ADDITIVE EFFECTS OF MULTIPLE *SEGREGATION DISTORTER* (*SD*) CHROMOSOMES ON SPERM DYSFUNCTION IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

A portion of the *Segregation distorter* (*SD*) chromosome, including both the *Sd* and *E(SD)* loci, has been moved by insertional translocation from *SD Roma* into Y^L. This *Dp(2;Y)SD* chromosome shows a negligible reduction in its ability to cause dysfunction of Rsp^s-bearing sperm when compared to the parent *SD* chromosome, suggesting that *SD* can still act effectively, even when removed from its normal second chromosome milieu, and that its activity level does not depend on pairing with a normal autosomal homologue. Male genotypes have been constructed using this *Dp(2;Y)SD* along with a standard *SD* chromosome (either *SD Roma* or $R(SD\text{-}36)\text{-}1^{bw}$) and a third chromosome suppressor of *SD* (*TM6*) in all possible three-way combinations. The observed level of *SD*-mediated dysfunction in each case is most compatible with a model that assumes that all *SD* elements act additively (in terms of *M*, the probit transformation of the probability of sperm dysfunction), rather than multiplicatively. The additive action of *SD* elements contrasts with the independent response to *SD* activity exhibited by multiple Rsp^s copies.

SEGREGATION distorter (*SD*) second chromosomes of *Drosophila melanogaster* are recovered in excess of Mendelian expectations in the sperm of SD/SD^+ males (SANDLER, HIRAIZUMI and SANDLER 1959). This form of meiotic drive (SANDLER and NOVITSKI 1957) apparently results from the dysfunction of some proportion of the SD^+-bearing gametes (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DEMARCO 1967). At the ultrastructural level, sperm dysfunction is associated with failure to undergo both the normal histone transition (KETTANEH and HARTL 1980) and sperm individualization (TOKUYASU, PEACOCK and HARDY 1977). Strength of drive in heterozygous males can be measured either as the fraction of *SD*-bearing gametes (k) recovered or as the absolute probability of survival of an SD^+-bearing sperm (R).

SD chromosomes apparently consist of a complex of loci, including *Sd*, where the meiotic drive of *SD* is presumed to originate, and that BRITTNACHER and GANETZKY (1983) have mapped to the basal euchromatin of *2L* (37D2-6 of the salivary gland chromosome map). The target for *Sd* action is *Responder*

(*Rsp*), which is located close to the centromere in *2R*. When *SD* chromosomes are isolated in nature they generally bear Rsp^i alleles insensitive to *Sd* action, whereas SD^+ chromosomes that are affected by *Sd* carry sensitive Rsp^s alleles. In addition to these major loci, there are also a number of others that may act as polygenic modifiers of *Sd* action (HARTL and HIRAIZUMI 1976; LYTTLE 1979; HIRAIZUMI, MARTIN and ECKSTRAND 1980). These are of varying strength and are located throughout the genome, the most important being *Enhancer of SD* [*E*(*SD*)], which is located proximal to *lt* in the *2L* heterochromatin (BRITTNACHER and GANETZKY 1984).

The molecular mechanism for segregation distortion is as yet unknown, although several models have been advanced to explain the large body of data that has accumulated in the more than 25 years since its discovery. Early work (reviewed in HARTL and HIRAIZUMI 1976) suggested that proper pairing of *SD* with its sensitive homologue in prophase I of meiosis was necessary for drive to occur; this led to the hypothesis that *SD* caused breakage of SD^+ chromosomes (SANDLER, HIRAIZUMI and SANDLER 1959). Such a model is supported by the observations of HIRAIZUMI (1961, 1962) that SD^+ chromosomes which surivived *SD* action often carried subvitals or even lethals, and of CROW, THOMAS and SANDLER (1962), who reported increased rates of X-ray-induced recombination in SD/SD^+ males. Sperm receiving chromosomes damaged by *SD* were assumed to fail in development, leading to their subsequent dysfunction. While these observations have remained unexplained, other phenomena associated with *SD* and originally assumed to arise from chromosome pairing have subsequently been explained by allelic variation within the *SD* system. This has served to reduce the attractiveness of models requiring such pairing as the basis for segregation distortion.

More recent models of *SD* action have generally postulated that the *Sd* locus is responsible for a product that is transported or diffuses to the vicinity of *Rsp*, and there interacts with Rsp^s alleles to give sperm dysfunction. One model views *Sd* as coding for a defective protein product that "poisons" regulatory multimers, interfering with their ability to properly bind to Rsp^s to *prevent* subsequent dysfunction of any sperm carrying it (HARTL 1973). A second model assumes that *Sd* is a neomorphic mutation responsible for a product that directly binds to Rsp^s to *cause* sperm dysfunction (GANETZKY 1977); whereas a third model also assumes that the *Sd* product causes sperm dysfunction, but does this by dislodging the regulatory product of a second locus [*M*(*SD*)] from Rsp^s (HIRAIZUMI, MARTIN and ECKSTRAND 1980). The second and third models predict that, as long as *Sd* product is initially present in rate-limiting amounts, measures of segregation distortion should vary directly with *Sd* copy number. However, the first model makes no clear prediction concerning the dependence of sperm dysfunction on *Sd* dose, since this would vary according to the nature of the interaction among the putative multimer subunits and its effect on the binding of the regulatory protein to the *Responder* locus.

Superimposed on these models of the qualitative nature of the *Sd-Rsp* interaction have been attempts to include the quantitative effects on drive strength

arising from a host of background genetic and environmental variation. MIKLOS and SMITH-WHITE (1971) demonstrated that the excessive male-to-male variation exhibited by *SD* lines with intermediate (k = 0.7–0.8) values of drive could be explained by assuming that the proportion of surviving sperm was a threshold character. The recovery probability for a Rsp^s sperm class would then be dependent on the relative position of some underlying distribution of sperm liability vis-à-vis a mortality threshold (see Figure 1a). Moreover, if the underlying distribution is normal, then recovery proportions for a sperm class (= R) can be related to a mean liability value (= M, synonymous with the "make" value of MIKLOS) by the probit transformation. MIKLOS (1972a,b) and others (DENELL and MIKLOS 1971; LYTTLE 1979) were able to show that a number of genetic and environmental phenomena that affected *SD* strength behaved uniformly across *SD* lines and were additive in their effects when measured on the M scale. It should be noted that the assumption of normality is meant to be heuristic only, rather than implying any biological mechanism. For example, any underlying distribution of sperm liability that is symmetric and unimodal might be expected to give similar results.

Until recently, a major impediment to proper testing of such models has been the difficulty of isolating the effects of individual loci, since the observed drive in a given male is an ensemble property of the several major loci and their associated modifiers. Although tedious recombinational dissection of *SD* chromosomes has been used as a first approach, it has been applied to only a small number of chromosomes. Moreover, it is difficult to adequately control for genetic variability arising from recombination outside of the region of interest, an important consideration when one is measuring weak drive effects. To avoid this problem, a protocol developed in this laboratory (LYTTLE 1984) has been used to produce site-specific insertional translocations from *SD* chromosomes. This yields *Dp(2;Y) copies of Sd*, *E(SD)* and Rsp^s, and these, in turn, have been used to test differing combinations of possible allelic alternatives of *SD* elements (*cf.* LYTTLE, BRITTNACHER and GANETZKY 1986). In addition, such duplications allow for complicated combinations of varying doses of these same elements, which may be expected to help elucidate the molecular and biological nature of *SD* activity, and in particular, distinguish among the various models already proposed.

The present report presents the results from a series of tests using such a *Dp(2;Y) Sd E(SD)* chromosome inserted into a number of male genetic backgrounds, variable for the presence or absence of one of two different standard *SD* second chromosomes, as well as for a third chromosome carrying a major suppressor of *SD* activity. Consideration of the behavior of the *Dp(2;Y)* alone bears on the question of whether *SD* chromosomes must pair with their normal homologues in order to cause drive, whereas consideration of all possible pairwise combinations of the several elements is used to test whether multiple *SD* chromosomes are mutliplicative or additive in their effect on segregation distortion, and whether suppressors act at *Sd* or *Rsp*. Finally, we consider what model of *SD* action is best supported by the data.

MATERIALS AND METHODS

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 (see Table 2), similar in strength to that reported for *Su(1)SD* (KATAOKA 1967).

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

R(SD-36)-1^{bw} = *R-1*, a standard intermediate strength *SD* chromosome (k = 0.881, see Table 2) derived by recombination from one of the original chromosomes (*SD-36*) isolated in Madison, Wisconsin (HARTL 1974). Carries two nonoverlapping inversions in *2R*, *In(2R)45C-F;49A* and *In(2R)NS* = *In*(2R)52A2-B1;*56F9-13*. Its genotype is *Sd E(SD) Rsp*^{i}.

SD Roma, *bw* = *ROM*, a standard intermediate strength *SD* chromosome (k = 0.832; see Tables 1 and 2) derived by recombination from *SD Roma* (BRITTNACHER and GANETZKY 1983). It carries no inversions. Its genotype is *Sd E(SD) Rsp*^{i}.

T(Y;2)B10, $B^s Y$ y^+; *SD Roma*, a translocation broken in Y^L and 36D2-3 in *2L*, induced by 4500 r of gamma rays.

T(Y;2)B10-4, $B^s Y$ y^+; *SD Roma*, *al dp*, an insertional translocation of region 36D2-3;40 into the *Y*. This was derived as a resealing of *T(Y;2)B10* [see LYTTLE (1984) for method of construction] and inserts the autosomal material into Y^L at the site of the original *B10* breakpoint. The recessive mutants *al* and *dp* were subsequently added to *2L* by recombination. The rearrangement can be resolved into *Dp(2;Y)B10-4* (= *Dp(2;Y)SD*, since both *Sd* and *E(SD)* are included) and *Df(2L)B10-4*.

Dp(2;Y)G, an insertional translocation of chromosome region 36A;40 into the *Y* chromosome (LINDSLEY and GRELL 1968). Covers *Df(2L)B10-4* and carries Sd^+ $E(SD)^+$.

MEASUREMENT OF SEGREGATION DISTORTION

The statistic k = proportion of Rsp^i-bearing sperm recovered from Rsp^i/Rsp^s heterozygous males (who also carry *Sd*) is commonly used as a measure of drive strength against *cn bw* for either *SD* or *Dp(2;Y)SD* chromosomes. It is assumed that Rsp^i alleles are unaffected by *Sd* in these crosses.

The k values of individual *SD* males were determined by matings with 2–3 *cn bw* females. The matings were generally brooded after 8 days, the parents being discarded after 8 more days. Eclosing progeny were counted for 19 days from each culture. Except where otherwise noted, only female progeny are scored for segregation, in order to avoid possible bias arising from the viability effects of the *Dp(2;Y)SD* chromosome in males. Mean k values for a given line were calculated from the weighted averages of the values for at least 20 males.

However, for purposes of analysis it is often preferable to use one of two transformations of k;

$$z = (2k - 1)/k$$

or

$$R = 1 - z = (1 - k)/k = (\text{number } Rsp^s \text{ progeny})/(\text{number } Rsp^i \text{ progeny}).$$

Here, z = the proportion of SD^+ sperm rendered dysfunctional, and R is a measure of the proportion of such sperm surviving. Since k itself is actually an observational transformation of the real biological event of dysfunction being measured by R or z, it is clear that these statistics are more appropriate for dealing quantitatively with segregation distortion (MIKLOS and SMITH-WHITE 1971; MIKLOS 1972a,b; LYTTLE 1979). In particular, k values are not additive, and their variance is generally dependent on the magnitude of the mean k value of a line. To remedy this, MIKLOS (1972a,b) has suggested using the probit transformation of z as the proper metric for *SD* activity. He assumes that there is an underlying biological variable involved in *SD* action that can be thought of as a normally distributed "liability" to sperm dysfunction. If this liability (called "make" by MIKLOS) exceeds some threshold value in the primary spermatocyte, then the Rsp^s-bearing sperm produced will be rendered dysfunctional (see Figure 1a). The mean liability of these sperm in an *SD* male can be equated to z by the probit transformation

$$z = \int_{-\infty}^{M^*} N(0,1)dx,$$

where M^* is the difference between the mean liability and the threshold of dysfunction, expressed in unit normal deviates, and $N(0,1)$ is the unit normal density function. To avoid negative values, $M = M^* + 5$ is used in calculations, and this is called the "probit" of z (FISHER and YATES 1963). Consequently, there exists a function, call it $f(R) = M$, and its inverse, $f^{-1}(M) = R$, which allow us to pass from one of these values to the other, with z as an intermediate.

A quantitative modifier of drive is assumed to act by shifting the mean liability by a fixed amount. While this results in a fixed effect on M, its effect on z, R and k will obviously vary, depending on the base strength of the *SD* line being modified. For example, examination of Figure 1b shows that the effect on k of a given shift along the M axis differs considerably according to the starting value of M, and is maximum for intermediate values of M. Standing the problem on its head, we might argue that an element in the *SD* system must be acting additively if it has a uniform effect on M, but differing effects on z, R and k, depending on the strength of the *SD* line tested. In fact, many of the genetic and environmental variables known to affect *SD* strength appear to act additively on the M scale (HARTL and HIRAIZUMI 1976).

RESULTS

Table 1 presents the results when *SD Roma*, *Dp(2;Y)SD* and the rearrangement forms connecting them are tested for *SD* strength. It is clear that the *T(Y;2)B10* break in *SD Roma* has not affected *SD* activity (compare lines 1 and 2). Further, when *Sd* and *E(SD)* are placed on the *Y* chromosome, they are still able to induce dysfunction of Rsp^s at a level nearly as high as the original *SD* chromosome (compare lines 1 and 5). The observed difference of ΔM = 0.42 can be explained on the basis of loss of weak enhancers known to be

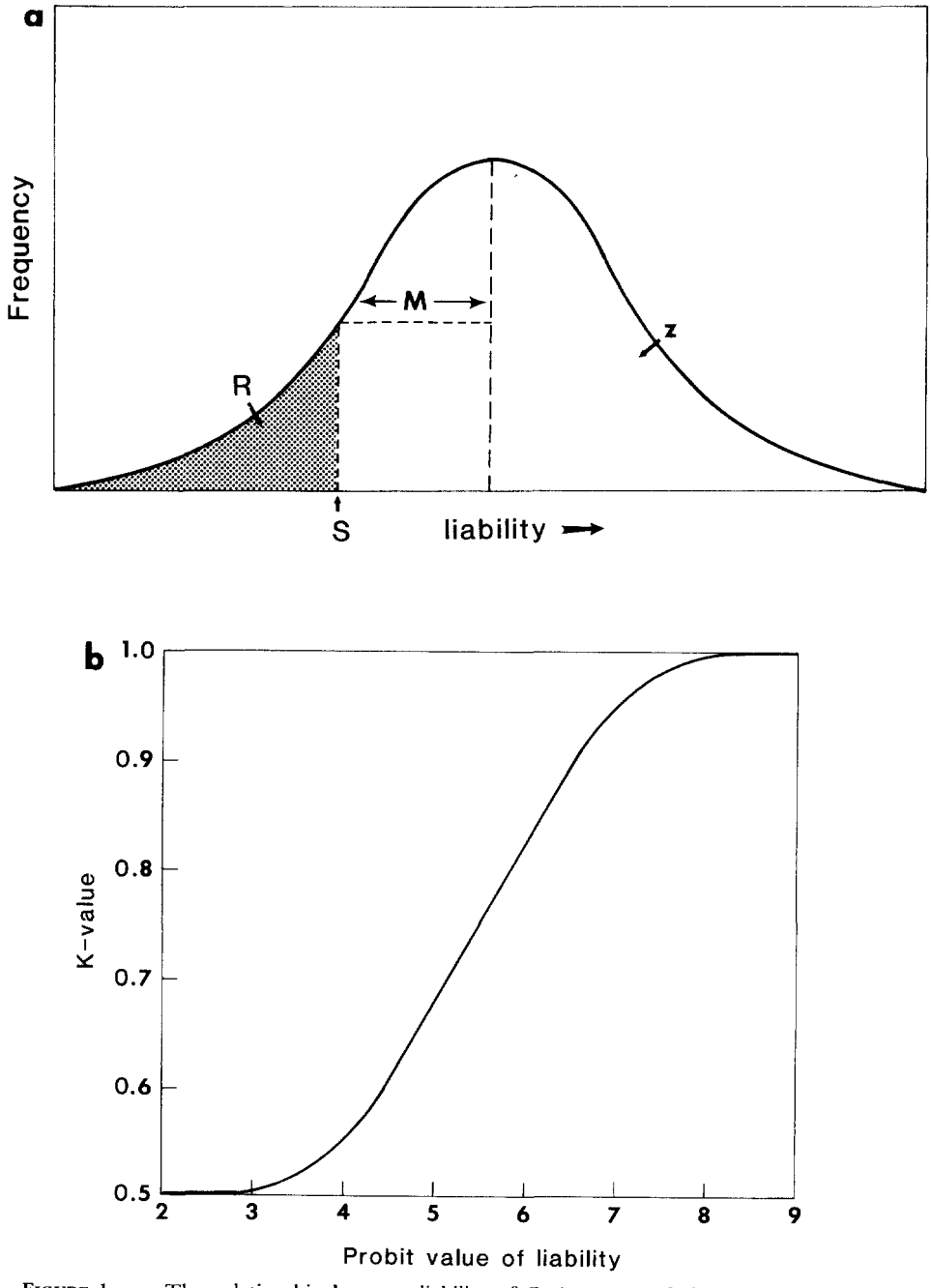

FIGURE 1.—a, The relationship between liability of *Rsp*^{s} sperm and the probability (*z*) of exceeding the threshold of dysfunction *S*. The position of the distribution is measured by *M*, and the proportion of surviving sperm is given by *R*. b, The relationship between the probit value of liability (measured as *M*) and fraction of *Rsp*^{s} progeny (*k*). Note that small changes in *k* at either end of the distribution result in large changes on the probit scale.

TABLE 1

Segregation distortion in rearrangements of *SD Roma*

SD line	Progeny		Drive strength			
	SD	SD^+	*k*	*z*	*R*	*M*
1. *SD Roma, bw/cn bw*	3394	683	0.832	0.798	0.202	5.84
2. *T(Y;2) B10/cn bw*	2677	556	0.828	0.793	0.207	5.82
3. *T(Y;2) B10-4/cn bw*	468	322	0.590	0.305	0.695	4.49
4. *Dp(2;Y)G+Df(2L) B10-4/cn bw*	309	215	0.590	0.305	0.695	4.49
5. *Dp(2;Y) B10-4;S^2CyO cn bw/cn bw*	895	309	0.743	0.654	0.346	5.40

Measurement of *k* and its statistical transformations are performed as described in MATERIALS AND METHODS, except that in crosses involving *T(Y;2)* lines, only *SD* sons and SD^+ daughters can be scored.

associated with distal *2R* (*cf.* HARTL and HIRAIZUMI 1976). Moreover, control crosses of *CyO/cn bw* males by *cn bw* females show a significantly reduced viability for the *CyO* chromosome (292 *CyO*:374 *cn bw* females recovered). If the *CyO* progeny class from line 5 is multiplied by 374/292 to correct for viability, then *k* and *M* rise to 0.743 and 5.62, respectively, and the difference between line 5 and lines 1 and 2 become even less significant ($\Delta M = 0.22$). In most crosses reported here, however, viability differences between the segregating second chromosomes are of negligible magnitude, and correction for them would only complicate maximum likelihood estimation procedures and increase the variances of estimates without providing any offsetting again in resolving power. Consequently, further calculations and comparisons will employ the uncorrected raw data only.

On the basis of lines 1, 2 and 5, therefore, we would conclude that *Sd* activity is effectively independent of its position in the genome, as argued by LITTLE, BRITTNACHER and GANETZKY (1986). The results from lines 3 and 4, however, are less easily understood. For line 3, the drive is significantly weaker than that observed for line 5, despite the fact that the same *Dp(2;Y)SD* is present in both. One might suspect that the lowered recovery of *T(Y;2)* males is at least partially due to the lowered vability of the twice-irradiated *SD Roma* chromosome; yet, when *Sd* is removed from the system in line 4, there is no evidence of any such residual viability effect. In fact, both lines show identical *k* values, as if *Sd* were inactive in the former. It is tempting to attribute the lowered drive to interference in proper *Rsp* pairing caused by the breakpoint near the centromere of *Df(2L)B10-4*, since this *Df* is common to both lines. However, the *CyO* balancer chromosome also has breakpoints near *Rsp* that would interfere with pairing; yet, line 5 exhibits quite normal drive activity. Moreover, preliminary tests with *Dp(2;Y)Rsp^s* and *Dp(2;f)*Rsp^s chromosomes (T. W. LYTTLE, unpublished results; J. G. BRITTNACHER and B. GANETZKY, unpublished results) show that these remain fully susceptible to *SD*-mediated dysfunction, despite suffering greater structural disruption than the lines reported here. Leaving aside the puzzling results of lines 3 and 4, however, it is clear that the *SD* elements themselves can still function quite well when re-

moved from their normal second chromosome milieu and placed at some distance from the *Rsp*^{s} locus at which they must act to cause dysfunction. As a final piece of evidence arguing that *SD* operates without requiring pairing with its target chromosome, it should be noted that only 3 of 7089 females recovered from *Dp(2;Y)SD* fathers in this study were *XXY* exceptions, demonstrating that pairing and segregation of the paternal *X* and *Dp* chromosomes were quite normal. None of the subsequent crosses described below use the full *T(Y;2)B10-4* rearrangement, but employ only the *Dp(2;Y)B10-4* chromosome. Consequently, they are similar in genetic structure to line 5 of Table 1, and for purposes of further analysis, data from this cross alone are used to measure the contribution of *Dp(2;Y)SD* to drive strength.

Table 2 presents the observed *k* values and their appropriately transformed *z*, *R* and *M* values, when *Dp(2;Y)SD* is combined with other *SD* elements. All genotypes tested include a standard *X*, second (= *cn bw*), and third chromosome, but vary as to whether *Dp(2;Y)SD* or a normal *Y*, *SD* or *CyO*, and *TM6* or another normal third, are present as the respective homologues. This allows for testing a number of male genotypes that include at least one *Sd* copy. Since there are six possible three-way combinations for each standard *SD* second chromosome (*ROM* and *R-1*) satisfying this requirement, this gives a total of ten tests (two of which are common to both sets). The last two columns of the figure give the net effect on drive strength, measured on the *M* scale, for both the *Dp(2;Y)SD* and *TM6*. The four independent estimates of ΔM_D all agree remarkably well, suggesting that the effect of adding an extra copy of *Sd* and *E(SD)* is approximately $\Delta M_D = 1.10$. Conversely, when we make the same comparisons on the *k* or *z* scales, we see no such consistent effect for the duplication. Four of the five observed ΔM_T values are similarly homogeneous, but an apparent outlier value is obtained from the comparison of lines i and j, where $\Delta M_T = 2.90$. This value is less reliable, however, because even a small measurement error for the very low *k* value of line j may result in large measurement errors for ΔM_T, as evident in the relationship shown in Figure 1b, lower left. In fact, if the actual drive strength were only as high as $k = 0.53$, *M* rises to 3.78 and ΔM_T drops to -1.62, within the range of the other ΔM_T measurements. The best estimate of the effect of the *TM6* suppressor, therefore, ranges from $\Delta M_T = -1.46$, excluding the outlier value, to $\Delta M_T = -1.79$, including it.

DISCUSSION

We have seen that, with one possible exception, the enhancing effects of a duplicate *Sd E(SD)* complex and the suppressing effects of *TM6* are consistent with their acting additively on the *M* scale. Moreover, these effects are quite uniform over a wide range of background drive strengths ($0.570 < k < 0.984$ in Table 2) and are independent of the *SD* chromosomes used in combination with *Dp(2;Y)SD*. This last point may be important, since HARTL (1973) suggested the possibility of complementation among *Sd* alleles of different geographic origin. Here, we see no qualitative differences in behavior for *ROM* + *ROM* and *ROM* + *R-1* combinations.

TABLE 2

The effect of various doses of *SD* elements on the strength of segregation distortion

	Paternal chromosomes			Progeny (females)			Drive strength				
	Dp	*Rsp*^{i}	*TM6*	*Rsp*^{i}	*Rsp*^{s}	*k*	*z*	*R*	*M*	ΔM_D	$-\Delta M_T$
a	+	*R-1*	−	1529	25	0.984 ± 0.004	0.984	0.016	7.14	1.04	1.25
b	−	*R-1*	−	6723	910	0.881 ± 0.010	0.859	0.136	6.10		
c	+	*R-1*	+	984	182	0.844 ± 0.026	0.815	0.185	5.89	1.16	1.37
d	−	*R-1*	+	3358	2034	0.623 ±0.010	0.394	0.606	4.73		
e	+	*ROM*	−	728	16	0.978 ± 0.008	0.978	0.022	7.01	1.17	1.69
f	−	*ROM*	−	3386	683	0.832 ± 0.013	0.798	0.202	5.84		
g	+	*ROM*	+	1339	503	0.727 ± 0.017	0.624	0.376	5.32	1.00	1.53
h	−	*ROM*	+	1826	1371	0.570 ± 0.014	0.248	0.752	4.32		
i	+	*CyO*	−	895	309	0.788[a] ± 0.021	0.654	0.346	5.40		2.90
j	+	*CyO*	+	237	342	0.469[a] ± 0.022	0[a]	1.000	2.50		

The genotype of each line (a–j) is described in terms of whether *Dp(2;Y)SD* and *TM6* are present (+) or absent (−), and the type of *SD* or *Rsp*^{i} second chromosome present. In all cases the homologous second chromosome is *cn bw*, the standard *Rsp*^{s}. Strength of drive is measured by the statistics *k*, *z*, *R* and *M*, as defined in MATERIALS AND METHODS. The effects of the *Dp* in enhancing, and of *TM6* in suppressing, drive are measured on the *M* scale by the ΔM values, calculated between appropriate lines and shown in the last two columns.

[a] Assuming *k* has a lower limit of 0.5.

Models that envision the effect of *SD* on spermatogenesis to be indirect (*e.g.*, causing a qualitative alteration of a regulatory process acting on *Rsp*) clearly would not predict that all elements in the *SD* system, particularly multiple copies of *Sd* itself, should show additivity or multiplicativity of action. However, models that assume that *SD* acts directly at *Rsp* to cause subsequent sperm dysfunction are quite compatible with such simple quantitative relationships. Therefore, among the models of *SD* action that do not depend on chromosome pairing, the data presented here tend to exclude the HARTL (1973) model (at least in its current form). Consequently, our further analysis will be confined to a model of *SD* action that takes the form of those of GANETZKY (1977) or HIRAIZUMI, MARTIN and ECKSTRAND (1980) in viewing *Sd* product as acting as Rsp^s to directly cause dysfunction. The data presented here do not speak to the differences between these two particular models, since with the possible exception of i and j, all lines described in Table 2 are genotypically identical for the *2R* base, where HIRAIZUMI *et al.* place their putative *M(SD)* locus. Without genotypic differences at *M(SD)*, the two models make identical predictions for *SD* behavior. However, it is possible to use the data to provide information both on the parameters of *SD* activity and the nature of the quantitative interaction among the various elements of segregation distortion under either model.

Accepting the idea that *Sd* acts to cause Rsp^s dysfunction, there are at least three possible models of quantitative interaction for variable doses of *Sd E(SD)*, *Rsp* and drive suppressors, such as *TM6*. (For this analysis, it is assumed that *E(SD)* is an integral part of the *SD* system, rather than simply another quantitative modifier.) The three models are discussed below, and the results expected under each are summarized in Table 3.

A. Each *Sd* acts at *Rsp* independently (*i.e.*, multiplicatively), and *TM6* suppresses the expression of each *Sd* individually: In this case, the survival of Rsp^s in the presence of both *SD* and *Dp(2;Y)SD* is expected to be equal to the product of the survival probabilities against each *SD* alone. For example, the value of R_g for *Dp(2;Y)Rsp;SD Roma/cn bw; TM6/+* (line g) is expected to be the product of the *R* values for *SD Roma/cn bw; TM6/+* (line h) and *Dp(2;Y)SD; S^2 CyO cn bw/cn bw; TM6/+* (line j); although in practice, the *R* values for a group of lines are estimated by an iterative maximum likelihood procedure. Thus, $\hat{R}_h$, $\hat{R}_j$ and $\hat{R}_g$ are obtained simultaneously from the six progeny classes of lines h, g and j recorded in Table 2. These can be used to generate expected values of *k* for the six progeny classes, and their goodness-of-fit can be measured by the calculation of a χ^2 statistic that has 1 degree of freedom (there are three lines each with one independent observation, but two parameters are estimated from these same data). The appropriate χ^2 values to test the fit for each of the four lines (a, c, e and g) carrying multiple doses of *SD* are listed to the right in Table 3. It is clear that model A gives a very poor fit to the data, with the observed recoveries of Rsp^s-bearing sperm from males with two *SD*s consistently much lower than expected; that is, two *SD* complements cause more sperm dysfunction than expected under this purely multiplicative model.

TABLE 3

Testing the fit for three models of element interaction

	MLEs obtained under model			Goodness-of-fit of model		
Observed (Table 2)	A	B	C	A	B	C
k_a 0.984 ± 0.004	0.963	0.970	0.988	$\chi^2_1 = 25.8^{***}$	$\chi^2_2 = 83.3^{***}$	$\chi^2_1 = 2.2$
k_c 0.844 ± 0.026	0.742	0.764	0.819	$\chi^2_1 = 176.0^{***}$		$\chi^2_1 = 4.9^{*}$
k_e 0.978 ± 0.008	0.944	0.952	0.969	$\chi^2_1 = 21.3^{***}$	$\chi^2_2 = 36.5^{***}$	$\chi^2_1 = 2.3$
k_g 0.727 ± 0.017	0.674	0.688	0.760	$\chi^2_1 = 100.7^{***}$		$\chi^2_1 = 11.9^{**}$
k_b 0.881 ± 0.010	0.885	0.890				
k_d 0.623 ± 0.010	0.645					
k_f 0.832 ± 0.013	0.839	0.845				
k_h 0.571 ± 0.014	0.600					
k_i 0.743 ± 0.021	0.765, 0.771[a]	0.786, 0.801[a]				
k_j 0.409 ± 0.022	0.613, 0.579[a]					

Comparison of observed values of meiotic drive with maximum likelihood estimates obtained under three models of element interaction. Model A assumes that *SD* chromosomes interact multiplicatively and that suppressors act on each *Sd* independently. Model B also assumes that *SD*s interact multiplicatively but that suppressors act to protect *Rsp*^{s} alleles from dysfunction. Model C assumes that all elements interact additively on the *M* scale.

[a] Two independently derived MLEs. * Significant at the $P = 0.05$ level; ** Significant at the $P = 0.001$ level; *** Significant at the $P \ll 0.001$ level.

B. Each *Sd* acts at *Rsp*^{s} independently, while *TM6* protects *Rsp*^{s}: Here, the probability of at least one *Sd* product binding to *Rsp*^{s} to cause dysfunction can be thought of as being first assessed by multiplying individual R values as in model A, above. The effect of the *TM6* suppressor would then be to additvely shift the distribution of *Rsp*^{s} liabilities (or, alternatively, change the threshold of dysfunction, S), resulting in a reduction in the probability of dysfunction. For the same example (line g) used for model A, we would first multiply R_f and R_j in order to calculate the effects of the two *SD* complexes, find the transformation of their product on the M scale, modify this value by the value $\Delta M_T = -1.53$ measured independently from line h, then retransform the result to generate the final R_g value under model B (in terms of earlier notation, $R_g = f^{-1}\,(f(R_f \times R_j) - M_T)$. In this case, the maximum likelihood estimates are obtained from simultaneous iteration over the eight progeny classes obtained from line i and three of the four lines involving *ROM* (f, g and e). Finally, these are used to calculate $\hat{k}$ values that yield a goodness-of-fit χ^2 statistic with 2 degrees of freedom. Note that this statistic is roughly analogous to the sum of the χ^2 values obtained for lines e and g under model A. Again, it is clear from Table 3 that this mixed multiplicative-additive model also fails to explain the observed data, although it comes closer than did model A.

C. The effects of all *SD* elements are additive: For this case, we obtained the maximum likelihood estimate of drive strength for a particular line by simply adding the best independent estimates of ΔM_T and ΔM_D, where appropriate, to the observed M value of the standard *SD*. For the example of line g, R_g is computed by retransforming the value obtained when M_f (for the *SD Roma* standard) is modified by $\Delta M_D = 1.17$ and $\Delta M_T = -1.53$, the most direct independent estimates for the additive effects of *Dp(2;Y)SD* and *TM6*. (It should be noted that, for both models B and C, I choose to employ these values, rather than the mean values of $\Delta M_D = 1.10$ and $\Delta M_T = -1.46$, both because the means combine data from two different *SD* lines (however similar) and because they are not independent of the data sets being tested. In any case, the use of these mean values does not alter the qualitative conclusions of the goodness-of-fit tests.) The χ^2 statistics demonstrate a much better fit to this model of the observed data, with only one of the four comparisons giving a clearly significant value for the test statistic. Even in this case, the MLE value of $\hat{k}_g = 0.760$ is not very far from the observed value of 0.727 ± 0.017. It is worth noting that, with large data sets, significant statistical deviations of this magnitude may arise from even very small biological differences. In general, however, the *R-1* lines (a and c) give a slightly better fit than is obtained from *ROM* (lines e and g).

The results of Table 3, combined with the homogeneity of ΔM_D and ΔM_T values observed in Table 2, produce a compelling argument for simple additive action of *SD* elements. This raises the intriguing question as to whether *Sd* might be just another strong additive element, similar in kind to *E(SD)* and the suppressor borne by *TM6*. The observation by HARTL (1980) that some *SD* chromosomes may carry multiple copies of *Sd* offers support for this possibility. We cannot address this question directly here, since in this analysis *Sd*

and *E(SD)* are confounded in their effects. However, there is good evidence that deleting *Sd* alone is enough to eliminate segregation distortion (BRITTNACHER and GANETZKY 1984). This argues that *Sd* has a qualitative, not quantitative, effect on meiotic drive, although a quantitative enhancer of sufficient strength might mimic this result.

One alternative is that *Sd* is merely the trigger for segregation distortion, the strength being determined by the combination of modifier alleles present. In this case, the effect of the *Dp(2;Y)SD* chromosome in enhancing *ROM* or *R-1* activity may be a consequence of its *E(SD)* allele alone. Apparently, it will ultimately be necessary to construct genotypes lacking *Sd*, but with several copies of *E(SD)*, in order to resolve whether *Sd* is a necessary prerequisite for segregation distortion or is simply another very strong modifier.

A companion study by LYTTLE, BRITTNACHER and GANETZKY (1986) demonstrates that multiple Rsp^s copies are effectively independent in their susceptibility to *SD*-mediated sperm dysfunction, in contrast to the additive action of multiple *SD* copies demonstrated here. This serves to emphasize the possible qualitative differences between the *Rsp* locus and other elements of the *SD* system. The difference could be critical to our understanding of segregation distortion. For although it seems clear that modifiers of *SD* are behaving additively, it is not yet possible to determine whether their action is to modulate *Sd* expression (equivalent to moving the threshold of dysfunction, *S*, in Figure 1a) or to modify Rsp^s to change its susceptibility to *Sd* (equivalent to moving the distribution of liabilities with respect to *S*).

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