The Independent Distorting Ability of the *Enhancer of Segregation Distortion, E(SD)*, in *Drosophila melanogaster*

Rayla Greenberg Temin

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

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ABSTRACT

Segregation distortion is a meiotic drive system, discovered in wild populations, in which males heterozygous for an *SD* chromosome and a sensitive SD^+ homolog transmit the *SD* chromosome almost exclusively. *SD* represents a complex of three closely linked loci in the centromeric region of chromosome 2: *Sd*, the *Segregation distorter* gene; *E(SD)*, the *Enhancer of Segregation Distortion*, required for full expression of drive; and *Rsp*, the target for the action of *Sd*, existing in a continuum of states classifiable into sensitive (Rsp^s) and insensitive (Rsp^i). In an SD/SD^+ male which is *Sd E(SD)* Rsp^i/Sd^+ $E(SD)^+$ Rsp^s, the *Sd* and *E(SD)* elements act jointly to induce the dysfunction of those spermatids receiving the Rsp^s chromosome. By manipulating the number of copies and the position of the Enhancer region, I demonstrated that: (1) *E(SD)*, whether in its normal position or translocated to the *Y* chromosome, is able to enhance the degree of *Sd*-caused distortion in a dosage-dependent manner; (2) even in the absence of *Sd*, the *E(SD)* allele in two doses can cause significant distortion, in Sd^+ or *Df(Sd)*-bearing genotypes; (3) quantitative differences among Enhancers of different sources suggest allelic variation at *E(SD)*, which could account at least in part for differences among wild *SD* chromosomes in strength of distortion; (4) *E(SD)/E(SD)*-mediated distortion, like that of *Sd*, is directed at the *Rsp* target, whether *Rsp* is on the second or the *Y* chromosome; (5) *E(SD)*, like *Sd*, is suppressed by an unlinked dominant suppressor of *SD* action. These results show that *E(SD)* is independently capable of acting on *Rsp* and is not a simple modifier of the action of *Sd*. *E(SD)* provides an example of a *trans*-acting gene embedded in heterochromatin that can interact with another heterochromatic gene, *Rsp*, as well as parallel the effect of a euchromatic gene, *Sd*.

SEGREGATION distortion is a naturally occurring system of meiotic drive in *Drosophila melanogaster* controlled by the *SD* gene complex on the second chromosome. In a notable departure from the Mendelian expectation, males heterozygous for Segregation Distorter transmit the *SD* chromosome in great excess over a sensitive SD^+ homolog. The original discovery and description of *SD* is documented in HIRAIZUMI and CROW (1960); HIRAIZUMI, SANDLER and CROW (1960); SANDLER, HIRAIZUMI, and SANDLER (1959); and SANDLER and HIRAIZUMI(1959, 1960b); the *SD* system is reviewed in HARTL and HIRAIZUMI (1976); CROW (1979); SANDLER and GOLIC(1985) and TEMIN *et al.* (1991). The basis for the distorted transmission ratio is an induced dysfunction of those spermatids that receive the SD^+ chromosome during meiosis, manifested as a set of striking morphological aberrancies during spermiogenesis, including the failure of chromatin to condense (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DE MARCO 1967; TOKUYASU, PEACOCK and HARDY 1977).

The *SD* complex comprises three loci straddling the centromeric region of chromosome 2: *Sd*, the *Segregation distorter* gene; *Rsp*, the *Responder*, which serves as the target site for the action of *Sd*; and *E(SD)*, the *Enhancer of Segregation Distortion*, a gene required for the full expression of drive (Figure 1). *Rsp* exists in a continuum of states ranging from insensitive to supersensitive, but the strains used for this paper fall into three nonoverlapping classes, sensitive (Rsp^s), insensitive (Rsp^i), and supersensitive (Rsp^{ss}) (MARTIN and HIRAIZUMI 1979; HIRAIZUMI, MARTIN and ECKSTRAND 1980; HIRAIZUMI and THOMAS 1984; TEMIN and MARTHAS 1984; LYTTLE, BRITTNACHER and GANETZKY 1986). *SD* chromosomes isolated from nature are *Sd E(SD)* Rsp^i, carrying the insensitive Responder and the Enhancer of *SD*. A chromosome whose transmission is reduced in the presence of *SD* carries a sensitive Responder. Thus, a sensitive SD^+ chromosome is typically Sd^+ $E(SD)^+$ Rsp^s, and an insensitive SD^+ chromosome, one not distorted by *SD*, is Sd^+ $E(SD)^+$ Rsp^i. In males heterozygous for *SD* and a sensitive SD^+ homolog (*Sd E(SD)* Rsp^i/Sd^+ $E(SD)^+$ Rsp^s), the *SD* chromosome, itself insensitive to the distorting action of the *Sd* locus, disrupts the maturation of those spermatids harboring the Rsp^s allele. If the linkage phase of *Sd* and *Rsp* is reversed, as in *Sd* Rsp^s/Sd^+ Rsp^i

This paper is dedicated to the fond memory of LARRY SANDLER, who inspired this interest in meiotic drive.

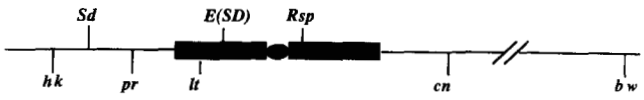

FIGURE 1.—The *SD* complex. The Segregation Distorter complex (*SD*) is comprised of the three major loci *Sd*, *E(SD)* and *Rsp*. *Sd*, the Segregation distorter gene is in the proximal euchromatin of *2L* (left arm). *E(SD)*, the Enhancer of Segregation Distortion, and *Rsp*, Responder, are in the centric heterochromatin of *2L* and *2R* (right arm), respectively. Euchromatin is represented by a line and heterochromatin by hatched rectangles. The black oval represents the centromere. The closely linked markers used in the analysis of *SD* are *hk*, for hook (location 53.9 on the genetic map of chromosome 2); *pr*, for purple (54.5); *lt*, for light (55.0); *cn* (57.5), for cinnabar; and *bw*, for brown (104.5). *SD* represents the distorting chromosome and *Sd*, the distorting gene. The chromosome is not drawn to scale in that the *SD* complex is greatly enlarged.

males, it is still the Rsp^s bearing sperm which are rendered dysfunctional (HARTL 1974, 1975). In addition to the three major loci, there are naturally occurring modifiers on certain of the *SD* chromosomes, particularly in *2R* (*e.g.*, SANDLER and HIRAIZUMI 1960a; MIKLOS and SMITH-WHITE 1971; HIHARA 1974; HIRAIZUMI, MARTIN and ECKSTRAND 1980). Characteristically, although not inevitably, *SD* chromosomes carry inversions, pericentric and/or paracentric in the right arm, preserving the tight linkage between *Sd* and the genes that promote its transmission. In fact, *SD* chromosomes are classified by their structural differences, for example as the *SD-5* or the *SD-72* inversion type (SANDLER, HIRAIZUMI and SANDLER 1959).

Molecular analyses of *Sd* and *Rsp* have revealed a particular alteration associated with each gene. The *Sd* locus has been cloned by POWERS (P. A. POWERS in TEMIN *et al.* 1991), who discovered a 5-kb tandem duplication uniquely associated with *SD* chromosomes and located within the polytene band 37D5, the known map region of *Sd* (GANETZKY 1977; BRITTNACHER and GANETZKY 1983). The *Rsp* locus, demonstrated by genetic analysis to be extended and subdivisible (LYTTLE 1989), was cytologically mapped by PIMPINELLI and DIMITRI (1989) to a specific band in the *2R* heterochromatin whose size is highly correlated with sensitivity level, suggesting that *Rsp* is a reiterated genetic element. Molecular cloning by WU *et al.* (1988) has shown that the copy number of a 120-base pair repeat sequence is correlated with *Rsp* sensitivity.

This paper will focus on the *Enhancer* locus, using a genetic approach to characterize in detail the properties of *E(SD)* and its role in segregation distortion. Since *E(SD)* in nature is typically associated with the closely linked *Sd*, the effects of these two loci at the base of *2L* are confounded in wild *SD* chromosomes. Recombinational and deletional analyses to resolve these components have shown that each plays an important role, although the mechanism of their combined or even individual action is not understood. HARTL (1975), to explain the behavior of two subsets of $Sd\ Rsp^s$ recombinant chromosomes distinguished by the magnitude of their self-distortion in the presence of an $Sd^+\ Rsp^i$ homolog, proposed the existence of an enhancing modifier in the centromeric region present in one subset of the sensitive recombinants but absent in the other. The $Sd\ Rsp^s$ recombinant subset presumed to harbor the modifier exhibited very strong self-distortion when heterozygous with $Sd^+\ Rsp^i$; the other subset, lacking the putative modifier, showed weaker self-distortion. This modifier, which exerts its effect in *cis* or in *trans* to *Sd* or to *Rsp*, corresponds to *E(SD)*, identified by GANETZKY(1977) during an analysis of γ-ray induced revertants of the *SD* chromosome. In that study, a class of partial revertants was recovered that resulted from the deletion of an element in the centric heterochromatin of *2L* near *lt*. This was in contrast to a set of complete revertants that resulted from deletion of the *Sd* gene, in the euchromatin just distal to *pr*. BRITTNACHER and GANETZKY (1984) enlarged the collection of radiation-induced partial revertants by screening for *lt* deletions in several additional *SD* strains and found *E(SD)* on all *SD* chromosomes examined and in the same location, proximal to *lt*. SHARP, HILLIKER and HOLM (1985) placed the *E(SD)* of yet a different *SD* strain proximal to *lt* as well, although in a slightly different location with respect to certain of the essential genes in the *2L* centric heterochromatin.

The several models for segregation distortion (*e.g.*, HARTL 1973; GANETZKY 1977; HIRAIZUMI, MARTIN and ECKSTRAND 1980; LYTTLE 1986) generally invoke a binding of *Sd* product with *Rsp*, thereby initiating a series of events that culminate in sperm dysfunction. As for the relationship between *Sd* and *E(SD)*, their measurably different capacities in drive strength, as demonstrated by the deletion analyses cited, have argued for a more central role for *Sd*. Thus, it has been proposed that *E(SD)* might intensify the aberrant effect by controlling the level of *Sd* expression or the efficiency of binding at *Rsp*, but that basically *E(SD)* is a modifier of the primary action of *Sd* (GANETZKY 1977; BRITTNACHER and GANETZKY 1984). On the other hand, *Sd* and *E(SD)* share certain properties; both are *trans*-acting dominant neomorphs with respect to meiotic drive and both display certain dosage effects (BRITTNACHER and GANETZKY 1984), hinting at a possible similarity in underlying function. In fact, *E(SD)*, like *Sd*, was shown to be able to distort on its own, in the report by SHARP, HILLIKER and HOLM (1985) that certain recombinant $Sd^+\ E(SD)\ Rsp^i$ chromosomes exhibited a small but measurable degree of drive, particularly when heterozygous with a supersensitive ($Sd^+\ E(SD)^+\ Rsp^{ss}$) homolog. This evidence

for independent distortion by *E(SD)* contrasted with the results on the complete revertants in which *SD* chromosomes deleted for *Sd* but retaining *E(SD)* exhibited no distortion at all (BRITTNACHER and GANETZKY 1984). The opposing results may depend on the parental *SD* chromosome or on the method for generating the *E(SD)*-retaining derivative lacking *Sd*. SHARP, HILLIKER and HOLM (1985) suggested that an *E(SD)*-bearing chromosome that is Sd^+, although not one that is *Df(Sd)*, is the type that preserves residual distorting capability.

The present study was undertaken to further elucidate the genetic behavior of *E(SD)* and to ascertain whether *E(SD)* can ever produce strong distortion independently of *Sd*. If so, does this require the *E(SD)* chromosome to be Sd^+ rather than deleted for *Sd*? By using a variety of rearrangements in which *E(SD)* has been disengaged from *Sd* it is possible to explore the properties of *E(SD)* in multiple dose, especially in genotypes lacking *Sd*. These experiments provide new data to support the hypothesis of independent action by *E(SD)*. Instead of being simply a modifier of the drive activity of *Sd*, *E(SD)* has distorting potential of its own in the absence of *Sd*. Thus, *Sd* is not an absolute prerequisite for drive, and *E(SD)*, like *Sd*, is capable of acting, directly or indirectly, on the *Rsp* target.

MATERIALS AND METHODS

Chromosomes: The examples referred to are all in Figure 2, which provides a summary diagram of the *SD*-derivative chromosomes used in this study. For a more complete description of markers, see LINDSLEY and GRELL(1968).

SD chromosomes, Sd E(SD) Rsp^i (example 1): *SD* chromosomes isolated from nature by TEMIN and MARTHAS (1984) include *C132* and *C11* from Sonoma County, California, and *M202* and *M325* from Madison, Wisconsin. Of the *SD-72* inversion type (SANDLER, HIRAIZUMI and SANDLER 1959), they carry the pericentric *In(2LR)39D;42A* and the paracentric *In(2R)NS = In(2R)52A2-B1;56F9-13*.

SD-Mad, isolated from a natural population in Madison, Wisconsin, by R. G. TEMIN in 1979, carries the same inversions as *SD-72* but differs from it in being fully viable and fertile in both sexes when homozygous. It is a strong distorter, giving $k = 0.99$, where k = proportion of *SD* progeny among total progeny of an *SD/cn bw* male (see discussion of k values below).

SD-Roma, an inversion-free *SD* chromosome isolated in Rome, Italy (NICOLETTI and TRIPPA 1967), is a moderate distorter, with k values that vary substantially among sublines, from approximately 0.80 to 0.90. Because of its greater variability and susceptibility to background effects (in contrast with the strong *SD* lines), *SD-Roma* was retested in each experiment.

SD-Roma bw is a recombinant derivative of *SD-Roma*, also inversion free (BRITTNACHER and GANETZKY 1983).

SD-R1,bw is the *R(SD-36)-1,bw* of HARTL (1974), a recombinant derivative of *SD-36*, which is an *SD-5* type (SANDLER, HIRAIZUMI and SANDLER 1959), carrying two nonoverlapping paracentric right arm inversions: *In(2R)45C-F;49A* and *In(2R)NS*.

SD^+ laboratory chromosomes with different sensitivities to SD action, of genotype Sd^+ $E(SD)^+$ and varying at Rsp:

cn bw [Sd^+ $E(SD)^+$ Rsp^s] is the standard sensitive chromosome (example 2).

lt pk cn, a supersensitive chromosome, and *lt pk cn bw*, produced by recombination between *lt pk cn* and *cn bw* (BRITTNACHER and GANETZKY 1984), are both Sd^+ $E(SD)^+$ Rsp^{ss}.

Rsp^{i16}, cn bw [Sd^+ $E(SD)^+$ Rsp^i] is a radiation-induced derivative of the *cn bw* chromosome. It is completely insensitive to the action of *SD* and, although deleted for the *Rsp* locus, is homozygous viable and fertile (GANETZKY 1977). See example 9.

In(2L)Cy, Cy b pr cn [Sd^+ $E(SD)^+$ Rsp^s], denoted as "*Cy R cn*," is a sensitive chromosome derived by HARTL (1974) from a double recombination (in the *pr-Rsp* and the *cn-bw* intervals) between the insensitive *In(2L)Cy, Cy b pr* (= *In(2L)22D1-2;33F5-34A1*) and the sensitive *cn bw*. Although the *Cy R cn* chromosome carries the Rsp^s allele from the *cn bw* chromosome, it is somewhat more sensitive than is *cn bw*, presumably due to modifiers. For *SD-R1,bw/cn bw* males, $k = 0.80$, whereas for *SD-R1,bw/Cy R cn* males, $k = 0.95$ (HARTL 1980). This chromosome is kept balanced with *In(2LR)Gla*. Its *SD* genotype is represented by example 2.

Derivatives of SD chromosomes carrying one or two, but not all, of the components of the native SD complex:

1. Chromosomes derived by recombination within the *SD* complex.

R(Cy)40 bw [Sd^+ *E(SD)* Rsp^i] is an Enhancer-bearing nondistorting, insensitive chromosome (example 3) derived by recombination between *SD-R1,bw* (example 1) and *Cy R cn* (example 2), in the *pr-E(SD)* interval (HARTL 1974). *R(Cy)40 bw* is *In(2L)Cy, Cy b pr* cn^+ *In(2R)45C-F;49A, In(2R)NS*.

Cy ReR cn [Sd^+ *E(SD)* Rsp^s], example 5, is an Enhancer-bearing, nondistorting sensitive chromosome in which the *E(SD)* traces back to *SD-36*, a strongly distorting *SD-5* type(see *SD-R1,bw* and *R(Cy)40 bw*, above). *Cy ReR cn* (cited in LYTTLE, BRITTNACHER and GANETZKY 1986) was derived by D. HARTL (unpublished results) as the *Cy cn* progeny of *R(Cy)40 bw/R(cn)-10* mothers [= Sd^+ *E(SD)* Rsp^i/*Sd E(SD)* Rsp^s] by a crossover in the centromeric region [see HARTL (1974, 1975) for derivation of *R(cn)-10*, example 4]. Since the two parental *E(SD)*-bearing chromosomes (examples 3 and 4) are themselves recombinants, the *Cy cn* derivative is a re-recombinant, signified by *ReR*. All experiments are repeated with two independent isolates, *Cy ReR cn-4* and *Cy ReR cn-5*, both found by D. HARTL (unpublished results) to be extremely sensitive to distortion. Molecular analysis (P. POWERS, personal communication) confirms the absence of the *Sd*-specific 5-kb duplication. During the construction of *Cy ReR cn*, certain modifiers of *SD* were eliminated, such as *Si(SD)*, associated with bw^+ in *2R* of some of the wild *SD* chromosomes (SANDLER and HIRAIZUMI 1960a). The *M(SD)* of *SD-R(cn)-14* (HIRAIZUMI, MARTIN and ECKSTRAND 1980), if also present on *R(Cy)40 bw*, would have been eliminated by the crossover that gave rise to *Cy ReR cn*. The *Cy ReR cn* chromosome, like its progenitor *Cy R cn* (see above), is *In(2L)Cy, Cy b* Sd^+ *pr* Rsp^s *cn*, but differs from it by the substitution of the *E(SD)* region for the $E(SD)^+$ region. *Cy ReR cn* is kept balanced with *In(2LR)Gla*.

2. *SD* revertants generated during radiation (and in one case, EMS) mutagenesis of the *SD-5*, *SD-72*, *SD-Mad* or *SD-Roma* chromosomes (GANETZKY 1977; BRITTNACHER and GANETZKY 1983, 1984). The revertants are classified as to whether they confer complete ($k = 0.50$) or partial (reduced k) reversion of the *SD* phenotype.

The complete revertants (SD^R) are of two types: cytologically deleted or not. Those with cytologically visible dele-

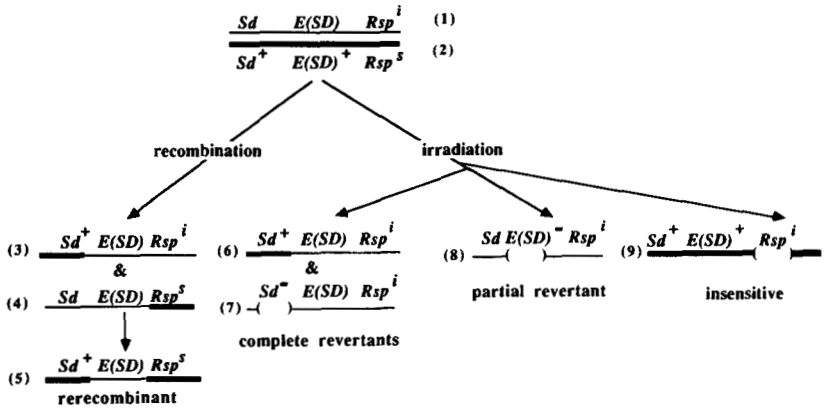

FIGURE 2.—The *SD* and the *SD*$^+$ chromosomes and derivatives. (1) The *SD* chromosome, *Sd E(SD) Rsp*^{i}, is represented by a horizontal line; examples include *SD-Mad*, *SD-Roma(bw)*, and *SD-R1,bw*. (2) The *SD*$^+$ chromosome, *Sd*$^+$ *E(SD)*$^+$ *Rsp*^{s}, represented by a hatched rectangle, is *cn bw*. Another chromosome of this genotype regarding the *SD* complex is *Cy R cn*, used as a control in many of the experiments. Derivatives by recombination between an *SD* and an *SD*$^+$ chromosome are (3) *Sd*$^+$ *E(SD) Rsp*^{i}, an insensitive nondistorter, specifically *R(Cy)40 bw*, and (4) *Sd E(SD) Rsp*^{s}, a self-distorter, specifically *R (cn)-10* (HARTL 1975). The re-recombinant type, (5) *Sd*$^+$ *E(SD) Rsp*^{s}, of which there are two isolates, *Cy ReR cn-4* and *Cy ReR cn-5*, was derived by recombination between (3) and (4), by D. HARTL (unpublished results). Derivatives (6) through (9) arose during radiation mutagenesis (GANETZKY 1977; BRITTNACHER and GANETZKY 1983, 1984). The complete *SD* revertants (*SD*^{R}) are either recombinant (6), *Sd*$^+$ *E(SD) Rsp*^{i} (namely, *SD-5*^{R2} and *SD-5*^{R7}) or deleted for the *Sd* locus (7), *Df(Sd) E(SD) Rsp*^{i} (namely, *SD-Mad*^{R77}, *SD-Mad*^{R68}, *SD-Roma*^{R2}*bw*, *SD-Roma*^{R14}*bw* and *SD-Roma*^{R57}*bw*). The partial revertant (8), *Sd Df[E(SD)]Rsp*^{i}, is deleted for the *E(SD)* locus, as in the *SD*^{lt} derivatives. The insensitive derivative (9), *Sd*$^+$ *E(SD)*$^+$ *Rsp*^{i}, deleted for the *Rsp* locus, is *Rsp*^{i16}, *cn bw*. A gap represents the region deleted.

tions that remove both *Sd* and *pr* are *SD-Mad*^{R77}, *SD-Mad*^{R68}, *SD-Roma*^{R2}*bw*, *SD-Roma*^{R14}*bw* and *SD-Roma*^{R57}*bw* (BRITTNACHER and GANETZKY 1983). These are represented by example 7 as *Df(Sd) E(SD) Rsp*^{i}. The *SD-5*^{R37}*lt* derivative is cytologically deleted for the *Sd* locus but is *pr*$^+$ (GANETZKY 1977); sometime after its original isolation *SD-5*^{R37} acquired a *lt* mutation, shown by molecular methods to be a deletion (R. DEVLIN, personal communication). Complementation analysis with the lethal markers of HILLIKER (1976) in the *2L* heterochromatin indicates that *SD-5*^{R37}*lt* is also deleted for *E(SD)*, and is therefore *Df(Sd) Df[E(SD)] Rsp*^{i} (R. G. TEMIN, unpublished results).

The complete revertants not cytologically deleted for the *Sd* region are *SD-5*^{R2} and *SD-5*^{R7} (GANETZKY 1977). They are *Sd*$^+$ *E(SD) Rsp*^{i} (example 6), established by molecular analysis of linked polymorphic sites to have acquired the *Sd*$^+$ allele of a *cn bw* homolog by a rare exchange event in *SD/cn bw* males (P. A. POWERS, in TEMIN *et al.* 1991).

SD-Mad$^{ER8.2}$ (*Sd*$^+$ *E(SD) Rsp*^{i}) is a nondeleted complete revertant generated by EMS mutagenesis (J. BRITTNACHER, unpublished results) that contains a novel *Eco*RI site within the region of the molecularly ascertained *Sd* duplication (P. A. POWERS, in TEMIN *et al.* 1991).

The partial revertants, deleted for *E(SD)* and *lt*, are *SD-5*^{lt1}, *SD-5*^{lt3}, and *SD-5*^{lt36}, originally named *SD-5*$^{Rev-1}$, *SD-5*$^{Rev-3}$, and *SD-5*$^{Rev-36}$, respectively (GANETZKY 1977), and *SD-Roma*^{lt1}, *SD-Roma*^{lt5}*bw*, *SD-Roma*^{lt8}*bw*, *SD-Roma*^{lt59}*bw*, *SD-Mad*^{lt73}, and *SD-72*^{lt23} (BRITTNACHER and GANETZKY 1984). See example 8, *Sd Df [E(SD)] Rsp*^{i}.

3.Insertional translocations of one or more of the *SD* components into the *Y* chromosome:

Dp(2;Y) B10–4, B^s*Yy*$^+$, *Sd E(SD)* is an insertional translocation of cytological region 36D2-3; 40 from the base of *2L* of *SD-Roma* into *Y*^{L}, constructed by LYTTLE (1984, 1986).

Dp(2;Y) C5–3, B^s*Yy*$^+$, *Sd* is an insertional translocation of *Sd*, but not of *E(SD)*, from *SD-NH2* into *Y*^{L}, constructed by LYTTLE (1984). *SD-NH2* is a strong distorter (HIRAIZUMI and NAKAZIMA 1967).

Dp(2;Y) CB25-4, B^s*Yy*$^+$, *Rsp*^{s} is an insertional translocation into *Y*^{L} of proximal *2R* centric heterochromatin containing *Rsp*^{s} from the *cn bw* chromosome, constructed by LYTTLE (1989).

Dp(2;Y) R10, B^s*Yy*$^+$, *Df(Sd) E(SD)* is a derivative of *Dp(2;Y) B10–4, B*^{s}*Yy*$^+$, *Sd E(SD)*, the isolation of which will be described below.

Other chromosomes:

In(3LR)TM6, ss$^-$*Ubx*^{67b} *Su(SD)* is a derivative of the standard multiply inverted third chromosome *TM6* balancer that carries a dominant suppressor of *SD* activity (LYTTLE 1986).

In(2LR) SM6b dpl^{vl} *Roi Cy O cn*^{2P}*bw* or *Cy O pr cn*^{2} are used as the balancers for the *SD* chromosomes or the *SD* derivatives. They are referred to collectively as *Cy O*.

Construction of a *Y* chromosome carrying *E(SD)*: A *Y·E(SD)* chromosome was derived as a radiation-induced deletion of *Sd* from *Dp(2;Y)B10-4 B*^{s} *Y, Sd pr*$^+$ *lt*$^+$ *E(SD) y*$^+$, by screening for loss of the closely linked *pr*$^+$ marker. *Dp(2;Y)B10–4 B*^{s} *Y, Sd pr*$^+$ *lt*$^+$ *E(SD) y*$^+$*; pr cn* males were irradiated with 4500 rad of γ-rays and mated, in groups of 15–20, to *pr cn* females, in vials. The irradiated males were discarded 4 or 5 days later. The sons of three broods of females per each original mating vial were screened for the appearance of exceptional orange eyed (*pr cn*) males among the expected *pr*$^+$ *cn* males. Polytene chromosomes in the progeny of these exceptional males were examined for deletions of the *Sd* locus that left the *E(SD)* region intact (R. KREBER, personal communication). Of 2815 males scored, 31 orange (*pr cn*) males were recovered, of which 14 were fertile. Of the fertile orange-eyed males, 12 had deletions that included *E(SD)*. The two remaining candidates were cytologically deficient for the *Sd* region but retained the lethal complementation groups *40-18*$^+$ and *lt*$^+$, distal to *E(SD)*, as well as *56-15*$^+$, proximal to *E(SD)*. Thus, both of these derivatives appear to have the constitution *Dp(2;Y) Df(Sd) E(SD)*. Males carrying each of these *Y* chromosome constructs were backcrossed to *lt pk cn* females for at least six generations to rid the stocks of possible induced mutations on other chromosomes. Molecular analysis with the *Sd* probe indicates that *Dp(2;Y) E(SD)* lacks the 12-kb *Eco*RI

fragment characteristic of *Sd* (P. POWERS, personal communication).

The tests for measuring segregation distortion: To measure segregation ratios, single *SD**-bearing males were mated to two *cn bw* females per vial, in 20–25 replicate vials. *SD** refers to a native *SD* chromosome or to a derivative carrying one or more components of the *SD* complex. After 4 days the parents were transferred to a fresh vial, and discarded four days later. The offspring from each vial were counted through day 18 after the parents had been introduced into that vial. All experiments were carried out at 25°.

The degree of segregation distortion is expressed as a *k* value, the proportion of *SD**-bearing progeny among the total progeny. The *k* value for each male is adjusted for differential viability effects by using a weighted average viability estimate obtained in the reciprocal cross. Since the *SD** chromosome is expected to segregate normally in females, any departure from $k = 0.50$ in the female tests is attributed to differential viability effects of the two homologs. The relative viability effect was estimated by W = number of SD^+ progeny/number of *SD** progeny from the control females, using the total flies in each phenotypic class summed over 10–20 replicate tests. The corrected *k* value for each SD^*/SD^+ male is computed as

k_c = number of *SD** progeny/[number of *SD** progeny + (number of SD^+ progeny/W)].

The unweighted mean k_c for the 20–25 replicate males of each SD^*/SD^+ type is reported as $k \pm 2$ SE. This is the statistic used in Tables 1, 3, 5 and 8.

For the insertional translocation of *Sd E(SD)* or *Sd* into the *Y* chromosome (Table 2), an analogous computation is made, although in this case, since *Sd* is on the *Y*, the *k* value measures the relative transmission of a particular second chromosome which is SD^+, compared to an SD^+ homolog. If Rsp^a and Rsp^b mark two second chromosomes that may differ in allelic status at the *Rsp* locus, the sensitivity level of the chromosome of interest is determined from the following pair of crosses, according to the procedure of LYTTLE, BRITTNACHER and GANETZKY (1986):

Cross 1. *X/Dp(2;Y) Sd [E(SD)]* ; Sd^+ *E(SD)* Rsp^a/Sd^+ *E(SD)*$^+$ Rsp^b ♂ × 2 *cn bw* ♀♀

Cross 2. *X/Y;* Sd^+ *E(SD)* Rsp^a/Sd^+ *E(SD)*$^+$ Rsp^b ♂ × 2 *cn bw* ♀♀.

In this experiment the allelic status of Rsp^b is known and serves as a benchmark in ascertaining the relative sensitivity of Rsp^a, specifically on the *Cy ReR cn* chromosome. The males tested in crosses 1 and 2 where Rsp^b is either Rsp^{ss} (*lt pk cn*) or Rsp^s (*cn bw*), are generated as follows: P_0 *Dp(2;Y) Sd [E(SD)]; lt pk cn* males are mated to *cn bw* females to give *Dp(2;Y) Sd [E(SD)]; lt pk cn/cn bw* sons. These are mated to *Cy ReR cn/Gla* females to generate *Dp(2;Y) Sd [E(SD)]; Cy ReR cn/lt pk cn* or *Dp(2;Y) Sd [E(SD)]; Cy ReR cn/cn bw* sons for the individual *k* tests. The *Y* chromosome carrying either *Sd E(SD)* or *Sd* provides the distorting background (cross 1). The control males carry a normal *Y* chromosome (cross 2). In the series where Rsp^b is Rsp^s, the P_0 females are Rsp^{i16}, *cn bw*.

Crosses 1 and 2 produce daughters which are genetically identical, and they are used to estimate the relative transmission of the *Cy ReR cn* (Rsp^a) chromosome from their respective fathers. Data on the sons are not used because of reduced viability owing to hyperploidy of the *Dp(2;Y)*. To represent the transmission ratio describing each test male in cross 1, *k* is computed as the number of Rsp^a daughters/number of (Rsp^a + Rsp^b) daughters. Since a normal *Y* chromosome does not distort, cross 2 provides a direct measure of viability differences between the Rsp^a and Rsp^b chromosomes. *W* is computed from [the number of Rsp^b/number of Rsp^a] daughters summed over cross 2 and applied as a viability correction to the individual *k* values in cross 1, as in the above formula for k_c. Thus, cross 2 is used to adjust for viability in the same way as a reciprocal cross was used earlier, but without the problem of female recombination.

For the data in Table 4 on the effect of the *Y·E(SD)*, first in the presence of *Sd*, the following pair of crosses are directly compared:

Cross 1. *X/Dp(2;Y) E(SD); Sd E(SD)** Rsp^i/Sd^+ *E(SD)*$^+$ Rsp^s ♂ × 2 *cn bw* ♀♀

Cross 2. *X/Y; Sd E(SD)** Rsp^i/Sd^+ *E(SD)*$^+$ Rsp^s ♂ × 2 *cn bw* ♀♀

where *E(SD)** signifies either *E(SD)* or *Df(E(SD))*. The Sd^+*E(SD)*$^+$ Rsp^s homolog is *cn bw*. To generate the test males, *Dp(2;Y) E(SD); cn* males are crossed to *Cy O/SD** females, where *SD** is an *SD* chromosome or an *SD* chromosome deleted for *E(SD)*. The F_1 *Dp(2;Y) E(SD); SD*/cn* sons are crossed to *cn bw* females to generate F_2 males for the *k* tests.

When the *Dp(2;Y) E(SD)* chromosome is used to supply the second or third dose of *E(SD)* in the absence of *Sd* (Table 6), the *k* values in the following pair of crosses are directly compared:

Cross 1. *X/Dp(2;Y) E(SD);* Sd^R*E(SD)* Rsp^i/Sd^+ *E(SD)*^{x} $Rsp^{s(s)}$ ♂ × 2 *cn bw* ♀♀

Cross 2. *X/Y;* Sd^R*E(SD)* Rsp^i/Sd^+ *E(SD)*^{x} $Rsp^{s(s)}$ ♂ × 2 *cn bw* ♀♀.

Sd^R*E(SD)* Rsp^i is a complete revertant, where Sd^R signifies either Sd^+ or *Df(Sd)*. On the homolog, *E(SD)*^{x} is either *E(SD)* or *E(SD)*$^+$. To generate the test males, *Dp(2;Y) E(SD); cn* (or for the controls, normal *Y* bearing) males are crossed to *Cy O/*Sd^R*E(SD)* Rsp^i females, and the F_1 *Dp(2;Y) E(SD);* Sd^R*E(SD)* Rsp^i*/ cn* sons are crossed to Sd^+ *E(SD)*^{x} $Rsp^{s(s)}$ females.

As in general when marked *Y* chromosomes with *SD* element inserts are used, only the data on daughters of *Y·E(SD)* (or control *Y*) males enter into *k* value computations, to avoid any viability effects of the hyperploid *Y*. Further, in experiments with the *Y·E(SD)* the absolute *k* values for *Y·E(SD)* and *Y* bearing males are directly compared, and no viability correction is applied.

When the insertional translocation of Rsp^s into the *Y* is used (Table 7), the data are given in terms of the sex ratio. If distortion occurs and this Rsp^s is the target site, then some proportion of sperm receiving such an Rsp^s *Y* may undergo dysfunction, whereas sperm receiving the *X* will mature normally (LYTTLE 1989). The proportion of daughters is ascertained in the following pair of crosses, where Sd^R is Sd^+ or *Df(Sd)*:

Cross 1. *X/Y·*Rsp^s ; Sd^R*E(SD)* Rsp^i/Sd^R*E(SD)* Rsp^i ♂ × 2 *cn/cn* ♀♀. The male has two doses of *E(SD)* and carries Rsp^s on the *Y*.

Cross 2. *X/Y* ; Sd^R*E(SD)* Rsp^i/Sd^R*E(SD)* Rsp^i ♂ × 2 *cn/cn* ♀♀. The male has two doses of *E(SD)* but a normal *Y* chromosome.

To generate the *Y·*Rsp^s males for cross 1, *Y·*Rsp^s; *cn bw* males are mated to *Cy O/*SD^{RA} females to give *Y·*Rsp^s;SD^{RA}*/ cn bw* sons, which are mated to *Cy O/*SD^{RB} females. SD^{RA} and SD^{RB} represent two different *SD* derivatives in which the *Sd*

of a native *SD* chromosome has been replaced by a deletion of *Sd* or by Sd^+. The *Y·Rsp*^{s}; SD^{RA}/SD^{RB} sons (and, for certain of the controls, their *Y·Rsp*^{s}; SD^{RB}/*cn bw* brothers) are crossed to *cn* females, either *cn bw*, or in some cases, *pr cn*. (It was necessary at this point to depart from the usual protocol of using exclusively *cn bw* females for *k* tests, in order to distinguish the classes inheriting one or the other of the SD^R homologs when one of them was *Df(pr)*, as in *SD-Mad*^{R77}, *SD-Roma*^{R14}, and *SD-Roma*^{R57}). The strength of drive is measured by the proportion of *daughters* (which are the non-*Rsp*^{s} class) among the total progeny, so as to be analogous with *k* values.

To measure the effect of the *Y·Rsp*^{s} itself on the sex ratio, in the absence of any distortion by two doses of *E(SD)*, segregation ratios were measured in *Y·Rsp*^{s} males carrying one or no dose of *E(SD)*, by substituting the appropriate second chromosome homolog. These were compared with the same males but carrying a normal *Y*.

In the test for suppression (Table 8), males that were homozygous for *E(SD)* were generated from the following series: *Cy* O/SD^R;+/+ males were mated with *cn bw;TM6 Su(SD)/e* females. Their SD^R/*cn bw;TM6 Su(SD)/Su(SD)*$^+$ sons were crossed with *Cy ReR cn/Gla;*+/+ females to give SD^R/*Cy ReR cn;TM6 Su(SD)/*+ and their SD^R/*Cy ReR cn;Su(SD)*$^+$/+ brothers for the individual *k* tests. Males with *SD* components in other doses and configurations were generated from the analogous series of crosses.

Other statistical procedures: When pooled means are given in Tables 1, 3, 4, 6 and 7 or in the text they are based on the arcsin transformation (HALD 1952), which spreads out the values near 0 or 1 and makes the variance less dependent on the mean. The procedure was to average the arcsin *k* and then convert back to the original units by the reverse transformation.

RESULTS

The effect of *E(SD)* in the presence of *Sd*: To illustrate the action of *E(SD)* in an *Sd* genome, *SD* chromosomes or derivatives deleted for *E(SD)* are used in combination with SD^+ homologs that are *E(SD)* or *E(SD)*$^+$, or in combination with the *Y·E(SD)* construct.

The effect of E(SD) in its normal second chromosome location: To study the dosage effect of *E(SD)* in a genome containing the native *SD* complex, several *SD* chromosomes (*Sd E(SD) Rsp*^{i}) are made heterozygous with a series of SD^+ homologs whose genotype at *E(SD)* and at *Rsp* varies (Table 1). The *SD* chromosomes chosen for this analysis include, along with one strong distorter, several moderate distorters so as to be able to demonstrate heightened distortion with added elements. The results show that when an *SD* chromosome of such a set segregates from the sensitive *cn bw* homolog [Sd^+ *E(SD)*$^+$ *Rsp*^{s}], the mean *k* value is 0.930, and when it segregates from the supersensitive *lt pk cn (bw)* [Sd^+ *E(SD)*$^+$ *Rsp*^{ss}], *k* increases to 0.984. Distortion likewise increases (to $k = 0.999$ or 0.980) when the homolog is *Cy ReR cn* [Sd^+ *E(SD) Rsp*^{s}], either the *ReR-4* or *ReR-5* isolate, respectively, even though *Cy ReR cn* carries the *Rsp*^{s} allele derived from *cn bw*. Therefore, adding an extra dose of *E(SD)* has at least the same impact as increasing the sensitivity of the *Rsp* locus itself. The comparable result is found for *SD-Roma* (see Table 3, rows 1 and 2).

Although the combination of *E(SD)* and *Rsp*^{s} is functionally indistinguishable from *Rsp*^{ss} in the foregoing test, a distinction can be made between these two genotypes by using a *Y* chromosome that contains an insertional translocation of *SD* (either *Sd E(SD)* or *Sd* alone), following the procedure of LYTTLE, BRITTNACHER and GANETZKY (1986). When such a *Y* chromosome is present in combination with two SD^+ chromosomes that differ at *Rsp*, as in *Y·SD*; *Rsp*^{a}/*Rsp*^{b} males, any *trans*-acting modifiers of distortion such as enhancers or suppressors will have the same effect on both second chromosomes, whereas the *cis*-acting *Rsp* specifically affects the transmission of the homolog on which it is located. The relative sensitivities of the two SD^+ chromosomes to distortion by the *Y·SD* depend exclusively on their *Rsp* alleles. The SD^+ chromosome carrying the less sensitive *Rsp* is transmitted preferentially, regardless of any linked *trans*-acting modifiers that might be present.

To confirm the *Rsp* status of *Cy ReR cn*, the recovery of this chromosome was measured relative to a series of homologs with *Rsp* alleles of different but known sensitivities, in the test described (Table 2). In the presence of the *Y·Sd E(SD)* (Table 2, top), *Cy ReR cn* is transmitted preferentially when it segregates from *Rsp*^{ss} ($k = 0.895$ for *ReR-4* or 0.948 for *ReR-5*), indicating that the *Rsp* on *Cy ReR cn* is not as sensitive as the *Rsp*^{ss} allele. When *Cy ReR cn* segregates from *Rsp*^{s}, the two homologs are recovered in about equal frequencies, showing that the intrinsic sensitivity of the *Rsp* on *Cy ReR cn* corresponds to *Rsp*^{s} on the *cn bw* chromosome, as expected. When it segregates from an *Rsp*^{i} chromosome, the *Cy ReR cn* chromosome is recovered with markedly reduced frequency ($k = 0.017$ or 0.027), consistent with the presence of an *Rsp* that is more sensitive than *Rsp*^{i}. These results confirm that it is the extra dose of *E(SD)* on the *Cy ReR cn* chromosome that gives the elevated *k* values in Table 1, not a supersensitive *Rsp*.

Comparable results are obtained using a *Y·Sd* construct instead of the *Y·Sd E(SD)* (middle section, Table 2), except that distortion overall is reduced, as may be noted when the choice is between *Rsp*^{i} and *Rsp*^{s} as target sites (column 3). When *Cy ReR cn* segregates from *Rsp*^{i} in the *Y·Sd* background there is considerable distortion, but the *k* values (0.161 or 0.087) are not as drastic as in the *Y·Sd E(SD)* background. This is a consequence of one instead of two doses of *E(SD)*, confirming the dosage effect of *E(SD)* in an *Sd* genome.

When *Sd* is not present, in males with a normal *Y* (last section, Table 2), no systematic differences in the relative recoveries of the two second chromosome homologs were observed. Thus, there is no evidence

TABLE 1

Distortion in *SD/SD*$^+$ males where the *SD*$^+$ homolog varies at *E(SD)* and *Rsp*

SD [Sd E(SD) Rsp^i]	*SD*$^+$ chromosome			
	Sd$^+$ *E(SD)*$^+$ *Rsp^s* *cn bw*	*Sd*$^+$ *E(SD)*$^+$ *Rsp^{ss}* *lt pk cn (bw)*	*Sd*$^+$ *E(SD) Rsp^s* *Cy ReR cn-4*	*Sd*$^+$ *E(SD) Rsp^s* *Cy ReR cn-5*
C132	0.990 ± 0.008	0.998 ± 0.002	1.000 ± 0.001	0.994 ± 0.003
C11	0.912 ± 0.050	0.980 ± 0.011	1.000 ± 0	0.942 ± 0.031
M202	0.898 ± 0.054	0.965 ± 0.012	0.999 ± 0.001	0.980 ± 0.020
M325	0.952 ± 0.021	1.000 ± 0.001	0.995 ± 0.006	0.987 ± 0.009
SD-R1, bw	0.853 ± 0.028	0.935 ± 0.029	0.994 ± 0.002*	0.984 ± 0.005*
Mean *k*	0.930	0.984	0.999	0.980
E(SD) doses in *SD/SD*$^+$ ♂♂	1	1	2	2

Unweighted mean *k* values (±2 SE), corrected for viability, are given for males heterozygous for the *SD* chromosome named on the left and the *SD*$^+$ chromosome named at the top of each column. The *SD/SD*$^+$ males tested were the sons of *CyO/SD* mothers and either *cn bw*, *lt pk cn bw*, or *Cy ReR cn* fathers. The *SD* chromosomes *C132*, *C11*, *M202*, *M325*, of the *SD-72* inversion type, were isolated from natural populations in Sonoma County, California ("*C*" prefix), or Madison, Wisconsin ("*M*"). *SD-R1, bw* is a recombinant derivative of *SD-36*, an *SD-5* inversion type. * Uncorrected *k* value.

TABLE 2

Demonstration that the *Rsp* carried by the *Cy ReR* chromosomes is *Rsp^s*

Sd$^+$ *E(SD) Rsp^i*	*Sd*$^+$ *E(SD)*$^+$ *Rsp^x*			Doses of	
	lt pk cn (Rsp^{ss})	*cn bw (Rsp^s)*	*Rsp^{i16}, cn bw*	*Sd*	*E(SD)*
In presence of *Dp(2;Y) Sd E(SD)*					
Cy ReR cn-4	0.895 ± 0.035	0.531 ± 0.067	0.017 ± 0.010	1	2
Cy ReR cn-5	0.948 ± 0.015	0.532 ± 0.024	0.027 ± 0.014	1	2
In presence of *Dp(2;Y) Sd*					
Cy ReR cn-4	0.883 ± 0.024	0.476 ± 0.028	0.161 ± 0.062	1	1
Cy ReR cn-5	0.944 ± 0.014	0.511 ± 0.024	0.087 ± 0.023	1	1
In presence of normal *Y*					
Cy ReR cn-4	0.478 ± 0.010	0.534 ± 0.017	0.481 ± 0.013	0	1
Cy ReR cn-5	0.470 ± 0.014	0.522 ± 0.019	0.499 ± 0.011	0	1

Each *k* value (±2 SE) gives the mean proportion of *Cy ReR cn [Sd*$^+$ *E(SD) Rsp^i]* daughters, among all daughters, from *Sd*$^+$ *E(SD) Rsp^s/Sd E(SD)*$^+$ *Rsp^z* fathers carrying a *Y* chromosome with or without an insertional translocation. The *Y* chromosome is either *Dp(2;Y) B10-4 [Y·Sd-Roma E(SD)]* or *Dp(2;Y) C5-3 [Y·Sd-NH2]*, in which the *Sd* on the *Y* can cause distorted transmission of second chromosomes differing in sensitivity at *Rsp*. *Cy ReR cn-4* and *Cy ReR cn-5* are independently isolated re-recombinant chromosomes. The *k* values for *Dp(2;Y)* males were corrected for viability using the data on the control males with the normal *Y* (third section of table).

here for significant or consistent distortion by the single *E(SD)* on the *Cy ReR cn* chromosome acting by itself. Any departures from $k = 0.50$ in these control crosses can be attributed to viability differences.

In summary, the experiments described thus far show (1) that *E(SD)* can augment the action of *Sd* when supplied in *trans*, either from the *Y* or from a second chromosome homolog, (2) that the combination *E(SD) Rsp^s* can be distinguished from *Rsp^{ss}*, and (3) that the *Rsp* on the *Cy ReR cn* chromosome corresponds to *Rsp^s*. The presence of cytological markers in the centric heterochromatin of *2L* and *2R* that are characteristic of *E(SD)* and *Rsp^s*, respectively, has provided independent confirmation of the genotype of the *Cy ReR cn* chromosomes regarding these two regions (S. PIMPINELLI and P. DIMITRI, personal communication).

Table 3 addresses the questions of whether an *E(SD)* supplied in *trans* can functionally replace the *E(SD)* deleted in the *Sd Df(E(SD)) Rsp^i* derivatives and whether *E(SD)* alleles from different *SD* chromosomes are functionally equivalent. To provide the baseline values, the amount of distortion caused by an *Sd Df(E(SD)) Rsp^i* chromosome heterozygous with an *E(SD)*$^+$ sensitive homolog was measured (columns 1–3). The particular result depends on the parental *SD* source of the *Df(E(SD))* partial revertant and on the sensitivity of the *SD*$^+$ homolog. Certain of the *Df(E(SD))* derivatives were recovered from the strongly distorting *SD-5*, *SD-72* or *SD-Mad* chromosomes, which characteristically produce *k* values in the range of 0.99 (*e.g.*, GANETZKY 1977; BRITTNACHER and GANETZKY 1984). Others were derived from the moderate distorter, *SD-Roma*. Because of the lower and more variable *k* values of *SD-Roma*, and in order to monitor an increase in distortion by a native *SD* complex upon addition of an extra *E(SD)* (as in Table 1), *SD-Roma* and *SD-Roma bw* chromosomes from the parental stocks were retested in this experiment, giving, respectively, $k = 0.855$ and 0.904 with a *cn bw* homolog (column 1, rows 1 and 2).

The *SD* chromosomes deleted for *E(SD)* are substantially weakened in distorting ability. When the

TABLE 3

Restoration of distortion to *Sd Df [E(SD)] Rsp^i* males by the *E(SD)* supplied in *trans*

	SD^+ homolog *[Sd^+ E(SD)^x Rsp^x]*				
	E(SD)$^+$ Rsp^i cn bw	*E(SD)$^+$ Rsp^{ss} lt pk cn bw*	*E(SD)$^+$ Rsp^i Cy R cn*	*E(SD) Rsp^i Cy ReR cn-4*	*E(SD) Rsp^i Cy ReR cn-5*
Sd E(SD) Rsp^i					
Doses of *E(SD)*	1	1	1	2	2
SD-Roma	0.855 ± 0.033	0.990 ± 0.008	0.920 ± 0.022	1.000 ± 0	1.000 ± 0
SD-Roma, bw	0.904 ± 0.020	0.998 ± 0.003	0.959 ± 0.016	1.000 ± 0.001	1.000 ± 0.001
Sd Df [E(SD)] Rsp^i					
Doses of *E(SD)*	0	0	0	1	1
SD-Roma^{lt1}	0.637 ± 0.036	0.885 ± 0.027	0.780 ± 0.042	0.999 ± 0.001	1.000 ± 0
SD-Roma^{lt5}, bw	0.507 ± 0.021	0.562 ± 0.047	0.612 ± 0.033	0.937 ± 0.014	0.973 ± 0.008
SD-Roma^{lt8}, bw	0.510 ± 0.025	0.633 ± 0.048	0.623 ± 0.040	0.928 ± 0.021	0.969 ± 0.014
SD-Roma^{lt59}, bw	0.456 ± 0.031	0.531 ± 0.042	0.576 ± 0.051	0.991 ± 0.005	0.994 ± 0.005
SD-5^{lt1}	0.829 ± 0.020	0.965 ± 0.011	0.756 ± 0.042	0.997 ± 0.002	0.990 ± 0.006
SD-5^{lt3}	0.582 ± 0.041	0.821 ± 0.042	0.616 ± 0.054	0.991 ± 0.004	0.987 ± 0.007
SD-5^{lt36}	0.706 ± 0.028	0.863 ± 0.035	0.703 ± 0.084	0.998 ± 0.002	0.997 ± 0.002
SD-Mad^{lt73}	0.692 ± 0.027	0.746 ± 0.031	0.641 ± 0.047	0.997 ± 0.005	0.992 ± 0.004
SD-72^{lt23}	0.725 ± 0.031	0.875 ± 0.029	0.724 ± 0.045	0.995 ± 0.004	1.000 ± 0
Mean *k, Df[E(SD)]*	0.631	0.782	0.672	0.988	0.993

Each mean *k* value gives the proportion of *SD** progeny from *SD*/SD$^+$* males, where *SD** is *SD* or the *SD^{lt}* derivative named on the left and *SD$^+$* is named at the top of the column. *Cy R cn* is the *Cy cn Sd$^+$ E(SD)$^+$ Rsp^i* progenitor of the *E(SD)*-containing *Cy ReR cn* chromosome. The *SD*/SD$^+$* males were the sons of *CyO/SD** fathers and either *cn bw*, *lt pk cn bw*, *Cy R cn*, or *Cy ReR cn* mothers. The *k* values, ± 2 SE are corrected for viability. The dose of *E(SD)* in each group of *SD/SD$^+$* or *SD^{lt}/SD$^+$* males is indicated. Mean *k* values for the *Sd Df[E(SD)] Rsp^i* males are given in the bottom row.

E(SD)-deleted derivative segregates from *cn bw (Sd$^+$ E(SD)$^+$ Rsp^s)*, the mean $k = 0.631$, and when it segregates from *lt pk cn bw (Sd$^+$ E(SD)$^+$ Rsp^{ss})*, the mean $k = 0.782$. When *Cy R cn (Sd$^+$ E(SD)$^+$ Rsp^s)* is the homolog, the mean *k* value, 0.672, is slightly higher than for *cn bw*, although the Responder is the same *Rsp^s*. This result was not unexpected, however, since HARTL (1980) had reported that this *Cy* recombinant was carefully selected for sensitivity and was more sensitive than *cn bw*, presumably because of linked modifiers. In sum, deleting *E(SD)* from an *SD* chromosome has a considerable impact on the strength of distortion, although the presence of *Sd* alone is sufficient to cause some distortion.

When a copy of *E(SD)* is added, by supplying *Cy ReR cn (Sd$^+$ E(SD) Rsp^s)* as the homolog for the *Sd Df(E(SD)) Rsp^i* chromosome, there is a marked increase in distortion, to a mean *k* of 0.988 for *Cy ReR cn-4* or 0.993 for *Cy ReR cn-5* (columns 4 and 5, Table 3). In this situation the response of the *Cy ReR cn* homolog is clearly different not only from that of its *E(SD)$^+$ Rsp^s* progenitor (*Cy R cn*) but also from that of *E(SD)$^+$ Rsp^{ss}*, further distinguishing the effects of *E(SD)* from *Rsp* sensitivity. To summarize, an *E(SD)* derived from one *SD* chromosome type (*SD-5*) can functionally substitute in *trans* for the *E(SD)* of a different *SD* type (*SD-Roma*, *SD-Mad* or *SD-72*). For genotypes with one dose of *E(SD)*, *k* happens to be greater when *E(SD)* is in *trans* from *Sd*, as in the values just cited (0.988 or 0.993), than when *E(SD)* is in *cis* with *Sd*, in the control *SD/Cy R cn* males, where $k = 0.920$ or 0.959 (column 3, top 2 rows, Table 3), but this may reflect an allelic difference between the *E(SD)*s of *SD-5* origin and *SD-Roma*, respectively, rather than a positional influence.

The effect of the E(SD) insert in the Y chromosome: To manipulate *E(SD)* independently of the second chromosome and to increase the dosage still further, it was useful to construct a *Y* chromosome containing *E(SD)*. Two such chromosomes were isolated as radiation-induced deletions of *Sd* from the *Y* chromosome originally carrying an insertional translocation of *Sd E(SD)*, as described in MATERIALS AND METHODS. One of these derivatives, *Dp(2;Y)R10, lt$^+$ E(SD)*, was studied in detail; it is cytologically deficient for euchromatic bands 36D-38BC (the *Sd*-containing region) but preserves euchromatic bands 38 to 39 at the euchromatin-heterochromatin junction, with their normal pattern, and retains the EMS-defined lethals *40-18$^+$* and *56-15$^+$*, respectively distal and proximal to *E(SD)*.

The *Y·E(SD)* is able to enhance distortion in males carrying either an intact *SD* or an *SD* chromosome deleted for *E(SD)*, as shown in Table 4. When an *SD-Roma(bw)* chromosome containing the native *SD* complex (top two rows) segregates from a *cn bw(Rsp^s)* homolog in males with a normal *Y*, the mean $k = 0.926$. This increases to mean $k = 0.984$ in males with the *Y·E(SD)*. The added *E(SD)* on the duplication is effective, although it does not work quite as well as the *E(SD)* region on the *ReR* chromosome ($k = 1.000$, Table 3, top 2 rows, last 2 columns), either because of a possible allelic difference or a background effect or because the *E(SD)* was changed during the irradiation.

TABLE 4

The enhancing action of the *Y·E(SD)* in the presence of *Sd*

	Normal *Y*	*Y·E(SD)*	*Y·Sd*
Sd E(SD) Rsp^i	1 *Sd*: 1 *E(SD)*	1 *Sd*: 2 *E(SD)*	
SD-Roma	0.913 ± 0.018	0.972 ± 0.008	
SD-Roma, bw	0.938 ± 0.016	0.993 ± 0.003	
Mean *k*	0.926	0.984	
Sd Df(E(SD))Rsp^i	1 *Sd*: 0 *E(SD)*	1 *Sd*: 1 *E(SD)*	2 *Sd*: 0 *E(SD)*
SD-Roma^{lt1}	0.536 ± 0.028	0.759 ± 0.028	0.563 ± 0.026
SD-Roma^{lt5},bw	0.542 ± 0.025	0.766 ± 0.030	0.501 ± 0.019
SD-Roma^{lt8},bw	0.513 ± 0.020	0.786 ± 0.031	0.476 ± 0.028
SD-Roma^{lt59},bw	0.475 ± 0.034	0.717 ± 0.039	0.491 ± 0.021
SD-5^{lt1}	0.666 ± 0.025	0.948 ± 0.020	
SD-5^{lt3}	0.482 ± 0.034	0.693 ± 0.029	
SD-5^{lt36}	0.435 ± 0.065	0.923 ± 0.039	
SD-Mad^{lt73}	0.587 ± 0.021	0.665 ± 0.021	
SD-72^{lt23}	0.458 ± 0.107	0.816 ± 0.040	
Mean *k*	0.522	0.796	0.508

Each *k* value (±2 SE) is the mean proportion of *SD**/*cn bw (Sd$^+$ E(SD)$^+$ Rsp^s)* males with a normal *Y*, a *Y·E(SD)*, or a *Y·Sd*. *SD** is *SD* or *SD^{lt}Df(E(SD))*, as named. The *k* values, computed from daughters only, are not viability corrected. The *Sd:E(SD)* dosage for each group is given.

The *Y·E(SD)* is also able to restore higher levels of distortion to *E(SD)*-deleted *SD* chromosomes; the mean *k* increases from 0.522 in males with the normal *Y* to *k* = 0.796 in males with the *Y·E(SD)* (bottom section, Table 4). The enhancing effect of the *Y·E(SD)* is not limited to *SD-Roma* derivatives but also occurs with the *Df(E(SD))* derivatives of *SD-5*, *SD-Mad* and *SD-72*. The *Y·E(SD); Sd Df(E(SD)) Rsp^i* males (mean *k* = 0.796) do not produce as much drive as do males carrying the intact *Sd E(SD) Rsp^i* chromosome(mean *k* = 0.926), although the dosage of *SD* components is the same. This difference may reflect viability effects of the deletion in the hemizygous daughters, chosen to measure *k* values in these and other crosses involving an insert-bearing *Y* for reasons explained in MATERIALS AND METHODS.

Although there is a clear increase in drive when *E(SD)* is added, no such increase occurs when a second copy of *Sd* is added to an *Sd Df(E(SD)) Rsp^i* genotype (mean *k* = 0.508, last column, Table 4). This result is consistent with the "trigger hypothesis" proposed by LYTTLE (1986), whereby only a small amount of *Sd* product may be needed to activate the distortion mechanism, but the strength of distortion is proportional to the dose of *E(SD)*.

In sum, this experiment demonstrates that the candidate *Y* chromosome recovered in the mutagenesis screen for a deletion of *Sd* from the *Y·Sd E(SD)* construct does indeed retain *E(SD)*, as predicted from the preliminary cytological and complementation tests. The *Y·E(SD)* confers the characteristic properties of *E(SD)*, of action in *trans* and of increasing effectiveness with dose. The dosage effect of *E(SD)* in the presence of *Sd* is apparent whether the change is from one to two doses of *E(SD)* or from zero to one. The enhancing effect of an additional *E(SD)* contrasts with the lack of effect of an extra *Sd* supplied to an *Sd*-bearing genome, at least when the added component is on the *Y* chromosome.

The effect of *E(SD)* in the absence of *Sd*: To determine whether *E(SD)* in sufficiently high dosage can produce strong distortion even in the absence of *Sd*, genotypes were constructed that contained two or more doses of *E(SD)* but lacked the *Sd* allele. Three types of configurations were generated: (1) where both second chromosomes carried *E(SD)* and one of these provided the *Rsp^s* target (Table 5); (2) where the *Y·E(SD)* supplied one of the copies of *E(SD)*, and additional doses were on the second chromosome (Table 6); and (3) where both second chromosomes were *E(SD) Rsp^i* and the *Rsp^s* target was furnished by the *Y* (Table 7).

E(SD) Rsp^i/E(SD) Rsp^s genotypes: In this series the *Cy ReR cn (Sd$^+$ E(SD) Rsp^s)* chromosome was heterozygous with a completely reverted *SD* chromosome carrying *E(SD)* but lacking the *Sd* allele. Two classes of complete *SD* revertants (*SD^R*) were used; in one the *SD* chromosome was deleted for *Sd*, and in the other, it was *Sd$^+$* as a result of a rare recombinational event. Each experiment was repeated with the independent isolates *Cy ReR cn-4* and *Cy ReR cn-5*, giving essentially the same results. The transmission ratios in *E(SD)/E(SD)* males of a variety of combinations were compared with those in males having one dose of *E(SD)*. Table 5 shows that significant segregation distortion can occur in males carrying two doses of *E(SD)* even in the absence of *Sd*. The degree of distortion varies with the particular *SD* derivative tested. When *SD-*

TABLE 5

Distortion by two doses of *E(SD)* in the absence of *Sd*

	Sd^+ *E(SD)* Rsp^s		Sd^+ *E(SD)*$^+$ Rsp^s		Sd^+ *E(SD)*$^+$ Rsp^{st}
	Cy ReR cn-4	*Cy ReR cn-5*	*Cy R cn*	*cn bw*	*lt pk cn bw*
Doses of *E(SD)*	2	2	1	1	1
Df[Sd] E(SD) Rsp^i					
SD-Mad^{R77}	0.873 ± 0.040	0.890 ± 0.044	0.519 ± 0.028	0.533 ± 0.022	0.676 ± 0.033
*SD-Mad^{R68}**	0.772 ± 0.077	0.837 ± 0.056	0.511 ± 0.058	0.450 ± 0.085	0.475 ± 0.066
SD-Roma revertants					
SD-Roma^{R14}, bw	0.579 ± 0.036	0.576 ± 0.027	0.488 ± 0.019	0.563 ± 0.027	0.446 ± 0.033
SD-Roma^{R57}, bw	0.641 ± 0.038	0.535 ± 0.031	0.536 ± 0.022	0.521 ± 0.028	0.428 ± 0.031
SD-Roma^{R2}, bw	0.507 ± 0.024	0.570 ± 0.036	0.475 ± 0.022	0.510 ± 0.029	0.472 ± 0.022
Sd^+ *E(SD)* Rsp^i					
SD-5^{R2}	0.683 ± 0.033	0.724 ± 0.037	0.539 ± 0.021	0.539 ± 0.023	0.581 ± 0.027
SD-5^{R7}	0.721 ± 0.043	0.762 ± 0.032	0.555 ± 0.025	0.539 ± 0.016	0.526 ± 0.026
SD-Mad$^{ER8.2}$	0.674 ± 0.034	0.667 ± 0.054	0.582 ± 0.037	0.510 ± 0.032	0.514 ± 0.034
R(Cy)40, bw				0.475 ± 0.021	0.427 ± 0.031
Sd^+ *E(SD)* Rsp^s					
Cy ReR cn-4				0.523 ± 0.031	0.465 ± 0.053
Cy ReR cn-5				0.498 ± 0.022	0.484 ± 0.053
Df[Sd] Df[E(SD)] Rsp^i					
Doses of *E(SD)*	1	1	0	0	0
SD-5^{R37},lt	0.573 ± 0.025	0.571 ± 0.024	0.529 ± 0.022	0.514 ± 0.019	0.489 ± 0.021

Each k value (±2 SE) is the proportion of progeny inheriting the *SD* derivative from males heterozygous for the *SD* derivative named on the left and its SD^+ homolog named at the top of the column. SD^R is a complete *SD* revertant, carrying Sd^R, which is *Df(Sd)* or Sd^+. The Sd^R *E(SD)* Rsp^s/*Cy ReR cn* males and the respective controls are sons of *CyO*/Sd^R fathers and *Cy ReR cn*, *Cy R cn*, *cn bw*, or *lt pk cn bw* mothers. *Cy ReR cn* can appear here as an SD^+ chromosome or an *SD* derivative. k values are viability corrected. * For *Dp(2;Y)G; SD-Mad^{R68}* k is computed from daughters only. Dosages of *E(SD)* are given.

Mad was the source of the *SD* revertant chromosome, the k values for SD^R/*Cy ReR cn* males were as high as 0.890 (columns 1 and 2, rows 1 and 2). The k values were higher when the SD^R chromosome was deleted for *Sd [Df(Sd) E(SD) Rsp^i]*, as in rows 1 and 2, than when it was recombinant *[Sd^+ E(SD) Rsp^i]*, as in rows 6, 7 and 8, but this pattern was not consistent in the other relevant experiments (Tables 6–8). For *SD-Mad^R* or *SD-5^R* the mean k values for *E(SD)/E(SD)* males, whether Sd^+ or *Df(Sd)*-bearing, were significantly higher in all cases than the k values for males with only one dose of *E(SD)* (columns 3–5).

The control of particular interest is the test with *Cy R cn* (column 3), the progenitor of *Cy ReR cn* and genetically identical to it except for the *E(SD)* region (see MATERIALS AND METHODS). From the Mendelian ratios observed for *Cy R cn* males (mean $k = 0.526$) it may be concluded that this chromosome does not carry any effector gene(s) capable of initiating distortion in the absence of *Sd*, even though it may harbor some modifiers that raise sensitivity in the presence of *Sd* (Table 3). The differences in k values between *SD-Mad^R* or *SD-5^R* males carrying *Cy ReR cn* as opposed to *Cy R cn* are therefore most likely attributable to *E(SD)* itself.

E(SD) in the *SD-Roma* revertants (rows 3–5) may seem to be an exception, since there is not much difference between the k values for males with two doses of *E(SD)* and those with one. The mean k for *SD-Roma^{R}bw/Cy ReR cn-4* is 0.576 and for *SD-Roma^{R}bw/Cy ReR cn-5* is 0.560, whereas for *SD-Roma^{R}bw/Cy R cn*, the most appropriate control, the mean $k = 0.500$. Nevertheless, the differences between one and two doses of *E(SD)* are significant by analysis of variance. For *Cy ReR cn-4 vs. Cy R cn*, $F(1, 2) = 52.5$ ($P < 0.05$), and for *Cy ReR cn-5 vs. Cy R cn*, $F(1, 2) = 34.7$ ($P < 0.05$), whereas for *Cy ReR cn-4 vs. Cy ReR cn-5*, $F(1, 2) = 1.7$ (NS). The Enhancer in *SD-Roma* therefore does have an effect in the absence of *Sd*, although it is weaker than that of *SD-5* or *SD-Mad*, supporting the hypothesis of allelic variability among Enhancers.

From the data in Table 5, as in Table 2, there is no consistent evidence for significant distortion caused by one dose of *E(SD)* (columns 3–5) even when the single copy of *E(SD)* was of the "stronger" type, as on the *SD-Mad^R*, *SD-5^R*, or *Cy ReR cn* chromosome. The one exception is for *SD-Mad^{R77}/lt pk cn bw*, where $k = 0.676$; interestingly, this result was also found by BRITTNACHER and GANETZKY (1984), but they explained it in terms of an elevated k for this chromosome even when it segregated from an insensitive homolog.

In sum, significant distortion independent of *Sd* is observed with two doses of *E(SD)*, and the strength of this distortion varies with the *SD* chromosomal source of the *E(SD)*.

Y·E(SD) bearing genotypes: The *Y·E(SD)* construct provided an alternative to the *Cy ReR cn* chromosome for generating males with two or three doses of *E(SD)*

TABLE 6

k values in SD^R/SD^+ males with the $Y{\cdot}E(SD)$ as compared with a normal Y

	Normal Y	$Y{\cdot}E(SD)$
Df(Sd) E(SD)Rsp^i/Sd$^+$ E(SD)$^+$ Rsp$^{s(i)}$	1 *E(SD)*	2 *E(SD)*
SD-Mad^{R77}/cn bw	0.464 ± 0.020	0.490 ± 0.015
SD-Mad^{R77}/lt pk cn bw	0.455 ± 0.026	0.414 ± 0.015
SD-Roma^{R14}/cn bw	0.552 ± 0.028	0.529 ± 0.018
SD-Roma^{R14}/lt pk cn bw	0.442 ± 0.036	0.467 ± 0.018
SD-Roma^{R57}/cn bw	0.470 ± 0.016	0.498 ± 0.024
SD-Roma^{R57}/lt pk cn bw	0.429 ± 0.023	0.461 ± 0.023
Sd$^+$ E(SD)Rsp^i/Sd$^+$ E(SD)$^+$ Rsp$^{s(i)}$	1 *E(SD)*	2 *E(SD)*
SD-5^{R2}/cn bw	0.557 ± 0.019	0.609 ± 0.021
SD-5^{R2}/lt pk cn bw	0.604 ± 0.020	0.600 ± 0.023
SD-5^{R7}/cn bw	0.560 ± 0.027	0.575 ± 0.018
SD-5^{R7}/lt pk cn bw	0.515 ± 0.021	0.565 ± 0.010
Mean k values for 1 *E(SD) vs.* 2 *E(SD)*	0.505	0.521
Df(Sd) E(SD)Rsp^i/Sd$^+$ E(SD) Rsp^s	2 *E(SD)*	3 *E(SD)*
SD-Mad^{R77}/Cy ReR cn-4	0.705 ± 0.038	0.673 ± 0.020
SD-Mad^{R77}/Cy ReR cn-5	0.689 ± 0.030	0.620 ± 0.060
SD-Roma^{R14}/Cy ReR cn-4	0.623 ± 0.026	0.683 ± 0.025
SD-Roma^{R14}/Cy ReR cn-5	0.658 ± 0.025	0.679 ± 0.036
SD-Roma^{R57}/Cy ReR cn-4	0.511 ± 0.015	0.516 ± 0.015
SD-Roma^{R57}/Cy ReR cn-5	0.534 ± 0.016	0.518 ± 0.016
Sd$^+$E(SD)Rsp^i/Sd$^+$ E(SD) Rsp^s	2 *E(SD)*	3 *E(SD)*
SD-5^{R2}/Cy ReR cn-4	0.853 ± 0.028	0.799 ± 0.022
SD-5^{R2}/Cy ReR cn-5	0.917 ± 0.027	0.833 ± 0.020
SD-5^{R7}/Cy ReR cn-4	0.794 ± 0.021	0.795 ± 0.023
SD-5^{R7}/Cy ReR cn-5	0.793 ± 0.042	0.741 ± 0.048
Mean k values for 2 *E(SD) vs.* 3 *E(SD)*	0.717	0.690

k (±2 SE) is the mean proportion of progeny inheriting the SD^R(*Sd^{R}E(SD) Rsp^i*) chromosome from SD^R/Sd^+ *E(SD)^x Rsp$^{s(i)}$* males carrying either a normal Y or the $Y{\cdot}E(SD)$. *Sd^R* is *Df(Sd)* or *Sd$^+$*. The *Sd$^+$E(SD)$^+$* homolog *cn bw* is *Rsp^s* and *lt pk cn bw* is *Rsp^{ss}*. The k values are not corrected for viability since direct male to male comparisons are shown. The dosage of *E(SD)* is given.

to assay the effect of increased dosage of *E(SD)* in the absence of *Sd*. Table 6 shows that when the $Y{\cdot}E(SD)$ supplies the second or third copy of *E(SD)* to a genotype lacking the *Sd* allele there is no change in transmission ratio compared to that for males with a normal Y. The mean k values for 1 *vs.* 2 doses of *E(SD)* are 0.505 and 0.521, and for 2 *vs.* 3 doses are 0.717 and 0.690, when the extra *E(SD)* in each case is on the Y. This is in spite of the clear enhancing activity of the same $Y{\cdot}E(SD)$ in the presence of *Sd* (Table 4).

The data in Table 6 (bottom half) confirm the high degree of *Sd*-independent distortion incurred by two doses of *E(SD)* when the *E(SD)* on *Cy ReR cn* acts in conjunction with the *E(SD)* of a strong SD^R derivative, as in *SD-5^R/Cy ReR cn* males (last 4 rows, $k = 0.741 - 0.917$). Although the absolute k values cannot be compared directly with those in Table 5 because of different genetic backgrounds and because these k values are not corrected for viability (see MATERIALS AND METHODS), the results confirm the quantitative distinction noted between *SD-5^R* and *SD-Roma^R* (third section, Table 6) when each is heterozygous with *Cy ReR cn*.

A possible consideration in the experiments on independent *E(SD)* activity (Tables 5 and 6) is that in *E(SD)/E(SD)* males such as *SD-5^R/Cy ReR cn*, which do distort, *E(SD)$^+$* is absent. On the other hand, in $Y{\cdot}E(SD)$; *E(SD)/E(SD)$^+$* males, which fail to distort despite carrying two doses of *E(SD)*, the *E(SD)$^+$* allele is present. Is there an inhibition by *E(SD)$^+$*? The *SD-5$^{Rev\ 37}$lt* derivative, deleted for *E(SD)* as well as for *Sd*, permits a test of whether the $Y{\cdot}E(SD)$ can contribute to *Sd*-independent distortion in *E(SD)*-bearing males if they lack *E(SD)$^+$*. Substituting *SD-5$^{Rev\ 37}$lt* as the SD^R chromosome in an experiment analogous to that in Table 6 gives the following results: for $Y{\cdot}E(SD)$; *Df(Sd) Df[E(SD)] Rsp^i/Sd$^+$ E(SD) Rsp^s* males, with two doses of *E(SD)* but no *E(SD)$^+$*, $k = 0.528 \pm 0.036$ (*ReR-4*) or $k = 0.572 \pm 0.004$ (*ReR-5*). For males with the same pair of second chromosomes but a normal Y and therefore one dose of *E(SD)*, $k = 0.535 \pm 0.018$ (*ReR-4*) or $k = 0.544 \pm 0.024$ (*ReR-5*). The k values are still no greater in the $Y{\cdot}E(SD)$ males lacking *E(SD)$^+$* than in males with the normal Y, so there is no reason to believe that the absence of drive in $Y{\cdot}E(SD)$; *E(SD)/E(SD)$^+$* males free of *Sd* is due to suppression by *E(SD)$^+$*.

TABLE 7

Distortion of the *Y·Rsp*^{s} by two doses of *E(SD)* in the absence of *Sd*

		Proportion of daughters		
		Phenotype A	Phenotype B	All progeny
X/Y·Rsp^{s}; *Sd*^{R} *E(SD) Rsp*^{i}/*Sd*^{R} *E(SD) Rsp*^{i}:2 doses *E(SD)*				
R(Cy)40bw/SD-Mad^{R77}	(3)/(7)	0.842 ± 0.016	0.816 ± 0.017	0.828 ± 0.014
R(Cy)40bw/SD-5^{R2}	(3)/(6)	0.934 ± 0.009	0.946 ± 0.009	0.940 ± 0.008
R(Cy)40bw/SD-5^{R7}	(3)/(6)	0.761 ± 0.027	0.809 ± 0.019	0.790 ± 0.019
SD-Mad^{R77}/*SD-5*^{R2}	(7)/(6)	0.967 ± 0.011	0.955 ± 0.015	0.961 ± 0.013
SD-Mad^{R77}/*SD-5*^{R7}	(7)/(6)	0.842 ± 0.024	0.864 ± 0.023	0.852 ± 0.022
SD-Roma^{R57}/*SD-5*^{R2}	(7)/(6)	0.820 ± 0.020	0.834 ± 0.019	0.826 ± 0.017
SD-Roma^{R14}/*SD-5*^{R2}	(7)/(6)	0.853 ± 0.020	0.853 ± 0.020	0.854 ± 0.013
SD-Roma^{R57}/*SD-5*^{R7}	(7)/(6)	0.755 ± 0.031	0.826 ± 0.029	0.790 ± 0.027
	Mean *k*	0.86	0.87	0.86
X/Y; Sd^{R} *E(SD) Rsp*^{i}/*Sd*^{R} *E(SD) Rsp*^{i}: 2 doses *E(SD)*				
R(Cy)40bw/SD-Mad^{R77}	(3)/(7)	0.520 ± 0.017	0.486 ± 0.016	0.504 ± 0.010
R(Cy)40bw/SD-5^{R2}	(3)/(6)	0.568 ± 0.024	0.494 ± 0.016	0.523 ± 0.014
R(Cy)40bw/SD-5^{R7}	(3)/(6)	0.486 ± 0.017	0.488 ± 0.016	0.488 ± 0.014
SD-Mad^{R77}/*SD-5*^{R2}	(7)/(6)	0.499 ± 0.017	0.497 ± 0.018	0.500 ± 0.011
SD-Mad^{R77}/*SD-5*^{R7}	(7)/(6)	0.510 ± 0.029	0.497 ± 0.017	0.507 ± 0.014
	Mean *k*	0.52	0.49	0.50
X/Y·Rsp^{s}; *Sd*^{R} *E(SD) Rsp*^{i}/*Sd*^{R} *E(SD)* Rsp*^{x}: 1 dose *E(SD)*				
R(Cy)40bw/SD-5^{R37}	(3)/(10)	0.598 ± 0.043	0.579 ± 0.108	0.590 ± 0.071
R(Cy)40bw/Rsp^{i16}*cnbw*	(3)/(9)	0.594 ± 0.024	0.600 ± 0.026	0.598 ± 0.015
SD-Mad^{R77}/*Rsp*^{i16}*cnbw*	(7)/(9)	0.523 ± 0.015	0.546 ± 0.018	0.534 ± 0.011
SD-5^{R2}/*cnbw*	(6)/(2)	0.576 ± 0.015	0.684 ± 0.026	0.615 ± 0.016
SD-5^{R7}/*cnbw*	(6)/(2)	0.609 ± 0.014	0.670 ± 0.017	0.635 ± 0.012
SD-Mad^{R77}/*cnbw*	(7)/(2)	0.540 ± 0.019	0.582 ± 0.014	0.561 ± 0.011
SD-Roma^{R14}/*cnbw*	(7)/(2)	0.623 ± 0.021	0.592 ± 0.034	0.610 ± 0.023
SD-Roma^{R57}/*cnbw*	(7)/(2)	0.570 ± 0.022	0.596 ± 0.023	0.585 ± 0.018
	Mean *k*	0.59	0.60	0.59
X/Y; Sd^{R} *E(SD) Rsp*^{i}/*Sd*^{R} *E(SD)* Rsp*^{x}: 1 dose *E(SD)*				
R(Cy)40bw/SD-5^{R37}	(3)/(10)	0.521 ± 0.016	0.435 ± 0.021	0.485 ± 0.016
R(Cy)40bw/Rsp^{i16}*cnbw*	(3)/(9)	0.489 ± 0.015	0.507 ± 0.011	0.501 ± 0.008
	Mean *k*	0.51	0.47	0.49
X/Y·Rsp^{s}; *Df(Sd) Df [E(SD)] Rsp*^{i}/*Sd*$^{+}$ *E(SD)*$^{+}$ *Rsp*^{i}: 0 dose *E(SD)*				
SD-5^{R37}/*Rsp*^{i16}*cnbw*	(10)/(9)	0.553 ± 0.022	0.609 ± 0.020	0.583 ± 0.016
X/Y; Df(Sd) Df [E(SD)] Rsp^{i}/*Sd*$^{+}$ *E(SD)*$^{+}$ *Rsp*^{i}: 0 dose *E(SD)*				
SD-5^{R37}/*Rsp*^{i16}*cnbw*	(10)/(9)	0.422 ± 0.017	0.483 ± 0.014	0.459 ± 0.012

The mean proportion of daughters among all progeny of fathers carrying a *Y* with a *Rsp*^{s} insert or a normal *Y*, and two, one, or zero doses of *E(SD)*. *Sd*^{R} = *Sd*$^{+}$ or *Df(Sd)*; *Rsp*^{x} = *Rsp*^{s} or *Rsp*^{i}; *E(SD)** = *E(SD)*$^{+}$ or *Df(E(SD))*. Phenotype A refers to progeny inheriting homolog A and phenotype B refers to progeny inheriting homolog B, from males that carry homolog A/homolog B as named in left to right order. The numbers in parentheses refer to the genotypes of homologs A and B, respectively, as diagrammed in Figure 2, namely (2) = *Sd*$^{+}$ *E(SD)*$^{+}$ *Rsp*^{s}; (3) = *Sd*$^{+}$ *E(SD) Rsp*^{i}, by normal recombination; (6) = *Sd*$^{+}$ *E(SD) Rsp*^{i} recovered during a radiation experiment; (7) = *Df(Sd) E(SD) Rsp*^{i}; (9) = *Sd*$^{+}$ *E(SD)*$^{+}$ *Rsp*^{i}; and (10), see MATERIALS AND METHODS, = *Df(Sd) Df [E(SD)] Rsp*^{i}. The *k* values are not corrected for viability since direct comparisons are made between fathers with a normal *Y* or *Y·Rsp*^{s}.

The *Y·E(SD)* is simply not as effective as the *E(SD)*-bearing *Cy ReR cn*.

In sum, the *Y* chromosome carrying the *E(SD)* insert, which originates from *SD-Roma*, does not produce distortion when serving as the second or even third dose of *E(SD)* in males free of *Sd*. This contrasts with the ability of this *Y·E(SD)* to enhance distortion in genomes that carry *Sd* (Table 4). The *SD-Roma E(SD)* contained within the progenitor *Dp(2;Y) Sd E(SD)* likewise can enhance distortion (Table 2). Therefore, the action of this *E(SD)* when inserted in the *Y* chromosome is detectable only in an *Sd*-bearing genome. The *E(SD)* in its native location on the *SD-Roma* chromosome deleted for *Sd* can produce some distortion in the absence of *Sd* in conjunction with the *E(SD)* on *Cy ReR cn*, but it is very mild (Table 5). These properties are consistent with the hypothesis of quantitative variation due to allelic differences at *E(SD)*. The *SD-Roma E(SD)* is sufficiently weak that when translocated to the *Y* it cannot produce the more subtle *Sd*-independ-

ent distortion even though it can do so, to a slight but measurable degree, when in its normal second chromosome position.

Y· Rsp^s; E(SD) Rsp^i/E(SD) Rsp^i males: To determine whether distortion by two doses of *E(SD)* is similar in mechanism to that caused by *Sd*, experiments were done to investigate *Rsp^s* as the target of *E(SD)*-mediated distortion. Since *Rsp^s* can function as the site for *SD* action even when removed from the second chromosome, it was convenient to use an *Rsp^s*-bearing *Y* (constructed by LYTTLE 1989) as the potential target. To test whether *E(SD)* in double dose can induce dysfunction of *Y·Rsp^s* bearing sperm, males that carried *Rsp^i* as well as *E(SD)* on both second chromosomes were tested, so that the only *Rsp^s* present was that on the *Y*. There are a number of *SD* revertant or recombinant chromosomes, listed in Table 7, that can be made heterozygous with one another to produce the desired genotype.

If the double dose of *E(SD)* directs its action toward the *Rsp^s* on the *Y*, distortion of the sex ratio is expected, giving an excess of daughters. In Table 7, the relative transmission of the *X* and *Y* chromosomes in *X/Y·Rsp^s; Sd^R E(SD) Rsp^i/Sd^R E(SD) Rsp^i* males is compared with that in males carrying either a normal *Y*, in one set of controls, or only one dose of *E(SD)*, in another set of controls. *Sd^R* signifies *Df(Sd)* or *Sd$^+$*. In measuring the effect of drive on a sex chromosome the data are given as the proportion of daughters (among all progeny), to be analogous with *k* values, which represent the proportion of progeny developing from *Rsp^i* (*i.e.*, non-*Rsp^s*) sperm.

The mean proportion of daughters from *X/Y·Rsp^s; Sd^R E(SD) Rsp^i/Sd^R E(SD) Rsp^i* fathers is 0.86 (top section of Table 7), compared to 0.50 for *X/Y; Sd^R E(SD) Rsp^i/Sd^R E(SD) Rsp^i* fathers (middle section), each carrying two doses of *E(SD)*. This supports the hypothesis that *Rsp* is the site of action of *E(SD)*. The excess of daughters from *Y·Rsp^s* fathers is observed for eight different combinations and is found consistently in both phenotypic subclasses. This excess is partly due to a viability effect of the *B^s* marker linked to *Rsp^s* on the *Y·Rsp^s* chromosome (LYTTLE 1989). To avoid this complication, a second control was performed to measure the sex ratio among the progeny of *Y·Rsp^s* fathers carrying just a single dose of *E(SD)* and not, therefore, expected to produce distortion. When either an *E(SD)$^+$* or a *Df(E(SD))* chromosome is substituted for one of the *Sd^R E(SD) Rsp^i* homologs in the *Y·Rsp^s* fathers, the average proportion of daughters is 0.59 (third section, Table 7). Furthermore, when the dosage of *E(SD)* is zero in the *Y·Rsp^s* fathers, the proportion of daughters is 0.58 (section 5, Table 7). These values exceed the Mendelian expectation because of viability effects in the hyperploid males, but the deviation is much less than observed when two doses of *E(SD)* are present.

In sum, there is strong distortion of the sex ratio in favor of daughters (*i.e.*, 0.86 *vs.* 0.59) arising from *Y·Rsp^s; E(SD)/E(SD)* fathers, as expected if distortion by two copies of *E(SD)* is mediated via the *Rsp^s* on the *Y*. This experiment also shows that distortion by two doses of *E(SD)* does not require the presence of the *Cy ReR cn* chromosome in particular.

Suppression of *E(SD)/E(SD)* distortion by *Su(SD)*: Another test of the similarity in distortion caused by *E(SD)* and *Sd* was to ask whether a suppressor of segregation distortion in general also suppresses the effect of two doses of *E(SD)*. For this purpose, the dominant suppressor of distortion on the *TM6* chromosome (LYTTLE 1986) is used. The *E(SD)* distorting genotypes are heterozygous for an *SD^R* chromosome (*Sd^R E(SD) Rsp^i*) and *Cy ReR cn (Sd$^+$ E(SD) Rsp^s)*, with the addition of the third chromosome suppressor, as shown in Table 8.

This *Su(SD)* is capable of reducing the *k* value in *SD-Mad/cn bw* males from 0.980 to 0.734 (top row). When the effector components are in the alternate linkage phase, in *Sd Df[E(SD)] Rsp^i/Sd$^+$E(SD) Rsp^s* males, the *Su(SD)* likewise reduces the *k* value, from 0.999 to 0.860 (means of rows 2 and 3). When there is one dose of *Sd* and two of *E(SD)*, the *k* value is reduced from 1.000 to 0.989 by the suppressor(means of rows 4 and 5). Furthermore, the *Su(SD)* suppresses the activity of *Sd* alone: when an *SD* chromosome deleted for *E(SD)* segregates from *Rsp^{ss}*, the *k* value is reduced from 0.808 to 0.584 (row 6). Most importantly, distortion produced by two copies of *E(SD)* in the absence of *Sd* is also suppressed, from $k = 0.797$ to $k = 0.642$ (pooled for rows 7–12).

Therefore, the *TM6 Su(SD)* is not specific to one or the other of the effector loci but can affect either *Sd* and/or *E(SD)*, suggesting that distortion by *E(SD)* and by *Sd* share at least certain mechanistic properties.

DISCUSSION

***E(SD)* properties:** The Enhancer of Segregation Distortion is a member of the tripartite *SD* complex (*Sd E(SD) Rsp^i*) as it exists in wild populations. Neither *Sd* nor *E(SD)* has been found in nature without the other as far as has been investigated (*e.g.*, BRITTNACHER and GANETZKY 1984; LYTTLE, BRITTNACHER and GANETZKY 1986). This is not unexpected since the *SD* chromosomes with the strongest drive, those that are *Sd E(SD)*, would be at an advantage. To examine the genetic behavior of *E(SD)* separately from *Sd* and to manipulate its dose, a large repertoire of rearrangements carrying one or the other of the elements was used to generate a variety of genotypic configurations. When *Sd* is present, *E(SD)* intensifies the level of distortion in a dosage dependent pattern. Moreover, *E(SD)*, whether in its normal position on a second chromosome homolog or inserted into the *Y*

TABLE 8

The effect of a third chromosome *Su(SD)* on *SD* distortion and *E(SD)* distortion

Genotype	Doses *Sd*	Doses *E(SD)*	*Su(SD)*	*Su(SD)*$^+$
Sd E(SD) Rsp^i/Sd$^+$E(SD)$^+$Rsp^s	1	1		
SD-Mad/cnbw			0.734 ± 0.023	0.980 ± 0.010
Sd Df[E(SD)] Rsp^i/Sd$^+$E(SD) Rsp^s	1	1		
SD-Mad^{lt73}/Cy ReR cn-4			0.825 ± 0.026	0.999 ± 0.001
SD-72^{lt23}/Cy ReR cn-4			0.891 ± 0.033	0.998 ± 0.003
Sd E(SD) Rsp^i/Sd$^+$ E(SD) Rsp^s	1	2		
SD-Mad/Cy ReR cn-4			0.991 ± 0.008	1.000 ± 0
SD-Mad/Cy ReR cn-5			0.986 ± 0.009	0.999 ± 0.002
Sd Df[E(SD)] Rsp^i/Sd$^+$E(SD)$^+$Rsp^{ss}	1	0		
SD-Mad^{lt73}/lt pk cnbw			0.584 ± 0.022	0.808 ± 0.023
Df(Sd) E(SD) Rsp^i/Sd$^+$E(SD)Rsp^s	0	2		
SD-Mad^{R77}/Cy ReR cn-4			0.673 ± 0.021	0.726 ± 0.061
SD-Mad^{R77}/Cy ReR cn-5			0.652 ± 0.028	0.787 ± 0.066
Sd$^+$ E(SD) Rsp^i/Sd$^+$E(SD)Rsp^s	0	2		
SD-5^{R2}/Cy ReR cn-4			0.592 ± 0.033	0.791 ± 0.053
SD-5^{R2}/Cy ReR cn-5			0.692 ± 0.032	0.875 ± 0.034
SD-5^{R7}/Cy ReR cn-4			0.622 ± 0.029	0.856 ± 0.046
SD-5^{R7}/Cy ReR cn-5			0.617 ± 0.030	0.730 ± 0.043

Mean k values are given for *SD*/SD$^+$* males in the presence and absence of *Su(SD)*, a dominant suppressor of *SD* on chromosome *3* associated with *In(3LR)TM6*. *SD* (Sd* E(SD)* Rsp^i)* refers to variation either at *Sd* or *E(SD)*, as specified: *Sd** is *Sd* or *Df(Sd)* or *Sd$^+$*; *E(SD)** is *E(SD)* or *Df[E(SD)]* or *E(SD)$^+$*. The superscript *R* refers to a complete *SD* revertant, and *lt* to *Df(lt) Df(E(SD))*. k (±2 SE) is the proportion of *SD** progeny among the total, corrected for viability.

chromosome can, in *trans*, restore strong drive to *SD* chromosomes deleted for *E(SD)*. Notably, *E(SD)* can distort independently of *Sd* when present in sufficiently high dosage. In fact, k values of up to 0.80 or 0.90 were observed in males free of *Sd* but carrying two doses of *E(SD)* derived from strongly distorting *SD* chromosomes such as *SD-5* or *SD-Mad*. The *E(SD)* of the moderately distorting *SD-Roma* can also contribute to *Sd*-independent distortion, but its effect is much weaker. Allelic variation is suggested by the quantitative differences associated with the parental source of the *E(SD)*. There was no consistent difference between the *Df(Sd) E(SD) Rsp^i* and *Sd$^+$ E(SD) Rsp^i* derivatives in ability to distort when heterozygous with an *Sd$^+$ E(SD) Rsp^s* homolog, implying that *E(SD)* is not required to be physically contiguous with *Sd$^+$* material in order to produce drive in the absence of *Sd* (Tables 5, 6, 8). The drive observed in males homozygous for *E(SD)* may be similar in mechanism to that generated by *Sd* itself, in that (1) *Sd* and *E(SD)* are comparably affected by an unlinked dominant suppressor of *SD* action, and (2) *E(SD)*, like *Sd*, appears to focus its action on the *Rsp* target site. This was confirmed by the significantly reduced transmission of the *Y·Rsp^s* chromosome in the presence of a pair of *E(SD) Rsp^i* homologs lacking *Sd* (Table 7). Distortion of the sex-ratio in that experimental system also shows that drive by two doses of *E(SD)* is not restricted to genotypes carrying the *Sd$^+$ E(SD) Rsp^s* chromosome in particular.

The potential for inducing distortion is therefore not exclusive to the *Sd* gene, although how *Sd* and *E(SD)* interact to produce the complete or nearly complete drive observed in *SD/SD$^+$* males carrying both elements is far from understood. Do *Sd* and *E(SD)* both act directly at *Rsp* with the same mode of action albeit with different quantitative input, or do *Sd* and *E(SD)* perform qualitatively different functions? The evidence for two effector loci suggests an analogy with the mouse *t*-locus, where transmission ratio distortion involves four distorter genes acting on a responder (LYON 1984, 1986; SILVER and REMIS 1987). There, the distorters, some stronger than others, act additively, in *cis* or in *trans*, to raise the transmission of whichever chromosome carries the responder, so that transmission ratio distortion is greater when more distorter loci are present.

Quantitative effects of *Sd* and *E(SD)*: Several investigators have attempted to measure the contributions to distortion by individual components of the *SD* complex. Using deletion data, BRITTNACHER and GANETZKY (1984) estimated the contribution of each element on a simple additive model, reasoning that if the loci acted additively, then the decrease in distortion following deletion of an element should be equivalent to the amount of distortion caused by that element acting alone. To analyze the magnitude of a change in drive on a linear scale they used a probit transformation of $((2k - 1)/k)$, a value which measures

the proportion of Rsp^s sperm rendered non-functional and which ranges from 0 to 1.00 as *k* ranges from 0.50 to 1.00 (MIKLOS and SMITH-WHITE 1971; MIKLOS 1972). This analysis did not support a model of independent additive effects by *Sd* and *E(SD)*. The *SD* chromosomes retaining *E(SD)* but deleted for *Sd* were, in fact, complete revertants, so their observed *k* values were not as high as predicted from the decrease in drive following deletion of *E(SD)* from *SD* chromosomes. Similarly, the *SD* chromosomes retaining *Sd* but deleted for *E(SD)* (the partial revertants), while still distorting, gave *k* values that were not as great as predicted from the large reduction in drive shown by *SD* chromosomes deleted for *Sd*. BRITTNACHER and GANETZKY (1984) concluded that enhancement by *E(SD)* does not reflect a potential by *E(SD)* to distort on its own and in some additive mode. Rather, they favored a model whereby *E(SD)* is basically a modifier acting via the *Sd* locus, perhaps by regulating its expression. In the present study, representatives of the same sets of deletions were retested with *cn bw* and *lt pk cn bw* (Sd^+ $E(SD)^+$ $Rsp^{s(s)}$), as controls for the new tests. The relevant data on the partial revertants, deleted for *E(SD)* (Table 3), and on the complete revertants, deleted for *Sd* (Table 5), in the sets in which *E(SD)* is tested in a single dose, confirm the non-additivity of effects. Although this implies an *Sd-E(SD)* interaction of a more complex nature, it leaves open the question of whether *Sd* and *E(SD)* products in fact physically interact prior to acting at *Rsp*.

In another quantitative approach, LYTTLE (1986) used an insertional translocation of the *Sd E(SD)* region from the base of *2L* into the *Y* to demonstrate a simple additive action of multiple *SD* copies. This result was consistent with the notion that *Sd* is similar to *E(SD)*, with each element acting additively, but since *Sd* and *E(SD)* were both encompassed within the *Y·SD* it was not possible to separate out individual effects. LYTTLE (1986) further considers a model whereby the strength of distortion is determined by *E(SD)*, but the "trigger" to activate the expression of *E(SD)* is provided by the *Sd* product, required in just a small amount. This hypothesis is based on the observation that an extra dose of *Sd* alone on the *Y* had no effect when added to a genome already containing an intact *SD* complex (T. LYTTLE, unpublished results) whereas an extra dose of the *Y·Sd E(SD)* did cause *k* values to go up, implicating *E(SD)* as the source of the increased drive.

The evidence presented for independent and significant distortion by *E(SD)* in two copies speaks to each of the above models in sorting out the contributions of *Sd* and *E(SD)*. Whereas *E(SD)* is not required for distortion, it is sufficient for distortion in the absence of *Sd*. *E(SD)* is more than a simple modifier of the drive activity of *Sd* and it does not need to act through *Sd*, but can exert an action of its own at *Rsp*. Nor does *E(SD)* need to be switched on by *Sd*. The dosage requirements of the two loci do differ. The significant distortion seen with two doses of *E(SD)* although not with one dose implies that the *E(SD)* product needs to reach a threshold before it can distort at a level comparable to that caused by a single dose of *Sd* itself, on the average. Significant drive by two doses of *E(SD)* was also found by E. SHIMAKAWA (cited in TEMIN *et al.* 1991). The failure to detect consistent or significant distortion here by a single copy of *E(SD)* even with a supersensitive homolog (Table 5), in the many tests affording an opportunity for this to be statistically resolved, confirms the report of BRITTNACHER and GANETZKY (1984). However, since *E(SD)* drive is so hypervariable, an effect of a single copy of *E(SD)* in a sufficiently sensitive background is not ruled out. In fact, SHARP, HILLIKER and HOLM (1985) reported that in certain combinations and especially when *b pr lt pk cn* females, instead of the conventional *cn bw*, were used, *E(SD)* in one dose can produce distortion. T. LYTTLE (cited in TEMIN *et al.* 1991) also found a case of distortion by one copy of *E(SD)*.

Variation in *E(SD)* action with *SD*-chromosomal source: In addition to dosage, the other condition that can influence *E(SD)*-mediated drive is the source of the *E(SD)*, whether it is derived from a strongly or a moderately distorting *SD* chromosome. Different allelic states at the *E(SD)* locus may exist, "stronger," represented by the *SD-72*, *SD-Mad* or *SD-5* type, and "weaker," represented by *SD-Roma*. The alleles might make different amounts of product or products that differ in efficiency of binding, either with another protein (*Sd* or even Sd^+ protein) or with DNA (either at *Sd* or Sd^+ or more likely at *Rsp*). Or, two different functions may be implicated such that the *SD-Roma E(SD)* is attenuated in one of the functions. The preferred sites of action may differ; for example, the *SD-Roma E(SD)* might interact primarily with *Sd* (or *Sd* product) and only weakly with *Rsp*, whereas the *E(SD)* from *SD-5* (or *SD-Mad*) might interact efficiently with *Rsp* as well as with *Sd* (or *Sd* product). Structurally, *E(SD)* might be a repeated gene, perhaps in tandem in one allelic class and dispersed in the other. There is a suggestion in the literature that the *E(SD)* of *SD-Roma* (BRITTNACHER and GANETZKY 1984) and the *E(SD)* of *SD-5* (SHARP, HILLIKER and HOLM 1985), each proximal to *lt*, may be at somewhat different positions within the centric *2L* heterochromatin, although how this would affect gene expression is unknown.

Allelic variation at *E(SD)* could be responsible, at least in part, for the range in *k* values displayed by naturally occurring *SD* chromosomes, along with the right arm modifiers held in linkage disequilibrium by

the characteristic *2R* inversions. Such linked right arm modifiers, however, cannot entirely explain the high *k* values because *SD-VO17lt*, a strong distorter recently isolated from a Spanish population, lacks the *2R* inversions but has a *k* value of 0.99 (R. G. TEMIN and R. KREBER, unpublished results). Each *Sd* may have coevolved with its own particular *E(SD)*, such that once an *Sd* has captured an Enhancer that promotes the transmission of that *SD* chromosome, it becomes fixed by the close linkage at the base of *2L*, assisted in some cases by the pericentric inversion, as in *SD-72* or *SD-Mad*.

Effector elements inserted into the *Y*: Whereas distortion by *E(SD)* in the absence of *Sd* occurs when *E(SD)* is homozygous on the second chromosome, such drive is not seen in *Y·E(SD); E(SD)/E(SD)*$^+$ males, which also carry two copies of *E(SD)* (Table 6). Moreover, when the *Y·E(SD)* is added as the third dose to a strong *E(SD)/E(SD)* combination, there is no increase in the drive already produced. In contrast, the *Y·E(SD)* does restore distortion when furnished as the sole copy of *E(SD)* to males carrying an *E(SD)*-deleted derivative (*Sd Df[E(SD)] Rsp*^{i}) and it magnifies distortion when added as the second copy of *E(SD)* to an intact *SD* chromosome (*Sd E(SD) Rsp*^{i}) (Table 4). Furthermore, the same *E(SD)*, derived from *SD-Roma*, intensifies distortion when it is part of the *Y·Sd E(SD)* construct (Table 2). Therefore, just the fact of being inserted into the *Y* does not prevent *E(SD)* from enhancing drive when *Sd* is also in the genome, whether *Sd* is physically contiguous or not. These results imply two separate functions for *E(SD)*, one to enhance *Sd*, a function not impaired in the *Y·E(SD)*, and the other, to distort independently of *Sd*, which is impaired in the *Y·E(SD)*, at least when it is the putatively weaker *E(SD)* of *SD-Roma* that is transposed to the *Y*.

There is a suggestion, regarding *Sd*, that being embedded within *Y* heterochromatin may sometimes limit the drive potential of an isolated effector element. When an extra copy of *Sd* on the *Y* is added to a genome that already contains *Sd*, drive does not increase (Table 4, and T. LYTTLE, unpublished results) even though this *Y·Sd* by itself can induce considerable drive (Table 2). On the other hand, GANETZKY (1977) found that an additional dose of *Sd* on a second chromosome can increase drive substantially, from $k = 0.69$ for *Sd*$^+$ *E(SD)*$^+$ *Rsp*^{i}*/Sd E(SD) Rsp*^{s} males (one copy of *Sd*) to $k = 0.996$ for *Sd Df(E(SD)) Rsp*^{i}*/Sd E(SD) Rsp*^{s} males (in which a copy of *Sd* is added while at the same time a copy of *E(SD)*$^+$ is deleted). This increase is not likely to be due to relief of any suppression by *E(SD)*$^+$. The availability of an *SD* derivative that is *Df[E(SD)]* as well as *Df(Sd)* made it possible to check for the effect of *E(SD)*$^+$, as summarized under RESULTS. Removal of *E(SD)*$^+$ did not affect the segregation ratio, confirming the conclusion by BRITTNACHER and GANETZKY (1984) that *E(SD)*$^+$ is amorphic with regard to distortion. The dosage effects of *Sd* and *E(SD)* on distortion are therefore complex and appear to differ, even though the elements have comparable dosage effects on fecundity, whereby two doses are more severe than one (BRITTNACHER and GANETZKY 1983).

Implications of *E(SD)*-mediated distortion: This paper has presented new evidence supporting the hypothesis of independent action by *E(SD)*. Sufficient *E(SD)* product in a permissive genotype can simulate the effect of *Sd*. It is not known whether *E(SD)* perturbs the normal maturation of *Rsp*^{s} spermatids in a manner identical to *Sd*. Such sperm abnormalities have not yet been looked for in *E(SD)/E(SD)* males. The genetics point to a lesion in the same physiological process. Whatever the mechanism for segregation distortion, it has to explain how the proposed complexing of effector gene product(s) with *Responder* can have such a profound effect on sperm development. Chromatin fails to condense not only in the immediate vicinity of *Rsp* and not only on the second chromosome but in the entire nucleus of the *Rsp*^{s}-bearing spermatids, and tails fail to individualize from the syncytium, leading to specific gametic lethality. The *E(SD)* product could intervene at any one of several points. It might regulate euchromatic gene expression by complexing with the *Sd* (or *Sd*$^+$) gene or gene product. Alternatively, the *E(SD)* product might bind directly with *Rsp*, as proposed for *Sd*. If so, the *Sd* and *E(SD)* proteins might each be able to modify chromatin conformation at *Rsp* to render it more accessible to the binding of the other. The most effective binding at *Rsp* would transpire when both *Sd* and *E(SD)* products are available, possibly forming a complex that could interfere with other proteins vital for chromatin compaction as they accumulate in the *Rsp*^{s} cells during spermiogenesis (HENNIG 1985; BONACORRSI *et al.* 1988, 1990). Such a putative *Sd-E(SD)* complex at *Rsp* might act as a seed to sequester essential proteins and might form a blockade to the activities of such proteins. If there is a sufficiently large amount of *E(SD)* product, such a putative interference might be brought about even in the absence of *Sd* product.

If *Sd* and *E(SD)* are functionally similar in respect to binding at *Rsp*, it is not because the genes are highly related at the DNA sequence level. Southern blotting and *in situ* hybridization with chromosomes deleted for *Sd* but retaining *E(SD)* show that the *Sd* probe does not hybridize to the *E(SD)* region (POWERS in TEMIN *et al.* 1991). However, this does not rule out possible homology or analogy between *Sd* and *E(SD)* at the protein level.

Over and beyond its role in segregation distortion, *E(SD)* takes on interest as a *trans*-acting gene embedded in constitutive heterochromatin, adding to the

growing list of euchromatic-like genes in such regions that are being more fully described (*e.g.* HILLIKER, APPELS and SCHALET 1980; PIMPINELLI *et al.* 1985, 1986; SULLIVAN and PIMPINELLI 1986; MARCHANT and HOLM 1988a,b; DEVLIN *et al.* 1990; WAKIMOTO and HEARN 1990; MCKEE and KARPEN 1990). Whether *E(SD)* remains active when translocated to euchromatin or whether it must reside in (either centric or *Y*) heterochromatin in order to function has not yet been studied. This report shows *E(SD)* to be a heterochromatic gene that can produce the same end result as a euchromatic gene *(Sd)*, given sufficiently high dosage. *E(SD)* may act in concert with that euchromatic gene, perhaps by cooperative binding, and/or may interact with another heterochromatic gene, *Rsp*. Its structure and function will be important to analyze molecularly.

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