The Effect of Novel Chromosome Position and Variable Dose on the Genetic Behavior of the Responder (*Rsp*) Element of the Segregation Distorter (*SD*) System of *Drosophila melanogaster*

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ABSTRACT

In the Segregation distorter (*SD*) system of meiotic drive, a minimum of two *trans*-acting elements [*Sd* and *E(SD)*] act in concert to cause a certain probability of dysfunction for sperm carrying a sensitive allele at the *Responder* (*Rsp*) target locus. By employing a number of insertional translocations of autosomal material into the long arm of the *Y* chromosome, *Rsp* can be mapped as the most proximal locus in the 2R heterochromatin as defined both by cytology and lethal complementation tests. Several of these insertional translocations result in the transposition of *Rsp* to the *Y* chromosome, where its sensitivity remains virtually unaltered. This argues that *Rsp* is separable from the second chromosome centromere, that its behavior does not depend on its gross chromosomal position, and that meiotic pairing of the chromosomes carrying the various *SD* elements is not a prerequisite for sperm dysfunction. Several other translocations apparently leave both resulting chromosomes at least partially sensitive to *SD* action, suggesting that *Rsp* is a large subdivisible genetic element. This view is compatible with observations published elsewhere that suggest that *Rsp* is a cytologically large region of highly repetitive AT-rich DNA. The availability of *Y*-linked copies of *Rsp* also allows the construction of *SD* males carrying two independently segregating *Rsp* alleles; this in turn allows the production of sperm with zero, one or two *Rsp* copies from the same male. Examination of the relative recovery proportions of progeny arising from these gametes suggests that sperm with two *Rsp* copies survive at much lower frequencies than would be predicted if each *Rsp* acted independently in causing sperm dysfunction. Possible explanations for such behavior are discussed.

SEGREGATION distorter (*SD*) second chromosomes of *Drosophila melanogaster* are recovered in excess of Mendelian expectations in the sperm of many *SD*/*SD*$^+$ males (SANDLER, HIRAIZUMI and SANDLER 1959). *SD* chromosomes carry interacting alleles at several loci, including *Sd*, which BRITTNACHER and GANETZKY (1983) have mapped to the basal euchromatin of 2L (37D2-6 of the salivary gland chromosome map), and *E(SD)*, which is located nearby in the 2L heterochromatin (region h35 of the heterochromatic map, S. PIMPINELLI and P. DIMITRI, personal communication). It is at one or both of these loci that the meiotic drive of *SD* chromosomes is presumed to originate. The target for segregation distortion is *Responder* (*Rsp*), which has been placed near the centromere in 2R by recombinational and cytogenetic studies (GANETZKY 1977; SHARP, HILLIKER and HOLM 1986). In males carrying at least one copy of *Sd*, sperm containing *Rsp* may fail to achieve normal chromatin condensation, and thus become dysfunctional (*cf.* SANDLER and CARPENTER 1972; TOKUYASU, PEACOCK and HARDY 1977). In a series of studies, GANETZKY's laboratory (BRITTNACHER and GANETZKY 1983, 1984, 1989) has demonstrated that both *Sd* and *Rsp*^{s} (the standard sensitive allele of *Rsp*) behave as neomorphic mutations; that is, chromosomes deleted for either are formally indistinguishable from *Sd*$^+$ *Rsp*^{i} chromosomes (where the *Sd*$^+$ and *Rsp*^{i} alleles are defined by their inability to cause or react to segregation distortion, respectively). When recovered from nature, *SD* chromosomes carry *Sd*, but lack a sensitive *Rsp* form. *SD*$^+$ chromosomes by definition lack *Sd*, but may either be insensitive to segregation distortion (*i.e.*, functionally defined as carrying *Rsp*^{i}), or harbor any one of a number of differing *Rsp* forms, ranging from those showing weak sensitivity (*Rsp*^{si}) through standard (*Rsp*^{s}) to so-called "supersensitive" (*Rsp*^{ss}) allelic states. Strength of drive in *SD* males can be measured either as the fraction of gametes recovered (*k*) that carry the *Rsp*^{i} homolog, as the probability of survival (*R*) of a gamete carrying a sensitive *Rsp* allele, or as the probit (*M*) transformation of *R*.

The early work mapping *Rsp* to the second chromosome centric heterochromatin raised the possibility that *Rsp* was the centromere itself. Besides proving that it was the presence of *Rsp*^{s} in a sperm that led to

This paper is dedicated to the memory of LARRY SANDLER, whose influence as philosopher, scientist and friend will be greatly missed.

its dysfunction in *SD* males, SANDLER and CARPENTER (1972) provided an elegant, but indirect, test of this hypothesis. They measured the recovery, both in the presence and absence of *SD*, of gametes arising from Alternate, Adjacent I and Adjacent II meiotic segregations in males heterozygous for a *T(2;3)* rearrangement. The translocation was chosen to have breaks in the centric heterochromatin of a Rsp^s-bearing second and a standard third chromosome. The gist of their argument was: 1) one of the two Adjacent segregation types produces gametes carrying both Rsp^i and Rsp^s, and 2) though *SD* males produce few of these gametes, in non-*SD* males the recovery of this gamete class exceeds that of classes arising from the other type of Adjacent segregation. Therefore, 3) this more common gamete class must arise from the more likely Adjacent I segregation; such gametes necessarily carry only one second chromosome centromere, and this must be from the intact second chromosome. Since for this cross we know that the normal second chromosome carries Rsp^i, it follows directly from 1 and 3 that Rsp^s cannot also be a second chromosome centromere. However, the numbers involved in this test were small, and the accuracy of the interpretation depended critically on the assumption that the frequency of Adjacent I segregations in males exceeds that of Adjacent II. Moreover, in estimating gamete frequencies it was sometimes necessary to rely on indirect partitioning of classes of flies with similar phenotype into different genotypic classes.

In the present study, a more direct approach was employed to answer several questions about the structure and function of Rsp^s. Using the technique of fertility rescue mutagenesis [see LYTTLE (1984) for a complete technical discussion], a number of translocations were generated that insert 2R centric heterochromatin from a standard Rsp^s laboratory chromosome into a marked *Y* chromosome. By examining the sensitivity to *SD*-induced sperm dysfunction exhibited by the full insertional translocation, and comparing it to the sensitivity exhibited by the separate parts, it was possible to provide answers to the following questions. Is Rsp^s separable from the second chromosome centromere? Will Rsp^s function as a target for *SD* when removed to a new chromosomal position? Is Rsp^s divisible into subcomponents that may each remain sensitive to sperm dysfunction?

Moreover, if Rsp^s functions when present on a *Y* chromosome, this provides an extra Rsp^s copy that should segregate regularly in meiosis, but independently of the normal second chromosomes. Thus, from a single male genotype, it should be possible to obtain sperm carrying zero, one or two *Rsp* copies, in a 1:2:1 ratio. In an earlier study, LYTTLE (1986) used a similarly derived *Dp(2;Y)* chromosome carrying a copy of the *Sd E(SD)* complex to demonstrate that multiple doses of these elements were additive (when scaled by the probit transformation) in their ability to cause dysfunction of sperm carrying single copies of *Rsp*. LYTTLE, BRITTNACHER and GANETZKY (1986) used this same *SD*-duplicated *Y* chromosome to demonstrate that second chromosomes carrying different *Rsp* alleles behaved independently in their qualitative response to *SD*, but that each diluted the effect of *SD* on the other; that is, the effects of *Sd* and/or *E(SD)* apparently are produced in rate-limiting amounts. The availability of a Rsp^s-duplicated *Y* chromosome would allow a number of similar dose studies. The first results from such experiments are also reported here.

MATERIALS AND METHODS

Genetic stocks: The following *Drosophila melanogaster* chromosomes were used. For a complete description of individual mutants, see LINDSLEY and GRELL (1968).

cn bw, the standard SD^+ chromosome used in *SD* analysis. Carries the eye color mutants *cinnabar* (*cn*) and *brown* (*bw*). Its genotype is Sd^+ Rsp^s.

In(3LR)TM6, ss^- Ubx^{67b} = *TM6*, a standard multiply inverted third chromosome balancer obtained from J. F. CROW. Apparently, this derivative of *TM6* also carries a major suppressor of *SD* activity (LYTTLE 1986).

In(2LR)O, S^2 *Cy* cn^{2P} *bw* = *CyO*, a multiply inverted second chromosome used as a balancer. It carries the standard inversions of *In(2LR)O* with additional inversions added by L. CRAYMER. It behaves as Sd^+ Rsp^i.

Df(2R)M-S2-10, a second chromosome deficient for most of the 2R heterochromatin (see Figure 2).

y bb^{1158}, an *X* chromosome deficient for 82% of the centric heterochromatin. In combination with most simple *T(Y;2)* rearrangements (including *T(Y;2)CB25*, discussed below) this chromosome produces complete male sterility.

R(SD-36)bw-1, a standard intermediate strength *SD* chromosome (see cross 1, Table 3) derived by recombination from one of the original chromosomes (*SD-36*) isolated in Madison, Wisconsin (HARTL 1974). Its genotype is *Sd* $E(SD)Rsp^i$.

SD-79,bw, a standard strong *SD* chromosome (see cross 2, Table 1). The *bw* mutant was recovered from a hybrid dysgenic cross performed by K. GOLIC. *SD-79* itself was recovered from a sample provided from a natural population in Madison, Wisconsin, by W. ENGELS, and is identical in structure to an *SD-36* chromosome. Its genotype is *Sd* $E(SD)$ Rsp^i.

SD ARM28, a standard strong *SD* chromosome (see cross 2, Table 3) recovered from a sample provided from a natural population in Armidale, Australia, by S. BARKER. It is similar in structure to an *SD-72* type chromosome, and has genotype *Sd* $E(SD)$ Rsp^i.

Dp(2;Y)B10-4, $B^S Y^{y+}$, a derived *Y* chromosome carrying an insertion of cytological divisions 36D-40 from *SD ROMA* into Y^L. It is genetically duplicated for *Sd E(SD)* (LYTTLE 1986).

T(Y;2)CB25, $B^{S(v)} Y^{y+}$; *cn bw*, the parental translocation giving rise to all the secondary rearrangements discussed here. The translocation breakpoints are in 2R heterochromatin of the standard *cn bw* chromosome, proximal to all known genetic loci (see Figure 2), and in Y^L just proximal to the B^S marker. This combination of breaks causes the Bar phenotype to show strong position effect variegation.

Its genotype is Sd^+ Rsp^s, and it exhibits full sensitivity to segregation distortion (see cross 3, Table 1).

Measurement of segregation distortion: The statistic k = proportion of *SD*-bearing sperm recovered from *SD*/SD^+ heterozygous males is normally used as a measure of drive strength for *SD* chromosomes against a standard SD^+ chromosome, or, conversely, as a measure of the *Rsp* sensitivity of an SD^+ to a standard *SD* chromosome. However, since the *Sd* locus is *trans*-acting, while *Rsp* is *cis*-acting, k can be more appropriately defined as the proportion of Rsp^i-bearing sperm recovered from Rsp^i/Rsp^s male heterozygotes. This is especially important in this report, where Rsp^s is sometimes linked to the *Y* chromosome, and thus segregates independently of the *SD* chromosome itself.

For most situations we prefer to use a transformation of k:

$$R = (1 - k)/k$$

$$= (\text{number of } Rsp^s \text{ progeny})/(\text{number } Rsp^i \text{ progeny}).$$

Here, R is a measure of the proportion of Rsp^s gametes which survive *SD*-mediated sperm dysfunction (LYTTLE 1979) and is the most direct biological measure of the strength of drive. In employing these statistics, it is always assumed that the Rsp^i allele is unaffected by distortion [see HARTL and HIRAIZUMI (1976) for a summary of evidence supporting this view], and that the R value is therefore an absolute measure of gamete survival. In this report, three such R values can be calculated for a given cross: R_Y = survival of *Y*;*SD*-bearing sperm; $R_{A'}$ = survival of *X*;SD^+-bearing sperm; and $R_{Y+A'}$ = survival of *Y*;SD^+-bearing sperm, each measured relative to the recovery of *X*;*SD*-bearing sperm. When these values are transformed, they yield the values k_Y, $k_{A'}$ and $k_{Y+A'}$, respectively. Note that R ranges from 0.0 to 1.0 as k ranges from 1.0 to 0.5.

In a few cases, the sensitivity of a chromosome to distortion is so low that its k value approaches 0.5, and small viability differences associated with the segregating chromosomes may allow the sensitivity to go undetected. Conversely, such viability effects might lead to the incorrect inferral of sensitivity where it does not exist. In these situations it is necessary to correct for viability according to the methods described in LYTTLE, BRITTNACHER and GANETZKY (1986), in which *SD* effects are removed by scoring, in a reciprocal cross, the segregation of the Rsp^i and Rsp^s chromosomes from females. This provides a measure of the effects of viability independent of *SD*, and the recovery proportions from such a control cross can be used to adjust the progeny frequencies arising from the experimental cross where *SD* is active.

The segregation ratios of individual males (4–7 days old) were determined by matings of each with two to three *y*;*cn bw* females (for female viability controls, single females were mated with two to three *y*/*Y*;*cn bw* males). These were generally brooded after 7 days, the parents being discarded after a further seven days. Cultures were maintained at 25 ± 1° throughout. Eclosing progeny were counted for 19 days from the initiation of each culture. Mean R and k values reported in the tables are weighted averages unless otherwise stated.

Mutagenesis protocol: *T*(*Y*;2)*CB25*, $B^{S(v)}Y^{y+}$;*cn bw* males, 4 to 7 days old, were irradiated with 4500 r of γ-rays (approximately 2500 r/min) from a ^{60}Co source. These were then mated to *y* bb^{1158}/*FM7* virgin females in half-pint bottles (approximately 15 pairs each). Crosses were brooded and collections made such that only *T*(*Y*;2)/*y* bb^{1158} sons arising from irradiated sperm were retained, and these were subsequently mated to *y*;*cn bw* females, with approximately 25 pairs per standard shell vial. Although *T*(*Y*;2)*CB25*/*y* bb^{1158} males are always sterile, certain classes of secondary rearrangements involving *T*(*Y*;2)*CB25*, in combination with *y* bb^{1158}, yield males with completely restored fertility. Thus, any vials producing larvae within ten days after mating are assumed to contain a parental male carrying such a newly induced rearrangement. The rearrangement-bearing progeny can be recovered and subjected to a variety of further mapping tests, as outlined below [see LYTTLE (1984) for a complete discussion of this protocol and the rationale for its design]. Two classes of rearrangements involving *T*(*Y*;2)*CB25* are expected to be predominantly recovered, as illustrated in Figure 1. One class (termed "resealing") arises from the secondary reexchange of the chromosome arms involved in the original translocation, such that each arm is restored to its proper centromere. Since the secondary breakpoints are likely to be out of register with the original breaks, resealings generally result in insertions of chromosome 2 material into the *Y*, insertions of *Y* material into 2, or reciprocal insertions (as Figure 1A illustrates); inserted material will of necessity be from regions adjacent to the breakpoint positions of the original translocation. Since in this study we will be focusing on the first class of resealing, these lines are given the notation *Tp*(2;*Y*)*CB25-i* to reflect the fact that they are essentially equivalent to transpositions of autosomal material into the *Y*. Resealings can be induced only in sperm arising from Alternate segregation in *T*(*Y*;2)*CB25* fathers (Figure 1A). A second class of rearrangements (termed "carve-down") can be induced in sperm arising from Adjacent I segregation (see Figure 1B), which contain $Y^P 2^D$ and *cn bw*. Progeny arising from such sperm are normally inviable owing to 2R trisomy, but if enough of the interstitial 2R material is removed by appropriate radiation-induced breaks, viable progeny can result. Even though both these classes of sperm should, in combination with *y* bb^{1158}, lead to fertile male progeny, "carve-downs" should be distinguishable from resealings by their loss of the B^S marker.

Mapping of secondary rearrangements: Each of the *Dp*(2;*Y*) and *Df*(2*R*) chromosomes was tested for its ability to complement a bank of lethal alleles (*l*(2)*EMS31*, *l*(2)*EMS45-39*, *l*(2)*EMS45-73* and *l*(2)*EMS45-72*, *cf.* Figure 2) known to mark 2R heterochromatic loci (HILLIKER 1976). Complementation tests were carried out using the following crosses:

A) *EMS j*/*CyO*♀ × ♂ *Dp*(2;*Y*)*CB25-i*/

+ ;*Df*(2*R*)*CB25-i*, *cn bw*/*CyO*

B) *Df*(2*R*)*M-S2-10*/*CyO*♀ × ♂ *Dp*(2;*Y*)*CB25-i*/ + ;*EMS j*/*CyO*

A deficiency of non-Cy daughters from cross A indicates that the putative *Df*(2*R*) fails to complement a given lethal allele (and therefore overlaps the locus it defines). A further test is obtained by observing whether the complementary *Dp*(2;*Y*) component rescues non-Cy sons from cross B by providing the normal allele corresponding to *EMS j* (which is otherwise lethal in combination with *Df*(2*R*)*M-S2-10*). The absence of non-Cy flies in progeny from both crosses would indicate that a rearrangement breakpoint fell within the locus marked by *EMS j*, such that neither the *Dp* nor the *Df* chromosomes could provide a normal allele. For those lines which were not resealings, only *Dp*(2;*Y*)*CB25-i* was available, and *cn bw* replaced the deficiency chromosome; complementation maps for these lines were constructed using the results of cross B alone. The complementation maps so constructed appear in Figure 2, with open lines indicating the extent of deficiencies mapped by cross A, solid lines indicating the

corresponding extent of the duplications, as determined by cross B.

P. DIMITRI generously mapped the extent of some of the rearrangements cytologically, by sequential staining of larval neuroblasts with Hoechst 33258 fluorescence and giemsa. The resulting fluorescence and N-banding patterns [see GATTI, PIMPINELLI and SANTINI (1976) and PIMPINELLI, SANTINI and GATTI (1976) for protocols] can be used to determine rearrangement breakpoints with reference to a standard map of the 2R heterochromatin of *cn bw* (after PIMPINELLI and DIMITRI 1989). Their map is reproduced in Figure 2A. PIMPINELLI and DIMITRI have also demonstrated that Rsp^s is associated with bright Hoechst fluorescence in h39, and that the sensitivity of a chromosome to *SD* is strongly correlated with the size and fluorescent intensity of this band. As an example of the power of cytological mapping, the banding pattern obtained for one rearranged chromosome (*Dp(2;Y)CB25-4*) is presented in Figure 2B (photographs provided by P. DIMITRI).

RESULTS

Figure 2 and Table 1 summarize the genetic mapping and *SD* sensitivity tests for six resealings (*Tp(2;Y)CB25-4, 22, 24, 29, 31, 42*) recovered from *T(Y;2)CB25* as described in MATERIALS AND METHODS. In addition, two other secondary rearrangements are included (*Dp(2;Y)CB25-9* and *11*) that also passed the fertility screen, but apparently arose as simple deletions of most of the interstitial 2R material from the Y^P2^D portion of *T(Y;2)CB25* (*i.e.*, the "carve-down" of Figure 1B). Finally, data for the dicentric *Dp(2;Y)CB25-1* are also included. This chromosome was recovered from the same mutant screen, and is similar to the other lines in the second chromosome material which it carries, but apparently also carried most of a fourth chromosome, including the centromere. This line can be denoted structurally as $Y^S \cdot Y^L2Rh4 \cdot$, *CB25-1* and is discussed in full in AULT and LYTTLE (1988). This is the only one of the derived chromosomes associated with any significant level of sex chromosome nondisjunction (on the order of 5%), presumably owing to its dicentricity. For simplicity, as it is only the *Rsp* status of the *Y* chromosome that is generally being measured, the exceptional *XO* or *XXY* progeny produced by males carrying this or any of the other derived *Y* chromosomes have been pooled with the *XX* or *XY* class, respectively.

While all nine secondary rearrangements were mapped using lethal complementation tests, only *CB25-1, -4, -9,* and *-11* have yet been checked cytologically; therefore the physical extent of the others (*CB25-22, -24, -29, -31, -42*) has been inferred from combining complementation data with patterns of *Rsp* sensitivity. Moreover, the complementation and cytological maps can be tentatively aligned by virtue of the observation that *EMS45-73* has been located to band h44 (P. DIMITRI, personal communication). While the map order of the other lethals is well established (HILLIKER 1976), uncertainty as to their

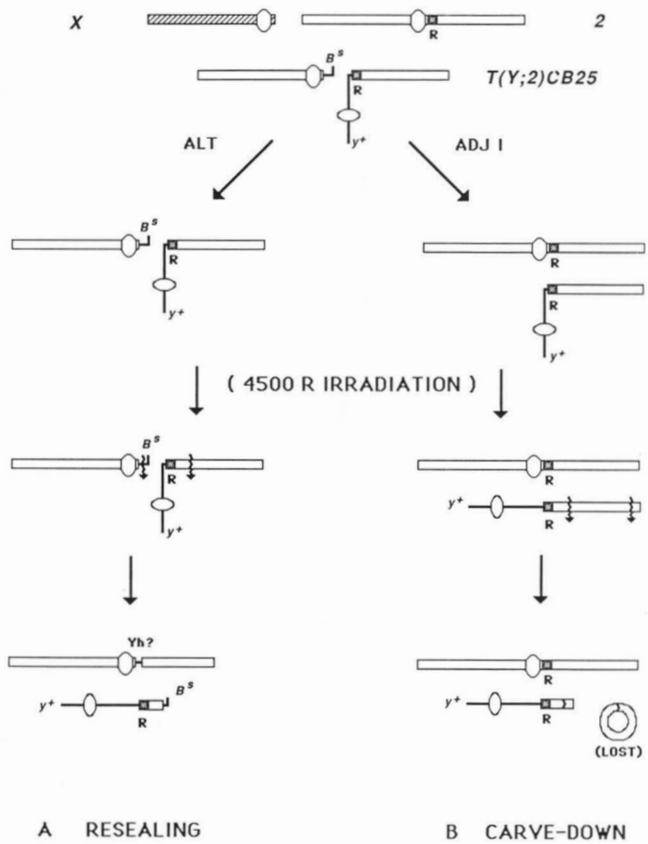

FIGURE 1.—Induction of secondary rearrangements. Rearrangements are induced in the sperm of *T(Y;2)CB25*, $B^{S(v)}Y^{y+}$;*cn bw/cn bw* males by γ rays. A, Resealings can occur in one class of sperm arising from Alternate segregation, and result in the rejoining of translocated arms to their original centromeres. Such an event may lead to the insertion of autosomal material into the *Y*, insertion of *Y* material into the autosome, or a combination of both outcomes (as depicted here). B, Carve-downs occur in one class of sperm arising from Adjacent I segregation (homologous centromeres disjoin). Here, the induction of a deficiency in the duplicated 2R arm appended to Y^L can reduce the level of 2R hyperploidy to a level which allows survival of the resultant progeny, the acentric piece being lost in the early zygotic divisions. Both these events yield fertile sons in the mutagenesis protocol.

exact cytological position is indicated by the brackets in Figure 2, which represent the current limit of resolution for placing these complementation groups (P. DIMITRI, personal communication). It is important to note that all four lines examined cytologically have a common breakpoint in h38, the N-band to which the centromere of chromosome *2* maps (*cf.* Figure 2B). It is this breakpoint that was used to place the original 2R break of *T(Y;2)CB25* to the position indicated in Figure 2.

It is clear from Table 1 that lines *CB25-1, 4, 9, 11, 22* and *24* carry *Y* chromosomes that are now sensitive to the action of *SD*, as indicated by their low recovery (R_Y) or high k_Y values compared to appropriate controls (crosses 1 and 2 of Table 1, and cross 2 of Table 2). In fact, the strength of drive operating against four of these six derived *Y* chromosomes is comparable

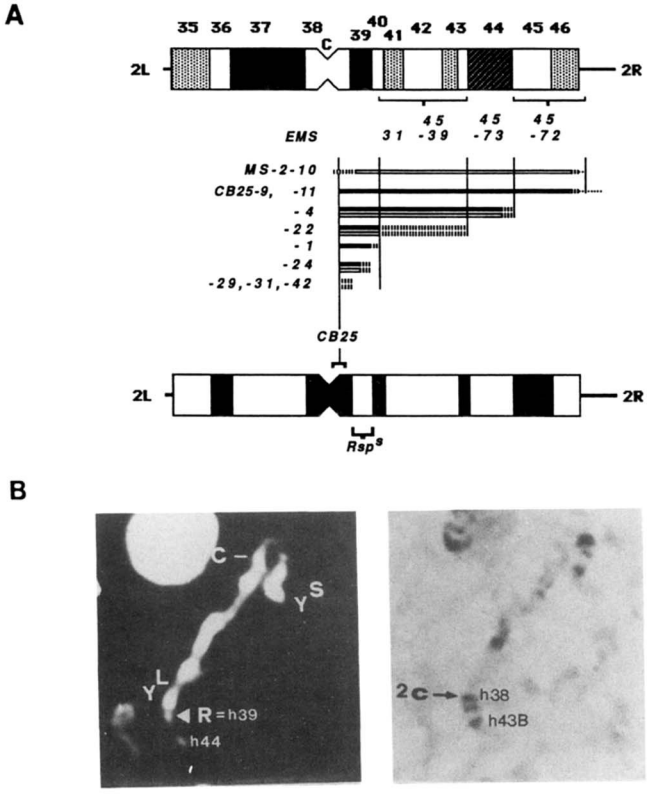

FIGURE 2.—Mapping Rsp^s in the 2R heterochromatin. A, Mapping secondary rearrangements to the Hoechst 33258 fluorescence (upper) and N-band (lower) maps of the 2R heterochromatin (after PIMPINELLI and DIMITRI 1989). For the Hoechst map, the darker the representation of the band, the more intense the fluorescence. Open and closed bars represent the extent of deficiencies and duplications, respectively, as determined by lethal complementation tests and by direct observation of larval neuroblasts to determine the presence of staining bands. Dashed lines represent uncertainty as to exact breakpoint. The locations of the lethals used for complementation tests appear below the Hoechst map, with brackets indicating uncertainty as to the precise location of the corresponding lethals (although their order is established). Euchromatin is represented by the thin lines outside the banded areas. The position of loci involved in segregation distortion are shown below the N-band map. The position of the original 2R breakpoint of *T(Y;2)CB25*, which is common to all secondary rearrangements, is shown by the vertical line. B, Larval neuroblasts showing *Dp(2;Y)CB25-4* stained sequentially for Hoechst 33258 (left) and N-banding (right). Numeric labels represent bands corresponding to the map of A. C represents the *Y* chromosome centromere; 2C represents material from the N-band (h38) corresponding to the position of the second chromosome centromere.

to that observed against Rsp^s in control crosses where that allele occupies its normal second chromosome position (compare R_Y in crosses 5–8 with $R_{A'}$ for control crosses 1 and 2). The apparent exceptions are *CB25-1* and *CB25-24*. In the former case, the increased *Y* recovery may simply reflect an inexplicable weakening in the strength of the *SD* chromosome used in the test, especially since the recovery of the *cn bw* chromosome in cross 4 is also enhanced. For *CB25-24*, there may be a real intrinsic reduction in sensitivity, as will be discussed later. These two cases notwithstanding, it is obvious that Rsp^s is able to function as a target for *SD* activity even when removed from its normal milieu deep in autosomal centric heterochromatin to a position on the tip of the long arm of the *Y* chromosome.

It is also worth noting that, at least for the case of *Tp(2;Y)CB25-4*, cytological preparations of spermatocytes reveal no evidence of meiotic pairing of the Rsp^s-bearing *Y* with the second chromosomes (AULT and LYTTLE 1988), nor is there any evidence that any of these derived lines (with the previously discussed exception of *CB25-1*) have an increase in the frequency of meiotic sex chromosome nondisjunction, as measured by the number of *XO* or *XXY* progeny (T. W. LYTTLE, unpublished data). These observations, taken in combination with similar results obtained for the *SD*-bearing *Y* chromosome of *Tp(2;Y)B10-4* [described in LYTTLE (1986), but designated *T(Y;2)B10-4* at that time] argue against the necessity for meiotic pairing of any of the elements in the *SD* system as a prerequisite for sperm dysfunction.

The mapping summary of Figure 2 clearly places Rsp^s in the most proximal portion of the 2R heterochromatin. This is in agreement with the data of BRITTNACHER and GANETZKY (1989), who have demonstrated that all known 2R heterochromatic complementation groups can be deleted from a sensitive second chromosome without removing Rsp^s activity, and with the work of PIMPINELLI and DIMITRI (1989) associating band h39 with *Rsp* activity. The present data go one step farther in demonstrating that Rsp^s is clearly separable from the centromere, as the second chromosomes of lines *CB25-4*, *-22* and *-24* would otherwise be acentric and should have been lost. However, it should be noted that this result does not formally exclude the possibility that Rsp^s and the centromere overlap, such that most or even all of Rsp^s could be removed while leaving some residual centromere function. Conversely, AULT and LYTTLE (1988) raise the possibility that the transposed Rsp^s material in *Tp(2;Y)CB25-4* may indeed have some mild centromeric activity of its own. This is suggested by the cytological observation in some spermatocytes of independent orientation of the Y^L tip to the poles.

Tp(2;Y)CB25-29, *-31* and *-42* (crosses 10–12 in Table 1) all have resealed *cn bw* second chromosomes that retain full Rsp^s sensitivity. In fact, for these three lines, the lethal complementation map of Figure 2 provides no rationale for deciding whether these are *Tp(2;Y)*, *Tp(Y;2)*, or reciprocal transposition events. The only basis for choosing the first designation is the evidence that each has some partial sensitivity associated with the *Y*, as indicated by the elevated k_Y values for these *Y* chromosomes when compared to the control values for $B^S Y^{y+}$ (cross 2). This could be attributable to a general decrease in viability for these derived *Y* chromosomes, but their similarity in structure to the

TABLE 1

Chromosomal location of *Rsp* in secondary rearrangements derived from *T(Y;2)CB25*

Male genotype:			Progeny from gamete class:				Strength of segregation distortion					
X/Y;	*A/A′*	*N*	*Y;A*	*X;A*	*Y;A′*	*X;A′*	R_Y	$R_{A'}$	$R_{Y+A'}$	k_Y	$k_{A'}$	$k_{Y+A'}$
1) *y/Y;*	*SD79,bw/cn bw*	56	1,939	1,862	28	57	1.041 (0.059)	0.038 (0.010)	0.023 (0.007)	0.482 (0.011)	0.968 (0.008)	0.980 (0.006)
2) *y/B^{S}Y^{y+};*	*SD79,bw/cn bw*	47	2,035	2,353	16	42	0.865 (0.031)	0.018 (0.004)	0.007 (0.003)	0.536 (0.009)	0.982 (0.004)	0.993 (0.003)
3) *y/Y^{p}2^{D},CB25;*	*SD ARM28/2^{P}Y^{D},CB25*	18	0	678	12	0			0.018 (0.008)			0.983 (0.007)
4) *y/DpCB25-1;*	*SD ARM28/cn bw*	212	3,729	15,285	129	2,251	0.244 (0.020)	0.147 (0.012)	0.008 (0.007)	0.804 (0.009)	0.872 (0.007)	0.991 (0.002)
5) *y/DpCB25-9;*	*SD79,bw/cn bw*	8	21	489	0	8	0.043 (0.021)	0.016 (0.009)	0	0.959 (0.018)	0.984 (0.009)	1.000
6) *y/DpCB25-11;*	*SD79,bw/cn bw*	10	14	439	0	3	0.032 (0.013)	0.007 (0.004)	0	0.969 (0.013)	0.993 (0.004)	1.000
7) *y/DpCB25-22;*	*SD79,bw/DfCB25-22*	20	32	780	12	737	0.041 (0.010)	0.945 (0.060)	0.015 (0.006)	0.961 (0.009)	0.514 (0.015)	0.985 (0.006)
8) *y/DpCB25-4;*	*SD79,bw/DfCB25-4*	43	86	2,042	37	1,398	0.042 (0.012)	0.685 (0.040)	0.018 (0.006)	0.956 (0.009)	0.593 (0.014)	0.982 (0.005)
9) *y/DpCB25- 24;*	*SD79,bw/DfCB25-24*	32	162	1,248	93	1,355	0.130 (0.023)	1.086 (0.049)	0.075 (0.015)	0.885 (0.015)	0.479 (0.011)	0.931 (0.011)
10) *y/DpCB25- 29;*	*SD79,bw/DfCB25-29*	15	347	533	5	1	0.651 (0.070)	0.002 (0.002)	0.009 (0.005)	0.606 (0.026)	0.998 (0.002)	0.990 (0.005)
11) *y/DpCB25- 31;*	*SD79,bw/DfCB25-31*	43	1,118	1,844	41	38	0.606 (0.026)	0.021 (0.005)	0.022 (0.005)	0.623 (0.010)	0.979 (0.005)	0.978 (0.004)
12) *y/DpCB25- 42;*	*SD79,bw/DfCB25-42*	19	560	836	22	23	0.670 (0.028)	0.028 (0.010)	0.026 (0.010)	0.599 (0.010)	0.973 (0.001)	0.975 (0.009)

Males of the appropriate *X/Y; A/A′* genotypic constitution were mated to *y; cn bw* females, and the progeny arising from each of the four independently segregating gamete classes were scored. The column labeled *N* denotes the number of parental males tested. Where appropriate, the standard error for each statistic is shown in parentheses. The computation of *R* and *k* values are described in MATERIALS AND METHODS. Cross 1 represents a control for strength of drive for *SD79,bw*, while cross 2 serves as a control for any viability effects arising from the B^S and y^+ markers, which are also present on the *Y* chromosome in crosses 3 and 8–12. Cross 3 presents the *Rsp* sensitivity of *T(Y;2)CB25*, the parental translocation used to generate the secondary rearrangements. Crosses 4–6 involve *Y* chromosomes carrying only y^+ and the viability control for these is discussed in the text. Cross 7, involving *Tp(2;Y)CB25-22*, contains a *Y* marked with y^+ while *Df(2R)CB25-22* carries the complementary B^S marker.

TABLE 2

Subdivisibility of *Rsp*

Male genotype:			Progeny from gamete class:				Strength of segregation distortion:	
X/Y;	*A/A'*	*N*	*Y;A*	*X;A*	*Y;A'*	*X;A'*	$R_{A'}$	$K_{A'}$ (corr)
1) *y/DpB10-4;*	*SD ARM28/cn bw*	35	1133	2357	1	7	0.003 (0.001)	0.997 (0.001)
2) *y/Y;*	*SD ARM28/cn bw*	24	1777	1802	1	4	0.002 (0.001)	0.998 (0.001)
3) *y/Y;*	*SD ARM28/DfCB25-4*	59	1725	1661	1033	1148	0.691 (0.035)	0.591 (0.562) (0.010)
4) *y/DpCB25-4;*	*SD ARM28/DfCB25-4*	28	57	2257	15	1487	0.659 (0.030)	0.603 (0.573) (0.011)
5) *y/DpB10-4;*	*SD ARM28/DfCB25-4*	15	582	898	216	234	0.261 (0.062)	0.793 (0.772) (0.031)
Viability Control (Females) *y;*	(reciprocal of cross 3) *SD ARM28/DfCB25-4*	36	1136	1122	1026	994	0.886 (0.063)	0.530 (0.016)
6) *y/DpB10-4;*	*SD ARM28/DfCB25-24*	62	1129	2291	336	917	0.400 (0.032)	0.714 (0.655) (0.015)
Viability Control (Females) *y;*	*SD ARM28/DfCB25-24*	13	344	211	320	160	0.758 (0.071)	0.569 (0.022)

Column headings are the same as in Table 1, except that $k_{A'}$ values corrected for viability are included in parentheses in the last column for crosses involving *Df(2R)CB25-4* and *Df(2R)CB25-24*, as described in MATERIALS AND METHODS. Crosses 1 and 2 represent controls for strength of distortion operating against a standard *Rsp* in males carrying two or one *SD* copies, respectively. Data from male progeny are not used for measuring strength of distortion because of the very low viability of *Dp(2;Y)B10-4*, which is hyperploid for 36BC-40 from chromosome 2.

control *Y* makes this argument less plausible. Taken at face value, these data raise the possibility that Rsp^s is subdivisible, such that the bulk of sensitivity has remained in its original position in the second chromosome, while some has been transposed to the *Y* chromosome.

More direct evidence for this notion of subdivisibility of Rsp^s comes from two of the three resealing lines in Table 1 (*Tp(2;Y)CB25-22*, *-4*, and *-24*, crosses 7–9 respectively) that show strong Rsp^s activity associated with the derived *Y*. For these, we have available the corresponding deleted second chromosomes that are presumed to now lack Rsp^s. We chose two of these deficient chromosomes (*Df(2R)CB25-4* and *-24*) for further study. The results from a series of crosses involving these lines are presented in Table 2, which includes a retest of the full *Tp(2;Y)CB25-4* with a new *SD* chromosome (cross 4), as well as similar data for males carrying only the *Df(2R)* from each of the two transpositions in combination with either one or two copies of the *SD* complex. Each $k_{A'}$ value is corrected for viability differences using the ratios of *SD* and *Df(2R)* progeny obtained from heterozygous females, where drive is not active (see MATERIALS AND METHODS). For *Tp(2;Y)CB25-4*, crosses 3 and 4 indicate that the resealed second chromosome still retains some residual sensitivity ($k_{A'}$ = 0.562 and 0.573, respectively) even after controlling for viability effects associated with the chromosome. Furthermore, this asymmetry of recovery becomes more exaggerated ($k_{A'}$ = 0.772) as drive strength is increased by the addition of an extra copy of the *Sd E(SD)* complex on *Dp(2;Y)B10-4*, even though the female progeny used to compute $k_{A'}$ are genotypically identical in each of these three crosses. This same decrease in recovery of the resealed autosome with increased *SD* dose can be seen for *Tp(2;Y)CB25-24*: compare the value of $k_{A'}$ from cross 6 of Table 2 to that of cross 9 in Table 1 (0.655 *vs.* 0.479). It is interesting to note that, in the standard tests of Table 1, *Df(2R)CB25-4* exhibits a weak residual sensitivity ($k_{A'}$ = 0.593, uncorrected for viability), while *Df(2R)CB25-24* appears to be insensitive ($k_{A'}$ = 0.479); conversely, the relative sensitivities appear to be reversed in Table 2. Nevertheless, it is clear that the sensitivity of each chromosome increases with increasing strength of *SD* background. The only logical explanation for this progression is that each of the two *Df(2R)* chromosomes retains some Rsp^s activity. Consequently, it follows that Rsp^s is a divisible element. It also must follow that some second chromosomes which are scored as Rsp^i in standard *SD* crosses, may in fact be revealed as partially sensitive when tested with multiple doses of *SD*. Certainly this is true for *Df(2R)CB25-24*. Finally, if *Tp(2;Y)CB25-24*

TABLE 3

Relationship between *Rsp* copy number and sensitivity of a gamete class to segregation distortion

Male genotype:		Progeny from gamete class:				Strength of segregation distortion						
X/Y; A/A′	*N*	*Y;Y*	*X;A*	*Y;A′*	*X;A′*	R_{Y}	$R_{A'}$	$R_{Y+A'}$	R'	k_{Y}	$k_{A'}$	$k_{Y+A'}$
1) *y/Y; R(SD36)bw-1/cn bw*	46	1,953	1,962	243	409	0.995	0.208	0.124		0.501	0.827	0.890
						(0.035)	(0.052)	(0.024)		(0.008)	(0.020)	(0.014)
2) *y/Y; SD ARM28/cn bw*	39	2,476	2,495	2	11	0.992	0.004	0.001		0.502	0.996	0.999
						(0.028)	(0.001)	(0.001)		(0.007)	(0.001)	(0.001)
3) *yDp1; R(SD36)bw-1/cn bw; TM6/+*	55	758	1,165	273	801	0.651	0.688	0.234	0.448	0.606	0.592	0.810
						(0.078)	(0.060)	(0.057)		(0.019)	(0.015)	(0.020)
	(a) 42	642	1,641	68	630	0.391	0.384	0.041	0.150	0.719	0.726	0.961
	(b) 36	831	1,043	119	560	0.797	0.537	0.114	0.428	0.557	0.651	0.898
	(c) 61	774	1,751	71	762	0.442	0.435	0.041	0.192	0.693	0.697	0.961
	(d) 55	864	1,646	87	701	0.525	0.426	0.053	0.224	0.656	0.701	0.950
4) *y/Dp1; R(SD36)bw-1/cn bw*	194	3,111	6,081	345	2,653	0.512	0.436	0.057		0.661	0.696	0.946
						(0.036)	(0.028)	(0.010)		(0.011)	(0.010)	(0.006)
5) *y/Dp4; R(SD36)bw-1/cn bw*	44	789	1,436	131	644	0.549	0.448	0.091	0.246	0.645	0.691	0.917
						(0.040)	(0.045)	(0.018)		(0.045)	(0.021)	(0.012)
	(a) 34	611	2,099	31	304	0.291	0.145	0.015	0.042	0.775	0.873	0.985
	(b) 60	1,200	3,857	51	630	0.311	0.163	0.013	0.051	0.763	0.860	0.987
	(c) 24	547	1,563	23	416	0.350	0.266	0.015	0.093	0.741	0.790	0.985
	(d) 48	1,041	4,731	15	683	0.220	0.144	0.003	0.032	0.820	0.874	0.997
	(e) 46	330	3,035	9	218	0.109	0.072	0.003	0.008	0.902	0.933	0.997
6) *y/Dp1; SD ARM28/cn bw*	212	3,729	15,285	129	2,251	0.244	0.147	0.008		0.804	0.872	0.991
						(0.020)	(0.012)	(0.007)		(0.009)	(0.007)	(0.002)
	(a) 23	175	816	5	60	0.214	0.074	0.006	0.016	0.824	0.931	0.994
	(b) 20	331	1,056	8	215	0.313	0.204	0.008	0.064	0.761	0.831	0.992
	(c) 38	525	3,226	6	263	0.163	0.082	0.002	0.013	0.860	0.925	0.998
7) *y/Dp4; SD ARM28/cn bw*	81	1,031	5,098	19	538	0.202	0.106	0.004		0.832	0.904	0.996
						(0.017)	(0.013)	(0.002)		(0.011)	(0.007)	(0.010)
8) *y/Dp1; SD ARM28/CyO*	47	91	3,161	66	2,893	0.029	0.915	0.023		0.972		0.978
						(0.006)	(0.047)	(0.005)		(0.006)		(0.005)

Crosses 3–7 demonstrate the interaction between two *Rsp′* copies (on *Y* and *A′*) and a single *SD* chromosome, while crosses 1 and 2 represent control crosses where a single *Rsp′* is present. For these experiments, both *Dp1* = *Dp(2;Y)CB25-1* and *Dp4* = *Dp(2;Y)CB25-4* are used as the *Rsp*-bearing *Y* chromosome. Cross 8 represents a further control for the effect of *SD* on a *Rsp′*-bearing *Y* alone, as the *CyO* chromosome carries no *Rsp′*. Crosses 3 and 4 are comparable, except that, in the former, males also carry the third chromosome balancer *TM6*, which reduces the strength of drive (see text). Crosses 4, 6 and 7 are recorded both as individual sets of replicates (the lettered rows) as well as summed to give a single pooled value. Column headings are as described for Table 1, except for the addition of R', which represents the expected value of $R_{Y+A'}$ under the assumption that multiple *Rsp′* copies have a multiplicative effect on sperm survival in *SD* males.

splits *Rsp*^{s} such that a sizable portion of it remains segregating with *Df(2R)CB25-24*, this could explain the apparent reduction in sensitivity exhibited by *Dp(2;Y)CB25-24* in cross 9, Table 1. In fact, there is evidence from the recent molecular analysis of the DNA structure of *Rsp*^{s} (WU *et al.* 1988) supporting the notion that both the *Dp(2;Y)CB25-24* and *Df(2R)CB25-24* chromosomes carry sizable numbers of copies of the basic 240 bp repeats associated with *Rsp*^{s}.

In testing the behavior of the *Rsp*^{s}-bearing *Y* chromosomes alone, it was useful to restrict analysis to *Dp(2;Y)CB25-1*, for which the most data exist, and *Dp(2;Y)CB25-4* (the derived *Y* chromosome from *Tp(2;Y)CB25-4*), which is the best mapped of the several rearrangement lines, both cytologically and genetically. Figure 2 shows that *Dp(2;Y)CB25-4* extends far enough distally to cover lethal *l(2)EMS45-73* [marking Group III of HILLIKER (1976)]. When *Dp(2;Y)CB25-4* is examined cytologically, it can be observed directly that the translocated material includes part of h38 (the N band containing the second chromosome centromere), extends through h39-h43, and ends somewhere in the Hoechst-positive band h44 (see Figure 2B), as predicted by the complementation map. Besides it precise mapping, *Tp(2;Y)CB25-4* had additional advantages; it had been used to characterize the divisibility of *Rsp*^{s} (Table 2), and it was known to exhibit normal patterns of segregation (see earlier arguments).

Table 3 summarizes the progeny data from several crosses involving fathers carrying either *Dp(2;Y)CB25-1* or *-4*, in a variety of *SD* backgrounds. Note that for three of the crosses (4, 6 and 7), a number of replicate experiments carried out at different times have been presented both individually (crosses labeled with lower case letters) and as pooled values. The pooled data from cross 6 have already been presented in Table 1, as cross 4. Since *R(SD-36)bw-1* is a weak *SD* chromosome (see $k_{Y+A'}$ in cross 1), and the third chromosome balancer *TM6* is known to carry a major suppressor of segregation distortion (LYTTLE 1986), these chromosomes in combination (cross 3) represent a very weak drive environment. Removing *TM6* increases *SD* strength (crosses 4–5); replacing *R(SD-36)bw-1* with *SD ARM28* raises background drive strength further (crosses 6 and 7), and removing the competing *Rsp*^{s} by replacing *cn bw* with an insensitive *CyO* homolog (cross 8) further concentrates the drive operating against *Dp(2;Y)CB25-1*. Therefore, in a trend analogous to that seen in Table 2 for the *Df* chromosomes, R_Y values for each *Y* chromosome decrease dramatically as background drive strength increases (*e.g.*, for *Dp(2;Y)CB25-1* compare crosses 3, 4, 6 and 8). One concern might be that the presence of 2R heterochromatin or other marker effects might be significantly affecting the viabilities of males carrying either of these *Dp(2;Y)* chromosomes, and thus biasing our estimates of R_Y and $R_{Y+A'}$ to the low side. However, an independent cross of *Dp(2;Y)CB25-1/y;cn bw* males by *y;cn bw* females yielded a male:female progeny ratio of 5539:4915, indicating that duplication for both 2R heterochromatin and a full fourth chromosome caused no reduction in the viability of that chromosome; further, cross 2 of Table 1 indicates that the B^S marker carried by *Dp(2;Y)CB25-4* could be responsible for only a mild viability depression. Since viability effects of this magnitude would not change any of the qualitative conclusions we reach in this report, the data is presented in an uncorrected form.

In those crosses (3–7) involving fathers carrying single copies of the *SD* complex, but two copies of *Rsp*^{s} (one in the normal 2R position, one transposed to the *Y*), normal independent assortment of the sex and second chromosomes should produce four equally frequent gamete classes: *X* + *SD* (with zero *Rsp*^{s} copies), *Y* + *cn bw* (with two *Rsp*^{s} copies), and *X* + *cn bw* and *Y* + *SD* (each with one *Rsp*^{s} copy). The survival of each *Rsp*^{s}-bearing gamete class can be measured relative to the *X* + *SD* class, which should not be subject to *SD*-induced sperm dysfunction. Note that it is the presence of this benchmark class of sperm carrying no copy of *Rsp*^{s} that allows measurement of absolute levels of sperm survival, in contrast to less informative measures of relative survival such as were employed in *Rsp* hierarchy tests of LYTTLE, BRITTNACHER and GANETZKY (1986). In practice, we estimate each *R* value by dividing the number of progeny arising from each of the *Rsp*^{s}-bearing gamete classes by the total number of *SD* daughters (representing the reference *X* + *SD* gamete class). These observed *R* values are presented in Table 3. The relative susceptibility of each individual *Rsp*^{s} copy to sperm dysfunction can be measured by R_Y and $R_{A'}$. Note that, for most such crosses, $R_{A'} < R_Y$, which suggests either that *Dp(2;Y)CB25-1* and *CB25-4* both carry a less than complete copy of *Rsp*^{s}; or that *Rsp*^{s} is slightly less sensitive when moved to its new position. The former interpretation is compatible with the notion that *Tp(2;Y)CB25-4* retains some *Rsp* sensitivity on the deficient second chromosome, as argued from the data of Table 2.

Now, if *SD* affects each *Rsp*^{s} copy independently, and the two *Rsp*^{s} copies are also independent in their effect on sperm dysfunction, then we expect:

$$R' = (R_Y)\cdot(R_{A'})$$

where R' represents the *expected* survival of the double *Rsp*^{s} sperm class, as opposed to the observed value $R_{Y+A'}$.

Departures from such independence could arise in

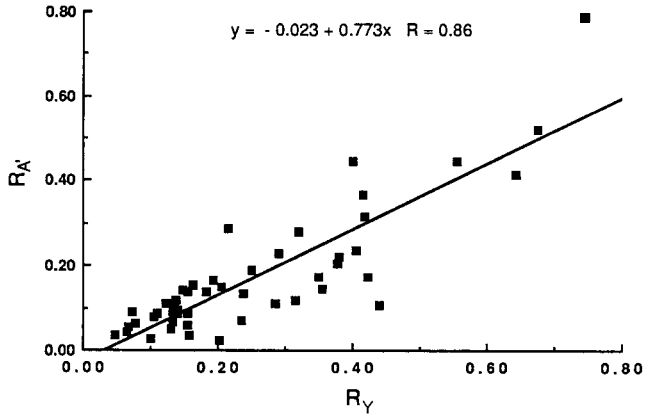

FIGURE 3.—Correlation between R_Y and $R_{A'}$ values for individual males. The recovery values for the two classes of single Rsp^s-bearing sperm from 48 individual males of genotype *y/Dp(2;Y)CB25-1; SD ARM28/cn bw* (see cross 6d, Table 3) are plotted. For these data the coefficient of correlation = 0.863. Note that, in general, $R_Y > R_{A'}$, indicating that the sensitivity of the Rsp^s-bearing *Y* chromosome is slightly reduced. There is a high intermale variation in drive strength (in excess of binomial), despite the fact that all fathers are presumed to be genetically identical.

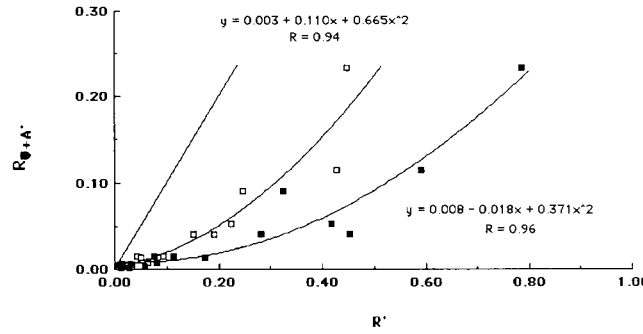

FIGURE 4.—Relationship of observed recovery of double Rsp^s-bearing sperm ($R_{Y+A'}$) to that expected (R') under a multiplicative model for Rsp^s effect on sperm survival. $R_{Y+A'}$ and uncorrected R' values (□) are taken from Table 3, and corrected R' values (■) were calculated as described in the text. The $R' = R_{Y+A'}$ line indicates the expected relationship if the probability of survival of sperm with two Rsp^s copies was equal to the cross product of the survival probabilities of sperm from the same male carrying single copies of Rsp^s. In general, observed survivals are much lower than expected, indicating a synergism between multiple Rsp^s copies.

a number of ways. For example, if there were a titration effect of two Rsp^s copies on a limited amount of *SD* product, such that the presence of one Rsp^s copy lowered the effective *SD* strength experienced by the other copy and therefore raised its survival probability, then we might expect $R' < R_{Y+A'}$. It is important to note that this same result could also arise purely as a statistical anomaly. This can be illustrated by Figure 3, which shows a scatter diagram of the R_Y and $R_{A'}$ values for the individual males whose pooled progeny produced the data of cross 6d. This plot is typical of standard *SD* crosses, where simple binomial variation combined with an inherent variable expressivity in the *SD* phenotype contribute to the scatter of *k* values obtained for males of presumably identical genotype. Now, suppose the survival probability of double-Rsp^s sperm in each male were in fact the simple cross product represented by R'. Then (for example), when weighted averages of R_Y and $R_{A'}$ are calculated by pooling progeny from males showing weak drive (*e.g.*, those in the upper right of the figure, which should give relatively high values of R') and males exhibiting strong drive (lower left, giving low values of R'), their cross product will tend to underestimate the actual weighted average of R' by an amount roughly equal to the intermale variance for either R_Y or $R_{A'}$ values (assuming that these are approximately equal). That is, if $R_Y \cdot R_{A'}$ is used to calculate the expected value R', we may inadvertently obtain an estimate actually biased to the low side, perhaps causing us to incorrectly reject the hypothesis of independent Rsp^s action. In essence, this would create an apparent *excess* survival of sperm carrying two Rsp^s copies; this situation is analogous to the apparent excess of homozygotes seen when one inadvertently pools a number of independent populations which vary in allele frequency (the Wahlund effect). For fourteen appropriate crosses, uncorrected values of R' appear in Table 3, while both corrected and uncorrected values of R' are plotted against $R_{Y+A'}$ in Figure 4. For each cross, the correction was carried out by taking the simple arithmetic average of the observed variances among males for R_Y and $R_{A'}$, then adding this quantity to the uncorrected R' value. Since the replicates of crosses 4, 6 and 7 provide independent estimates of the several *R* statistics, they have separately calculated R' values, and are presented as independent data points.

However, in point of fact the observed $R_{Y+A'}$ values are actually much *lower* than the expected R' values even without this correction, and the discrepancy is actually exacerbated after adjustment is made for the gametic Wahlund variance. It is clear from Figure 4 not only that $R' \gg R_{Y+A'}$ for each cross depicted, but that there is basically a simple second order relationship between the two variables. This apparent enhanced mortality of sperm carrying two Rsp^s copies might be explained in at least two ways. On the one hand, it might be that Rsp^s copies do not operate independently in their effect on sperm dysfunction; *i.e.*, they show positive epistasis. Conversely, the Rsp^s copies could be reacting independently, but when together in a meiocyte, each Rsp^s finds itself subjected to a higher *SD* background than either sees when alone. In this view, we might suppose that *SD* product is normally present in limiting amounts, its distribution throughout the syncytial meiotic products depending on the position of the various Rsp^s copies. Figure 5 illustrates this speculative interpretation schematically. In meiocytes where the Rsp^s copies have separated after first division (Figure 5B′), the *trans*-acting *SD* product is presumably spread uniformly

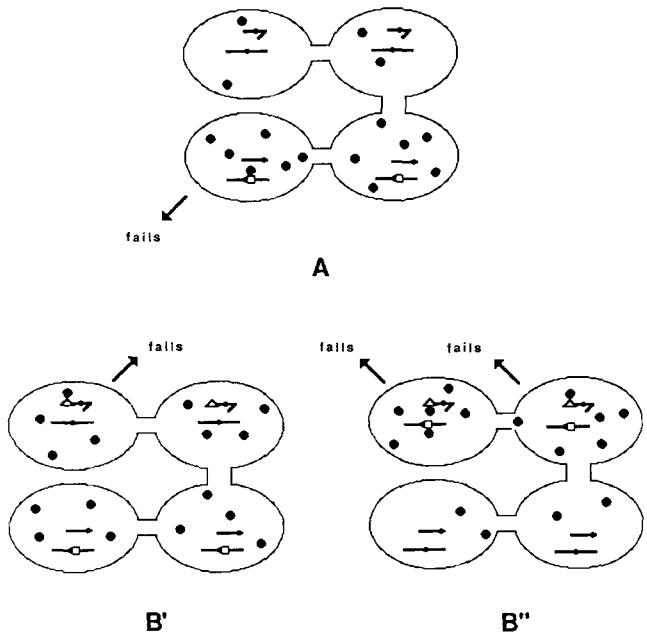

FIGURE 5.—Hypothetical model to explain the synergistic effect of Rsp^s copy number on sperm survival. The figure is a schematic representation of the syncytial products arising from male meiosis, with the four spermatocytes connected by cytoplasmic bridges. The *Y*, *X* and second chromosomes are represented, respectively, by the bent, short and long lines. The Rsp^s copies are denoted by □ and △, while the hypothetical product of the single *Sd* copy present in each genotype is denoted by ●. A, Spermatocytes from SD/Rsp^s. *Sd* product becomes concentrated in meiocytes carrying Rsp^s, resulting in a moderate probability of sperm dysfunction (arbitrarily set here at $R = 0.5$). B, Spermatocytes from $Dp(2;Y)Rsp^s;SD/Rsp^s$. Here, Rsp^s copies may segregate into different spermatocytes (B′) or cosegregate (B″). In the former case, the resulting even distribution of Rsp^s leads (hypothetically) to an even distribution of a transacting *Sd* product across the syncytium, giving a higher survival (average $R = 0.75$) for Rsp^s than obtained under case A. Under B″, the concentration of Rsp^s copies causes the *Sd* environment experienced by each Rsp^s to be similar to that under A, raising the probability for any given Rsp^s to cause sperm dysfunction, and giving a low sperm survival ($R \approx 0.25$). Since R_Y and $R_{A'}$ are calculated from meiocytes of type B′, while $R_{Y+A'}$ is obtained from meiocytes of type B″, this would cause $R' > R_{Y+A'}$, as happens for the data illustrated in Figure 4.

among the syncytial meiotic products. It is from gametes arising from these cells that R_Y and $R_{A'}$ values would be estimated. However, in meiocytes where the copies cosegregate (Figure 5B″), a concentration gradient of *SD* product might become established as the Rsp^s copies deplete the product locally, leading to a diffusion of *SD* product from neighboring meiocytes. In this case, each Rsp^s copy would be subjected to a stronger *SD* environment, each would therefore have a lower than expected survival probability, and the overall survival of the two-Rsp^s sperm would be dramatically decreased below expected, as is observed in the data of Table 3 and Figure 4. A similar asymmetrical distribution of *SD* product should also cause recovery of the Rsp^s-bearing *cn bw* second chromosome in a standard cross (*i.e.*, from a male of genotype *Sd E(SD)* $Rsp^i/++Rsp^s$, *cf.* Figure 5A) to be lower than its equivalent recovery in the sperm of males who also carry *Dp(2;Y)CB25-1* or *-4* (*e.g.*, in Table 3, compare $R_{A'}$ values for cross 1 with crosses 4 and 5, and values for cross 2 with crosses 6 and 7). A drop in recovery also occurs for *Dp(2;Y)CB25-1* when it is the only Rsp^s copy (compare R_Y for cross 8 with cross 6). Finally, the curvature of the regression lines relating R' to $R_{Y+A'}$ in Figure 4 could be interpreted as arising because the concentration gradient of *SD* product is more pronounced in strong *SD* backgrounds (*i.e.*, near the origin of the figure). All these observations are at least in qualitative agreement with what would be predicted if Rsp^s survival were inversely related to concentration of *SD* product, and asymmetrical distribution of Rsp^s copies led to asymmetrical concentration of *SD* product in the meiocytes. In general, therefore, these data support the notion that lowered recovery of double-Rsp^s sperm arises as a consequence of the altered nature of the *Sd*-Rsp^s interaction, rather than due to the subsequent epistatic interaction of the two *SD*-modified Rsp^s copies in their effect on sperm dysfunction. In particular, the latter model alone cannot adequately provide an explanation for both the low recovery of double-Rsp^s sperm and the simultaneous higher recovery of all classes of single-Rsp^s sperm from $Dp(2;Y)Rsp^s;SD/Rsp^s$ *cn bw* males.

DISCUSSION

Based on its cytological properties alone (PIMPINELLI and DIMITRI 1989) it is clear that Rsp^s is a large genetic locus. The additional facts that it is a cis-acting target for *SD*-mediated sperm dysfunction, and that it is susceptible to subdivision leads us to the further expectation that Rsp^s is not a structural gene coding for a protein, but some sort of target sequence in the DNA which is probably repetitive. This supports the view of WU *et al.* (1988), who have demonstrated that Rsp^s sensitivity is positively correlated with the number of copies of an AT-rich 240 bp sequence which is present in high copy number (700–3500 repeats) in the 2R heterochromatin of chromosomes sensitive to *SD*, but missing from insensitive chromosomes. However, the mapping results depicted in Figure 2 still present certain problems for a complete understanding of *Rsp* structure. For example, given that *Dp(2;Y)CB25-4* must carry all of the Hoechst-positive band h39, yet *Df(2R)CB25-4* still retains Rsp^s activity (Table 2), it becomes necessary to postulate that some sensitivity to distortion must reside outside h39. In fact, until it can be demonstrated that it is possible to obtain a resealed *cn bw* chromosome that is totally deficient in sensitivity to *SD*, the possibility remains that some *Rsp* activity may actually map to the short stretch of basal 2L heterochromatin remaining proximal to the *T(Y;2)CB25* breakpoint (or, less likely, in

2L or distal to h44 in 2R). Conversely, if *Rsp*^{s} represents a single cohesive stretch of DNA, the observation of residual sensitivity in *Df(2R)CB25-4* requires that at least some portion of the sensitivity be associated with N-band h38. In any case, the cytological, genetic, and molecular evidence are consistent in presenting a view of *Rsp*^{s} as being at minimum a large, highly repetitive stretch of DNA.

Our understanding of the mechanism by which the *SD-Rsp*^{s} interaction causes sperm dysfunction is by no means as clear, but the observations reported here and elsewhere add several important points that must be accommodated when models are constructed. First, it is clear that the ability of *Rsp*^{s} to act as a target does not depend critically on its position next to the second chromosome centromere; apparently it retains virtually the same ability to induce sperm dysfunction even when removed to the tip of Y^L. This almost certainly rules out any role for chromosome pairing as a prerequisite for segregation distortion. Second, models which presume that sperm dysfunction occurs only after *Rsp*^{s} is saturated with *SD* product, are effectively eliminated by the fact that sensitivity does not show a negative, but a positive correlation with *Rsp*^{s} size or number. In fact, the evidence presented here is that multiple *Rsp*^{s} copies appear to be synergistic, the survival probability of sperm carrying two *Rsp*^{s} copies being much lower than predicted by the cross product of the survival probabilities of the single *Rsp*^{s} sperm classes from the same male genotype. If this observation can be extrapolated downward to smaller *Rsp* targets, we might guess that an intact *Rsp*^{s} copy should have a lower recovery frequency than would be predicted from simultaneous measurements of the survival of its subdivided pieces. It is difficult to test that notion here with *Tp(2;Y)CB25-4* and *Tp(2;Y)CB25-24*, because the pieces resulting from subdivision are apparently of quite unequal size.

As discussed above, and illustrated in Figure 3, the strength of drive exhibited in a particular cross shows considerable heterogeneity among supposedly genetically identical males. The fact that there is a strong correlation ($r = 0.863$) between the recoveries of the two single *Rsp*^{s} sperm classes for a given male, would seem to suggest that this heterogeneity arises from a male to male variability in *SD* expression, rather than from a variability in the susceptibility of each *Rsp*^{s} copy to *SD* action (for example, by variable conformational changes in *Rsp*^{s} DNA). If the latter explanation were true, it would be difficult to see why chromosomally independent *Rsp*^{s} copies should covary so strongly in their susceptibility to segregation distortion.

Our understanding of the *SD* system is rapidly becoming more sophisticated. Now that DNA sequences from both *Sd* (P. POWERS and B. GANETZKY, unpublished data) and *Rsp*^{s} (WU *et al.* 1988) are available, it is clear that we will have the necessary tools to rigorously define the nature of the interaction between *SD* product and the *Rsp*^{s} target. At the same time, it will be necessary to turn more attention to the nature of that *SD* product, and the mechanism by which *Sd*, *E(SD)* and other elements on the *SD* chromosome interact to control its expression.

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