SUPPRESSOR SYSTEMS OF SEGREGATION DISTORTER (*SD*) CHROMOSOMES IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

YUICHIRO HIRAIZUMI AND ANITA M. THOMAS

Department of Zoology, The University of Texas at Austin, Austin, Texas 78712

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ABSTRACT

Several natural populations of *D. melanogaster* were investigated for the presence (or absence) of the Segregation Distorter (*SD*) chromosomes and their suppressor systems. The *SD* chromosomes were found, at frequencies of a few percent, in two independent samples taken in different years from a Raleigh, North Carolina, population, whereas no *SD* chromosomes were found in samples collected from several populations in Texas. The populations in these localities were found to contain suppressor *X* chromosomes in high frequencies (75% or higher). They also contained relatively low frequencies of partial suppressor or insensitive second chromosomes of varying degrees, but completely insensitive second chromosomes were practically absent in all populations examined. The frequencies of suppressor *X* chromosomes, as well as those of the partially insensitive or suppressor second chromosomes, were the same among the populations investigated. This suggests the possibility that the development of a suppressor system of *SD* in a population could be independent of the presence of an *SD* chromosome. Segregation distortion appeared to be occurring in natural genetic backgrounds, but the degree of distortion varied among males of different genotypes. There were many instances in which the *SD* chromosomes showed transmission frequencies from their heterozygous male parents that were smaller than 0.6 and, in several cases, even smaller than 0.5. The presence of a recessive suppressor, or suppressors, of *SD* in natural populations was suggested.

THE Segregation Distorter chromosome (*SD*) was first discovered in a natural population of *D. melanogaster* in Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959). Subseqent surveys have revealed that this chromosome is spread worldwide among natural populations of this species, although its frequency within each population is rather low, at a level of only a few percent. When males heterozygous for the *SD* chromosome are crossed to normal, SD^+/SD^+, females, usually 90% or more of the progeny recovered are found to contain the *SD* chromosome. Transmission frequency from the *SD* heterozygous female is normal. The distorted transmission ratio is due to dysfunction of the sperm bearing the SD^+ chromosome (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DE MARCO 1967; TOKUYASU, PEACOCK and HARDY 1972). A model of the molecular basis of segregation distortion

was originally proposed by HARTL (1973), modified later by GANETZKY (1977) and further modified by HIRAIZUMI, MARTIN and ECKSTRAND (1980). The *SD* system consists of at least two major elemens on the second autosome, *Sd* and *Rsp*. The product of *Sd* interacts with the *Rsp* locus to cause sperm dysfunction. *Rsp* is a multiple allelic locus such that a chromosome with a sensitive Rsp^s allele is distorted, but a chromosome with a completely insensitive Rsp^i allele is not distorted by *SD*. Distortion is also reduced by the presence of suppressors.

Population surveys have indicated that natural populations contain suppressors of *SD* in high frequencies (HIRAIZUMI, SANDLER and CROW 1960). Some of the suppressors were found to be *X*-linked, whereas others were associated with the autosomes (KATAOKA 1967; HARTL 1970; HARTL and HARTUNG 1975).

The purpose of the present study was to investigate the frequencies of the *SD* chromosomes and some of their suppressor systems, as well as the Rsp^i alleles, in natural populations of *D. melanogaster* in Texas and in some other natural populations of this species.

MATERIALS

Strains and chromosome lines of *D. melanogaster* used in the present study are listed below.

cn bw: a standard laboratory strain in which the second chromosome is marked with two recessive mutants, *cn* (cinnabar eyes, *2R*-57.5) and *bw* (brown eyes, *2R*-104.5). The *cn bw* phenotypes is white-eyed. This is a standard strain that has been used in our laboratory for various studies of segregation distortion.

In(2LR) Cy: a second chromosome balancer that carries a dominant marker *Cy* (Curly wing, *2L*-6.1) and an inversion in both left and right arms. This chromosome has been kept in this laboratory by repeated backcrosses to the standard *cn bw* females, and will be designated as *Cy*.

In(1)FM7, y^{31d}, w^a *B v*; *cn bw*: a strain containing a balancer *X* chromosome that carried a dominant marker *B* (Bar eyes, *1*-57.0) and several recessive markers. The autosomes are from the standard *cn bw* strain. This strain will be designated as *FM7*.

C(1)DX, In(1)dl-49—In(1)sc^8, *y f*; *cn bw*: a strain containing two *X* chromosomes attached in a tandem acrocentric configuration. Females in this strain carry a *Y* chromosome. The autosomes are from the standard *cn bw* strain. This will be referred to as the attached-*X* strain.

R(SD)cn-14: a second chromosome line having the genotype *Sd* Rsp^i. This chromosome carries the recessive marker *cn* and shows a moderate degree of segregation distortion when heterozygous with the *cn bw* chromosome in the male. This will be used as a "tester *SD*" throughout the present study and is abbreviated as *cn-14*. So far, no structural abnormality has been found in this chromosome. This chromosome has been kept in this laboratory by repeated backcrosses to the standard *cn bw* females.

Isofemale lines established from wild-inseminated females: *IC-i*, captured at Ithaca, New York, 1982; *RL(A)-i*, Raleigh, North Carolina, 1980; *RL(B)-i*, Raleigh, North Carolina, 1982; *BiT(B)-i*, Brownsville, Texas, 1980.

X and/or second chromosome lines isolated from wild males: *BiT(A)-i*, captured at Brownsville, Texas, 1970; *ST-i*, San Benito, Texas, 1975; *HAT-i*, Hiraizumi's backyard at Austin, Texas, 1978.

METHODS

A standard cornmeal-agar medium was used for all experiments at a room temperature of 23–24°. Mating schemes for four major sets of experiments are as follows.

Detection of the SD *chromosome in natural populations*: Males captured from the *HAT*, *BiT(A)* and the *ST* populations were individually crossed to two *cn bw* females. A single F_1 male from each

mating was backcrossed to two *cn bw* females to obtain several F_2 males heterozygous for *cn bw* and the wild second chromosomes. These F_2 males, five or more for each F_1 male, were then crossed individually to two *cn bw* females for F_3 progeny counts to determine whether the wild second chromosome in each of the F_1 males was *SD* or non-*SD* (*i.e.*, SD^+). For the isofemale lines from various localities, seven to ten males, sometimes more, were taken from each line, and each of them was examined as described before to determine whether *SD* chromosomes were present in any of the isofemale lines. The transmission frequency of a certain second chromosome from its heterozygous male parent is referred to as its *k* value.

Determination of sensitivities of the wild second chromosomes to SD *activity*: Some of the second chromosomes isolated from the *HAT* population and examined in *Detection of the* SD *chromosome in natural populations*, were made heterozygous with the *cn-14* chromosome in males. These were individually crossed to two *cn bw* females to examine the sensitivities of the *HAT* wild chromosomes to the *SD* activity of *cn-14*. For the isofemale lines, a single male from each line was crossed to two *cn bw* females. A single F_1 male from this mating was crossed to *cn-14/Cy* females to obtain *cn-14*/wild F_2 males. These males were then crossed individually to two *cn bw* females for F_3 progeny counts to examine the sensitivities of the wild second chromosomes. As a control, *cn-14/Cy* females were crossed to the standard *cn bw* males to obtain *cn-14/cn bw* progeny males. The reciprocal of this parental mating was also made, *i.e.*, *cn-14/Cy* males were crossed to the *cn bw* females to obtain *cn-14/cn bw* progeny males. These males were then crossed individually to two *cn bw* females to estimate the control *k* values for the *cn-14* chromosomes.

Presence of suppressor X *chromosomes in natural applications*: Some of the males captured in the *HAT* population were crossed individually to attached-*X*; *cn bw* females, and the F_1 males from each mating were backcrossed to attached-*X*; *cn bw* females. Backcross matings were repeated for at least five generations. Progeny males of the genotype, *X*(wild)/*Y*; *cn bw*, in which *X*(wild) is the *X* chromosome from the wild population, were then crossed to *FM7*; *cn bw* females to obtain *FM7*/*X*(wild); *cn bw* female progeny, which were then crossed to *cn-14/Cy* males. This cross produced *X*(wild)/*Y*; *cn-14/cn bw* progeny males, which were individually crossed to two *cn bw* females for progeny counts to estimate *k* values for the *cn-14* chromosomes. For the isofemale lines, a single male was taken from each of the lines to sample independent *X* chromosomes, and the males were treated following the same procedure as described earlier. As a control, males taken from the standard *cn bw* strain were subjected to the same mating procedures.

Transmission frequencies of the SD *chromosomes in a natural genetic background*: As shown in Table 1, four isofemale lines, namely, *RL(A)-4*, *RL(B)-32*, *RL(B)-38* and *RL(B)-72*, were found to contain

TABLE 1

Presence or absence of the SD *chromosomes in natural populations of* D. melanogaster

Populations	Year of collection	No. of chromosomes (isofemale lines) examined	No. of *SD* chromosomes (*SD*-containing isofemale lines)
Ithaca, New York	1982	(10)	(0)
Raleigh (A), North Carolina	1980	(13)	(1)
Raleigh (B), North Carolina	1982	(85)	(3)
Brownsville (A), Texas	1970	195	0
Brownsville (B), Texas	1980	(18)	(0)
San Benito, Texas	1975	88	0
Austin, Texas	1978	556	0
Harlingen, Texas	1970	154[a]	0
Brownsville, Texas	1979	(28)[b]	(0)

The figures shown in parentheses indicate the numbers of isofemale lines examined, and the figures without parentheses indicate the numbers of independent, wild-caught male-derived second chromosomes examined.

[a] Data reported by HIRAIZUMI (1971).

[b] Data reported by HIRAIZUMI and GERSTENBERG (1981).

SD chromosomes. Males and females from the *RL(A)-4* line were crossed individually to *cn bw* mates of the opposite sex. A group of 7–38 F_1 males was selected from each mating and individually crossed to two *cn bw* females. A single, heterozygous F_2 male was chosen from each mating and crossed to two *cn bw* females for F_3 progeny counts to examine the *k* value for the wild chromosome.

Also for this purpose, another set of experiments was conducted in a somewhat different way. Males from each of the four *SD*-containing isofemale lines were crossed to females from either the *RL(A)* or *RL(B)* isofemale lines that did not contain the *SD* chromosomes. F_1 males from each mating were then crossed individually to two *cn bw* females, and F_2 males from each of these matings were crossed individually to two *cn bw* females for F_3 progeny counts in order to determine whether the wild second chromosome in each of the F_2 males was an *SD* or SD^+ chromosome.

Males used in the experimental matings were in the age range of 1–3 days. The parental flies were allowed to produce progeny for 3 days in a culture vial and were then discarded. Progeny counts were made on the 15th or 16th day after the mating was initiated for the first count and again on the 18th or 19th day for the second count. Then, the culture vials were discarded. By this time, the progeny had eclosed exhaustively, without the possibility of inclusion of any progeny of the next generation.

RESULTS

Distribution of the SD *chromosomes among natural populations*: The presence of *SD* chromosomes in natural populations of *D. melanogaster* surveyed in this study, as well as those in Texas populations examined in the past, are summarized in Table 1. One striking observation is that the *SD* chromosome is totally absent among Texas populations. The number of independent second chromosomes tested was more than 1000, and the results can hardly be due to sampling chance. Not much can be said for the Ithaca population, since the number of isofemale lines examined was so small, but the Raleigh population, in both collections, certainly contained *SD* chromosomes. Since each *RL(A)* isofemale line had been kept in the laboratory for more than 1 yr before the tests were made, it is rather difficult to estimate accurately the frequency of *SD* chromsomes in their natural habitat from this collection. The second collection from this population, *RL(B)*, however, should reflect the frequency of *SD* in nature fairly well, since the tests were made within five generations after the isofemale lines were established. If it is assumed that each of the isofemale lines started with four second chromosomes, the minimum estimate of the *SD* chromosomes in nature might be about 2% for *RL(A)* and about 1% for *RL(B)*. Whatever is the correct frequency of *SD* chromosomes in the Raleigh population, the important point is that this population did, in fact, contain the *SD* chromosomes in a frequency of a few percent, which is comparable to those observed in other natural populations of this species; for example, it was about 3% in Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959), and about 1% in Odate, Japan (HIRAIZUMI and NAKAZIMA 1965). Thus, we now have two population groups of which one group (Raleigh) contains *SD* chromosomes, and the other group (Texas) does not contain *SD* chromosomes.

Sensitivity of the wild second chromosomes to SD *activity*: Sensitivity of the wild second chromosome was measured by calculating the *k* value for the *cn-14* chromosomes recovered among the F_3 progeny from the matings as described in METHODS. When the wild chromosome is completely insensitive (which is the case when the wild chromosome carries a completely insensitive Rsp^i allele),

k is 0.5, whereas k is 1.0 when the wild chromosome is completely sensitive. Results are shown in Table 2. It can be clearly seen that the four populations showed nearly the same k value distributions and that none of them appeared to contain completely insensitive Rsp^i alleles, at least not in high frequencies. HARTL and HARTUNG (1975) found that the Raleigh, North Carolina, population that they studied contained a high frequency of insensitive second chromosomes; 36 of 75 chromosomes caused the k value for their tester *SD* chromosome (*R-1*) to be smaller than 0.6, and a large fraction of them, indeed, appeared to carry the Rsp^i alleles. Since their tester *SD* chromosome was different from ours (*cn-14*), it is desirable to select an appropriate cutoff point of k for the *cn-14* chromosome, which is comparable to their $k = 0.60$. Let d be the proportion that the SD^+ chromosome is distorted in an SD/SD^+ male. The d value corresponding to $k = 0.60$ is 0.33. Since no k value for the standard *R-1/ cn bw* male was given in their study, we asume that it was 0.85 (HARTL 1977a). This k value corresponds to $d = 0.824$. Thus, the decrease in d, Δd, corresponding to the decrease in k from 0.85 to 0.60, is 0.491. Based on this Δd value and an average k of 0.95 for the standard *cn-14/cn bw* males, the comparable cutoff point of k for this study is calculated to be 0.65. Less than 10% of the wild second chromosomes from the Raleigh population fell into the k range smaller than this cutoff point. The present results certainly disagree with the observation reported by HARTL and HARTUNG. Our results are, in fact, in good agreement with those obtained for the Japanese population (KATAOKA 1967).

TABLE 2

The k *value distributions for the* cn-14*/wild males for 4 natural populations of* D. melanogaster

	Populations				
k Range	Ithaca	Raleigh (A)	Raleigh (B)	Austin	Brownsville (B)
0.500–0.549					1 (5.6)
0.550–0.599			2 (3.0)	3 (5.5)	0 (0.0)
0.600–0.649		1 (7.7)	2 (3.0)	5 (9.1)	1 (5.6)
0.650–0.699	1 (10.0)	0 (0.0)	3 (4.5)	5 (9.1)	0 (0.0)
0.700–0.749	0 (0.0)	1 (7.7)	5 (7.5)	1 (1.8)	0 (0.0)
0.750–0.799	0 (0.0)	2 (15.4)	4 (6.0)	2 (3.6)	1 (5.6)
0.800–0.849	0 (0.0)	0 (0.0)	7 (10.4)	1 (1.8)	0 (0.0)
0.850–0.899	1 (10.0)	0 (0.0)	2 (3.0)	4 (7.3)	1 (5.6)
0.900–0.949	3 (30.0)	2 (15.4)	4 (6.0)	7 (12.7)	1 (5.6)
0.950–1.000	5 (50.0)	7 (53.8)	38 (56.7)	27 (49.1)	13 (72.2)
Total	10	13	67	55	18
Average k	0.924	0.896	0.895	0.868	0.914

Control average k for the *cn-14 bw* males:
cn-14 from maternal parents; $k = 0.946 \pm 0.005$; no. of replications = 119.
cn-14 from paternal parents; $k = 0.966 \pm 0.005$; no. of replications = 104.

The k value was calculated for the *cn-14* chromosome. The figures in this table indicate the number of independent second wild chromosome lines falling in each k range, and the figures in parentheses are percentages.

It should be pointed out here that the *SD* chromosomes were found in the Raleigh population but not in the Texas populations; yet, the distribution of sensitivities of the wild second chromosomes remained the same between these two localities.

Suppressor-X chromosomes in natural populations: Males of the genotype, *X*(wild)/*Y*; *cn-14/cn bw*, were individually crossed to two *cn bw* females for progeny counts to estimate *k* values for the *cn-14* chromosomes. As a control, the *k* values were also examined for the *X(cn bw)/Y*; *cn-14/cn bw* males, in which *X(cn bw)* is the *X* chromosome from the standard *cn bw* strain. Results are shown in Table 3. As mentioned earlier, both Austin and Brownsville populations were free of the *SD* chromosomes, yet they contained rather high frequencies of *X* chromosomes which strongly, although not completely, suppress the *SD* activity of the *cn-14* chromosome. Only a small number of the wild *X* chromosomes were tested from the Raleigh population, but many of them showed strong suppression of *SD* activity. It should be noted, at this point, that the frequency of suppressor-*X* chromosomes in the Odate, Japan, population (where *SD* chromosomes were found) was 63–64% in 1963 and 84–85% in 1964, when 0.75 was used as a cutoff *k* value for suppression (KATAOKA 1967). Since KATAOKA, like HARTL and HARTUNG, used *R-1* as a tester *SD* chromosome, we calculated, in a similar way to that shown in the previous section, an appropriate cutoff point of *k* for our *cn-14* comparable to her cutoff point of $k = 0.75$; it was computed to be 0.81. Based on this cutoff point, the frequency of suppressor-*X* chromosomes was found to be 0.78 (46/

TABLE 3

The k *value distributions for the* X*(wild)*/Y; cn-14/cn bw *males for three natural populations of* D. melanogaster

k Range	Populations		
	Raleigh (A)	Austin	Brownsville (B)
0.500–0.549	1 (5.9)	4 (6.8)	1 (5.6)
0.550–0.599	6 (35.3)	9 (15.3)	3 (16.7)
0.600–0.649	2 (11.8)	13 (22.0)	6 (33.3)
0.650–0.699	3 (17.6)	11 (18.6)	2 (11.1)
0.700–0.749	0 (0.0)	5 (8.5)	2 (11.1)
0.750–0.799	1 (5.9)	3 (5.1)	1 (5.6)
0.800–0.849	0 (0.0)	4 (6.8)	0 (0.0)
0.850–0.899	0 (0.0)	4 (6.8)	1 (5.6)
0.900–0.949	1 (5.9)	4 (6.8)	1 (5.6)
0.950–1.000	3 (17.6)	2 (3.4)	1 (5.6)
Total	17	59	18
Average *k*	0.698	0.699	0.681

Control average *k* for the *X(cn bw)/Y*; *cn-14/cn bw* males:
$k = 0.956 \pm 0.004$; no. of replications = 80.

The *k* value was calculated for the *cn-14* chromosome. The figures in this table indicate the number of independent, wild *X* chromosome lines falling in each *k* range, and the figures in parentheses are percentages.

59) in the Austin, 0.83 (15/18) in the Brownsville and 0.76 (13/17) in the Raleigh population. Thus, the Odate, Japan, Texas and North Carolina populations contained suppressor-*X* chromosomes in comparable frequencies. In other words, natural populations of *D. melanogaster* contain suppressor-*X* chromosomes of *SD* in nearly the same frequencies, regardless of whether or not they contain *SD* chromosomes.

Transmission frequencies of the SD *chromosomes in natural populations*: The four Raleigh isofemale lines found to contain *SD* chromosomes will provide us with a good opportunity to estimate the *k* values of *SD* chromosomes in natural genetic backgrounds. The standard, average *k* value for the *SD* chromosomes in each line was estimated from the *cn bw* ♀ × *RL(A* or *B)-i/cn bw* ♂ matings. About 50 replications were made for each line, and the average *k* was found to be 0.998 ± 0.001, 0.997 ± 0.001, 0.994 ± 0.022 and 0.967 ± 0.006 for the *RL(A)-4*, *RL(B)-32*, *RL(B)-38* and *RL(B)-72* line, respectively. The first set of experiments was conducted by choosing 25 males and 18 females from the *SD*-containing isofemale line, *RL(A)-4*, and by following the procedures described in METHODS. Results are summarized in Table 4. Of the 25 parental

TABLE 4

The number of SD *and* SD^+ *chromosomes recovered among progeny of males and females taken from a* SD-*containing isofemale line,* RL(A)-4

Individual no.	From female parents				From male parents			
	SD	SD^+	Total	*k* for *SD*	*SD*	SD^+	Total	*k* for *SD*
1	9	10	19	0.474	13	12	25	0.520
2	7	12	19	0.368	19	0	19	1.000
3	0	30	30		29	1	30	0.967
4	8	13	21	0.381	0	18	18	
5	10	5	15	0.667	16	3	19	0.842
6	7	8	15	0.467	0	10	10	
7	7	16	23	0.304	7	1	8	0.875
8	18	17	35	0.514	10	10	20	0.500
9	5	12	17	0.294	7	0	7	1.000
10	8	12	20	0.400	9	3	12	0.750
11	0	29	29		18	0	18	1.000
12	0	32	32		13	1	14	0.929
13	10	12	22	0.455	0	22	22	
14	13	9	22	0.591	15	8	23	0.652
15	19	20	39	0.487	16	6	22	0.727
16	9	8	17	0.529	2	9	11	0.182
17	0	23	23		10	14	24	0.417
18	13	16	29	0.448	29	9	38	0.763
19					0	24	24	
20					7	11	18	0.389
21					15	3	18	0.833
22					22	0	22	1.000
23					20	1	21	0.952
24					15	6	21	0.714
25					18	4	22	0.818
Mean *k* for *SD*				0.456				0.754

males tested, four appeared to be of the SD^+/SD^+ genotype, and the remaining 21 were either of the SD/SD^+ or SD/SD genotypes. Among 18 parental females examined, four were found to be of the SD^+/SD^+ genotype, whereas the remaining 14 were all SD/SD^+ heterozygotes. There were no SD/SD homozygotes parental females. If it is considered that there is such a high frequency of *SD* chromosomes in this isofemale line, a considerable fraction of individual flies in this line should be *SD* homozygotes. Since all of the parental males and females taken from this line were fertile, the absence of *SD* homozygotes among female samples suggests that the *SD* chromosomes were homozygous lethal. At this point we shall assume that the *SD*-bearing parental males were all *SD* heterozygotes. The frequencies of *SD* progeny from *SD* heterozygous female parents varied somewhat, but they were statistically homogeneous ($\chi^2_{13} = 10.50$, $0.75 > P > 0.50$), and the overall *SD* to SD^+ ratio did not deviate from the theoretical 1:1 ($\chi^2_1 = 2.33$, $0.25 > P > 0.10$). The transmission frequency of *SD* chromosomes from heterozygous male parents, on the other hand, varied significantly among different parental males; *k* values ranged from 0.18 to 1.00 and gave an overall unweighted average k value of 0.754. At this point, one may wonder how strong is the resolving power of distinguishing the *SD* from the SD^+ chromosomes under the present experimental scheme. The *k* value for each tested F_2 male entered in Table 4, together with those presented in Table 6, which will be described later, were tabulated in Table 5. The *k* value distribution is clearly bimodal, and the separation into two groups, one distorting and the other not distorting, could be made relatively easily. In this study, the cutoff point was 0.8; if the *k* value was 0.8 or higher, the chromosome was judged as distorting and, if smaller than 0.8, nondistorting. There were a few cases of *k* between 0.7 and 0.8, which may be the lower tail of the distorting *k* distribution but were misclassified into the SD^+ class. However, the number of such cases was so small that any such misclassification, if any, would be negligible.

In the experiments described in Table 4, the possibility that some of the parental *SD* males were, in fact, *SD* homozygotes was not completely ruled out, although it appears to be rather unlikely. In order to eliminate this possibility and to obtain additional data, the second set of experiments was conducted as follows. Males from each of the four *SD*-containing isofemale lines were crossed to females from other lines without *SD* chromosomes, and the males from each mating were then subjected to the procedures employed in the first set of experiments. Results are shown in Table 6. The average *k* values for the *SD* chromosomes appeared to be heterogeneous among the four combinations; the *RL(B)-24* and *RL(B)-72* combination showed the average *k* distinctly higher than those in the other three combinations, suggesting that either the *SD* chromosomes in the *RL(B)-72* isofemale line were, under a natural genetic background, much stronger distorters than those in the other isofemale lines, or that this combination did not contain any suppressors of *SD* activity. In any event, it is clear that the *SD* chromosomes definitely show segregation distortion in natural populations, although there are suppressor systems of *SD* in the populations that reduce the degree of distortion consid-

TABLE 5

The number of males showing k *values in each interval shown*

k Interval	No. of males observed
1.00	643
0.95–0.99	183
0.90–0.94	78
0.85–0.89	21
0.80–0.84	10
0.75–0.79	2
0.70–0.74	2
0.65–0.69	13
0.60–0.64	32
0.55–0.59	63
0.50–0.54	119
0.45–0.49	166
0.40–0.44	173
0.35–0.39	165
0.30–0.34	105
0.25–0.29	55
0.20–0.24	21
0.15–0.19	12
0.10–0.14	2
0.05–0.09	1
0.00–0.04	2
Total	1868

The *k* values were calculated for the wild second chromosomes, either *SD* or *SD*$^+$, recovered among progeny of the mating, *cn bw* ♀ × wild/*cn bw* ♂. The tested males tabulated in this table were those shown in either Table 4 or Table 6.

TABLE 6

The number of SD *and* SD$^+$ *chromosomes recovered among progeny of the* cn bw ♀ × *wild* ♂ *mating, in which the wild male may be either* SD/SD$^+$ *or* SD$^+$/SD$^+$

Parental mating		Individual				
♀	♂	no.	*SD*	*SD*$^+$	Total	*k* for *SD*
RL(A)-3	*RL(A)-4*	1	25	0	25	1.00
		2	10	8	18	0.566
		3	0	21	21	
		4	0	24	24	
		5	0	21	21	
		6	26	0	26	1.000
		7	0	5	5	
		8	0	19	19	
		9	10	8	18	0.556
		10	0	24	24	
		11	12	0	12	1.000
		12	0	11	11	
Mean *k* for *SD*						0.822

See text for detailed information about the mating scheme.

TABLE 6—Continued

Parental mating		Individual				
♀	♂	no.	*SD*	*SD*$^+$	Total	*k* for *SD*
RL(B)-59	*RL(B)-32*	1	0	23	23	
		2	0	29	29	
		3	15	15	30	0.500
		4	21	1	22	0.955
		5	15	0	15	1.000
		6	0	31	31	
		7	21	13	34	0.618
		8	0	36	36	
		9	19	9	28	0.679
		10	15	1	16	0.938
		11	12	8	20	0.600
		12	15	16	31	0.484
Mean *k* for *SD*						0.722
RL(B)-9	*RL(B)-38*	1	13	2	15	0.867
		2	14	1	15	0.933
		3	0	19	19	
		4	5	12	17	0.294
		5	12	3	15	0.800
		6	0	9	9	
		7	0	13	13	
		8	0	11	11	
		9	17	0	17	1.000
		10	0	15	15	
		11	14	0	14	1.000
		12	5	5	10	0.500
		13	6	3	9	0.667
		14	8	4	12	0.667
Mean *k* for *SD*						0.748
RL(B)-24	RL(B)-72	1	13	0	13	1.000
		2	19	0	19	1.000
		3	0	16	16	
		4	11	0	11	1.000
		5	15	0	15	1.000
		6	14	0	14	1.000
		7	0	33	33	
		8	27	0	27	1.000
		9	23	1	24	0.958
		10	14	2	16	0.875
		11	15	0	15	1.000
		12	21	1	22	0.955
Mean *k* for *SD*						0.979

erably. Indeed, in many cases the *k* values for the *SD* chromosomes were reduced to 0.6 or smaller, in some cases even smaller than 0.5. Since the *k* values for *SD* from female parents were frequently found to be smaller than 0.5 (Table 4), the *k* values smaller than 0.5 for *SD* chromosomes from male parents could well be due to chance fluctuation. A somewhat reduced viability

of *SD* heterozygotes might also be responsible for this. It seems to be true, however, that the *k* values for the "original *SD* chromosomes" in natural populations could be reduced to 0.6 or smaller. This is a rather surprising observation since the original *SD* chromosomes have been found to be somewhat "resistant" to the effects of suppressors, and their activities are not suppressed to the extent found in this study; for example, KATAOKA (1967) reported that the suppressor-*X* chromosomes in a Japanese pouplation reduced the *k* value of a recombinant *SD* chromosome (*R-1*), a relatively weaker distorter, from 0.87 to 0.53, whereas they reduced the *k* value for the original *SD* chromosome (*SD-72*) from 0.98 to only 0.88. We shall come back to this point later.

DISCUSSION

HIRAIZUMI, SANDLER and CROW (1960) reported that a natural population of *D. melanogaster* at Madison, Wisconsin, where the *SD* chromosomes were originally discovered, contained a suppressor system, or systems, of the *SD* activity, and that several southern Japanese populations, although sample sizes were extremely small and therefore findings were not conclusive, did not contain *SD* chromosomes, nor were any suppressors of *SD* activity present. Based upon these observations, together with those of the *SD*-containing population cage experiments, they argued that, when *SD* chromosomes were introduced into a population that was previously free of *SD*, this population tended to develop a suppressing system of *SD* activity to reduce the burden caused by the presence of *SD* in high frequency. KATAOKA (1967) studied a natural population at Odate in northern Japan, where the Japanese *SD* chromosomes were first discovered (HIRAIZUMI and NAKAZIMA 1965), for suppressor systems of the *SD* activity. She found that the Odate population contained a high frequency of suppressors, most of which were associated with the *X* chromosomes. As mentioned earlier, HARTL and HARTUNG (1975) examined a natural population at Raleigh, North Carolina, and they found a high frequency of insensitive second chromosomes, many of which were due to the presence of Rsp^i alleles. They examined a total of 75 male-derived second chromosomes, none of which were *SD*, but they assumed that the *SD* chromosomes could still be present in that population even though they were not recovered, probably because of the small sample size. HARTL also examined an artificial population containing *SD* chromosomes and found that the frequency of insensitive Rsp^i alleles (Sd^+ Rsp^i chromosomes) in that population was as high as 79% (HARTL 1977b). Based upon these observations, they suggested that segregation distortion had a greater influence in determining the genetic structure of populations than had generally been appreciated, and that the presence of *SD* would force a population to accumulate suppressors of *SD*. The present observations raise some doubt about this generally accepted relationship between the presence of *SD* chromosomes and their suppressor system in a natural population. It was clearly shown in this study that the average sensitivity of the second chromosomes and the average suppressing effect of the *X* chromosomes to *SD* activity remained the same among populations with or without *SD* chromosomes. In other words, the presence of a

suppressor of *SD* in natural populations may not be due to the presence of *SD* in that population but, rather, is due to some other, as yet unknown, reason. Alternatively, it may be that the Texas populations had, in fact, contained *SD* until very recently, although at least more than 13 yr ago for the South Texas populations, and the suppressor systems found in Texas represent relics of the presence of *SD* chromosomes in the past. If this were the case, what genetic or environmental factor could cause such a sweep of the *SD* chromosomes from all of the Texas populations? Although such an occurrence seems highly unlikely, it is a possibility that, at this time, must remain open for future studies.

It is difficult to explain why HARTL and HARTUNG found a very high frequency of Rsp^i alleles in the Raleigh population, whereas we found a very much reduced frequency of Rsp^i alleles in the Raleigh population. To make this point clearer, the same *RL(A)-i* second chromosomes included in Table 2 were made heterozygous in males with the *R-1* chromosome, and the *k* values for *R-1* were measured. Results, together with those shown in Table 2, are summarized in Table 7. As might be expected, there was a highly significant, positive correlation in *k* between the two tester *SD* chromosomes ($r = +0.870$, d.f. = 11, $P < 0.01$). The overall average *k* for the *R-1/RL(A)-i* males was smaller than that of the control, *R-1/cn bw* males by 0.070 (= 0.891 − 0.821), whereas the difference between the *cn-14/RL(A)-i* males and the control *cn-14/*

TABLE 7

The k *value distributions for the* R-1/RL(A)-i *males*

Lines	*k* for *R-1/RL(A)-i* ♂	*k* for *cn-14/RL(A)-i* ♂
RL(A)-1	0.571 (7)	0.614 (12)
3	0.849 (7)	0.798 (11)
4	0.563 (7)	0.717 (10)
5	0.876 (7)	0.970 (11)
6	0.830 (4)	0.945 (12)
7	0.800 (6)	0.961 (10)
8	0.951 (7)	0.944 (9)
9	0.669 (7)	0.758 (11)
10	0.850 (7)	0.988 (12)
11	0.960 (7)	0.996 (10)
15	0.953 (7)	0.991 (10)
16	0.846 (7)	1.000 (10)
19	0.959 (7)	0.969 (12)
Average	0.821	0.896
Control average *k* values for the *R-1/cn bw* and the *cn-14/cn bw* males:		
	0.891 (105)	0.955 (223)[a]
	±0.007	±0.004

The *k* value was calculated for the *R-1* chromosome. The *k* value distributions for the *cn-14/RL(A)-i* males presented in Table 2 are also shown for each *RL(A)-i* line separately. Number of replications for each line is shown in parentheses.

[a] An average of the two $\bar{k}$'s shown in Table 2.

cn bw males was 0.059 (= 0.955 − 0.896). In terms of *d* as defined earlier, the difference was approximately 0.136 for the *R-1* and 0.095 for the *cn-14* chromosomes. These results suggest that the amount of decrease in *k* (or *d*) caused by insensitivity of the *RL(A)-i* chromosomes is comparable between the two tester *SD* chromosomes. Based on the average *k* value for the control, *R-1/cn bw* males (= 0.891), the cutoff point, corresponding to HARTL and HARTUNG'S $k = 0.60$, was computed to be 0.620. Only 15% (2/13) of the second chromosomes tested fell into the *k* range smaller than this cutoff point, and this frequency was significantly lower than that found by HARTL and HARTUNG ($\chi^2_1 = 4.77$, $P < 0.05$). There is, therefore, some difference in the frequency of insensitive second chromosomes between the two studies, but the present authors feel that the two studies are not mutually exclusive. Since the wild flies used by HARTL and HARTUNG and those used by the present authors were collected at different sites in Raleigh in different years, the observed difference may simply be a reflection of some degree of fluctuations in space and time. The difference might also be caused by some minor experimental conditions, such as age of the tested *SD* males and temperature in the laboratory. These factors are known to affect the *k* values of *SD* males considerably (MANGE 1968; SANDLER and HIRAIZUMI 1961; HIRAIZUMI 1969).

A final word should be given to the *k* values for *SD* chromosomes under a natural genetic background. The present observations clearly demonstrate that the average *k* value for the *SD* chromosome in nature is considerably larger than the Mendelian expectation of 0.5, so that segregation distortion is actually occurring in natural populations, although, due to the presence of suppressors, in considerably reduced degrees. There were many cases in which the *k* values for the *SD* chromosomes were suppressed to the level of 0.6 or smaller. Such strong suppressors of the "original, strong *SD* chromosomes" have not yet been reported, except for the completely insensitive Rsp^i alleles but, as shown in this study, such Rsp^i alleles are almost absent in the Raleigh population. The present observations, therefore, suggest that either the suppressor-*X* and the suppressor second chromosomes, when both were present together in the *SD* males, reduced the *k* values for the *SD* chromosomes to very low levels, or that the Raleigh population contained a strong, recessive suppressor or suppressors of *SD* that could not be detected in the standard tests in which *SD* males were examined after making them heterozygous with laboratory, suppressor-free chromosomes.

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Corresponding editor: D. L. HARTL