ON THE COMPONENTS OF SEGREGATION DISTORTION IN *DROSOPHILA MELANOGASTER*. II. DELETION MAPPING AND DOSAGE ANALYSIS OF THE *SD* LOCUS[1]

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ABSTRACT

Segregation distorter (*SD*) chromosomes are preferentially transmitted to offspring from heterozygous SD/SD^+ males owing to the induced dysfunction of the SD^+-bearing sperm. This phenomenon involves at least two major loci: the *Sd* locus whose presence is necessary for distortion to occur and the *Rsp* locus which acts as the site of *Sd* action. Several additional loci on *SD* chromosomes enhance distortion.——In a previous study deletions were used to map the *Sd* locus and to determine some of its properties. We have extended this analysis with the isolation and characterization of 14 new deletions in the *Sd* region. From our results we conclude (1) *SD* chromosomes contain a single *Sd* locus located in region 37D2-6 of the salivary gland chromosome map. Deletion of this locus in any of three *SD* chromosomes now studied results in complete loss of ability to distort a sensitive chromosome; (2) the reduced male fecundity observed in many homozygous *SD* or SD_i/SD_j combinations is due at least in part to the action of the *Sd* locus. The fecundity of these males can be substantially increased by deletion of one *Sd* locus. Thus, it is the presence of two doses of *Sd* rather than the absence of Sd^+ that produces the lowered male fecundity in *SD* homozygotes; (3) *Sd* behaves as a neomorph, whereas Sd^+, if it exists at all, is amorphic with respect to segregation distortion; (4) these results support a model in which the *Sd* product is made in limiting amounts and the interaction of this product with the *Rsp* locus causes sperm dysfunction. The *Sd* product appears to act preferentially at Rsp^s (sensitive-Responder) but may also act at Rsp^i (insensitive-Responder).

SEGREGATION distorter (*SD*) is a naturally occurring meiotic drive system located on the second chromosome in *Drosophila melanogaster* (SANDLER, HIRAIZUMI and SANDLER 1959). Males heterozygous for an *SD* chromosome can transmit this chromosome virtually to all of their offspring owing to the dysfunction of those sperm bearing the non-*SD* homologue (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DEMARCO 1967; NICOLETTI 1968; TOKUYASU, PEACOCK and HARDY 1972a,b). Generally, this distortion is measured in terms of a *k* value which is defined as the proportion of *SD*-bearing progeny among the total offspring. At the ultrastructural level, sperm dysfunction is first manifest as a failure of normal chromatin condensation in half the spermatids

[1] This paper is dedicated to the memory of GIOVANNI TRIPPA in recognition of his seminal contributions to the study of segregation distortion and whose untimely passing we deeply regret.

produced by *SD* heterozygotes (TOKUYASU, PEACOCK and HARDY 1977; HAUSCHTECK-JUNGEN and HARTL 1978).

Previous genetic analysis of *SD* chromosomes established that they contain two loci primarily responsible for the distortion phenomenon: the *Sd* locus, whose presence is necessary to cause distortion (SANDLER and HIRAIZUMI 1960; HIRAIZUMI and NAKAZIMA 1967), and the *Rsp* (Responder) locus which behaves as the site where *Sd* exerts its effect. Chromosomes carrying a sensitive allele of *Rsp* (Rsp^s) are subject to segregation distortion, whereas those bearing an insensitive allele (Rsp^i) segregate normally from an *SD* chromosome (HARTL 1973, 1974). In addition, a number of modifiers that alter the degree of segregation distortion are scattered throughout the genome. Two enhancers with major effects have been located on *SD* chromosomes closely linked to the *Sd* and *Rsp* loci (MIKLOS 1972; GANETZKY 1977; SHARP 1977; HIRAIZUMI, MARTIN and ECKSTRAND 1980).

Much previous work has involved attempts to dissect *SD* chromosomes by recombination which resulted in the initial identification and characterization of the *Sd* and *Rsp* loci (see HARTL and HIRAIZUMI 1976 for a review). The performance of such experiments and their interpretation have been complicated by the presence of inversions and lethal and sterile mutations on *SD* chromosomes. In addition, *Sd*, *Rsp* and the two major enhancers all lie within a genetic region about 3.4 map units in length, spanning the centric heterochromatin of chromosome 2. This has made it very difficult to identify and characterize the individual loci in their natural background. Partly for this reason, questions still exist concerning the number, location and function of the components of *SD* chromosomes 25 years after they were first discovered.

An alternative approach to recombinational dissection of *SD* chromosomes is to delete individually each of the loci involved in segregation distortion while leaving the rest of the chromosome intact (GANETZKY 1977). This approach was used to map the physical location of *Sd* to salivary chromosome region 37D-38B; *Rsp* to the proximal heterochromatin of *2R*; and a major enhancer, *E(SD)*, to the proximal heterochromatin of *2L*. These deletions were also useful in determining some properties of these loci (GANETZKY 1977; SHARP 1977). The work presented here extends this approach to a more detailed mapping and analysis of the *Sd* locus itself. The results indicate that *SD* chromosomes have a single *Sd* locus located in salivary region 37D2-6 which is primarily responsible for distorted segregation ratios in heterozygotes and for partial sterility of *SD* homozygotes. This sterility appears to depend on the number of copies of *Sd* present rather than homozygosity *per se*. Our results indicate that the presence of Sd^+ does not affect male fecundity or the degree of distortion; thus, Sd^+ appears either to have no function or one different from *Sd*. These results are discussed in respect to recent ideas concerning the genetic structure of *Sd* chromosomes and their mechanism of action.

MATERIALS AND METHODS

Chromosomes: cn bw was used as the sensitive tester chromosome in these studies. It was chosen from among a number of *cn bw* stocks because it gave the lowest variance in *k* values when tested

with *SD-Roma,bw*. Except where otherwise indicated, all *k* values given are measured using this chromosome as a tester.

SD-Roma,bw was produced by recombination between *SD-Roma* (=$SD^{R\text{-}1}$ of NICOLETTI and TRIPPA 1967) and *cn bw*. It has a $k = 0.88$ and is homozygous viable and female fertile. The chromosome carries a recessive male sterile mutation separable from the *Sd* locus that we mapped to the salivary chromosome region 38C6-10 using the deletions of WRIGHT, HODGETTS and SHERALD (1976), GANETZKY (1977) and deletions recovered in this study. Sterility caused by this mutation is covered by *Dp(2;Y)G*.

SD-72, recovered from natural populations in Madison, WI (SANDLER, HIRAIZUMI and SANDLER 1959), $k > 0.99$, carries a pericentric inversion, *In(2LR)39D;42A*, and a paracentric inversion in 2R, *In(2R)NS=In(2R)52A2-B1;56F9-13*.

SD-5, recovered in Madison (SANDLER, HIRAIZUMI and SANDLER 1959), $k > 0.99$ carries two nonoverlapping paracentric inversions in 2R, *In(2R)45C-F;49A* and *In(2R)NS*.

SD-NH2 was collected in Ohdate, Japan (HIRAIZUMI and NAKAZIMA 1965), $k > 0.99$, carries the same inversions as *SD-72* with an additional inversion, *In(2R)55E;60E*, imposed on *In(2R)NS*.

SD-Mad was collected in Madison in 1979 by RAYLA G. TEMIN, $k = 0.99$. It carries the same inversions as *SD-72* but unlike *SD-72* is fully viable and fertile in both sexes when homozygous.

Dp(2;Y)G is an insertional translocation of chromosome region 36A;40 into the Y chromosome (see LINDSLEY and GRELL 1968).

Euchromatic deletions and lethals: The euchromatic deletions of WRIGHT, HODGETTS and SHERALD (1976) and GANETZKY (1977) and the lethals of WRIGHT, BEWLEY and SHERALD (1976) were used to determine genetically the breakpoints of the newly induced deletions. T. R. F. WRIGHT supplied additional deletions with breakpoints in the *Sd* region, and P. GAY sent additional lethal mutations used in complementation mapping.

For a complete description of the markers used in this study, see LINDSLEY and GRELL (1968).

Nomenclature: In naming the deleted *SD* chromosomes, we considered it important to provide information about the nature of the deletion as well as the *SD* chromosome on which it was induced since all *SD* chromosomes may not be strictly equivalent. For each deleted chromosome we write the *SD* chromosome of origin followed by a superscript. Deletions that remove *Sd* are designated with an *R* (for revertant) in the superscript followed by an isolation number. The other deletions contain either *pr* or *hk* in the superscript to indicate which of these loci is deleted. To conform with this nomenclature, a previous *Sd* deletion named SD^{Rev}-*37* is now referred to as *SD-*5^{R37}. The deficiency associated with each deleted chromosome is given a corresponding designation in accord with standard nomenclature.

We use the name *SD-Roma* rather than $SD^{R\text{-}1}$ since the latter leads to confusion because *R* is used to refer to revertants and has also been used to denote recombinant derivatives of *SD* chromosomes, i.e., *R(SD)-1* of SANDLER, HIRAIZUMI and SANDLER (1959).

Screen for deletions in the Sd *region: SD-Roma,bw/cn bw, SD-72/cn bw* and *SD-Mad* males were irradiated with 3000–4000 rad of X rays and mated to *rdo hk pr cn* or *b pr lt pk cn* females. After 3 days, the irradiated males were discarded. The F_1 males were screened for the appearance of *hk* (hooked bristles) or *pr* (purple eyes). The putative deleted chromosomes were extracted with a balancer and examined in complementation tests with other deletions and lethals in the region to determine the presence and extent of any newly induced lesion. In cases in which the genetic tests indicated a deletion, salivary gland chromosomes were examined to confirm these results and to determine breakpoints.

Tests for the ability to distort: To test whether the *SD* chromosomes carrying newly induced deletions retained the ability to distort, ten males heterozygous for the deletion and the *cn bw* tester chromosome were singly mated to two *cn bw* females. Each male and two females were brooded 5 days, transferred to new food vials, and brooded another 7 days before being discarded. Offspring from each vial were counted through day 16. Differences in genetic background were minimized by repeated backcrosses to *cn bw* females for all chromosomes tested.

The ability of males to cause distortion is expressed in terms of *k* value, where *k* is the proportion of *SD*-bearing progeny among the total offspring. In practice, the *k* value is estimated using the adult progeny from the male normalized for viability differences. The viability of each chromosome was measured by taking advantage of the fact that *SD* chromosomes segregate normally in females.

Thus, departures from $k = 0.50$ in heterozygous females can be attributed to differences in relative viabilities of the chromosomes in question. The progeny produced by *SD/cn bw* females mated to *cn bw* males were counted following the same protocol as cited previously. In an effort to keep larval density constant, we still used two females per vial. The k values, corrected for viability, will be given ± two standard errors of the mean based on the empirical variance but do not include the added variance introduced by the viability estimates.

Tests of male fecundity in SD *homozygotes:* The fecundity of males of the genotype SD_i/SD_j and $Df(Sd)SD_i/SD_j$ was determined in a series of experiments using a protocol similar to that used to determine k values. The major difference was that 20 males were tested instead of ten. We observed that when brooded for a shorter period of time (e.g., MARTIN and HIRAIZUMI 1979; HIRAIZUMI, MARTIN and ECKSTRAND 1980) it is more difficult to distinguish males that are sterile from those with low fecundity or those with low fecundity from those that are normal. Males that gave no offspring were not included in the fecundity estimates. When possible, k values for one *SD* vs. the other were determined with the appropriate female controls.

RESULTS

Deletion mapping of Sd

In a previous screen for *SD*-revertants from a collection of X-irradiated *SD-5* chromosomes (GANETZKY 1977), one revertant was recovered which was associated with a cytologically visible deletion in the salivary chromosome region 37D2-38B1. Because this chromosome, $SD\text{-}5^{R37}$, caused no distortion, it was argued that reversion of *SD* and the cytological lesion were the consequence of the same X-ray-induced event and that the *Sd* locus was located within the confines of the deletion. These results were not entirely conclusive, however, because several other revertants were recovered whose behavior was identical with that of $SD\text{-}5^{R37}$ but which lacked any newly generated cytological lesion at the base of *2L*. Thus, the possibility remained that reversion of *SD* in $SD\text{-}5^{R37}$ was independent of the deletion.

In the present experiments, we generated a collection of new deletions at the base of *2L* in *SD* chromosomes in order to assign a definite location to the *Sd* locus. The deletion in $SD\text{-}5^{R37}$ is flanked on the left by the *hk* locus at 37B9-13 (WRIGHT *et al.* 1981) and on the right by the *pr* locus at 38B4-6 (WRIGHT, HODGETTS and SHERALD 1976; this work). To produce new deletions in this region we examined X-irradiated *SD* chromosomes for the loss of pr^+ or hk^+ (see MATERIALS AND METHODS). Recovery of these deletions was, therefore, not dependent on their behavior with respect to segregation distortion. We then examined the ability of each deletion generated in this way to distort the *cn bw* tester chromosome.

We recovered a total of 14 deletions: 11 uncovered *pr*, two uncovered *hk* and one uncovered both *hk* and *pr*. The cytological extent of each deletion is shown in Figure 1 and listed in Table 1. The excess of *pr* deletions relative to *hk* deletions appears significant since 11 of the 14 deletions were recovered from flies heterozygous for the *rdo hk pr cn* chromosome which is capable of detecting both *hk* and *pr* deletions (see MATERIALS AND METHODS). Whether the *pr* region is intrinsically more sensitive to X-ray-induced breakage or whether deletions in the *hk* region generally have reduced viability or fertility cannot at this point be answered. T. R. F. WRIGHT (personal communication) has noted

TABLE 1

The SD chromosomes and their derivatives containing X-ray-induced deletions

Chromosome	Deletion	Breakpoints	k
SD-72			0.993 ± 0.008
SD-72^{hk18}	*Df(2L)hk18*	*Df(2L)36E4-6;37B9-13*	0.978 ± 0.015
SD-72^{pr21}	*Df(2L)pr21*	*Df(2L)37E3-F1;38C6-10*	1.000 ± 0.000
SD-72^{pr26}	*Df(2L)pr26*	*Df(2L)37D5-6;38C8-10*	0.974 ± 0.015
SD-Mad			0.989 ± 0.005
SD-Mad^{pr69}	*Df(2L)pr69*	*Df(2L)38B1-2;38C5-6*	0.994 ± 0.004
SD-Mad^{R68}	*Df(2L)Sd^{R68}*	*Df(2L)37B3-7;38E3-5*	0.485 ± 0.018[a]
SD-Mad^{R77}	*Df(2L)Sd^{R77}*	*Df(2L)37D1-2;38C1-2*	0.573 ± 0.054
SD-Roma, bw			0.884 ± 0.037
SD-Roma^{hk39}, bw	*Df(2L)hk39*	*Df(2L)36F6-37A1;37D1-2*	0.845 ± 0.041
SD-Roma^{pr47}, bw	*Df(2L)pr47*	*Df(2L)38B1-2;38C1-2*	0.828 ± 0.039
SD-Roma^{pr49}, bw	*Df(2L)pr49*	*Df(2L)38B3-6;38C6-10*	0.809 ± 0.072
SD-Roma^{pr65}, bw	*Df(2L)pr65*	*Df(2L)38A3-5;38D3-5*	0.763 ± 0.083
SD-Roma^{pr67}, bw	*Df(2L)pr67*	*Df(2L)38A5-8;39C-E*	0.843 ± 0.044
SD-Roma^{R2}, bw	*Df(2L)Sd^{R2}*	*Df(2L)37D1-2;38D2-E1*	0.537 ± 0.048
SD-Roma^{R14}, bw	*Df(2L)Sd^{R14}*	*Df(2L)37D1-2;38C1-2*	0.512 ± 0.028
SD-Roma^{R57}, bw	*Df(2L)Sd^{R57}*	*Df(2L)37D1-2;38C1-2*	0.504 ± 0.032

The *k* values are the proportion of *SD* offspring from males heterozygous with the *cn bw* tester chromosome corrected for viability and reported ± 2 S.E.

[a] *Dp(2;Y)G;SD-Mad^{R68}/cn bw* males were tested; no correction for viability was made.

similar results with *SD^{+}* chromosomes suggesting this difference is not associated with *SD* chromosomes.

The *k* values of the unirradiated *SD* chromosomes and the deleted chromosomes derived from them are presented in Table 1. The *k* values of the deleted chromosomes fall into two discrete groups: (1) *k* values that do not depart significantly from the unirradiated nondeleted *SD* chromosomes and (2) *k* values close to the 0.50 expected for complete revertants. In no case did one of the deleted chromosomes exhibit an intermediate *k* value that would be indicative of residual distortion. No revertants were recovered from the *SD-72* chromosome, although the structurally similar *SD-Mad* chromosome yielded two revertants. Three remaining revertants were all induced in the *SD-Roma,bw* chromosome.

Comparison of the *k* values with the breakpoints of the deletions shows that deletions extending no further to the right than 37D2 (e.g., *SD-Roma^{hk39}*, *SD-72^{hk18}*) or to the left than 37D6 (e.g., *SD-72^{pr26}*, *SD-Roma^{pr67}*) do not have an appreciable effect on *k* value. Coversely, all five deletions with *k* values close to 0.50 include the region 37D2-37D6. These results argue strongly that the *Sd* locus is located in the chromosome interval 37D2-37D6, and that deleting *Sd* results in the complete loss of ability to distort. These conclusions are in agreement with the previous analysis of *SD-5^{R37}* (GANETZKY 1977).

As shown in Figure 1, five of the six *Sd* deletions have a left breakpoint in the region 37D1,2 that cannot be further resolved cytologically. To determine the order of the breakpoints, complementation tests were carried out using a collection of other deletions and EMS-induced lethal mutations in this region.

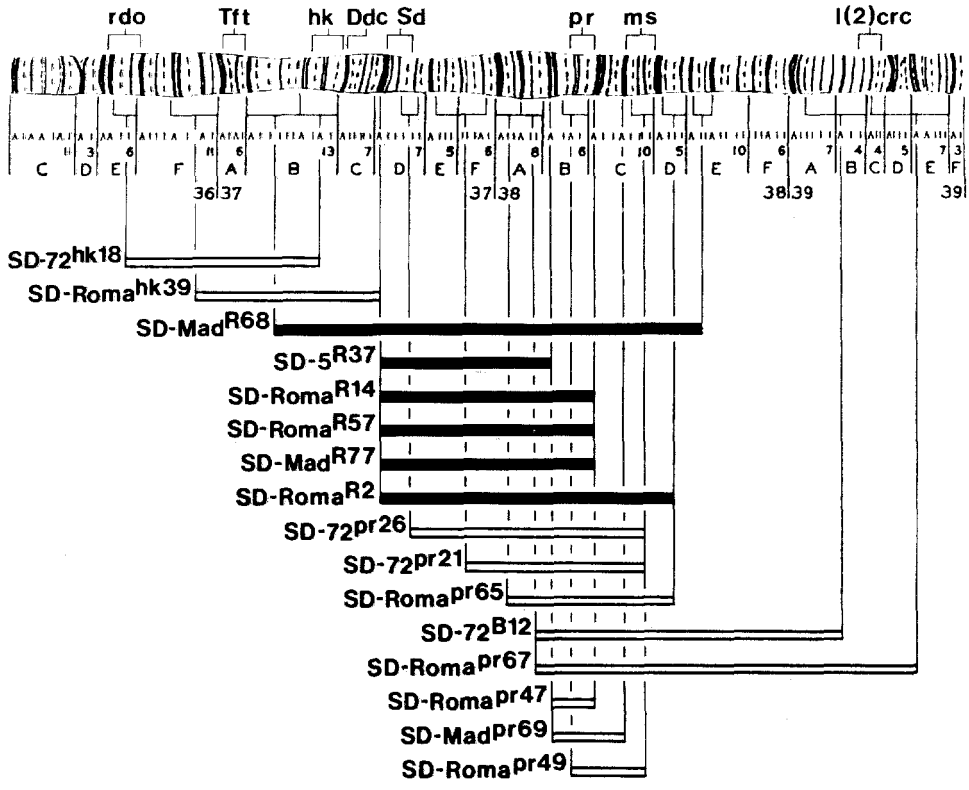

FIGURE 1.—Cytogenetic representation of the salivary gland chromosome region 36C–39F showing the extent of deletions in the *SD* chromosomes recovered in this study. Deletions shown with solid bars reverted the *SD* phenotype; deletions shown with open bars did not. The deletions *SD-5*^{R37} and *SD-72*^{B12} from GANETZKY (1977) are shown for reference. The marker *ms* is the recessive male-sterile mutation on the *SD-Roma* chromosome. The map of this region is based on BRIDGES (1942), and the locations of many of the markers are from WRIGHT, HODGETTS and SHERALD (1967) and T. R. F. WRIGHT (personal communication).

The resulting complementation map is shown in Figure 2. The complementation tests further resolve the left endopoint of the five *Sd* deletions examined. *SD-Roma*^{R57} is lethal in heterozygous combination with *l(2)37Cf* indicating that its breakpoint extends further to the left than the other revertants. *SD-Mad*^{R77} does not overlap *l(2)37Cf* but is lethal when heterozygous with *SD-Roma*^{hk39} and four other deletions extending into 37D1-2 from the left. The existence of another lethal complementation group deleted by *SD-Mad*^{R77} located to the right of *l(2)37Cf* and distal to the breakpoints of the other revertants is suggested by these results. Such a complementation group has not yet been defined by any EMS-induced mutations. The left endpoint of the three remaining revertants cannot be distinguished genetically or cytologically. These complementation tests, together with the results previously described, indicate that *Sd* must be located to the right of the proximal breakpoint of *SD-Roma*^{hk39} and the distal breakpoint in *SD-Roma*^{R14}, *SD-Roma*^{R2} and *SD-5*^{R37} and to the left of the distal

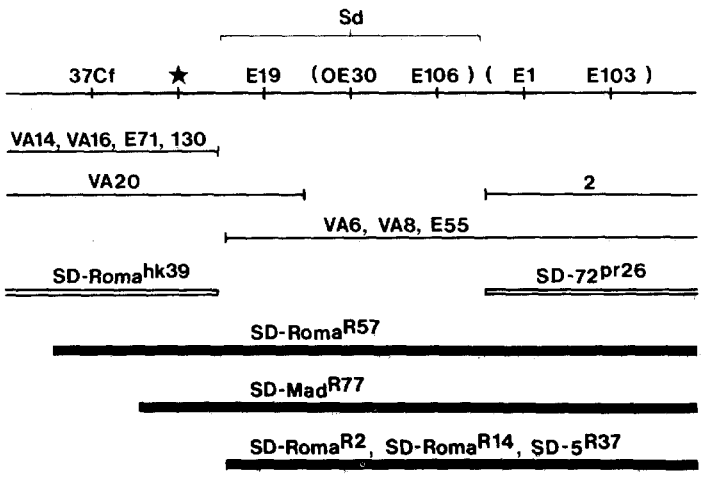

FIGURE 2.—Complementation map of the *Sd* region. The lethals shown are representative alleles from WRIGHT, BEWLEY and SHERALD (1976) and WRIGHT *et al.* (1981) except *OE30* which is from P. GAY (personal communication). They are in the region 37C7 to 37E1 based on lack of complementation with *Df(2L)VA19*, which removes 37C7 and extends to the right, and with *Df(2L)158*, which removes 37E1 and extends to the left. The extent of deletions in SD^+ chromosomes from WRIGHT, HODGETTS and SHERALD (1967) and T. R. F. WRIGHT (personal communication) are shown with lines. Deletions in *SD* chromosomes that reverted the *SD* phenotype are shown with solid bars; deletions that did not are shown with open bars. Deletions that gave identical complementation results are listed on the same line or bar. Lethals that could not be ordered are enclosed in parentheses. An extra lethal complementation group not identified by an EMS-induced allele is marked with a star.

breakpoint in *SD-72*^{pr26}. Based on both the cytological and complementation analyses of these chromosomes, we believe that this region extends from 37D2 to 37D6.

Dosage analysis of male fecundity

It has been previously reported that males bearing two *SD* chromosomes (e.g., *SD-5/SD-72*) in some instances have significantly reduced fecundity when compared with SD/SD^+ males (HARTL 1973; KETTANEH and HARTL 1980). Furthermore, with the use of recombinant *SD* chromosomes, HARTL (1973) was able to map this phenotype to the *Sd* locus. The partial sterility in such males may be due to the absence of Sd^+ (HARTL 1973) or, alternatively, to the presence of two doses of *Sd* (GANETZKY 1977). The distinction between these alternatives is important to the proposed models of *SD*.

Experiments to distinguish between these possibilities are not as straightforward as they might seem. What we really want to know is the proportion of dysfunctional sperm; however, fecundity is only an indirect measure of this at best. Discrepancies exist in the literature as to whether or not certain *SD* genotypes are fertile or sterile as males (see HARTL and HIRAIZUMI 1976). Some of these differences can be attributed to the lack of standardized procedures for measurements of fecundity and to varying uses of the labels, "fertile" and "sterile" (*cf.* HARTL 1973; GANETZKY 1977; HIRAIZUMI, MARTIN and ECKSTRAND 1980).

We measured male fecundity as the number of offspring per fertile male when brooded with two females over a 12-day period. Under these conditions, *SD/cn bw* males produce 120 to 180 offspring. This provided a reasonable compromise between expediency and accuracy. We have found these measurements to be fairly reproducible for males of a particular genotype and sufficiently reliable to reveal consistent and significant differences in fecundity. In our experiments, infertility of various *SD* genotypes appears to involve production of few offspring rather than none at all.

The fecundity of males bearing two intact *SD* chromosomes is compared in Table 2 with the fecundity of males that are genetically identical except that the *Sd* locus is deleted from one of the *SD* chromosomes. The results with *SD-5* and *SD-Roma,bw* are most clear-cut. For *SD-5/SD-Roma,bw* males only about 20 offspring/male were produced. However, when *Sd* was deleted from either *SD-5* or *SD-Roma,bw* the total number of progeny increased six-fold. Because fecundity can be increased in these males by deleting the *Sd* locus, it appears that it is the dosage rather than homozygosity of *Sd* that leads to lowered fecundity.

Similar but less striking changes in fecundity were observed with other combinations of *SD* chromosomes. There was about a two-fold increase in fecundity of *SD-Roma,bw/SD-72* and *SD-Roma,bw/SD-NH2* males when the *Sd* locus was deleted from the *SD-Roma,bw* chromosome (Table 2). Fecundity of *SD-5/SD-Mad* males also increased about two-fold when *Sd* was deleted from the *SD-5* chromosome, although deleting *Sd* from the *SD-Mad* chromosome has little, if any, effect on fecundity.

The fecundity of *SD-Mad/SD-72* and *SD-Mad/SD-NH2* males was higher than for the other combinations of intact *SD* chromosomes examined. Since *SD-Mad* is a strongly distorting chromosome it appears that fecundity effects in homozygotes and drive strength need not be directly correlated. In particular, *SD-Mad* has little effect on the fecundity component such that one dose and two doses of *Sd* for this chromosome are equivalent. HARTL (1973) reported similar differences among various *SD* chromosomes with respect to male fecundity in SD_i/SD_j combinations.

We conclude from these experiments that, for combinations of *SD* chromosomes that produce a significant reduction in male fecundity, this phenotype is associated with the *Sd* locus and the dosage of *Sd* rather than homozygosity *per se* is responsible. However, two doses of *Sd* in intact *SD* chromosomes do not invariably lead to reduction in male fecundity; the fecundity depends on the particular *SD* chromosomes involved.

In a previous analysis by HARTL (1973), the male fecundities of various SD_i/SD_j combinations fell into a pattern of fertility and sterility that was suggestive of intracistronic complementation. Since this form of complementation is generally assumed to involve an interaction of subunits in a multimeric protein (FINCHAM 1966), HARTL suggested that the product of the *Sd* locus is such a multimer. According to this view, SD_i/SD_j males could possibly be fertile if the two *SD* chromosomes were different (intracistronic complementation at the *Sd* locus) but should have lowered fecundity in true homozygotes since only

TABLE 2

The fecundity of SD_i/SD_j *and* $Df(Sd)SD_i/SD_j$ *males*

	SD-Roma, bw	*SD-Roma*^{R57}*, bw*	*SD-Mad*	*SD-Mad*^{R77}
SD-5	20 ± 4	125 ± 6	56 ± 12	87 ± 11
	(0.466 ± 0.046)	(0.512 ± 0.026)		
SD-5^{R37}	119 ± 7		115 ± 8	
	(0.442 ± 0.023)			
SD-72	66 ± 8	114 ± 9	87 ± 10	99 ± 11
	(0.477 ± 0.056)	(0.603 ± 0.026)		
SD-NH2	72 ± 8	145 ± 8	114 ± 12	116 ± 14
	(0.496 ± 0.037)	(0.501 ± 0.036)		

The fecundity is the number of offspring per fertile male using our standard *k* protocol. When determined, *k* values giving the proportion of the *SD-Roma* chromosome are reported in parentheses.

dissimilar subunits should be able to complement (FINCHAM 1966). However, the *SD* chromosomes studied by HARTL carried recessive lethals preventing the analysis of male fecundity in true homozygotes. MARTIN and HIRAIZUMI (1979) used recombinant *SD* chromosomes that were lethal free to show that Sd_iRsp^s/Sd_iRsp^s males showed no apparent reduction in fertility.

Two of the *SD* chromosomes used in this study are viable as homozygotes, enabling us to examine the fecundity of males homozygous or hemizygous for the *Sd* locus carried by these chromosomes. As shown in Table 3, the fecundity of homozygous *SD-Mad* males does not depart noticeably from *SD-Mad/cn bw* males (128 ± 7), and this level of fecundity did not increase in *SD-Mad/SD-Mad*^{R77} males. The high level of fecundity in such males obviously cannot be attributed to intracistronic complementation.

Males homozygous for *SD-Roma,bw* (and carrying a duplication that covers an unrelated male-sterile mutation—see MATERIALS AND METHODS) are fertile but have reduced fecundity. The fecundity of such males shows a significant increase when one of the chromosomes is deleted for *Sd*, whereas adjacent deletions that do not remove the *Sd* locus do not alleviate the reduced fecundity. Since the only difference between *SD-Roma,bw* males homozygous or hemizygous for the *Sd* locus is the number of copies of *Sd*, the increased fecundity of the hemizygote again must be attributed to dosage effects rather than to any type of subunit interaction.

We constructed males homozygous for the *SD-Roma,bw* chromosome but lacking any copies of *Sd* by using two deleted derivatives of *SD-Roma,bw*. The lethality which would otherwise be associated with homozygosity for deletions of this size was complemented in these crosses by a duplication of this region inserted into the Y chromosome (see MATERIALS AND METHODS). The fecundity of these males (Table 3) was essentially indistinguishable from hemizygotes. Only the *SD-Roma*^{R14}*/SD-Roma*^{R57} genotype failed to show an increase in fecundity compared with *SD-Roma,bw* homozygotes. The low fecundity of this particular combination is most likely extrinsic to the *Sd* locus since *SD-Roma*^{R14}*/SD-Roma*^{R2} and *SD-Roma*^{R57}*/SD-Roma*^{R2} do exhibit significantly increased fecundity. Our interpretation of these results is that our experimental

TABLE 3

The fecundity of homozygous SD *males*

Male genotype	Copies of *Sd*	Offspring/male
SD-Roma, bw/SD-Roma, bw	2	65 ± 9
SD-Roma, bw/SD-Roma^{hk39}, bw	2	78 ± 11
SD-Roma, bw/SD-Roma^{pr65}, bw	2	79 ± 11
SD-Roma, bw/SD-Roma^{R2}, bw	1	95 ± 4
SD-Roma, bw/SD-Roma^{R14}, bw	1	108 ± 9
SD-Roma, bw/SD-Roma^{R57}, bw	1	115 ± 5
SD-Roma^{R2}, bw/SD-Roma^{R14}, bw	0	123 ± 6
SD-Roma^{R2}, bw/SD-Roma^{R57}, bw	0	132 ± 15
SD-Roma^{R14}, bw/SD-Roma^{R57}, bw	0	62 ± 8
SD-Mad/SD-Mad	2	117 ± 12
SD-Mad/SD-Mad^{R77}	1	107 ± 6

The fecundity is the number of offspring per fertile male from our standard *k* protocol. All *SD-Roma* males had *Dp(2;Y)G* which covers the male sterile located near *pr*.

procedure for measuring male fecundity is probably insufficient to detect differences due to a single dose of *Sd* (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DEMARCO 1967); two doses of *Sd* increase the level of sperm dysfunction to a point where lowered fecundity becomes apparent.

One interpretation of the lowered fecundity of SD_i/SD_j males is that each *SD* chromosome renders the sperm carrying its homologue dysfunctional (HARTL and HIRAIZUMI 1976; KETTANEH and HARTL 1980). This mutual dysfunction hypothesis implies that each *SD* chromosome is insensitive to its own action but sensitive to the action of a different *SD* chromosome. Our results do not support this interpretation. If mutual dysfunction were occurring, then significant departures in *k* value from 0.50 would be expected when one of the *Sd* loci is deleted. Specifically, in Table 2, the *SD-Roma,bw/SD-5^{R37}* males would have a *k* value much higher than 0.50 (in favor of *SD-Roma,bw*), whereas for *SD-Roma^{R57}/SD-5*, *SD-Roma^{R57}/SD-72* and *SD-Roma^{R57}/SD-NH2* it would be much less than 0.50 (in favor of the nondeleted *SD* chromosome). Clearly, this did not occur—the *SD* chromosome deleted for *Sd* was not distorted by the intact *SD* chromosome.

DISCUSSION

The location of the Sd locus

Since the discovery of *SD* there has been considerable uncertainty over the exact location of the *Sd* locus (HARTL and HIRAIZUMI 1976; HARTL 1980). Virtually all of the previous studies involve dissection of *SD* chromosomes via recombination—a method that has a number of inherent problems that make the localization of the relevant loci difficult. These include the presence of inversions in the region of interest, the problem of scoring *Sd* when separated by recombination from *Rsp^i* and the various enhancers of *Sd*, the possibility of unequal recovery of reciprocal classes of recombinants for reasons unrelated to the *SD* system, and the imprecision of extrapolating physical location from

recombination frequency. As the present study illustrates, most of these problems can be eliminated by using deletions to map the *SD* elements.

Analysis of our deletions that revert the *SD* phenotype indicates that a single major component, the *Sd* locus, is responsible for causing segregation distortion. This component is located in region 37D2-6 of the salivary chromosome map which corresponds to about 2-54.1 on the standard recombination map [by interpolation between *hk* (2-53.9) and *pr* (2-54.5)].

Based on the recovery of recombinants between the markers *b* (2-48.5) and *cn* (2-57.5), TANZARELLA *et al.* (1972) reported a map position for *Sd* of 2-52.9 ± 1.0. Although the actual mapping data are not provided, the locus mapped by these workers probably corresponds to the one we have localized.

More recently, HARTL (1980) has suggested that the unequal recovery of reciprocal recombinants from a derivative of *SD-36* (an *SD-5* type) could be explained by the existence of a second *Sd* locus "inseparable from *pr* and perhaps even to its right." Our results provide no support for this interpretation because the ability of an *SD* chromosome to distort is not affected by euchromatic deletions at the base of 2L which do not remove the *Sd* locus and segregation of *SD* chromosomes with the *Sd* locus deleted do not deviate from Mendelian expectations. Deletions of *Sd* have now been generated in three structurally different *SD* chromosomes (*SD-5*, *SD-Roma* and *SD-Mad*), and all fail to distort a sensitive homologue. Thus, if the chromosome analyzed by HARTL (1980) does have two copies of *Sd*, it is most probably a peculiar feature of that chromosome and not a general feature of *SD* chromosomes.

The nature of the Sd *locus*

The results of GANETZKY (1977) and the more extensive data reported here argue that *Sd* behaves as a neomorph (MULLER 1950), whereas Sd^+ is the null condition with respect to segregation distortion. Operationally, we are unable to distinguish Sd^+ from a deletion of *Sd* by its effects either on *k* value or male fecundity. Additional evidence supporting this view comes from experiments showing that increasing the dosage of Sd^+ by means of a duplication has no effect on *k* value or fecundity in $Sd/Sd^+/Sd^+$ or $Sd/Sd/Sd^+$ males (B. GANETZKY and M. E. ULBER, unpublished observations).

It has been known for some time that the *Sd* locus is responsible for the reduced fecundity observed in many SD_i/SD_j males (HARTL 1973). However, we find the fecundity of homozygous *SD* or SD_i/SD_j males increases after the deletion of *Sd*. This argues that the low fecundity was not due to the absence of an Sd^+ function in *Sd* homozygotes but results from the presence of two copies of *Sd*. The implication is that the *Sd* product *actively* causes some deleterious effect in its interaction with *Rsp* leading to sperm dysfunction. Furthermore, the *Sd* product must be made in limiting amounts relative to its target of action such that the effect of two doses of *Sd* is distinguishable from a single dose.

Electron microscopic observations of sperm development in SD_i/SD_j males have revealed a pattern of defects qualitatively similar to that observed in SD/SD^+ males, but affecting the majority of developing sperm rather than just half

(PEACOCK, TOKUYASU and HARDY 1972; KETTANEH and HARTL 1980). These observations suggest a mechanism of mutual dysfunction in which each *SD* chromosome in an SD_i/SD_j male could cause the dysfunction of sperm carrying the other *SD* (KETTANEH and HARTL 1980). An alternative view expressed by PEACOCK and MIKLOS (1973) is that the reduced fecundity of SD_i/SD_j males may not depend directly on effects of the *SD* system itself.

Our observation that deleting the *Sd* locus can alleviate the reduced fecundity in SD_i/SD_j males indicates that the original low fertility is a concomitant of the *SD* system and, thus, supports the mutual dysfunction hypothesis. However, this hypothesis must be somewhat modified. If each *SD* chromosome in an SD_i/SD_j male were causing the dysfunction of the spermatid receiving the other *SD* chromosome, then the increased fecundity observed when *Sd* is deleted in one of the *SD* chromosomes should result in preferential recovery of the nondeleted *SD* chromosome. We observed an equal recovery of the deleted and nondeleted chromosomes. Thus, each *SD* chromosome in SD_i/SD_j males contributes to its own dysfunction as well as to that of its homologue. This further suggests that insensitivity to distortion of a chromosome bearing Rsp^i is relative rather than absolute. Under certain conditions, *Sd* can apparently act even at its own Rsp^i locus to cause dysfunction, although it acts preferentially at Rsp^s. An alternative possibility is that reduced fecundity in SD_i/SD_j males results from an effect of *Sd* that does not involve the *Rsp* locus at all.

The mechanism of distortion

HARTL (1973) presented the first explicit molecular model for the mechanism of *SD* action. He proposed that the *Sd* product was a multimeric regulatory protein whose binding at the *Rsp* site was necessary for a chromosome to be included in a functional gamete. Subsequently, GANETZKY (1977) proposed an alternative model in which the binding of an *Sd* product at the *Rsp* locus initiated the event that led to dysfunction. GANETZKY ascribed no function to Sd^+ and further proposed that the *Sd* product was made in limiting amounts relative to available binding sites. Our observations that deletion of *Sd* reverts the *SD* phenotype and that males bearing one *vs.* two doses of *Sd* have differences in fecundity support the latter model.

The suggestion that the *Sd* product is made in limiting amounts follows from other considerations as well. Our data indicate that an *SD* chromosome is not actually immune to its own effect but that non-Mendelian recovery of an *SD* chromosome when segregating from a sensitive homologue is due to preferential action of *Sd* at Rsp^s rather than Rsp^i. If the *Sd* product is made in unlimited amounts, then in *Sd* Rsp^i/Sd^+ Rsp^s males, excess *Sd* product could bind at Rsp^i leading to the dysfunction of the spermatid receiving this chromosome. But this event would limit the fitness advantage that the *SD* chromosome might otherwise enjoy. It is more likely to suppose that the *Sd* locus is expressed in such a way that dysfunction of a sensitive homologue occurs without exceeding a threshold that could lead to self-distortion.

Recently, HIRAIZUMI, MARTIN and ECKSTRAND (1980) proposed a third model for *SD* which incorporated features of the previous two. They identified a locus

referred to as *M(SD)* that enhanced *k* values of an *SD* chromosome and proposed that binding of the product of this locus at the *Rsp* site was necessary for normal sperm development and that the *Sd* product interfered with this binding causing dysfunction. Their results can also be explained in the context of our model where the various enhancers of *SD* such as *E(SD)* (GANETZKY 1977) and *M(SD)* control the amount of *Sd* product made.

The high fecundity of homozygous *SD-Mad* males is somewhat unusual. In previous cases in which it has been possible to test true *SD* homozygotes they have usually had low fecundity (HARTL and HIRAIZUMI 1976). Several *SD* chromosomes have been found to be quite fecund in SD_i/SD_j combinations (*cf.* TRIPPA, LOVERRE and CICCHETTI 1980), although many others are not very fecund in such combinations (HARTL 1973). The reason for the fecundity differences among *SD* chromosomes is not clear. It may be due to allelic variation at the *Sd* and *Rsp* loci or it may involve differences in the presence of or response to various modifiers of *Sd* (*cf.* HIRAIZUMI, MARTIN and ECKSTRAND 1980). In any case, the fecundity of males homozygous or hemizygous for *Sd* would appear to rule out the possibility of intracistronic complementation at the *Sd* locus.

Finally, earlier views of segregation distortion (e.g., HARTL 1973; HIRAIZUMI, MARTIN and ECKSTRAND 1980) imply that *Sd* represents a mutation in a system that is involved in the normal control of sperm development. However, there is no direct evidence that Sd^+ or any of the components of segregation distortion subserve a normal role in spermatogenesis. The fact that Sd^+ behaves as an amorph with respect to segregation distortion indicates that if there is an Sd^+ function it is probably qualitatively different from that of *Sd*. *Sd* is similar to other dominant neomorphic mutations in that the severity of its effect depends on the number of mutant alleles and that it is revertible by deletion. Thus, *Sd* could represent a mutant allele in which a normal gene is inappropriately expressed in place, in time or in amount. It is also possible that *Sd* represents a foreign element inserted in the Drosophila genome and Sd^+ is the absence of this element. This notion does not rule out the likely possibility that *SD* operates by parasitizing existing mechanisms that regulate communcation between homologues and control gene expression at a chromosomal level. The most striking feature about *SD* chromosomes is the remarkable regularity and efficiency with which they exert their effect. Thus, they provide a unique opportunity to elucidate the way these regulatory mechanisms normally operate.

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