On the Components of Segregation Distortion in *Drosophila melanogaster*. IV. Construction and Analysis of Free Duplications for the *Responder* Locus

John G. Brittnacher and Barry Ganetzky[1]

Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT

Male Drosophila heterozygous for an *SD*-bearing second chromosome and a normal homolog preferentially transmit the *SD* chromosome to their offspring. The distorted transmission involves the induced dysfunction of the sperm that receive the SD^+ chromosome. The loci on the *SD* chromosome responsible for causing distortion are the *Sd* locus the the *E(SD)* locus. Their target of action on the SD^+ chromosome is the Rsp^s locus. Previous studies of Rsp^s indicated that deletion of this locus rendered a chromosome insensitive to the action of *SD* and mapped Rsp^s physically within the centric heterochromatin of *2R*. In this study we have constructed a collection of marked free duplications for the centromeric region of a second chromosome that carried Rsp^s. The heterochromatic extent of each duplication as well as its sensitivity to distortion was determined. We found that Rsp^s is the most proximal known locus within the *2R* heterochromatin. Furthermore, our results demonstrate that the presence of Rsp^s is not only necessary but sufficient to confer sensitivity to distortion irrespective of its association with an intact second chromosome or one that pairs meiotically with an *SD* chromosome. By use of these duplications we increased the usual dosage of Rsp^s relative to *SD* to determine whether there was any competition for limited amounts of *SD* [and/or *E(SD)*] product. When two Rsp^s-bearing chromosomes are present within the same spermatocyte nucleus an *SD* chromosome is capable of causing efficient distortion of both. However, at least in some cases the degree of distortion against a given Rsp^s was reduced by the presence of an extra dose of Rsp^s indicating that there was some competition between them. The bearing of these results on present models of segregation distortion are discussed.

SEGREGATION distorter (*SD*) chromosomes in *Drosophila melanogaster* are transmitted from *SD*/SD^+ males in great excess over the Mendelian expectation as a consequence of the induced dysfunction of those sperm receiving the SD^+ homolog (SANDLER, HIRAIZUMI and SANDLER 1959; NICOLETTI, TRIPPA and DEMARCO 1967; HARTL, HIRAIZUMI and CROW 1967; reviewed by HARTL and HIRAIZUMI 1976; SANDLER and GOLIC 1985). The molecular details of this dysfunction are not yet understood but it appears to involve a failure of chromatin condensation in those spermatid nuclei that contain the SD^+ chromosome (NICOLETTI 1968; TOKUYASU, PEACOCK and HARDY 1977). Failure of chromatin condensation leads to subsequent defects in the maturation of these spermatids.

Dissection of *SD* chromosomes by recombination and by analysis of deletions has led to the identification and cytological localization of the major loci responsible for distortion. These include the *Sd* (*Segregation distorter*) locus in region 37D2-6, the *E(SD)* [*Enhancer of (SD)*] locus in the *2L* centric heterochromatin, and the *Rsp* (*Responder*) locus in the *2R* centric heterochromatin (GANETZKY 1977; BRITTNACHER and GANETZKY 1983, 1984; SHARP, HILLIKER and HOLM 1985). The *Sd* and *E(SD)* loci are jointly responsible for producing a high level of distortion; deletion of *Sd* renders an *SD* chromosome incapable of causing distortion, whereas deletion of *E(SD)* reduces the strength of distortion but does not eliminate it completely. The *Rsp* locus behaves as the target of distortion. Various alleles of the *Rsp* locus have been distinguished based on their sensitivity to distortion (MARTIN and HIRAIZUMI 1979, HIRAIZUMI, MARTIN and ECKSTRAND 1980; TEMIN and MARTHAS 1984; LYTTLE, BRITTNACHER and GANETZKY 1986). *SD* chromosomes as well as some SD^+ chromosomes carry an insensitive *Rsp* allele (Rsp^i). The allele carried by the standard *cn bw* tester chromosome is called *Rsp* sensitive (Rsp^s). In addition, chromosomes that carry a supersensitive *Rsp* (Rsp^{ss}) allele have been identified that are even more sensitive to distortion than the standard *cn bw* tester chromosome. Deletion of Rsp^s from an SD^+ homolog renders that chromosome completely insensitive to distortion. Analysis of these components of the *SD* system has given rise to models of

[1] To whom correspondence should be addressed.

This paper is dedicated to the fond memory of LARRY SANDLER whose inspiration, guidance and friendship will be sorely missed.

distortion proposing that products specified by *Sd* [and/or *E(SD)*] act with deleterious effect at Rsp^s or Rsp^{ss} but not at Rsp^i loci to cause sperm dysfunction (GANETZKY 1977, LYTTLE, BRITTNACHER and GANETZKY 1986). The nature of the proposed interaction between *Sd* and *Rsp* and the immediate consequences of such an interaction remain unknown.

The generation of chromosomal deficiencies that deleted each of the individual loci involved in segregation distortion circumvented many of the problems associated with recombinational dissection of *SD* chromosomes and facilitated the analysis of the functional role of each of these components. More recently, the construction of insertional translocations that place *Sd* alone or together with *E(SD)* into the *Y* chromosome have provided a useful new set of experimental tools (LYTTLE 1986, LYTTLE, BRITTNACHER and GANETZKY 1986). These insertional translocations enable the construction of a new array of genotypes containing different combinations of the possible allelic alternatives of the *SD* elements and permit the relative dosage of these elements to be varied. Analysis of such genotypes has revealed further information about the mechanism of distortion.

To extend this type of experimental investigation of the *SD* system, we have constructed and analyzed small, free chromosome duplications that carry Rsp^s. Characterization of the genetic extent of these duplications has enabled us to refine the localization of *Rsp* within the *2R* centric heterochromatin relative to that obtained from the previous deletional analysis. In addition, we used the duplications to increase the dosage of sensitive *Rsp* elements relative to *Sd* to test the possibility that the *Rsp* loci would compete for a limited amount of *Sd* product. The results are discussed in light of our present understanding about the mechanism of segregation distortion. Recently, LYTTLE (1989) has succeeded in generating insertional translocations of the *Rsp* locus into the *Y* chromosome and has used these translocation to perform analyses similar to those described here.

MATERIALS AND METHODS

Chromosomes: For a complete description of the various markers see LINDSLEY and GRELL (1968).

cn bw was used as the standard sensitive tester chromosome in measuring the drive strength of *SD* chromosomes. It was also used as the starter chromosome in construction of the free duplications.

$In(2LR)lt^{G10}$,*cn bw* = *In(2LR)40;59F3* and $In(2LR)$-lt^{G16}, *cn bw* = *In(2LR)40;60E4* are pericentric inversions with one break proximal to the *lt* locus on *2L* and show variegated expression of lt^+. They were recovered in a screen for *lt* mutations following irradiation of the *cn bw* chromosome at 4000 rad.

C(2)EN, bw sp = *C(2R2L·2L2R)* a compound chromosome that contains two complete second chromosomes attached to a single centromere (NOVITSKI, GRACE and STROMMEN 1981).

lt pk cn bw is a supersensitive responder (Rsp^{ss}) chromosome derived by recombination between *lt pk cn* and *cn bw* (BRITTNACHER and GANETZKY 1984).

Rsp^{i16},*cn bw* (=Rsp^{ins}-*16* of GANETZKY 1977) was derived by radiation mutagenesis of the *cn bw* chromosome. It is homozygous viable and fertile and is completely insensitive to the action of *SD* (GANETZKY 1977).

SD-72 was isolated from a natural population in Madison (SANDLER, HIRAIZUMI and SANDLER 1959) and carries a pericentric inversion, *In(2LR)39D;42A*, and a paracentric inversion, *In(2R)NS* = *In(2R)52A2-B1;56F9-13*.

SD-Mad is an *SD-72* type chromosome recently isolated from a Madison population (TEMIN and MARTHAS 1984). It carries the same inversions as *SD-72* but *SD-Mad* homozygotes are viable and fertile in contrast with *SD-72*, which is lethal when homozygous.

SD-Roma, bw was produced by recombination between *SD-Roma*(=$SD^{R\text{-}1}$ of NICOLETTI and TRIPPA 1967) and *cn bw* (BRITTNACHER and GANETZKY 1984). The *SD-Roma* chromosome carries no structural rearrangements and is viable when homozygous.

Complementation tests with heterochromatic lethals: The details of the construction of the free duplications are described in the results. To assess the heterochromatic extent of each free duplication, tests were performed to determine which of the heterochromatic lethal complementation groups identified by HILLIKER (1976) were covered by the duplications. For these tests *y;l(2)EMS-i/In(2LR)O, Cy/Dp(2;f),*y^+ males were crossed to *y;l(2)EMS-i/In(2LR)O, Cy* females. The appearance of viable y^+;Cy^+ progeny among the offspring of the cross indicated that the lethal mutation being tested was covered by the duplication.

Tests of sensitivity of free duplications to distortion by *SD*: In theory, a male with one copy of a free duplication should transmit the duplication to half of his offspring. We found, in practice, that even from non-*SD* males the duplications were transmitted to fewer progeny than expected, presumably because of decreased viability or meiotic loss. To quantify the reduced recovery associated with each duplication we measured recovery values (R_{Dp}) as follows: for each duplication, ten males of genotype *y;cn bw/cn bw/ Dp(2;f),*y^+ were mated individually by two *y;cn bw/cn bw* females in a vial at 25° for 5 days, and the offspring counted through day 18. This mating protocol was also used for all subsequent experiments. R_{Dp} for each duplication was then defined as

$$R_{Dp} = [y;cn\ bw/cn\ bw/Dp(2;f),y^+ \text{ offspring}] \div [y;cn\ bw/cn\ bw \text{ offspring}]$$

where a duplication that had no effects on viability and had no meiotic loss would have a value of 1.00. Recovery values are reported for each duplication in Table 1.

In subsequent crosses in which the sensitivity of the duplications to *SD* was measured, it was necessary to correct the observed number of offspring for the reduced recovery of the duplications seen in non-*SD* males. For example, *y;SD/cn bw/Dp(2;f),*y^+ males were mated to *y;cn bw/cn bw* females using the standard protocol and four classes of offspring were counted that were derived from sperm containing: (1) an *SD* chromosome alone, (2) a free duplication with an *SD* chromosome, (3) a *cn bw* chromosome alone, and (4) a free duplication with a *cn bw* chromosome (*cf.* Figure 3). Recovery values were calculated using the class of *SD* offspring as the standard since sperm containing the *SD* chromosome alone are not expected to undergo any dys-

TABLE 1

List of free duplications for the centromeric region of the second chromosome generated in this study, their extent and their recovery values from non-*SD* males

Duplication	Chromosome order	Region of *2R* heterochromatin covered				R_{Dp}
		I	II	III	IV	
Dp(2;f)e24	y^+Y^S|21C6-23A1|42D1·40|59F3-60F	+	+	+	+	0.654 ± 0.049 (677)
Dp(2;f)e57	y^+Y^S|21C6-21D3|41F1·40|59F3-60F	+	+	+	+	0.875 ± 0.078 (687)
Dp(2;f)e97	y^+Y^S|21C6-21E1|42A1·40|59F3-60F	+	+	+	+	0.710 ± 0.053 (699)
Dp(2;f)e1	y^+Y^S|21C6-21D2|41·40|59F3-60F	+	+	+	−	0.670 ± 0.084 (740)
Dp(2;f)e29	y^+Y^S|21C6-21D1|41·40|59F3-60F	+	+	+	−	0.543 ± 0.049 (677)
Dp(2;f)e44	y^+Y^S|21C6-22A1|41·40|59F3-60F	+	+	+	−	0.499 ± 0.028 (687)
Dp(2;f)e58	y^+Y^+|21C6-22A1|41·40|59F3-60F	+	+	+	−	0.595 ± 0.062 (664)
Dp(2;f)e61	y^+Y^S|21C6-22A1|41·40|59F3-60F	+	+	+	−	0.646 ± 0.070 (699)
Dp(2;f)e74	y^+Y^S|21C6-22A3|41·40|59F3-60F	+	+	+	−	0.692 ± 0.092 (674)
Dp(2;f)e87	y^+Y^S|21C6-22A1|41·40|59F3-60F	+	+	+	−	0.556 ± 0.046 (685)
Dp(2;f)e5	y^+Y^S|21C6-22A1|41·40|59F3-60F	+	+	−	−	0.598 ± 0.082 (695)
Dp(2;f)e51	y^+Y^S|21C6-22A4|41·40|59F3-60F	+	+	−	−	0.634 ± 0.051 (691)
Dp(2;f)e55	y^+Y^S|21C6-22C1|41·40|59F3-60F	+	+	−	−	0.566 ± 0.054 (724)
Dp(2;f)e72	y^+Y^S|21C6-22D1|41·40|59F3-60F	+	+	−	−	0.567 ± 0.070 (679)
Dp(2;f)e83	y^+Y^S|21C6-24D1|41·40|59F3-60F	+	+	−	−	0.506 ± 0.053 (255)
Dp(2;f)e70	y^+Y^S|21C6-22B3|41·40|59F3-60F	−	−	−	−	0.670 ± 0.067 (631)
Dp(2;f)f45	y^+Y^S|21C6-25B1|41·40|60E4-60F	+	+	+	+	0.737 ± 0.060 (638)
Dp(2;f)f82	y^+Y^S|21C6-22A1|41F1·40|60E4-60F	+	+	+	+	0.855 ± 0.091 (651)
Dp(2;f)f29	y^+Y^S|21C6-21D1|41·40|60E4-60F	+	+	+	−	0.878 ± 0.072 (720)
Dp(2;f)f52	y^+Y^S|21C6-25D1|41·40|60E4-60F	+	+	+	−	0.821 ± 0.045 (733)
Dp(2;f)f53	y^+Y^S|21C6-22B3|41·40|60E4-60F	+	+	+	−	0.768 ± 0.039 (712)
Dp(2;f)f62	y^+Y^S|21C6-24D2|41·40|60E4-60F	+	+	+	−	0.808 ± 0.111 (673)
Dp(2;f)f74	y^+Y^S|21C6-21E1|41·40|60E4-60F	+	+	+	−	0.717 ± 0.059 (740)
Dp(2;f)f77	y^+Y^S|21C6-22A1|41·40|60E4-60F	+	+	+	−	1.021 ± 0.075 (841)
Dp(2;f)f84	y^+Y^S|21C6-23C1|41·40|60E4-60F	+	+	+	−	0.724 ± 0.051 (715)
Dp(2;f)f6	y^+Y^S|21C6-25B1|41·40|60E4-60F	+	+	−	−	0.619 ± 0.037 (588)
Dp(2;f)f15	y^+Y^S|21C6-23D1|41·40|60E4-60F	+	+	−	−	0.843 ± 0.073 (652)
Dp(2;f)f48	y^+Y^S|21C6-25B1|41·40|60E4-60F	+	+	−	−	0.689 ± 0.030 (586)
Dp(2;f)f49	y^+Y^S|21C6-24D2|41·40|60E4-60F	+	+	−	−	0.696 ± 0.048 (738)
Dp(2;f)f67	y^+Y^S|21C6-22F1|41·40|60E4-60F	+	+	−	−	0.718 ± 0.073 (800)
Dp(2;f)f80	y^+Y^S|21C6-22A1|41·40|60E4-60F	+	+	−	−	0.794 ± 0.049 (964)

The duplications designated with the letter "*e*" in their stock number were all derived from $In(2LR)lt^{G10}$; those designated with the letter "*f*" were all derived from $In(2LR)lt^{G16}$. The division of the *2R* heterochromatin into separate regions is based on the analysis of HILLIKER (1976). The heterochromatic extent of each duplication was determined by complementation tests with lethal mutations from each of the complementation groups as described in MATERIALS AND METHODS. From Region I, *l(2R)EMS-31* was used; from Region II, *l(2R)EMS45-39*; from region III, *l(2R)EMS45-73*; and from region IV, *l(2R)34-2*. The visible marker *rl* is located in region II and the marker *uex* is located in region III. R_{Dp} is the ratio of y^+;*cn bw* to *y*; *cn bw* flies in crosses of *y;cn bw/cn bw/Dp(2;f)*,y^+ males to *y*, *cnbw/cnbw* females. Each value represents an unweighted average from ten males ± the standard error. The number of flies counted for each duplication is given in parentheses.

function. The recovery value of a duplication from an *SD* male was then defined as

$$R_{Dp;SD} = (y;SD/cn\ bw/Dp(2;f)y^+ \text{ offspring} \div R_{Dp}) \div (y;SD/cn\ bw \text{ offspring}).$$

Dividing the number of *y;SD/cn bw/Dp(2;f)*y^+ offspring by R_{Dp} gives the number of *y;SD/cn bw/Dp(2;f)*y^+ offspring corrected upwards for the previously measured viability reduction associated with a duplication.

$R_{Dp;cn\ bw}$ was similarly defined as

$$R_{Dp;cn\ bw} = (y;cn\ bw/cn\ bw/Dp(2;f)y^+ \text{ offspring} \div R_{Dp}) \div (y;SD/cn\ bw \text{ offspring}).$$

Transmission of the *cn bw* chromosome (or one of its derivatives) was measured as

$$R_{cn\ bw} = (y;cn\ bw/cn\ bw \text{ offspring}) \div (y;SD/cn\ bw \text{ offspring})$$

or as

$$k = (y;SD/cn\ bw \text{ offspring}) \div (y;cn\ bw/cn\ bw + y;SD/cn\ bw \text{ offspring}).$$

For these *k* values, only the non-duplication-bearing offspring were included in the calculation.

RESULTS

Construction of free duplications: The general scheme for the construction of *Rsp*-bearing free duplications is diagrammed in Figure 1. To construct free duplications containing the centromeric region

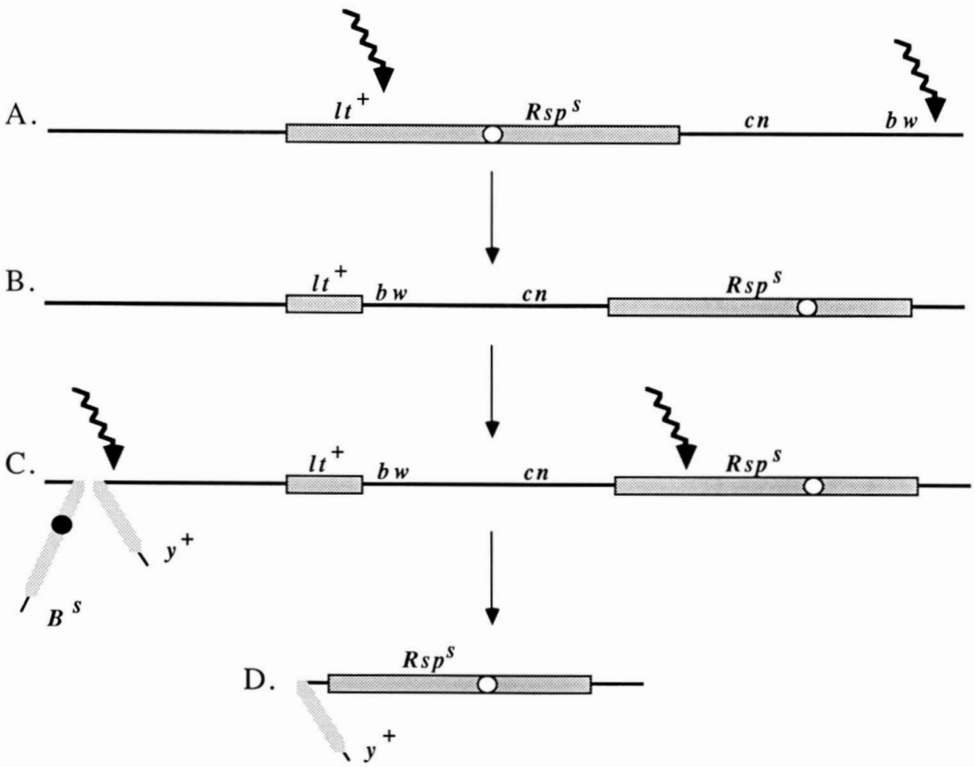

FIGURE 1.—General scheme for the construction of free duplications for chromosome 2. Stippled bars represent heterochromatin of chromosome 2 and the *Y* chromosome; thin black lines represent euchromatin. **A,** A standard Rsp^s-bearing *cn bw* chromosome was irradiated and screened for new *lt* mutations. Two of these mutations were associated with pericentric inversions with breaks near *lt* and distal to *bw* as shown in **B**. The inverted chromosomes were recombined with $T(Y;2)L124, B^s y^+$, which is broken in distal *2L*, to place a y^+ marker on the inverted chromosomes. The recombined inverted chromosomes are diagrammed in **C**. The y^+-marked inverted chromosomes were irradiated to delete most of the euchromatic portion of the second chromosome. These deletions were selectively recoverable as free duplications, diagrammed in **D**, in offspring that received *C(2)EN* from their mother. See text for further details.

of the second chromosome, we took advantage of pericentric inversions that moved the centromere to the distal tip of *2R* (Figure 1, A and B). Among a group of X-ray induced *lt* variegating mutations generated on the standard *cn bw* tester chromosome, two chromosomes were found to be pericentric inversions with one break proximal to lt^+ in the *2L* heterochromatin and the other break distal to *bw* on *2R*. These two inversions, $In(2LR)lt^{G10}$, *cn bw* and $In(2LR)lt^{G16}$, *cn bw*, were as sensitive to distortion by *SD* chromosomes as the parental *cn bw* chromosome.

To provide a convenient genetic marker to follow the duplications after their construction, we placed y^+ onto the tip of *2L* by isolating recombinants between the inverted chromosomes and $T(Y;2)L124$, $B^s y^+$, which is broken in distal *2L* at 21C4-6 and in Y^s of $y^+ Y B^s$ (LINDSLEY *et al.* 1972). A single exchange event in *2L* moved the y^+-capped tip of the translocation onto each of the inverted chromosomes. The resulting recombinant chromosome is illustrated in Figure 1C. The distalmost portion of the *2L* tip was attached to the proximal portion of the *Y* chromosome marked with B^s and was lost by segregation at a later step in the construction of duplications. The recombined inversions were again tested for sensitivity to *SD* and were found to remain as sensitive as the parental *cn bw* chromosome.

Free duplications were then recovered by irradiating males (3000–4000 rad) carrying the y^+-marked inverted chromosomes (Figure 1, C and D) and mating them to *y*; *C(2) EN*, *bw sp* females (NOVITSKI, GRACE and STROMMEN 1981). These females produce two kinds of gametes with respect to the second chromosome: diplo-*2* and nullo-*2*. Therefore, when crossed to unirradiated males bearing normal chromosomes *2*, these females produce very few viable offspring because most of the zygotes will be aneuploid for chromosome *2*. The rare survivors are usually triploids or result from nondisjunction of chromosome *2* in the male. However, sperm bearing free duplications resulting from deletion of most of the euchromatic portion of the inverted chromosomes can give rise to viable offspring when they fertilize diplo-*2* eggs. Thus, duplication-bearing offspring can be selectively recovered. In practice, about one in seven of the viable y^+ offspring produced by irradiated males yielded a recoverable free duplication.

For subsequent experiments it was necessary to remove the free duplications from the *C(2)EN* background into free chromosome *2* stocks. For this purpose we crossed $y;C(2)EN;Dp(2;f)y^+$ males with $y; mei^{S332}$, *cn* females. Because of the nondisjunction caused by mei^{S332} (DAVIS 1971), diplo-*2* and null-*2* eggs are generated, which allow the recovery of viable progeny resulting from the fusion of diplo-*2* eggs with sperm bearing a *Dp(2;f)* but no other second chromosome material. Duplication-bearing male offspring ($y;mei^{S332}$, cn/mei^{S332}, $cn;Dp(2;f)y^+$) from that cross were mated to $y;l(2)EMS\text{-}31,bw/In(2LR)O,Cy$ females. The *l(2)EMS-31* mutation is a recessive lethal located in the *2R* heterochromatin (HILLIKER 1976). Male and female progeny from that mating were inter-

crossed to establish balanced *y;l(2)EMS-31,bw/l(2)EMS-31,bw;Dp(2;f)y*$^+$ stocks for all of the duplications except the smallest, *Dp(2;f)e70*, which does not cover the lethal mutation (see below). The euchromatic extent of each duplication was determined by cytological analysis of polytene chromosomes (Figure 2).

The heterochromatic content of each duplication was assessed in complementation tests with the recessive lethal mutations in the *2L* and *2R* heterochromatin isolated by HILLIKER (1976). As expected, none of the duplications covered *l(2)EMS 56-3*, which is at the *lt* locus, confirming the break proximal to *lt*$^+$ in both inversions used to generate the duplications.

The *2R* heterochromatic content of each duplication is expected to vary depending on the location of the radiation-induced breakpoint in its construction. The results of complementation tests with the four most proximal lethal complementation groups in *2R* for each of the duplications are summarized in Table 1. The most proximal lethal (group I) is covered by all of the duplications except *Dp(2;f)e70*, which therefore has a *2R* break proximal to all previously identified loci. Five of the duplications covered all of the lethal complementation groups in the *2R* heterochromatin and were broken in the proximal portion of the *2R* euchromatin. The remaining duplications cover some but not all of the lethal complementation groups enabling the heterochromatic *2R* break to be located between a pair of adjacent complementation groups.

The recovery frequency of each duplication when transmitted from *y;cn bw/cn bw/Dp(2;f),y*$^+$ males is shown in Table 1 as R_{Dp}. All of the duplications were associated with some degree of meiotic loss or inviability since the recovery values were generally much less than 1.00. In general, the recovery values for the *e*-series duplications tended to be lower than for the *f*-series. Otherwise, no significant trends in the recovery values with respect to the location of the breaks or the size of the duplication are apparent. The duplications were mitotically stable since no cuticular mosaicism for *y* was observed.

Sensitivity of duplications to *SD*: To assay for the presence of *Rsp*^{s}, the sensitivity of each duplication to *SD* was measured in crosses of *y;SD/cn bw/Dp (2;f)y*$^+$ males to *y;cn bw* females. A measure of sensitivity of each duplication can be obtained in these crosses from the recovery value ($R_{Dp;SD}$). This value is the ratio of offspring that carry both the *SD* chromosome and the duplication to those that carry the *SD* chromosome without the duplication (Figure 3). For a duplication completely insensitive to distortion this ratio should be 1.00 (after correcting for any viability deficit associated with the duplication). Conversely, a duplication that is completely sensitive to distortion would have an $R_{Dp;SD}$ value close to zero. The results of these crosses, shown in Table 2, indicate that all 31 of the duplications are sensitive to distortion. *Dp(2;f)e87* appears to be somewhat less sensitive than the other duplications. No other marked differences in sensitivity among the duplications are apparent nor is there any apparent correlation between the sensitivity of a duplication and its heterochromatic or euchromatic size. Of particular interest is the fact that *Dp(2;f)e70*, which is deleted for all the known lethal complementation groups in the heterochromatin, still remains completely sensitive to distortion.

Is the sensitivity of the duplications the same as that of the parental *cn bw* chromosomes from which they are derived? Because the free duplications, unlike an intact homolog, do not segregate regularly from the *SD* chromosome, it is not possible to calculate *k* values for the duplications that are exactly comparable to those measured for intact second chromosomes. However, *k* values for the duplications can be estimated from crosses of *y;SD/Rsp*^{i16}*,cn bw/Dp(2;f)y*$^+$ males to *y; cn bw* females if the reasonable assumption is made that reduction of the *y;SD/cn bw/Dp(2;f)y*$^+$ and *y;Rsp*^{i}*, cn bw/cn bw/Dp(2;f)y*$^+$ classes of offspring relative to the *y;SD/cn bw* and y;*Rsp*^{i}*,cn bw/cn bw* sibs (after appropriate viability corrections have been made) is owing to distortion against the *Rsp*^{s}-bearing duplication. In that case, a *k* value can be calculated as the proportion of offspring lacking a free duplication divided by the total progeny. From data presented in Table 3, mean *k* values (corrected for viability) of the duplications can be calculated as 0.992, 0.950, and 0.719 with *SD-72*, *SD-Mad* and *SD-Roma,bw*, respectively. These values are somewhat lower than the standard *k* values we measured against the *cn bw* chromosome, which were 1.000, 0.994 and 0.928 (Table 4). These results suggest that the duplications may be somewhat less sensitive to distortion by *SD* than the *cn bw* chromosome from which they were derived. Although it appears that the reduction in sensitivity is greater for *SD-Roma, bw* than for *SD-72* or *SD-Mad*, *k* values are nonlinear measurements of the amount of sperm dysfunction and it is difficult directly to compare changes in *k* value for chromosome of very different drive strength. To avoid this problem, probit transformation of *k* values (MIKLOS and SMITH-WHITE 1971) is often used to provide a linear metric of sperm dysfunction (MIKLOS 1972; HARTL and HIRAIZUMI 1976, LYTTLE 1979, 1986). When such a transformation is performed on the above *k* values, the data suggest that the sensitivity of the duplications to distortion is reduced to approximately the same degree (about 1.13, 0.89 and 1.14 probits, respectively) for all of the *SD* chromosomes tested. In any case, whereas the free duplications are somewhat less sensitive to distortion than an intact chromosome, the magnitude of this reduction is small.

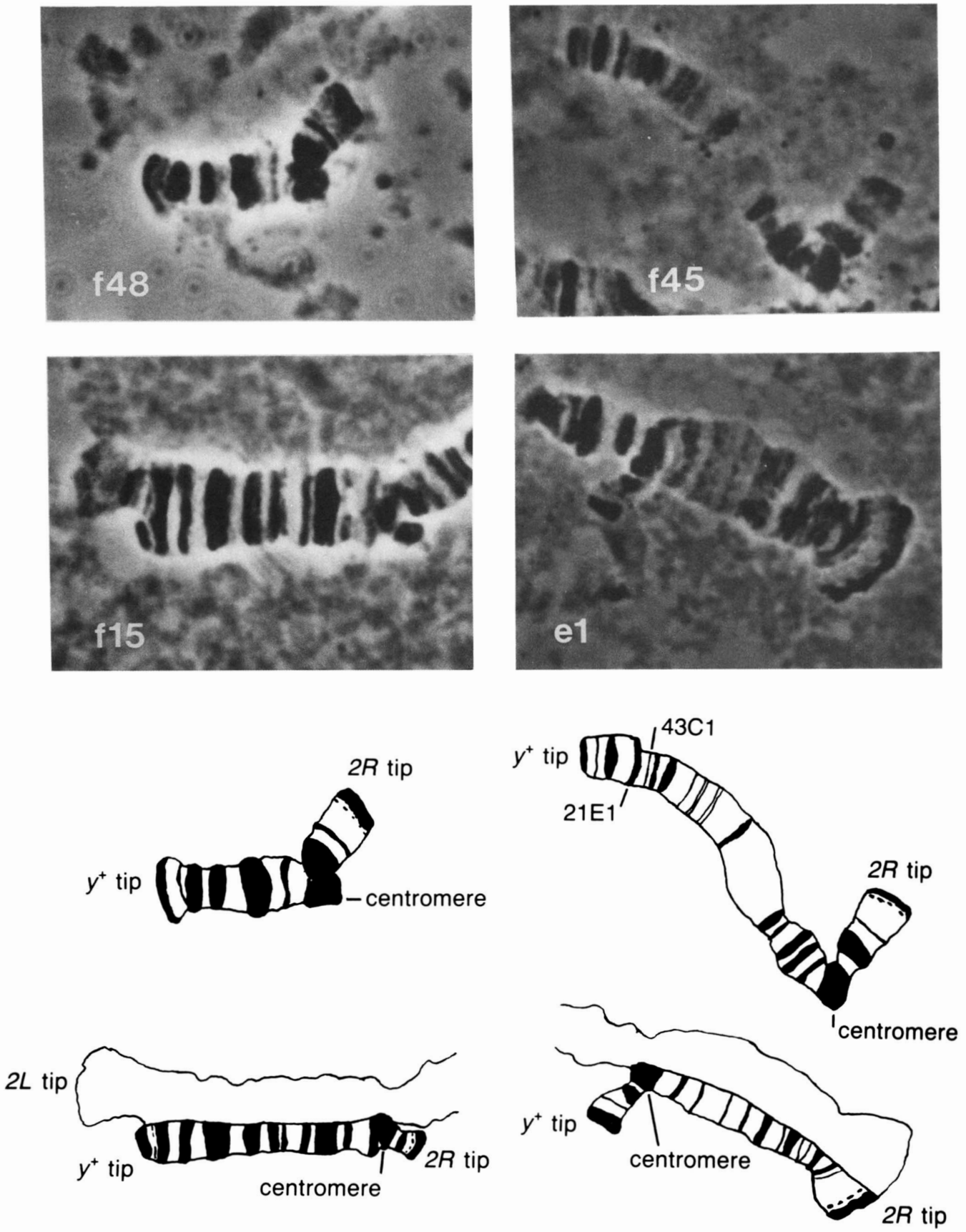

FIGURE 2.—(Upper) Photomicrographs of salivary chromosome squashes of representative examples of the free duplications produced in this study. (Lower) Drawings showing the interpretation of the salivary chromosome banding pattern for each of the free duplications pictured above.

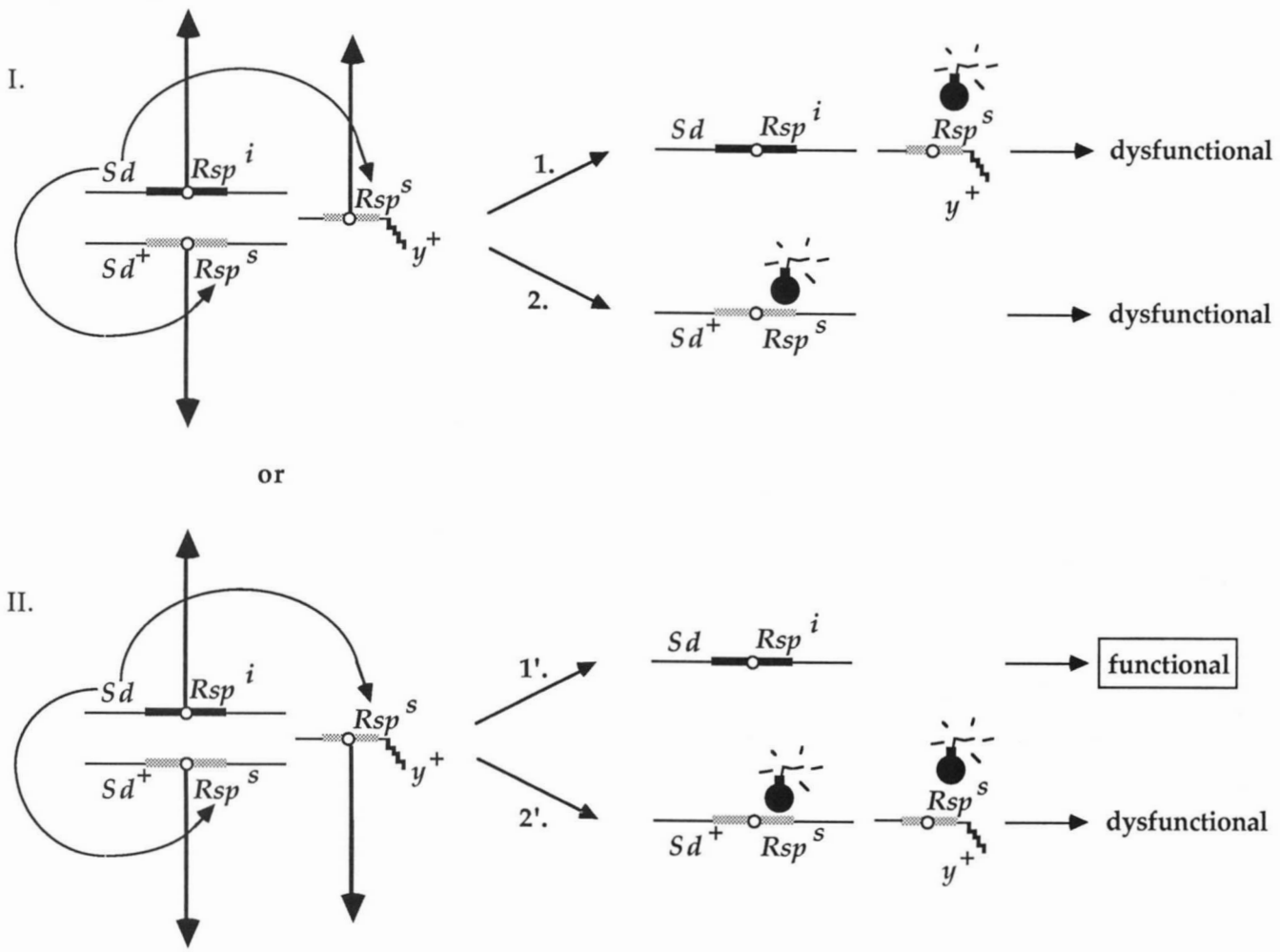

FIGURE 3.—The action of *SD* and the patterns of segregation and sperm recovery in an *SD/cnbw* male also carrying a *Rsp*^{s}-bearing free duplication. The *SD* chromosome is presumed to act upon both its homolog and the duplication at some point early in meiosis. Subsequently, the *SD* chromosome segregates from its homolog while the free duplication segregates randomly relative to the *SD* chromosome. In diagram I, the duplication segregates to the same pole as the *SD* chromosome giving rise to spermatid products 1 and 2. In diagram II, the duplication segregates to the same pole as the non-*SD* homolog generating spermatid products 1′ and 2′. In the absence of any distortion the four classes of spermatids should be recovered with equal frequency. However, the action of *SD* at either *Rsp*^{s} in the nucleus is presumed to be capable of causing the dysfunction of a spermatid that receives a *Rsp*^{s}-bearing chromosome element. Only spermatid class 1′ is expected to be fully functional. This class provides a standard against which the recovery of each of the other spermatid classes can be compared to provide a metric of distortion. $R_{Dp;SD}$ is a measure of the recovery of spermatids of class 1 relative to 1′. R_{cnbw} is a measure of the recovery of spermatids of class 2 relative to class 1′. $R_{Dp;cnbw}$ is a measure of the recovery of spermatids of class 2′ relative to class 1′. If the non-*SD* homolog carries an insensitive *Rsp* (e.g. *Rsp*^{i6}), both class 2 and class 1′ spermatids are expected to be fully functional, but the remaining two classes will still be dysfunctional.

The results therefore indicate that the presence of *Rsp*^{s} is sufficient to render a chromosomal element sensitive to distortion by *SD* and that meiotic pairing is not a prerequisite for distortion to occur.

Distortion in the presence of competing *Rsp* loci: From previous studies it was proposed that the *Sd* product might be made in limited amount that could be competed for by the available *Rsp* loci (GANETZKY 1977; BRITTNACHER and GANETZKY 1983). Using the free duplications it was possible to examine this idea by measuring distortion in *SD/cn bw/Dp(2;f)* males, which carry two doses of *Rsp*^{s}, in the presence of an intact *SD* chromosome. The results of these crosses are presented in Table 3. There are several points in these data worth noting: First, it appears that the activity provided by a single dose of *Sd* is sufficient to act effectively against two *Rsp*^{s} loci in the same spermatocyte nucleus. Thus, with *SD-72*, the *Rsp*^{s}-bearing duplication is eliminated 99% of the time ($R_{Dp;SD}$ = 0.010) while the *cn bw* chromosome is being simultaneously eliminated with an efficiency of over 99% (R_{cnbw} = 0.002). Similar results are seen with *SD-Mad* and *SD-Roma* although, since these are weaker distorters than *SD-72*, the recovery values both for the duplications and the *cn bw* chromosome are increased. Second, with each of the three *SD* chromosomes the sensitivity of the duplications is somewhat less than that of *cn bw* (compare $R_{Dp;SD}$ and $R_{cn\ bw}$) in agreement with results presented above. The relative difference in recovery values for the duplications compared with

TABLE 2

Results of crossing *y;SD/cnbw;Dp(2;f)y*$^+$ males with *y;cnbw* females

Duplication	y^+,*SD*	*y,SD*	y^+,*cn bw*	*y,cn bw*	$R_{Dp;SD}$
Dp(2;f)e24	5	461	0	3	0.017 ± 0.007
Dp(2;f)e57	11	581	0	3	0.023 ± 0.014
Dp(2;f)e97	12	593	0	6	0.025 ± 0.018
Dp(2;f)e1	6	541	0	2	0.014 ± 0.007
Dp(2;f)e29	32	457	1	10	0.137 ± 0.036[a]
Dp(2;f)e44	6	462	0	1	0.022 ± 0.013
Dp(2;f)e58	33	564	0	5	0.101 ± 0.035[a]
Dp(2;f)e61	6	448	0	7	0.013 ± 0.008
Dp(2;f)e74	4	432	0	2	0.009 ± 0.007
Dp(2;f)e87	92	473	2	41	0.395 ± 0.182[a]
Dp(2;f)e5	6	463	0	0	0.024 ± 0.012
Dp(2;f)e51	2	497	0	2	0.007 ± 0.005
Dp(2;f)e55	30	549	0	8	0.074 ± 0.041
Dp(2;f)e72	6	448	0	7	0.003 ± 0.003
Dp(2;f)e83	19	648	0	0	0.063 ± 0.023[a]
Dp(2;f)e70	0	581	0	4	0.000 ± 0.000
Dp(2;f)f45	16	628	0	8	0.039 ± 0.039
Dp(2;f)f82	0	674	0	0	0.000 ± 0.000
Dp(2;f)f29	2	728	0	0	0.004 ± 0.003
Dp(2;f)f52	13	531	0	8	0.034 ± 0.029
Dp(2;f)f53	15	397	0	3	0.054 ± 0.018
Dp(2;f)f62	3	456	1	0	0.013 ± 0.007
Dp(2;f)f74	30	634	3	19	0.060 ± 0.039
Dp(2;f)f77	5	600	0	4	0.008 ± 0.005
Dp(2;f)f84	1	395	0	0	0.003 ± 0.003
Dp(2;f)f6	0	628	0	0	0.000 ± 0.000
Dp(2;f)f15	1	685	0	2	0.001 ± 0.001
Dp(2;f)f48	0	426	0	0	0.000 ± 0.000
Dp(2;f)f49	5	705	0	6	0.010 ± 0.004
Dp(2;f)f67	7	743	0	3	0.014 ± 0.006
Dp(2;f)f80	17	732	0	9	0.032 ± 0.014

$R_{Dp;SD}$ is the ratio of y^+,*SD* to *y,SD* flies after correcting the former class for viability using the R_{Dp} values from Table 1. Each value represents the unweighted average from seven to ten males ± the standard error. A duplication insensitive to the action of *SD* is expected to have an $R_{Dp;SD}$ value of 1.00. The *SD-72* chromosome was used except where noted otherwise.

[a] *SD-Mad* was used.

the *cn bw* chromosome is least in the presence of the weakly distorting *SD-Roma* chromosome. Third, the *Dp; cn bw* class of offspring resulting from gametes bearing two doses of *Rsp*^{s} has the lowest recovery in these crosses. The observed recovery values for this class of offspring corresponds closely to the values predicted if *SD* acts independently at both *Rsp*^{s} loci present in the same nucleus [*i.e.*, $R_{\text{expected}} = (R_{Dp;SD})x(R_{cn\ bw})$].

We also measured recovery values when the duplications were competing against the *lt pk cn bw* chromosome, which bears a supersensitive *Rsp* locus, in *SD/lt pk cn bw/Dp(2;f)* males. The results (Table 3) clearly indicate that the *lt pk cn bw* chromosome is preferred over the duplications as a target for the action of *SD*. Regardless of the overall strength of distortion caused by a particular *SD* chromosome, the recovery of the duplication was at least tenfold greater than that of the *lt pk cn bw* chromosome (compare columns 1 and 3). Nonetheless, *SD* did not act on the supersensitive chromosome to the exclusion of any effect on the duplication since the $R_{Dp;SD}$ values all revealed substantial distortion of the duplication by *SD* in these males (column 1). However, for all three *SD* chromosomes, the $R_{Dp;SD}$ values were higher in *SD/lt pk cn bw/Dp(2;f)* males than in *SD/cn bw/Dp(2;f)* males (e.g. compare rows 1 and 2 for column 1 of *SD-72*). This result may indicate that when present in the same primary spermatocyte nucleus as a *Rsp*^{s}-bearing duplication, a supersensitive chromosome is a better competitor for *Sd* product than a *Rsp*^{s} chromosome, thereby enabling the duplications to be recovered at slightly greater frequencies when *lt pk cn bw* rather than *cn bw* is also segregating.

Unlike the situation in *SD/cn bw/Dp(2;f)* males, the observed recovery values for those offspring that received both the duplication and the supersensitive *lt pk cn bw* chromosome from their *SD/lt pk cn bw/Dp(2;f)* fathers do not appear to agree well with the recovery values predicted if *SD* were acting independently on *lt pk cn bw* and the duplications. Instead, the recovery values for the *Dp;lt pk cn bw*-bearing offspring are only slightly less than for those offspring receiving the *lt pk cn bw* chromosome alone. The results in this case seem to indicate that when two *Rsp* alleles of very different sensitivity are present in the same spermatid, its subsequent dysfunction depends primarily on the action of *SD* on the most sensitive *Rsp* allele present in the nucleus.

Recovery values for the duplications in the presence of an insensitive homolog (*Rsp*^{i16} *cn bw*) are also shown in Table 3. For *SD-72* and *SD-Mad* it is of interest to note that the value of $R_{Dp;SD}$ decreases in order as the non-*SD* homolog carries a *Rsp*^{ss}, *Rsp*^{s} or *Rsp*^{i} allele, respectively. In *SD/Rsp*^{i16} *cn bw/Dp(2;f)* males the duplication is expected to be the only sensitive target to distortion whereas competing targets are available in *SD/cn bw/Dp(2;f)* and *SD/lt pk cn bw/Dp(2;f)* males. Thus, the observed recovery of the duplication from *SD/Rsp*^{i16} *cn bw/Dp(2;f)* males relative to recovery from *SD/cn bw/Dp(2;f)* or *SD/lt pk cn bw/Dp(2;f)* males also gives some indication of a quantitative reduction in distortion when extra doses of *Rsp* in the same nucleus are able to compete for the action of *SD*. With *SD-Roma*, in contrast to the results just described, $R_{Dp;SD}$ is higher in *SD/Rsp*^{i16} *cn bw/Dp(2;f)* males than in *SD/cn bw/Dp(2;f)* or *SD/lt pk cn bw/Dp(2;f)* males. Why *SD-Roma* behaves differently in this respect from the other two *SD* chromosomes is unclear. Another point that can be noted in the data from *SD/Rsp*^{i16} *cn bw/Dp(2;f)* males is that the $R_{Dp;SD}$ and $R_{Dp;cn\ bw}$-values are in each case very similar to each other. This result demonstrates that the sperm dysfunction caused by the action of *SD* at *Rsp*^{s} is the same irrespective of whether an *SD* or *SD*$^+$ chromosome is

TABLE 3

Sperm recovery from *SD* males when *Rsp*^{s}-bearing free duplications are segregating in presence of *SD*$^+$ chromosomes of varying sensitivity

Male genotype	Number of offspring				Recovery values		
	y^+, *SD*	*y*, *SD*	y^+, *cn bw*	*y*, *cnbw*	$R_{Dp;SD}$	$R_{Dp;cnbw}$[a]	R_{cnbw}[a]
y; *SD-72/lt pk cn bw/Dp(2;f),y*$^+$	116	7397	5	13	0.022 ± 0.008	0.001 ± 0.001	0.002 ± 0.001
y; *SD-72/cn bw/Dp(2;f),y*$^+$	37	5296	1	12	0.010 ± 0.003	0.000 ± 0.000	0.002 ± 0.001
y; *SD-72/Rsp*^{i16}*,cn bw/Dp(2;f),y*$^+$	26	3445	18	3070	0.009 ± 0.005	0.006 ± 0.004	0.895 ± 0.019
y; *SD-Mad/lt pk cn bw/Dp(2;f),y*$^+$	814	6505	29	46	0.176 ± 0.055	0.006 ± 0.004	0.008 ± 0.005
y; *SD-Mad/cn bw/Dp(2;f),y*$^+$	327	7003	3	104	0.076 ± 0.002	0.001 ± 0.000	0.016 ± 0.006
y; *SD-Mad/Rsp*^{i16}*,cn bw/Dp(2;f),y*$^+$	145	3151	112	3058	0.058 ± 0.012	0.044 ± 0.010	0.966 ± 0.040
y; *SD-Roma/lt pk cn bw/Dp(2;f),y*$^+$	1535	6054	41	79	0.368 ± 0.051	0.009 ± 0.003	0.014 ± 0.004
y; *SD-Roma/cn bw/Dp(2;f),y*$^+$	775	3220	140	639	0.352 ± 0.047	0.068 ± 0.027	0.212 ± 0.040
y; *SD-Roma/Rsp*^{i16}*, cn bw/Dp(2;f),y*$^+$	996	3165	912	2994	0.468 ± 0.065	0.428 ± 0.054	0.950 ± 0.025

Males of the indicated genotype were crossed to *y;cn bw* females. For each series of crosses the number of offspring represent pooled data from the following duplications: *e1*, *e5*, *e57*, *e72*, *f6*, *f15*, *f29*, *f62* and *f67*. The recovery values are the unweighted means from the pooled data ± the standard error. The $R_{Dp;SD}$ and $R_{Dp;cnbw}$ values are corrected for viability differences using the R_{Dp} values from Table 1.

[a] Depending on the particular cross, $R_{Dp;cnbw}$ and R_{cnbw} represent the corresponding recovery values when the non-*SD* homolog is *lt pk cn bw*, *cn bw* or *Rsp*^{i16}*cn bw*.

TABLE 4

***k* values against the indicated *SD*$^+$ test chromosome in *SD* males lacking a *Rsp*^{s}-bearing free duplication compared with the *k* values in sib males that do carry such a duplication**

Male genotype	Number of offspring		*k*	χ^2
	SD	*cn bw*[a]		
SD-72/lt pk cn bw	1796	1	0.999 ± 0.001	
SD-72/lt pk cn bw/Dp(2;f)y$^+$	7397	13	0.998 ± 0.001	1.37
SD-72/cn bw	4119	1	1.000 ± 0.000	
SD-72/cn bw/Dp(2;f)y$^+$	5296	12	0.998 ± 0.001	6.86*
SD-72/Rsp^{i16}*,cn bw*	995	953	0.512 ± 0.012	
SD-72/Rsp^{i16}*,cn bw/Dp(2;f)y*$^+$	3445	3070	0.528 ± 0.005	1.95
SD-Mad/lt pk cn bw	2109	1	1.000 ± 0.000	
SD-Mad/lt pk cn bw/Dp(2;f)y$^+$	6505	46	0.992 ± 0.004	12.68**
SD-Mad/cn bw	2181	12	0.994 ± 0.003	
SD-Mad/cn bw/Dp(2;f)y$^+$	7003	104	0.984 ± 0.005	11.41**
SD-Mad/Rsp^{i16}*,cn bw*	1172	1221	0.489 ± 0.010	
SD-Mad/Rsp^{i16}*,cn bw/Dp(2;f)y*$^+$	3151	3058	0.510 ± 0.010	2.17
SD-Roma/lt pk cn bw	1642	10	0.994 ± 0.005	
SD-Roma/lt pk cn bw/Dp(2;f)y$^+$	6054	79	0.987 ± 0.004	5.37*
SD-Roma/cn bw	1427	107	0.928 ± 0.021	
SD-Roma/cn bw/Dp(2;f)y$^+$	3220	639	0.831 ± 0.025	84.58**
SD-Roma/Rsp^{i16}*, cn bw*	595	586	0.505 ± 0.017	
SD-Roma/Rsp^{i16}*, cn bw/Dp(2;f)y*$^+$	3165	2992	0.514 ± 0.007	0.40

Males of each of the indicated genotypes were to *y;cn bw* females and *k* values against the *SD*$^+$ homolog were calculated as the number of *SD* progeny divided by the total of *SD* and *cn bw* progeny. For the duplication-bearing males the data are extracted from Table 3, but only the offspring that did not inherit a free duplication were used to calculate the *k* values against a given test chromosome. Standard errors are shown.

[a] Depending on the cross, *cn bw* refers to the *lt pk cn bw*, the *cn bw* or the *Rsp*^{i16}*cn bw* homologs.

* $P < 0.05$; ** $P < 0.001$.

present in the same spermatid nucleus as the *Rsp*^{s} locus.

The experiments presented in Table 3 all indicate that when two *Rsp*^{s} alleles (or a *Rsp*^{s} and a *Rsp*^{ss} allele) are present in the same spermatocyte nucleus, a single dose of *Sd* and *E(SD)* can cause the effective distortion of both. However, the data also suggest that the degree of distortion against *Rsp*^{s} could be reduced by the presence of another *Rsp*^{s} (or *Rsp*^{ss}) allele in the same nucleus indicating that these alleles may be competing for the action of *SD*. To examine this possibility further and to quantify the competitive effect, we asked whether distortion of a given *SD*$^+$ homolog was reduced by the presence of a competing duplication

in distorting males. In Table 4 we compare *k* values against various test chromosomes in *SD/Rsp*^{x}*/Dp(2;f)* males with the corresponding *k* values in sib males (*SD/Rsp*^{x}) lacking the duplication. As expected, *Rsp*^{i16} *cn bw* is insensitive to distortion and the *k* value is not changed when the parental male carries a duplication. When the competing chromosome is *lt pk cn bw*, the presence of the duplication in the distorting males results in a decrease in *k* value and this decrease is significant for the *SD-Mad* and *SD-Roma* chromosomes. Since the latter two *SD* chromosomes are weaker distorters than *SD-72*, the effect of competition may be most apparent in situations where the strength of distortion is low enough to permit the detection of small changes in *k*. The competitive effect becomes still more apparent when the two *Rsp* alleles are of about equal sensitivity; for all *SD* chromosomes tested the *k* values against *cn bw* are clearly reduced by the presence of a competing duplication and the amount of reduction increases with decreasing strength of the *SD* chromosome (Table 4).

DISCUSSION

The previous isolation of deletions insensitive to the action of *SD* provided the first cytological localization of the *Rsp* locus. A region of heterochromatin was identified whose presence was necessary for a chromosome to be sensitive to *SD* (GENETZKY 1977). The construction of free duplications that are completely sensitive to *SD* confirms the previous mapping of *Rsp* and demonstrates that this region of heterochromatin is sufficient to confer sensitivity to distortion. Although the deletions localized the *Rsp* locus to the heterochromatin of *2R*, there remained some uncertainty about the precise location of *Rsp* within the heterochromatin. The present analysis removes this uncertainty. All 31 of the duplications that we recovered carry *Rsp*^{s} despite the fact that the construction scheme did not select for breaks in any particular region of *2R* heterochromatin. Furthermore, complementation tests indicated that the smallest of these duplications did not cover any of the vital loci in the *2R* heterochromatin suggesting that most or all of this heterochromatin had been deleted. Cytological analysis of this duplication in mitotic chromosome preparations confirms this interpretation (PIMPINELLI and DIMITRI 1989). Thus, the *Rsp* locus maps proximal to all previously known loci in the *2R* heterochromatin, a conclusion that is compatible with and clarifies the results of the deletional studies. Whether the location of *Rsp* so close to the centromere is functionally significant or merely fortuitous remains to be determined.

The sensitivity of the duplications permits some formal conclusions about the mechanism of distortion. It is clear that neither the recognition of *Rsp*^{s} nor the functional consequences of the action of *SD* at its target site require that *Rsp*^{s} be physically associated with an intact second chromosome. We cannot yet rule out, however, the possibility that the DNA sequences that immediately flank *Rsp* are important to its function. Furthermore, the gametic lethality engendered by interaction of *SD* with *Rsp*^{s} is dominant in that the resultant sperm dysfunction is not in any way ameliorated by the simultaneous presence of a complete and intact *SD* chromosome in the same secondary spermatocyte nucleus as the *Rsp*^{s}-bearing duplication.

The duplications also enabled us to vary the dosage of *Rsp*^{s} to gain further insight about competition between *Rsp* loci for the available *Sd* product. It was previously suggested that the *Sd* product is made in limited amount relative to the number of *Rsp* loci available in a normal diploid male. There were several observations that led to this idea. It had been noted that males heterozygous for some combinations of *SD* chromosomes (*e.g.*, *SD-5/SD-72*) had a very marked reduction in fecundity relative to *SD/SD*$^{+}$ males (HARTL 1974; GANETZKY 1977). Furthermore, ultrastructural studies indicated that the reduction in fecundity was associated with the production of dysfunctional sperm whose defect resembled that seen in *SD/SD*$^{+}$ males, but affecting the majority rather than just half of the developing sperm (PEACOCK, TOKUYASU and HARDY 1972; KETTANEH and HARTL 1980). We found that by deleting the *Sd* locus from either of the two *SD* chromosomes, the fecundity of *SD-5/SD-72* males was restored to a level comparable to that of *SD/SD*$^{+}$ males (GANETZKY 1977; BRITTNACHER and GANETZKY 1983). In contrast, the addition of extra doses of *Sd*$^{+}$ to *SD-5/SD-72* males did not restore fecundity (B. GANETZKY, unpublished results). Therefore, the reduced fecundity in *SD-5/SD-72* males was not owing to the absence of *Sd*$^{+}$ but apparently resulted from the presence of two doses of *Sd*. The pronounced difference in fecundity between males carrying one dose of *Sd* or two was consistent with limited expression of the *Sd* product.

This conclusion was also consistent with the observation that *Sd Rsp*^{s}*/Sd*$^{+}$*Rsp*^{s} males are fertile (with $k \approx 0.5$) despite the fact that the *Sd Rsp*^{s} chromosome is capable of causing substantial sperm dysfunction as indicated by its self-distortion in *Sd Rsp*^{s}*/Sd*$^{+}$*Rsp*^{i} males (HARTL 1974). If *SD* were acting with full effectiveness at both *Rsp*^{s} loci in *Sd Rsp*^{s}*/Sd*$^{+}$*Rsp*^{s} males, these males should be nearly sterile. However, if both *Rsp*^{s} loci were competing for a limited amount of *Sd* product such that in any one spermatocyte *Sd* acted at only one of the two *Rsp*^{s} loci with equal probability, the observed results could be accounted for.

In the experiments reported here there is additional

evidence for competition between *Rsp* loci for the action of *Sd*. Thus, the $R_{Dp;SD}$ values for the Rsp^s-bearing free duplications tended to be highest when a Rsp^{ss} chromosome was segregating in the same primary spermatocyte and least when a Rsp^i chromosome was segregating. Presumably, the Rsp^{ss} chromosome was the preferred target in the former case and the Rsp^s-bearing duplication was in the latter.

Nonetheless, although the results with the duplications are in general accord with the notion of competition for a limited amount of *Sd* product, the quantitative effect of this competition is less than we might have predicted. For example, in *SD-72/cn bw/Dp(2;f)* males more than 99% of the sperm containing either the duplication or the *cn bw* chromosome were not recoverable. Clearly, even if the Rsp^s loci carried by the duplication and the *cn bw* chromosome are acting to buffer each other to some extent against distortion, the single dose of *Sd* is capable of producing very strong distortion against both Rsp^s-bearing elements simultaneously. It is hard to reconcile this result with the fertility of $Sd\ Rsp^s/Sd^+Rsp^s$ males because if the single dose of *Sd* in this case were equally effective in distorting the two classes of Rsp^s-bearing sperm, these males should be nearly sterile. Apparently, distortion against the two Rsp^s loci is different in *SD/cn bw/Dp(2;f)* males compared with $Sd\ Rsp^s/Sd^+Rsp^s$ males, but the basis of this difference is not self-evident. The explanation may involve the fact that the intrinsic drive strength of the $Sd\ Rsp^s$ chromosome, as measured by its self-distortion when segregating from an Sd^+Rsp^i homolog is lower than a nonrecombined *SD* chromosome. As described in the RESULTS, our experiments indicated that competition between Rsp^s loci was most apparent when distortion was of intermediate strength. Another relevant consideration may be the meiotic behavior of free duplications compared with an intact chromosome. Although meiotic pairing is not a prerequisite for distortion, perhaps competition between two Rsp^s loci is most effective when the chromosomes carrying these loci are paired. If so, a Rsp^s locus carried by a free duplication, which segregates independently of the *cn bw* chromosome in *SD/cn bw/Dp(2;f)* males, would be a less effective competitor than the same Rsp^s carried on an intact second chromosome.

In any case, it appears that none of the currently proposed models for the mechanism of *SD* is sufficient to explain fully all the observations described above. Despite the recent advances in elucidating the details of segregation distortion essential pieces of the puzzle still seem to be missing. Perhaps the molecular analyses of the *Sd* (P. POWERS and B. GANETZKY, unpublished results) and *Rsp* (WU *et al.* 1988) loci now underway will help provide the missing clues.

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LITERATURE CITED

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