} *cn bw* males by *cn bw* females and also in the reciprocal cross. The Rsp^{ins-1} chromosome has already been shown (Table 2) to be completely insensitive to distortion by a normal *SD* chromosome.

The results of this cross are shown in Table 7. Several points of interest emerge from these data. First, consistent with the expectation, the observed segregation ratios in the male cross and the reciprocal cross are now similar for all the *SD* revertants, whereas in the previous set of crosses (Table 6), the observed segregation ratios for SD^{Rev}-*1, 3* and *-36* were consistently much higher in the male cross than the reciprocal cross. Secondly, if observed k values in the male cross are corrected in the same way as before, the k values for all the *SD* revertants are

TABLE 7

The results of crosses of (A) SD^{Rev}-i/Rsp^{ins}-1 cn bw ♂♂ × cn bw ♀♀ *and (B) the reciprocal cross*

Chromosome tested	Type of cross	Phenotype of progeny		k*
		+	*cn bw*	
SD^{Rev}-1	A	1269	1740	0.55
	B	561	951	
SD^{Rev}-2	A	1585	1593	0.49
	B	1378	1328	
SD^{Rev}-3	A	1097	1648	0.47
	B	837	1097	
SD^{Rev}-7	A	1284	1434	0.46
	B	1095	1062	
SD^{Rev}-16	A	1487	1428	0.50
	B	941	897	
SD^{Rev}-19	A	1362	1702	0.46
	B	1362	1454	
SD^{Rev}-36	A	1060	1244	0.46
	B	963	948	
SD^{Rev}-37	A	1074	1392	0.47
	B	963	1093	
SD-5	A	1740	1715	0.48
	B	1543	1423	

* k values in A crosses corrected for viability differences as described previously.

approximately the same and close to 0.50. Most importantly, it will be noticed that those revertants with values close to 0.50, when segregating from a sensitive homolog, show no change in k value in this cross. However, in the case of *SD^{Rev}-1*, *-3*, and *-36*, the k values are higher when segregation is from a sensitive rather than insensitive homolog. Identical results are obtained if the *Rsp^{ins-1} cn bw* chromosome was replaced in these crosses by *In(2LR) CyO*, another insensitive homolog (Table 8).

These experiments therefore imply that there is a distinction to be made among the *SD* revertants. The class containing *SD^{Rev}-1*, *-3*, and *-36* retain a residual ability to distort, which, as expected, is eliminated if segregation is from an insensitive homolog. This suggests that this class of *SD* revertants may not be mutant at *Sd* itself. The remaining five revertants behave alike in these two sets of crosses and show no ability to distort in any circumstance—behavior consistent with a deletion or inactivation of the *Sd* locus.

If we represent the original *SD* chromosome as *Sd Rsp^{ins}*, then an *SD* chromosome reverted by loss of the *Sd* site will still carry *Rsp^{ins}*. As already discussed there, is a specific test for *Rsp^{ins}*, namely the ability of the chromosome to induce self-distortion of an *Sd Rsp^{sens}* homolog. The results from crosses of *SD^{Rev}/ Sd Rsp^{sens}* males by *cn bw* females and the reciprocal crosses are given in Table 9. In all but one case, *SD^{Rev}-16*, the *SD* revertants induce complete self-distortion

TABLE 8

Results of crosses of (A) SD^{Rev}-i/In (2LR) Cy0 ♂♂ × cn bw ♀♀ *and (B) the reciprocal cross*

Chromosome tested	Type of cross	Phenotype of progeny		k^*
		+	*Cy*	
SD^{Rev}-*1*	A	558	1161	0.46
	B	616	1094	
SD^{Rev}-*2*	A	1200	1684	0.49
	B	817	1090	
SD^{Rev}-*3*	A	756	1520	0.46
	B	878	1529	
SD^{Rev}-*7*	A	1061	1684	0.46
	B	1071	1455	
SD^{Rev}-*16*	A	1147	1772	0.45
	B	861	1110	
SD^{Rev}-*19*	A	1109	1846	0.45
	B	647	896	
SD^{Rev}-*36*	A	458	918	0.44
	B	1020	1616	
SD^{Rev}-*37*	A	990	1693	0.52
	B	688	1250	
SD-5	A	1086	1493	0.53
	B	812	1248	

* k values in A crosses corrected for viability differences as described previously.

of the *Sd* Rsp^{sens} chromosome. The amount of self-distortion in these crosses is much greater than that induced by the Rsp^{ins} mutations described earlier, even though the *Sd* Rsp^{sens} chromosome is the same in both crosses. This difference does not necessarily reflect a difference in behavior of the induced Rsp^{ins} mutations and the Rsp^{ins} carried by the reverted-*SD* chromosomes, but more likely is the result of differences in other genes carried on the chromosomes. Thus, for SD^{Rev}-*1*, *-3*, and *-36*, which retain some ability to distort and may, in fact, still carry *Sd*, there would be two *Sd* loci in SD^{Rev}/*Sd* Rsp^{sens} males, both acting at the sensitive-*Rsp* locus of the *Sd* Rsp^{sens} chromosome. The other *SD* revertants, which may have lost the *Sd* locus, should still carry the complete set of enhancers of distortion present on an *SD* chromosome (SANDLER and HIRAIZUMI 1960a; MIKLOS 1972a) and partly lacking on the suicide chromosome since it is an exchange product (HARTL 1975). The addition of these enhancers should enable the *Sd* locus carried on the suicide chromosome to operate at full capacity. The outcome in either of these cases is complete self-distortion of the suicide chromosome. The Rsp^{ins} mutations induced in the *cn bw* chromosome are derived from non-distorters and lack enhancers of distortion, so that they should be unable to produce complete self-distortion.

TABLE 9

Test for the ability of SD^{Rev} *chromosomes to induce self-distortion of* R(cn)-10 (= Sd Rsp^{sens}). *The cross is: (A)* SD^{Rev}-i/R(cn)-10 ♂ ♂ × cn bw ♀ ♀ *and (B) the reciprocal cross*

Chromosome tested	Type of cross	Phenotype of progeny		k^*
		cn	+	
SD^{Rev}-*1*	A	10	959	0.004
	B	2055	942	
SD^{Rev}-*2*	A	0	1789	0.00
	B	1065	958	
SD^{Rev}-*3*	A	7	2223	0.002
	B	1655	1117	
SD^{Rev}-*7*	A	6	2157	0.002
	B	940	704	
SD^{Rev}-*16*	A	1494	1153	0.54
	B	736	677	
SD^{Rev}-*19*	A	1	2254	0.0004
	B	676	572	
SD^{Rev}-*36*	A	4	757	0.004
	B	2063	1411	
SD^{Rev}-*37*	A	6	1670	0.003
	B	469	388	
SD-5	A	0	486	0.00
	B	311	352	

* *k* values are given as the proportion of *R(cn)-10* bearing progeny. Corrections made for viability differences in A crosses as before.

In any case, whatever the cause of this difference between the Rsp^{ins} *cn bw* and the SD^{Rev} chromosomes, the important point here is that all of the revertants except SD^{Rev}-*16* induce suicide behavior, demonstrating that they still carry Rsp^{ins}.

SD^{Rev}-*16* behaves differently from the others and thus defines a third class of revertant. In this case, the suicide chromosome is recovered at least as often as the *SD* revertant. This outcome would be explained if SD^{Rev}-*16* carried a newly induced suppressor of distortion, or mutation from Rsp^{ins} to Rsp^{sens}. In the latter case, the original *Sd* Rsp^{ins} chromosome would now be *Sd* Rsp^{sens}, which is itself a suicide combination of genes. If SD^{Rev}-*16* were such a chromosome, it should have distorted itself in the cross of SD^{Rev}-*16*/Rsp^{ins-1} *cn bw* males by *cn bw* females and given a *k* value under 0.50, which it did not. Another test of this possibility is to ask whether SD^{Rev}-*16*, but not the other revertants, can be distorted by another *SD* chromosome. The results from crosses of *SD-72 bw*/SD^{Rev} males to *cn bw* females, and the reciprocal crosses are shown in Table 10. It is evident that the *k* value in each case is close to 0.50, indicating that all of the revertants, including SD^{Rev}-*16*, are insensitive to distortion. This experiment rules out the possibility that SD^{Rev}-*16* carries a sensitive responder and suggests rather, that it carries a suppressor of distortion. SD^{Rev}-*16* has been tested further, in preliminary

TABLE 10

The results of crosses of (A) SD^{Rev}*-i*/SD-72 bw ♂♂ × cn bw ♀♀ *and (B) the reciprocal cross*

Chromosome tested	Type of cross	Phenotype of progeny		k^*
		+	*bw*	
SD^{Rev}*-1*	A	919	1169	0.57
	B	214	361	
SD^{Rev}*-2*	A	734	754	0.52
	B	1081	1188	
SD^{Rev}*-3*	A	789	794	0.53
	B	795	899	
SD^{Rev}*-7*	A	1311	1341	0.52
	B	1285	1405	
SD^{Rev}*-16*	A	917	1026	0.49
	B	752	814	
SD^{Rev}*-19*	A	1187	1198	0.52
	B	753	816	
SD^{Rev}*-36*	A	383	666	0.41
	B	300	357	
SD^{Rev}*-37*	A	1013	1138	0.49
	B	299	322	
SD-5	A	469	440	0.54
	B	1167	1303	

* *k* values are given as the proportion of SD^{Rev} or *SD-5* offspring among total, and corrected in A crosses for viability differences as described previously.

studies, to determine if a distorting chromosome could be recovered following removal of the suppressor by recombination. So far, a recombinant derivative of SD^{Rev}*-16* has not been recovered that shows any ability to distort. Since recombination along the right arm of this chromosome is effectively eliminated by the pair of *SD-5* inversions present on this arm, only *2L* has been studied in these recombination tests. Thus, it is possible that a suppressor of distortion has been induced somewhere on *2R*. Alternatively, it is possible that the mutational event is at or near the *Sd* site itself, and the two have not been separated yet by recombination. Further tests should allow these two possibilities to be distinguished.

A point of interest in the last experiment is the fecundity of males carrying the different *SD* revertants heterozygous with *SD-72*. These fecundities and that of *SD-5*, calculated as the ratio of total progeny to the number of males tested, are listed in Table 11. HARTL (1969; 1973) has demonstrated that males heterozygous for two different *SD*-bearing chromosomes are much less fertile than controls and that the *Sd* locus is responsible for this sterility. In agreement with results of HARTL, the productivity of the combination of two *SD* chromosomes (*SD-5* and *SD-72*) is seen to be greatly reduced. Of interest here is the observation that the fecundity of SD^{Rev}*-7*, *-16*, *-19*, and *-37* males is appreciably higher than that of *SD-5* males, while SD^{Rev}*-3*, and *-36* males show the same low fecundity as *SD-5*.

TABLE 11

Fecundity of* SD^{Rev} *and* SD-5 *males when heterozygous with the indicated homolog*

Chromosome tested	Number of progeny per male when homolog is:	
	SD-72	*Rsp^{ins}-1 cn bw*
SD^{Rev}-1	116	201
SD^{Rev}-2	106	212
SD^{Rev}-3	61	183
SD^{Rev}-7	176	181
SD^{Rev}-16	194	194
SD^{Rev}-19	159	204
SD^{Rev}-36	48	177
SD^{Rev}-37	143	164
SD-5	34	181

* These fecundities are calculated by dividing the total number of progeny (results shown in Tables 7 and 10) by the number of fertile males.

SD^{Rev}-1, and *-2* males exhibit intermediate fecundity. Those revertants that are most fertile are those that behaved as deletions or inactivations of *Sd*, while those that have the same low fertility as *SD-5* are those that show residual distortion and therefore likely retain the *Sd* locus. The revertants with intermediate levels of fertility are ambiguous in that one of these, *SD^{Rev}-1*, behaves as an incompletely reverted *SD*, while the other, *SD^{Rev}-2*, is indistinguishable from the completely reverted *SD* chromosomes. Nonetheless, the overall pattern provides additional evidence in support of the division of the *SD* revertants into different classes.

It should be pointed out that the data presented above were not derived from experiments specifically designed to measure male fertility as were the more precise experiments of HARTL (1969, 1973). However, there are several reasons to believe that these data accurately measure male fecundity. First, the numbers correspond well with those of HARTL for similar chromosome combinations. Second, the numbers are reproducible. For example, in the cross involving *SD^{Rev}/Sd Rsp^{sens}* males, a similar pattern of fecundities as that shown in Table 11 is observed. Finally, the differences in fecundity are specifically related to the *SD* system, because the fecundity of the incompletely reverted *SD* chromosomes increases when segregation is from an *Sd^{+} Rsp^{ins}* homolog, a situation where no distortion occurs. (Male fecundities for this cross shown in Table 11 demonstrate that all the revertants have the same level of fertility.)

In summary, the eight *SD* revertants can be divided into at least three classes: (1) *SD^{Rev}-1*, *-3*, and *-36*, which retain some ability to distort and thus presumably still carry *Sd*; (2) *SD^{Rev}-16*, which shows no distortion but fails to induce suicide behavior, suggesting it carries a suppressor of *Sd*; and (3) *Sd^{Rev}-2*, *-7*, *-19*, and *-37*, which show no distortion and whose behavior in a variety of crosses is consistent with a deletion or inactivation of *Sd*.

Having divided the *SD* revertants into these categories it remains to determine the location of any associated chromosomal lesion. Of particular interest are those

revertants that appear to be mutant at the *Sd* locus itself, as they may reveal the cytological location of *Sd*. To this end, the *SD* revertants were analyzed in complementation tests with morphological markers and deficiencies of known cytological location and by examination of the polytene chromosomes.

Complementation tests with *lt*, an eye color mutation in or near the proximal heterochromatin of *2L*, revealed that SD^{Rev}-*1*, -*3*, and -*36* lacked the lt^+ function. Further complementation analysis with a series of deletions for the heterochromatin of *2L* gave the complementation map shown in Figure 2. These results indicate that SD^{Rev}-*1*, -*3*, and -*36* all carry overlapping deletions for the base of *2L* that include lt^+ and the normal alleles of flanking lethals.

The salivary chromosomes of SD^{Rev}-*36* are normal (except for the pair of inversions on *2R* carried by the original *SD-5* chromosome and present on all the revertants). The most proximal band that can be clearly resolved at the base of *2L* is 40A (Figure 4a); thus the deletion carried by SD^{Rev}-*36* is proximal to this, in agreement with the location of *lt* at 40 B-F (LINDSLEY and GRELL 1968). Similarly, no deletion was visible at the base of *2L* in either SD^{Rev}-*3* or SD^{Rev}-*1*, indicating that the distal-most breakpoint does not extend into the euchromatin.

SD^{Rev}-*3* does carry a small distal inversion on *2L* with breakpoints at 29E-30A; 34A-B; this almost certainly is the result of a second X-ray-induced aberration and not relevant to the segregational properties of SD^{Rev}-*3*, because SD^{Rev}-*3* behaved similarly to SD^{Rev}-*36* and SD^{Rev}-*1* and these three revertants have only the

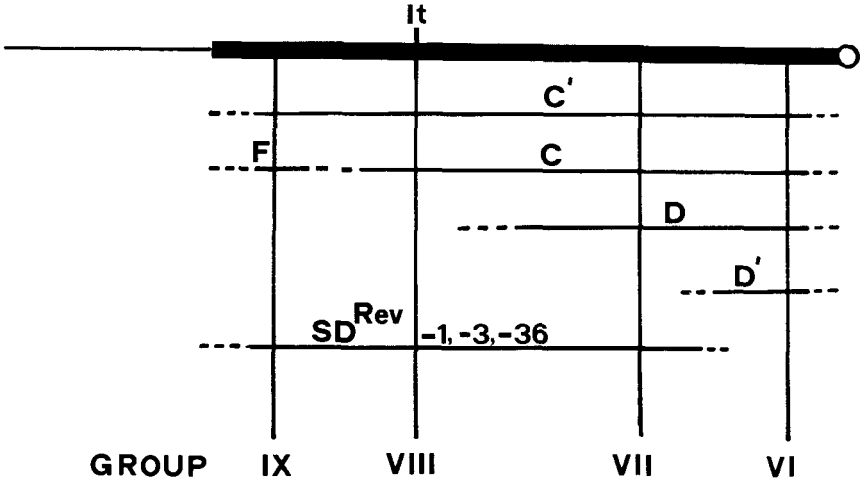

FIGURE 2.—Complementation map of SD^{Rev}-*1*, -*3*, and -*36* with deletions and EMS-induced lethal loci in *2L* heterochromatin (after HILLIKER 1976, his Figure 5). The centromere is indicated by the open circle and the heterochromatin by the heavy black line. The extent of the various deficiencies is indicated by the horizontal solid lines; the dashed lines represent the regions where the breakpoint cannot be specified precisely. The vertical lines indicate the relative position of the EMS-induced lethal loci designated by the Roman numerals below. An overlap between a deficiency and a lethal locus signifies that this heterozygous combination is lethal. Two deficiencies are lethal in heterozygous combination if they are both drawn as overlapping a common lethal locus; otherwise the heterozygous combination is viable.

deletion near *lt* in common. SD^{Rev}*-1* is involved in a translocation with the tip of *3R* translocated to the base of the right arm of the second chromosome. Translocation tests confirm that SD^{Rev}*-1*, but none of the other revertants, carries a translocation. This translocation too is surely the result of a second X-ray-induced event and, for the same reasons as in the case of SD^{Rev}*-3*, does not influence the segregational behavior of SD^{Rev}*-1*.

The conclusion from the genetic and cytological examination of SD^{Rev}*-1*, *-3*, and *-36* is that there is a locus carried by *SD* chromosomes at the base of *2L* near *lt*, which behaves as a strong enhancer of distortion; we name it *E(SD)*. This locus is deleted in SD^{Rev}*-1*, *-3*, and *-36*; it is the absence of *E(SD)* that accounts for the reduced, but not entirely eliminated, ability to distort.

The other *SD* revertants were not mutant at *lt*, nor did they carry newly induced lethals contained in any of the heterochromatic *2L* deficiencies tested. To determine whether any of these revertants carried detectable X-ray-induced lesions elsewhere on the base of *2L*, they were examined in complementation tests with a series of deletions that cover the base of *2L* from 36F to 40A (Figure 3). SD^{Rev}*-2*, *-7*, *-16*, and *-19* all proved fully viable in combination with each of the deficiencies, implying the absence of any newly induced lethals in this region. The salivary chromosomes of these revertants confirmed this result; there were no

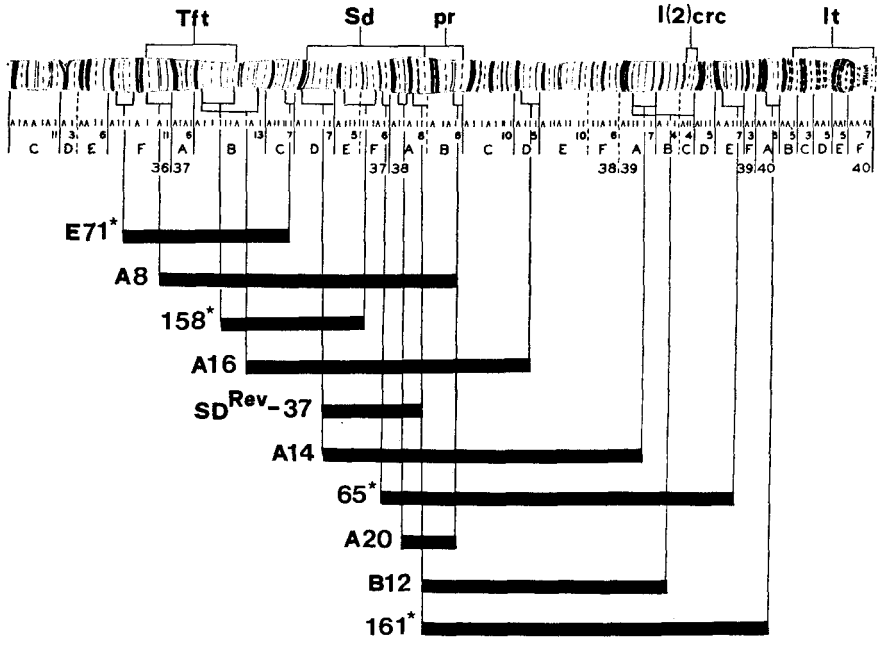

FIGURE 3.—Diagrammatic representation of the base of *2L* (after WRIGHT, HODGETTS and SHERALD 1976, their Figure 2) showing the cytological extent of the deficiencies used in this study. The location of several genes is also shown above the chromosome. Those deletions which are indicated as overlapping are lethal in heterozygous combination. Deletions marked with a star were kindly supplied by DR. R. HODGETTS; the others were recovered in the present study.

FIGURE 4.—Salivary gland preparations of some of the chromosomes analyzed in these studies:

(a) *SD^{Rev}-36*. This chromosome illustrates the normal banding pattern at the base of *2L*. There is no cytologically detectable lesion associated with this chromosome, although it carries a proximal deletion as explained in the text.

(b) *SD^{Rev}-37* = *Df(2L)*37D2–7; 38A6-B2. Segment between arrows is deleted in the deficient homolog.

(c) *Df(2L)pr^{B12}SD-72* = *Df(2L)*38A-B; 39A-D. Segment between black arrows is deleted in deficient homolog. The bracket indicates that the breakpoint in this region is uncertain. Also shown is the pericentric inversion associated with *SD-72*. The left breakpoint is between 39D and 39E (upper double arrow) and the right breakpoint is at 42A (bottom double arrow).

detectable lesions at the base of *2L* (or elsewhere in the chromosome complement). In the case of these revertants, the event that caused the loss of the ability to distort is not associated with any cytologically detectable chromosomal alteration. Thus, SD^{Rev}*-2*, *-7*, and *-19* all behave as deletions or inactivations of *Sd* (Sd^{Rev}*-16* probably carries a suppressor of *SD*; see above), yet none is associated with a mutational lesion at the base of *2L* that is detectable either cytologically or in complementation tests. It is possible, therefore, that these represent "point" mutations at the *Sd* locus, but it is not yet possible to eliminate alternative explanations.

The remaining revertant, SD^{Rev}*-37*, is more informative. The results of complementation tests with SD^{Rev}*-37* are shown in Figure 3. These tests indicate that SD^{Rev}*-37* is a deletion, since it overlaps two deletions which do not themselves overlap. Examination of the polytene chromosomes (Figure 4b) shows that this was indeed the case; the breakpoints of the deletion are 38A6-B2 on the right and 37D2-7 on the left. Genetically, this deletion is from just to the left of *pr* to just to the right of *Tft*. SD^{Rev}*-37* was characterized on genetic grounds as a revertant with properties consistent with a deletion or inactivation of *Sd*. Together with the cytological demonstration of a deletion at the base of *2L*, this strongly argues that both are the consequence of the same event and therefore that the cytological location of the *Sd* locus is in the region spanned by the SD^{Rev}*-37* deletion.

DISCUSSION

The Rsp *locus:* Five chromosomes that carried X-ray-induced mutations to insensitivity to distortion were recovered in these studies. These were classified as mutations at the *Rsp* locus, resulting in a change from Rsp^{sens} to Rsp^{ins}, based on the following evidence. The mutation conferring insensitivity, in each case: (1) is located between *pr* and *cn*; (2) does not cause distortion of a sensitive homolog; (3) results in insensitivity to distortion by three different *SD* chromosomes; and (4) is capable of inducing the suicide response in an *Sd* Rsp^{sens} homolog. Complementation tests with three of the Rsp^{ins} *cn bw* chromosomes that were homozygous lethal indicated that all three shared a common lethal in the centric heterochromatin of *2R* and that at least two of these chromosomes are deleted in this region. From this it is inferred that the *Rsp* locus is in the centric heterochromatin of *2R*, and that a deletion for the *Rsp* locus results in a Rsp^{ins} phenotype. This agrees with, and extends, previous information on the location of the *Rsp* site. The first evidence on this point came from the recombination studies of SANDLER and HIRAIZUMI (1960b) who showed that the locus of insensitivity was between *pr* and *cn*, although they could not determine whether it mapped to *2L* or *2R*. Moreover, SANDLER and HIRAIZUMI were unaware that the *SD* chromosome that they used carried a pericentric inversion, though they did realize that there was a chromosome aberration in the region causing a ten-fold reduction in map distance between *pr* and *cn*. They imagined that it was the aberration itself that produced insensitivity because insensitivity was genetically inseparable from the reduction in the *pr–cn* map distance. After LEWIS (1962) reported the

presence of a pericentric inversion on that *SD* chromosome but not on other *SD* chromosomes, the data were reinterpreted to mean that insensitivity was due to a locus within the pericentric inversion, rather than to the aberration itself (HIRAIZUMI and NAKAZIMA 1967; SANDLER and CARPENTER 1972).

Because the breakpoints of the pericentric inversion are at 39–40 and 42A (LEWIS 1962), the *Rsp* locus must be located within these limits. SANDLER and CARPENTER (1972) showed that the *Rsp* site was not located in salivary regions 41 to 43A, because a *Y* chromosome into which this material was inserted was not distorted, while the deficient second chromosome from which the piece was removed was distorted. Thus, the best information available had narrowed the location of the *Rsp* site to between 39-40 and 41, which agrees exactly with the location of *Rsp* reported here.

The analysis of the Rsp^{ins} *cn bw* chromosomes in these studies indicates that the location of *Rsp* is in the heterochromatin of *2R* between HILLIKER's (1976) Group I and Group III lethal loci. One difficulty with this placement of the *Rsp* locus is that some chromosomes, including $Df(2R)M\text{-}S2^{10}$ and *Df(2R)A'*, apparently deleted for the region (Figure 1), are sensitive to distortion (Table 4). There are several possible explanations of this apparent inconsistency. One is that the *Rsp* site is not located in exactly the same place in chromosomes of different origin. For example, a rearrangement entirely limited to the heterochromatin would normally be indetectable, but could change the location of the *Rsp* locus. The possibility that *Rsp* has the capacity to remove itself from the chromosome and reintegrate at another nearby locus also cannot be eliminated; indeed, MINAMORI (1970) has reported behavior of this type for the extrachromosomal element, delta, another distorting system. Another possibility is that the *Rsp* site actually occupies a location different from that shown on the complementation map in Figure 1 (between the Group I and Group III lethal loci). The critical chromosome in this analysis is $Rsp^{ins\text{-}1}$, which is represented in Figure 1 as a deletion contained in this region. However, because $Rsp^{ins\text{-}11}$ and $Rsp^{ins\text{-}51}$ are deletions which extend through the most proximal known locus on *2R*, it may be that *Rsp* is actually located in the most proximal portion of the *2R* heterochromatin, and that $Rsp^{ins\text{-}1}$ is not a deletion but a double "hit" of some type, with one "hit" at *rl* and a more proximal one at the *Rsp* locus, producing insensitivity.

If this notion is correct, *Rsp* may be located in the region just adjacent to the centromere such that a break between the centromere and *Rsp* is expected to be rare. As a consequence, in the construction of a compound second chromosome both *C(2L)* and *C(2R)* would carry *Rsp*, and only rarely would a heterochromatic deletion isolated as a detachment from these compounds be deleted for *Rsp*, thus explaining the sensitivity of these deletions. Because the breakpoints of a deletion proximal to the Group I locus (Figure 1) cannot be specified with precision, it may be the case that those deletions that are insensitive extend more proximally than those that are sensitive, accounting for the differences seen among the deletions tested.

At this point it is not possible to decide in favor of any of these possibilities. However, the analysis of the X-ray-induced Rsp^{ins} mutations themselves is self-

consistent, and the data clearly suggest that the *Rsp* site is located somewhere in the heterochromatin of *2R*, and that a deletion for this locus renders the chromosome insensitive to distortion. How general this is for second chromosomes different from the *cn bw* chromosome can be determined only by mapping X-ray-induced mutations to insensitivity on such chromosomes.

The mapping of *Rsp* to the heterochromatin, a region sparse in structural genes, is consistent with the views of SANDLER and CARPENTER (1972) and HARTL (1973), who picture *Rsp* as a regulatory site rather than a structural gene. The question that arises is what is the function of the *Rsp* site? As HARTL (1973) has pointed out, the facts that transcription ceases after meiosis (OLIVIERI and OLIVIERI 1965; HENNIG 1967; GOULD-SOMERO and HOLLAND 1974) and that sperm almost devoid of chromosomes complete development and function normally (MULLER and SETTLES 1927; MCCLOSKEY 1966; LINDSLEY and GRELL 1969), argue against the idea that the effect of *SD* can be to cause the SD^+ chromosome to fail to produce a normal product, which failure results in sperm lethality. One is forced to the opposite view, namely, that the inactivation of genes during spermiogenesis is necessary for the completion of the process and that the action of *SD* is to cause genes on its homolog to remain active; it is this unscheduled activity that causes the affected sperm to be inviable. The electron microscopic studies of TOKUYASU, PEACOCK and HARDY (1976), showing the failure of the chromatin to condense in the SD^+ nuclei, is consistent with this idea. Moreover, this would explain the dominance of sensitivity apparent in the dysfunction of SD/SD^+ gametes (SANDLER and CARPENTER 1972).

If these ideas are correct, the *Rsp* locus is a site of considerable importance, upon whose activity or inactivity the state of the rest of the chromatin in the sperm nucleus depends. The evidence presented here indicates that a deletion of the *Rsp* site is equivalent to Rsp^{ins}, which in respect of the ideas discussed above, suggests that the complete absence of *Rsp* prevents the unscheduled activity. Formally, the *Rsp* locus has some similarity to an operator or promoter region.

This leads to a consideration of what the "normal" function of the Rsp^{sens} locus might be. The only effect that can be definitely attributed to Rsp^{sens} is that it causes a chromosome carrying it to be distorted by *SD*, a peculiar reason to exist. HARTL (1973) has argued that Rsp^{sens} must be part of a normal regulatory mechanism operating during spermiogenesis, and that *Sd* and Rsp^{ins} are mutations in this system. This is quite appealing, but direct evidence indicating a function for the Rsp^{sens} locus in the absence of *Sd* is lacking. The recovery of X-ray-induced Rsp^{ins} mutations that are homozygous viable and fertile seems to indicate that the Rsp^{sens} function is dispensable. In view of this, it is puzzling why any second chromosome isolated from nature would show sensitivity to distortion, rather than having disposed of the Rsp^{sens} locus completely. One possibility is that the Rsp^{ins} mutants that are homozygous viable and fertile are not completely deleted for the *Rsp* locus, but represent changes in base sequence or partial deletions of the *Rsp* locus. This might affect the interaction of *Rsp* with *Sd* but still allow it to perform its indispensible functions.

The E(SD) *locus:* Based on the following criteria, three of the eight *SD*-rever-

tants define a distinct class, not mutant at the *Sd* locus, but with reduced distortion: (1) a reduced, but not entirely eliminated, ability to distort; (2) reduced fecundity in combination with another *SD* chromosome, a characteristic of homozygosity for *Sd*; (3) a newly induced deletion that includes the lt^+ locus and extends into the centric heterochromatin of *2L*.

It seems clear that these revertants are not mutant at the *Sd* locus, not only from their segregational properties, but also because of the location of the mutational event. SANDLER and HIRAIZUMI (1960b) demonstrated that the *Sd* locus is distal to the leftmost breakpoint of the pericentric inversion carried by *SD-72*. LEWIS (1962) reported that this breakpoint is at 39-40. I have confirmed this and narrowed the location of the break to 39D-E (Figure 4c). Because *lt* is proximal to this (LINDSLEY and GRELL 1968), deletions extending proximal to *lt* do not include *Sd*. Direct cytological analysis of these revertants indicate that the deletion defined by complementation tests must be proximal to 40A. Thus, these revertants define a new component of the *SD* system, located in or near the centric heterochromatin of *2L*, whose behavior is that of strong enhancer of *Sd*; it has been given the name *E(SD)*.

This *E(SD)* would not have been evident in the previous studies of the *SD* region by SANDLER and HIRAIZUMI (1960b) and HIRAIZUMI and NAKAZIMA (1967), because their analyses dealt with specifying whether a recombinant product of an *SD* and SD^+ chromosome pair could distort a sensitive homolog and whether it was sensitive to distortion. These criteria are insufficient to recognize the presence of *E(SD)* because any exchange that separates *Sd* from *E(SD)* also separates *Sd* from Rsp^{ins}, producing a chromosome of the genotype *Sd* $E(SD)^+$ Rsp^{sens}, while an exchange to the other side of *E(SD)* produces an *Sd E(SD)* Rsp^{sens} bearing recombinant. Both of these recombinants would be sensitive to distortion, but neither would distort a sensitive homolog, owing to the fact that both carry Rsp^{sens} (HARTL 1974; 1975). MIKLOS (1972a) has proposed the existence of positive modifiers of distortion at the base of the second chromosome; these too must differ from *E(SD)* because MIKLOS deduces the existence of these modifiers from a re-examination of the data of SANDLER and HIRAIZUMI (1960b) and HIRAIZUMI and NAKAZIMA (1967), which, as just discussed, are insufficient to reveal the presence of *E(SD)*.

The presence of *E(SD)* does, however, suggest itself in HARTL's (1975) work. The *Sd* Rsp^{sens} chromosomes that he recovered fall into two distinct classes with respect to suicide behavior—those that are complete self-distorters ($k = 0.00$) and those that self-distort less strongly ($k = 0.15 - 0.20$). The magnitude of the difference in k for these chromosomes corresponds quite closely to the reduction in k observed in the present study for those *SD* revertants missing *E(SD)*. Moreover, HARTL (1975) specifically reports that those *Sd* Rsp^{sens} recombinants recovered from an exchange distal to *pr* [that of necessity separates *Sd* from *E(SD)* because, as discussed in the next section, *Sd* is located to the left of *pr*] are always in the class that are incomplete self-distorters, while *Sd* Rsp^{sens} recombinants from an exchange between *pr* and *cn* may show either complete or partial self-distortion. HARTL notes that these results suggest the existence of an enhancer of dis-

tortion located in the centrometric region, so that an exchange between *Sd* and Rsp^{ins} is almost always between *Sd* and the enhancer. It is likely that this enhancer of distortion corresponds to the locus defined by SD^{Rev}-*1*, -*3*, and -*36* in the present studies.

Further evidence on the existence and location of *E(SD)* has been provided by SHARP and HOLM (personal communication), who screened for X-ray-induced *lt* deletions in *SD-5* and found that several of these had reduced *k* values.

An examination of HARTL's data (1975, his Table 3) suggests that *E(SD)* enhances distortion in repulsion as well as in coupling with the *Sd* locus. It was mentioned above that, owing to the location of *E(SD)*, most *Sd* Rsp^{sens} recombinants will not carry *E(SD)*; the complementary product, on the other hand, should therefore usually carry both *E(SD)* and Rsp^{ins}. If *E(SD)* is capable of acting in repulsion, we would expect *Sd E(SD)*$^+$ Rsp^{sens}/Sd^+ *E(SD)* Rsp^{ins} heterozygotes to show the same amount of self-distortion as *Sd E(SD)* Rsp^{sens}/Sd^+ *E(SD)*$^+$ Rsp^{ins} heterozygotes. Males of the first genotype are represented by the more weakly self-distorting *Sd* Rsp^{sens} recombinants heterozygous with their complementary recombinant product. Males of the second genotype are constructed by making a completely self-distorting *Sd* Rsp^{sens} chromosome heterozygous with a homolog carrying Rsp^{ins} but not *E(SD)*. *In(2L + 2R)Cy bw* is a chromosome of the latter type, carrying a naturally occurring Rsp^{ins} (HARTL 1975) but, because it is not derived from an *SD* chromosome, in all probability not carrying *E(SD)*. The *k* value in these males is about 0.17, which corresponds closely to the *k* values of 0.15 — 0.20 observed for males of the first type.

Finally, based on the analysis above, males which may be represented as *Sd E(SD)* Rsp^{sens}/Sd^+ *E(SD)* Rsp^{ins} are shown by Hartl to have a *k* value of 0.00 (complete self-distortion). Males represented by *Sd E(SD)*$^+$ Rsp^{sens}/Sd^+ *E(SD)* Rsp^{ins} show very little, if any, distortion. Thus, there is a dosage effect of *E (SD)*; *Sd* in the presence of two copies of *E(SD)* distorts more strongly than in the presence of one copy, whether this copy is in coupling or repulsion with *Sd*, and this in turn results in more distortion than the absence of *E(SD)*.

The Sd *locus*: Of the eight X-ray-induced nondistorting *SD-5* chromosomes recovered in these studies, four appear to be mutations at the *Sd* locus itself. The evidence for this conclusion is that these four revertants exhibit: (1) complete loss of ability to distort; (2) insensitivity to distortion by another *SD* chromosome; and (3) increased fecundity, relative to an unirradiated *SD-5* chromosome, when heterozygous with *SD-72*.

Of these four, only one (SD^{Rev}-*37*) was associated with a detectable lesion; the lesion is a deficiency with breakpoints at 37D2-7 and 38A6-B2. It seems most reasonable to conclude that the *Sd* locus is between these breakpoints. Because only one of the *SD* revertants has a detectable lesion in this region, the possibility that the deficiency associated with SD^{Rev}-*37* is unrelated to the reversion of the *SD* phenotype cannot be eliminated. However, this placement of *Sd* is in agreement with evidence from other sources and helps explain heretofore puzzling results from earlier recombination studies of *SD* chromosomes.

The important new piece of information inherent in this placement is that *Sd*

is very close to, but to the left of *pr*, rather than to the right as previously proposed (SANDLER and HIRAIZUMI 1960b; HIRAIZUMI and NAKAZIMA 1967; SANDLER and CARPENTER 1972). This is suggested not only by the deficiency associated with SD^{Rev}-*37*, but also by the recovery of a *pr* deletion in the *SD-72* chromosome (Figure 4c) that deletes almost all of the material between *pr* and the pericentric inversion, but does not affect the *SD* phenotype. Other evidence supports the contention that *Sd* is to the left of *pr*. HARTL (1975) reports the recovery of *Sd* Rsp^{sens} (suicide) chromosomes as recombinants from *R-1/In (2L)Cy, Cy b pr cn* females, where *R-1* (which stands for "recombinant-1" and is unrelated to SD^{R-1} = *SD-Roma*) is an *SD* chromosome of the *SD-5* type, carrying both *2R* inversions but no pericentric inversion, and *In(2L)Cy, Cy b pr cn* is a chromosome sensitive to distortion. An *Sd* Rsp^{sens} combination is produced by an exchange between the *Sd* and Rsp^{ins} loci carried on *R-1*. What is significant is that some of the suicide chromosomes have picked up the markers *pr* and *cn*. The simplest explanation of this result is that *Sd* is between *b* and *pr*, and that these recombinants result from a single exchange between *Sd* and *pr*, picking up Rsp^{sens} and *cn* in the process. If *Sd* were proximal to *pr*, a triple crossover is required to produce recombinants of this type. TANZARELLA *et al.* (1972) report that SD^{R-1} maps genetically at 52.9 on the second chromosome. Although the data on which this mapping is based are not reported, their location is consistent with *Sd* being to the left of *pr*.

HARTL's (1974) recent recombination experiments using *Tft*, a marker 1.3 map units to the left of *pr*, demonstrated that *Tft* is distal to *Sd*, and therefore that *Sd* is located between *Tft* and *pr*. The cytological location of *Tft* is between 36F10 and 37B7, and *pr* is at 38A8-38B6 (WRIGHT, HODGETTS and SHERALD 1976). This corresponds closely to the cytological location of *Sd* proposed above, based on the limits of the deficiency associated with SD^{Rev}-*37*. Thus, the evidence strongly favors the view that SD^{Rev}-*37* is a deletion of the *Sd* locus.

Models of segregation distortion: The analysis of *SD* revertants makes it possible to rule out one recent suggestion concerning the *SD* system. Based on similarities between *SD* and the sc^4sc^8 drive system, PEACOCK and MIKLOS (1973) suggested that, "the different *Sd* alleles could be deficiencies of varying extents." The fact that *SD* is X-ray revertable, while SD^+ chromosomes in which deletions along the entire base of the second chromosome have been induced show no tendency whatever to distort, makes this notion untenable.

The possibility that the *SD* system involves an abnormality in the transition from lysine-rich to arginine-rich histones that occurs in Drosophila spermiogenesis has been raised (TOKUYASU, PEACOCK and HARDY 1976). The localizations of *Sd* and *Rsp* reported here show that neither is at the site of the histone genes, which are at 39D-E (PARDUE *et al.* 1972). This does not, however, necessarily invalidate the notion because, in the first place, the *SD* system could be involved in the regulation of the histone transition event, though neither *Sd* nor *Rsp* code for histones, and, in the second place, only the somatic histone genes have been localized by *in situ* hybridization; structural genes for the sperm histones may be different and located elsewhere. It may in this context be worth noting

that the *Sd* region is shown on BRIDGES' (1935) salivary map to pair ectopically with the region containing the histone genes. Direct evidence lending further support to the idea of a connection between *SD* and the histones has been provided recently by KETTANEH and HARTL (1976), who have shown by cytochemical techniques that the histone transition does not occur in males homozygous for *SD*.

The results described here also bear on an important prediction of HARTL's (1973) model of segregation distortion. Briefly, HARTL's model is that *Sd* codes for a multimeric regulatory protein that must bind with the *Rsp* region in order for normal spermiogenesis to occur. Regulatory Sd^+/Sd^+ homomultimers can bind to both Rsp^{sens} and Rsp^{ins}, resulting in neither distortion nor sperm dysfunction; Sd^+/Sd heteromultimers bind only at Rsp^{ins}, resulting in the gametic lethality of Rsp^{sens}-bearing spermatids; *Sd/Sd* homomultimers bind neither at Rsp^{sens} nor Rsp^{ins}. This model accounts for distortion in *Sd* Rsp^{ins}/Sd^+ Rsp^{sens} males, suicide behavior in *Sd* Rsp^{sens}/Sd^+ Rsp^{ins} males, the absence of distortion in *Sd* Rsp^{ins}/Sd^+ Rsp^{ins} males, and the near sterility of homozygous *SD* males.

A prediction from this model is that males carrying *Sd* and a deficiency for *Sd* would be sterile or nearly so, because only *Sd/Sd* homomultimers can be produced. However, it was observed in these studies that the various deletions at the base of *2L* shown in Figure 3 in combination with an *SD* chromosome are male fertile.

Even more revealing is the finding that SD^{Rev}-*37*, which we have argued is a deletion of *Sd*, has *greater* fecundity in combination with *SD-72* than does the unirradiated *SD-5* chromosome (Table 11). This implies that the sterility of homozygous *SD* males is not the consequence of a *failure* of an *Sd/Sd* homomultimer to carry out a required function, but suggests, rather, that each *SD*-bearing chromosome is *actively* doing something deleterious to the other, causing both to become gametic lethals. A deletion of *Sd* on one chromosome would then restore partial fertility.

This leads to a model of distortion quite similar to that of HARTL's, but with the following revision: rather than the binding of an *Sd* product at the *Rsp* locus being a necessary step in spermiogenesis, it is this binding that *causes* sperm dysfunction (perhaps by preventing the chromosomal inactivation which would otherwise occur). A function is not ascribed to the Sd^+ locus in this model; indeed, Sd^+ may simply be the absence of *Sd*. Finally, it is assumed that the *Sd* product binds more readily to Rsp^{sens} than to Rsp^{ins} and that the amount of *Sd* product is limiting with respect to the number of binding sites available. In the simplest case, each *Sd* locus may be thought of as producing a single molecule of product and each *Rsp* locus capable of binding one such molecule. For simplicity we will not concern ourselves here with the possible role of *E(SD)*, or other enhancers or suppressors of *SD*, except to note that they might well influence the degree to which the *Sd* product binds at *Rsp* (HARTL 1973).

The model accounts, in a straighforward way, for distortion and suicide behavior. In addition, it can explain several other observations. Thus, if the *Sd* product, when not competed for by a Rsp^{sens} locus, can bind to a Rsp^{ins} site; in

Sd Rsp^{ins}/Sd^{+} Rsp^{ins} males there should be gametic dysfunction of either one homolog or the other but not both from any one spermatocyte. This would result in a k value of 0.50, but in a reduction in fecundity of one-half; this is the result reported by HARTL (1969). In homozygous *SD* males, two copies of the *Sd* product would be produced, both of which are free to attach at an unbound *Rsp* site, resulting in the near sterility seen in these males. Those cases reported by HARTL (1973) that show partial complementation for fertility are understandable if the affinity of an *Sd* product for the *Rsp^{ins}* site varies depending upon the particular *Sd* and *Rsp^{ins}* in question.

If the *Sd* locus were deleted or inactivated, a homozygous *SD (i.e., Sd$_i$ Rsp^{ins}/Sd$_j$ Rsp^{ins})* combination would be converted to *Sd Rsp^{ins}/[Sd] Rsp^{ins}*, which in this model is the equivalent of *Sd Rsp^{ins}/Sd^{+} Rsp^{ins}*. The fertility of these males should be only half that of normal males, but should be much higher than that of homogygous *SD* males. Thus, the model explains the increased fertility of *SD-72/SD^{Rev}-37* males compared to *SD-72/SD-5* males.

It will be noticed that many features of the proposed model bear a strong resemblance to a recent model of mammalian *X*-chromosome inactivation (BROWN and CHANDRA 1973). That model also invokes the existence of a locus which produces a single "informational entity" (corresponding to the *Sd* product in the present model) that binds to a specific locus (equivalent to *Rsp*), on either of the two *X* chromosomes present in the nucleus causing that *X* chromosome to remain active while its homolog is inactivated. These ideas of BROWN and CHANDRA formed the basis of the revision of the HARTL (1973) model suggested here.

It is apparent that segregation distortion and *X*-chromosome inactivation share many similarities. In both systems, two homologs, initially equivalent, have different fates; one of the homologs remains active while the other becomes inactive. In both cases, the state of the entire chromosome appears to depend ultimately on a primary effect at a single regulatory site (CATTANACH, POLLARD and PEREZ 1970; RUSSELL and MONTGOMERY 1970). There are even alleles of a controlling element in mice with the property that an *X* chromosome carrying such an allele is more likely than its homolog to be inactivated (CATTANACH 1975). The apparent similarities between the two systems may not be fortuitous. LIFSCHYTZ and LINDSLEY (1972) have pointed out the significance of *X*-chromosome inactivation as a normal step in spermiogenesis in male heterogametic organisms, and suggest that the inactivation of *X* chromosomes in the somatic cells of female mammals evolved from this system. It is not unreasonable to imagine that a controlling system also exists to bring about the inactivation of the autosomes for the completion of spermiogenesis, and that *SD* represents an anomaly in this system.

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