CYTOGENETIC ANALYSIS OF AN *SD* CHROMOSOME FROM A NATURAL POPULATION OF *DROSOPHILA MELANOGASTER*[1]

G. TRIPPA, A. LOVERRE AND R. CICCHETTI

Istituto di Genetica, Facoltà di Scienze, Città Universitaria, 00185 Roma, Italy

Manuscript received October 15, 1979
Revised copy received January 25, 1980

ABSTRACT

The discovery and the cytogenetic characterization of a new *SD* (Segregation Distorter) chromosome *2* from a natural population in Ranna (Sicily, Italy), SD^{Ra}, are reported. The main features of this chromosome are as follows: (a) it contains an Sd^{Ra} gene with a moderate degree of segregation distortion ($\bar{k} = 0.72$), (b) a recessive female sterile gene, $fs(2)^{TLM}$, responsible for modifications of the morphology and structure of the testes and ovaries is located at 89.7, (c) SD^{Ra}/SD^{Ra} males and females are viable but sterile, the females due to homozygosis of $fs(2)^{TLM}$ and the males because of homozygosis of a region containing the *Sd* locus, and (d) *SDi/SDj* combinations are fertile, thus suggesting that the different *Sd* factors found in natural populations constitute a multiple allelic series.——These data may indicate that each population containing *SD* chromosomes has evolved its own genetic architecture for the complex *SD* system, with specific modifiers and perhaps different *Sd* genes. The possibility of reconstructing the evolutionary pattern of the SD^{Ra} chromosome in the natural Ranna population after the model of CHARLESWORTH and HARTL (1978) and CROW (1979) is considered.

ALMOST all natural populations of *Drosophila melanogaster* sampled for cases of meiotic drive (ZIMMERING, SANDLER and NICOLETTI 1970) possess a small percent of Segregation Distorter (*SD*) second chromosomes (SANDLER, HIRAIZUMI and SANDLER 1959). Segregation distortion is characterized by the fact that males heterozygous for an *SD* chromosome and an SD^+ chromosome produce an excess of *SD*-bearing individuals, often 95% or more. The extent of segregation distortion by different *SD* chromosomes is measured by k, which is defined as the proportion of *SD* individuals in the total progeny (SANDLER, HIRAIZUMI and SANDLER 1959). The reciprocal cross, SD/SD^+ females, leads to a 50% recovery of the two classes of gametes, indicating an absence of distortion in females.

Segregation distortion in males is caused by dysfunction of sperm bearing the SD^+ chromosome. This has been directly demonstrated by fecundity studies on heterozygous *SD* males and control males, in which SD/SD^+ males were only half as fertile as SD^+/SD^+ control males (NICOLETTI, TRIPPA and DE MARCO 1967; HARTL, HIRAIZUMI and CROW 1967), thus implying that spermatozoa

[1] This work was partially supported by a Grant from Consiglio Nazionale delle Ricerche (C.N.R.) and performed under Progetto finalizzato "Biologia della Riproduzione: Controllo della gametogenesi" of the C.N.R.

with an SD^+ chromosome are rendered nonfunctional by the action of the *Sd* gene. Furthermore, electron microscopic analysis has shown that, for different cases of *SD*, during spermiogenesis in heterozygous males a series of characteristic anomalies are present that are consistent with the dysfunctional sperm hypothesis (NICOLETTI 1968; TOKUYASU, PEACOCK and HARDY 1972a,b; 1977).

The genetic dissection of segregation distortion has made it possible to identify various elements of the *SD* system. They are:

Sd: the locus responsible for segregation distortion of second chromosomes in Sd/Sd^+ males, which maps in the euchromatin of the left arm to the left of *pr* (2-54.5) (TANZARELLA *et al.* 1972; GANETZKY 1977).

Rsp: Responder, associated with Sd and situated in the centromeric heterochromatin of *2R* (SANDLER and HIRAIZUMI 1960; HARTL (1973). HARTL (1973) introduced this term, which is also used by GANETZKY (1977), for the two allelic forms, Rsp^{ins} (Responder insensitive) and Rsp^{sens} (Responder sensitive). However, the data so far available indicate that a whole series of alleles exist at this locus, which results in different expressions of the same *Sd* factor (HARTL 1973; TRIPPA and LOVERRE 1975).

St(Sd): Stabilizer of *Sd*, localized at the extremity of *2R*; this locus determines the intensity of *SD* expression (SANDLER and HIRAIZUMI 1960; DENELL and JUDD 1968).

Besides elements composing the *SD* system, which are to a greater or lesser extent necessary for segregation distortion to occur, a whole series of other factors characterizing the different examples of *SD* have also been identified. These include the presence of characteristic inversions on the *SD* chromosome, recessive lethals, and modifiers of *Sd* and SD^+ second chromosomes differently sensitive to dysfunction by *Sd* (see TRIPPA and LOVERRE 1975; HARTL and HIRAIZUMI 1976).

Despite the great amount of information on the *SD* phenomenon, the precise mechanism by which *Sd* operates is still unknown. The only molecular model so far proposed and accepted in its general lines is that of HARTL (1973).

It is, therefore, useful to characterize the genetic structure of each new *SD* chromosome discovered and to describe its behavior, so as to compare the different *SD* systems. In this paper the genetic structure and the main features of *SD*-Ranna (SD^{Ra}), a chromosome recovered from a natural population in Ranna (Sicily, Italy), are described.

MATERIALS AND METHODS

Description of strains: (1) *y*; $SD^{Ra}/SM5$; *st*. The SD^{Ra} chromosome is fully described in CONCLUSIONS; *SM5* is a balancer for chromosome *2* and is homozygous lethal. (2) *y*; $SD^{Ra}/SM5$; *TM2*/+. *TM2* is a balancer for chromosome *3* and is homozygous lethal. (3) *y*; *bw-5*; *st-5*, an isogenic line selected from a *y*; *bw*; *st* stock for high sensitivity to *SD* chromosomes, by using the *y*; *SM5*; *TM2/T(2;3)S9*, *bw e* strain. (4) Ore-R, a standard stock used as a source of wild-type chromosomes. (5) *Sp Bl L Pin/SM5*, a balanced-lethal chromosome *2* stock carrying the dominant *Sp*, *Bl*, *L* and *Pin* markers. (6) $SD^{R-1}/bw\text{-}5$; *st-5*. The SD^{R-1} chromosome, isolated from a natural population from Rome (NICOLETTI and TRIPPA 1967; SANDLER *et al.* 1968), does not appear to be associated either with inversions detectable by salivary chromosome analysis or

with recessive lethals. The k value for $SD^{R-1}/bw\text{-}5$ males is 0.99. (7) *SD-72/SM5*. The *SD-72* chromosome, recovered from a natural population from Madison, Wisconsin (SANDLER, HIRAIZUMI and SANDLER 1959), carries a pericentric inversion *In(2LR)*39–40; 42A and a paracentric inversion in *2R*, *In(2R)NS* = *In(2R)*52A2–B1; 56F9–13 (LEWIS 1962). It is a strong distorter, with a k value of approximately 0.99.

Further details on genetic markers, inversions and balancer chromosomes may be found in LINDSLEY and GRELL (1968).

Isolation of the SD^{Ra} *chromosome*: The cross scheme used for detecting *SD* chromosomes from natural populations is shown in Figure 1.

Fertility of males and females homozygous for the SD^{Ra} *chromosome*: Matings between 3 SD^{Ra}/SD^{Ra} males and 3 Ore-R and 3 *y*; *bw-5*; *st-5* females were observed in single vials, along with reciprocal matings. Three *y*; *bw-5*; *st-5* females were crossed with 2 Ore-R or *y*; *bw5*; *st-5* males as a control. Males and females were from 1 to 3 days old.

Mated females were designated at random for sperm or progeny counts. Sperm counts were performed on female storage organs stained with aceto-orcein.

For progeny counts, single females were placed in vials to see whether eggs had been laid, and then the progeny were counted between the 14th and 18th day after eclosion.

Fertility test of SDi/SDj *combinations*: To test the fertility of the genotypes "homozygous" for SD chromosomes of different origin, matings between 3 virgin SD^{Ra}/SD^{R-1} or $SD^{Ra}/SD\text{-}72$ females and 2 Ore-R and 2 *y*; *bw-5*; *st-5* males were observed in single vials. Likewise, 3 SD^{Ra}/SD^{R-1} or $SD^{Ra}/SD\text{-}72$ males with 3 Ore-R females and, as the control, 3 Ore-R females with 2 Ore-R and 2 *y*; *bw-5*; *st-5* males were observed during mating. About half the females were dissected for sperm count following the above reported procedure. Progeny counts were done on the remaining mated females.

Cytological examinations: Cytological examination of the structures of the internal genital organs of males and females of the different genotypes studied was carried out with Zeiss Tessovar equipment on fresh *in toto* preparations of newly hatched males and females.

Mapping the sterility factor(s): In order to map the factor(s) responsible for the sterility of SD^{Ra}/SD^{Ra} homozygous individuals, *Sp Bl L Pin*/SD^{Ra} females (produced by crossing $SD^{Ra}/SM5$ males with *Sp Bl L Pin*/*SM5* females) were mated with $SD^{Ra}/SM5$ males. This mating produced *recombinant chromosome*/SD^{Ra} males and females, which were crossed with individuals of the *y*; *bw-5*; *st-5* stock and tested for fertility.

All fertility tests were done on standard corn-meal-agar-glucose food. All experiments were performed at 24° ± 1°.

RESULTS AND DISCUSSION

Detection of the SD^{Ra} *chromosome:* Of 279 second chromosomes from the Ranna (Sicily, Italy) wild population of *Drosophila melanogaster*, 11 (0.04) were found to be *SD* chromosomes. One of these *SD* chromosomes was further studied because F_1 +/*bw-5*; +/*st-5* individuals showed a dark brown eye phenotype, rather than the expected wild phenotype. Moreover, F_1 males crossed with *y*; *bw-5*; *st-5* females segregated only two phenotypes: dark brown and a new eye color, pale yellow. These results are in agreement with the hypothesis that the wild chromosome *2* carries both an isoallele of the *bw* gene and an *SD* factor with a strong distorting effect on the segregation of the second chromosomes ($k = 1.00$); consequently, only two of the four phenotypes expected in the above cross are recovered. These two phenotypes were thus interpreted as due to the +/*bw-5*; +/*st-5* and +/*bw-5*; *st-5*/*st-5* genotypes, respectively. In order to confirm this hypothesis, males of both the dark brown and the pale yellow eye phenotypes were therefore tested again according to the scheme in Figure 1.

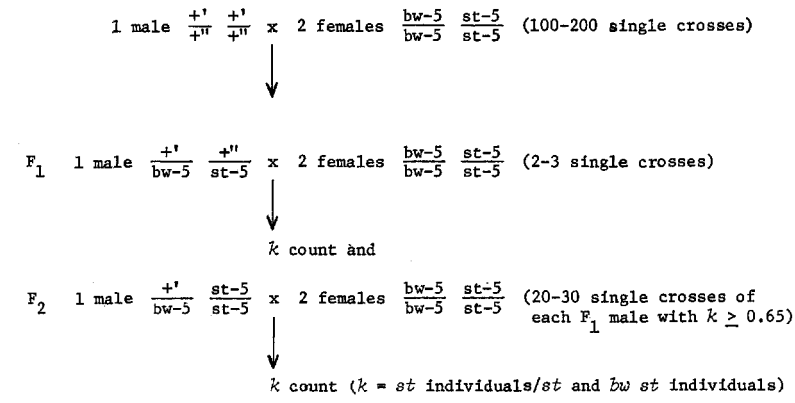


FIGURE 1.—Crossing procedures to detect abnormal segregation of chromosomes *2* and *3*.

Surprising enough, we found that while males with the dark brown phenotype confirmed the type of segregation seen for the eye color phenotypes and therefore still had k values of 1.00 ($n = 141$; $N = 15.688$), males with the pale yellow phenotype segregated two classes of eye color: pale yellow and white (*bw-5*/*bw-5*; *st-5*/*st-5*) with $k = 0.72$ ($n = 105$; $N = 11.325$).

Further tests confirmed these results and their interpretation. The different k values for males with +/*bw-5*; +/*st-5* and +/*bw-5*; *st-5*/*st-5* genotypes depend on the presence of the wild chromosome *3*. The second and third chromosomes extracted from a male from the Ranna population have been therefore termed SD^{Ra} and III^{Ra}, respectively. The role of the III^{Ra} chromosome in modifying the *Sd* action has already been described elsewhere (TRIPPA and LOVERRE 1975).

Analysis of the fertility of SD *homozygous males and females:* In the study of the SD^{Ra} chromosome, it was not possible to obtain a homozygous strain despite the fact that SD^{Ra}/SD^{Ra} individuals were normally produced in the $SD^{Ra}/SM5$ strain and did not show any evident morphological anomalies. In order to clarify the causes of sterility of the SD^{Ra}/SD^{Ra} homozygotes, reciprocal crosses were performed using SD^{Ra}/SD^{Ra} males and females with different genotypes. The results are shown in Table 1.

Within three hr, 17 of the 45 SD^{Ra}/SD^{Ra} males had mated with Ore-R females (7) and *y*; *bw-5*; *st-5* females (10), the average duration of mating being not unlike that of the control males (about 15 min); 20 of the 48 SD^{Ra}/SD^{Ra} females had mated with Ore-R males, the average duration of mating being again similar to that of the controls and 26 of the 29 *y*;*bw-5*; *st-5* control females had mated with Ore-R males.

As far as the males homozygous for the SD^{Ra} chromosome are concerned, dissection of the five females mated with them showed that their storage organs were completely empty. Evidently, the male had either not transmitted sperm or not produced any. This second hypothesis proved to be correct: dissection of these males showed rudimentary dark yellow testes (Figure 2) containing no sperm.

TABLE 1

Fertility analysis of SD^{Ra}/SD^{Ra} *males and females and control after observed mating*

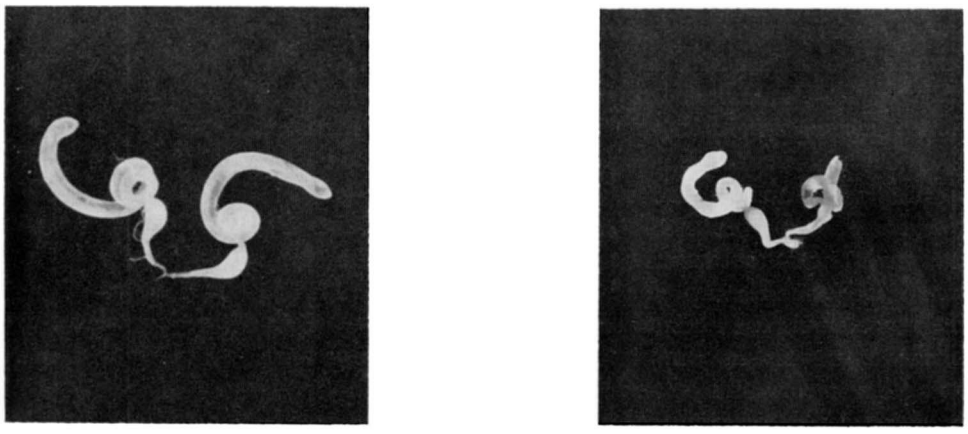
Genotype	No.	Mated with Ore-R	Mated with *y;bw-5;st-5*	Presence of sperm*		Females examined for Egg delay		Progeny production	
		Females		Yes	No	Yes	No	Yes	No
♂ SD^{Ra}/SD^{Ra}	45	7	10	0	5	11	1	0	12
		Males							
♀ *y;bw-5; st-5*	29	26	0	9	1	13	3	13	3
♀ SD^{Ra}/SD^{Ra}	48	20	0	10	0	0	10	0	10

* Average number of sperm (S) transferred to the storage organs of females and average number of progeny (P):

		S	P
♀♀ *y; bw-5; st-5*	inseminated by ♂♂ SD^{Ra}/SD^{Ra} =	0	0
♀♀ Ore-R	inseminated by ♂♂ SD^{Ra}/SD^{Ra} =	0	0
♀♀ SD^{Ra}/SD^{Ra}	inseminated by ♂♂ Ore-R =	852.3	0
♀♀ *y; bw-5; st-5*	inseminated by ♂♂ Ore-R =	838.2	167.2

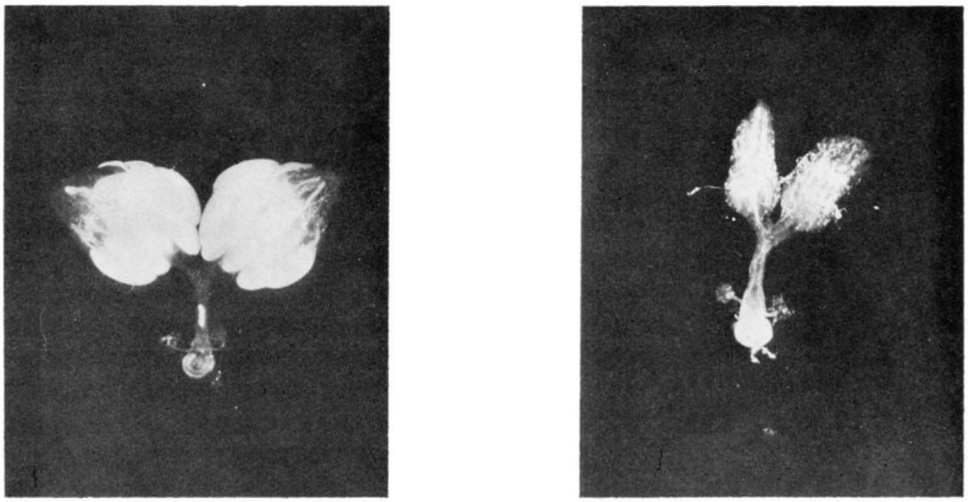
Ten of the 20 mated SD^{Ra}/SD^{Ra} females were dissected. The ovaries and the sperm storage organs were examined and the number of stored sperm counted. Insemination and storage were normal; the seminal receptacles and spermathecae were full, with an average number of sperm equal to 852.3. The ovaries were reduced in volume as compared with those of females of the *y; bw-5; st-5* strain of the same age and contained no mature eggs (Figure 3). The remaining 10 SD^{Ra}/SD^{Ra} females fertilized by Ore-R males were placed in single vials to lay eggs. One of these 10 females died and the other nine laid no eggs even after 14 days when the progeny of the control crosses had already hatched. Dissected on the 15th day after mating, the SD^{Ra}/SD^{Ra} females still had numerous sperm in the seminal receptacles.

Finally, dissection of 10 of the 26 *y; bw-5; st-5* control females showed that nine had their storage organs full of sperm (average number of sperm = 838.2), whereas one had no sperm. The remaining 16 females examined for progeny



A B

FIGURE 2.—Testes of (a) normal and (b) $fs(2)^{TLM}$ homozygous males (×80).



A B

FIGURE 3.—Ovaries of (a) normal and (b) *fs(2)*^{TLM} homozygous females (×80).

count layed eggs, but only 13 gave progeny (average number of flies = 167.2).

The sterility of homozygous individuals is due to the fact that the males produce no sperm and the females no eggs. To determine whether the sterility in the two sexes was due to the homozygosis of the *Sd* factor or to that of one or more recessive genes localized on the *SD*^{Ra} chromosome, the fertility of males and females "homozygous" for *SD* chromosomes of different origin was checked. Also in this case observed matings were performed. The results are presented in Table 2.

TABLE 2

Fertility analysis of SD^{Ra}/SD^{R-1} *and* SD^{Ra}/SD-72 *males and females and control after observed mating*

Genotype	No.	Mated with Ore-R	Mated with *y;bw-5;st-5*	Inspection of internal genital organs	Females examined for* Egg delay Yes	Egg delay No	Progeny production Yes	Progeny production No
		Females						
♂ *SD^{Ra}/SD^{R-1}*	60	20	0	Normal testes, motile sperm and normal sperm bundles	10	0	10	0
♂ *SD^{Ra}/SD-72*	60	20	0		9	1	9	1
		Males						
♀ Ore-R	30	12	0	Normal ovaries; storage organs containing sperm	6	0	6	0
♀ *SD^{Ra}/SD^{R-1}*	30	14	0		7	0	7	0
♀ *SD^{Ra}/SD-72*	30	6	0		3	0	3	0

* Average number of sperm (S) transferred to the storage organs of females and average number of progeny (P):

			S	P
♀♀ Ore-R	inseminated by ♂♂ *SD^{Ra}/SD^{R-1}*	=	118.3	40.6
♀♀ Ore-R	inseminated by ♂♂ SD^{Ra}/*SD-72*	=	103.1	36.2
♀♀ Ore-R	inseminated by ♂♂ Ore-R	=	818.3	187.5
♀♀ *SD^{Ra}/SD^{R-1}*	inseminated by ♂♂ Ore-R	=	768.2	87.0
♀♀ *SD^{Ra}/SD-72*	inseminated by ♂♂ Ore-R	=	809.4	59.7

Sixty SD^{Ra}/SD^{R-1} and 60 $SD^{Ra}/SD\text{-}72$ males were crossed with Ore-R females. Within about two hr, 20 SD^{Ra}/SD^{R-1} and 20 $SD^{Ra}/SD\text{-}72$ males had mated; the average duration of mating was about 12 min. Thirty SD^{Ra}/SD^{R-1}, 30 $SD^{Ra}/SD\text{-}72$ and 30 Ore-R females crossed with Ore-R and *y*; *bw-5*; *st-5* males were tested. Within four hr, 14, six and 12, respectively, had mated; the average duration of mating was about 15 min. About half of the SD^{Ra}/SD^{R-1} and $SD^{Ra}/SD\text{-}72$ males and mated females were dissected, and it was established that in these genotypes the "homozygosis" of *Sd* does not determine the malformation of male and female internal genital organs. Some of the Ore-R females mated with the *SD* males and some of the control females were dissected from sperm counts. Finally, the morphology of the sperm bundles and the motility of the sperm were studied in males of the different genotypes. A progeny count was performed for the remaining females. All types of crosses were fertile, with an average number of progeny of 40.6 individuals for the SD^{Ra}/SD^{R-1} males, 36.2 for the $SD^{Ra}/SD\text{-}72$ males; 87.0 for the SD^{Ra}/SD^{R-1} females, 59.7 for the $SD^{Ra}/SD\text{-}72$ females and 187.5 for the control.

An interesting result is related to the segregation ratio of the *SDi/SDj* males. Since the $SD^{Ra}/bw\text{-}5$ genotype can be distinguished from the $SD^{R-1}/bw\text{-}5$ and $SD\text{-}72/bw\text{-}5$ genotypes by the presence of the *bw* isoallele on the SD^{Ra} chromosome, it was possible to follow the segregation of the two *SD* chromosomes in the *SDi/SDj* males. No significant departures from $k = 0.50$ were observed; this result is quite similar to those previously observed in *SDi/SDj* males by SANDLER, HIRAIZUMI and SANDLER (1959) and HARTL (1969, 1973).

These data would seem to exclude the possibility that the rudimentary morphology of the gonads and the nonproduction of gametes is due to the homozygosis of the *Sd* factor, unless it is supposed that Sd^{Ra}, Sd^{R-1} and *Sd-72* are mutations in different cistrons and therefore complement in the *trans* position.

The sterility in both sexes could, however, be due to a recessive sterility factor(s) associated with Sd^{Ra}. If the anomalies observed in the two sexes depend on a single factor, this would be one of the rare cases in which a single factor is responsible for sterility in both sexes in Drosophila. SANDLER (1977), in a study of a euchromatic region on chromosome *2*, found a mutant, *mfs48*, that proved to be sterile as both homozygous females and homozygous males. Such genes might control some process of gametogenesis or differentiation of the gonads common to both sexes.

Mapping the sterility factor(s): In order to localize the sterility factor(s) on the SD^{Ra} chromosome, the fertility of males and females heterozygous for an SD^{Ra} chromosome and different crossovers derived from *Sp Bl L Pin*/SD^{Ra} females crossed with $SD^{Ra}/SM5$ males was examined. The results are reported in Table 3. First, they show, as expected, that there is a decrease in the frequency of crossing over in the regions between the four dominant markers; the decrease was more marked in the *Sp-Bl* region (13.2 instead of 32.8 centimorgans; 121 recombinants in 916 individuals). This results suggested that an inversion might be present on the left arm of the SD^{Ra} chromosome. Analysis of the salivary chromosomes of SD^{Ra}/SD^{+} larvae showed that there is a small paracentric inver-

TABLE 3

Fertility test on F_1 individuals from the cross
♀ ♀ Sp Bl L Pin/SD^{Ra} × ♂ ♂ SD^{Ra}/SM5 (*see text for details*)

Type of chromosome	Females		Males		Total
	Fertile	Sterile	Fertile	Sterile	
+ + + +	—	95	—	89	184
Sp Bl L Pin	69	6	77	5	157
Sp	—	14	1	25	40
Bl L Pin	24	—	5	1	30
Sp Bl	—	49	47	2	98
L Pin	38	5	—	56	99
Sp Bl L	28	31	38	4	101
Pin	35+37*	41+31*	—	59	135
Sp L Pin	4	—	—	7	11
Bl	—	12	1	1	14
Sp Pin	—	—	—	—	—
Bl L	4	7	11	4	26
Sp Bl Pin	1	3	6	2	12
L	5+16*	1+17*	—	3	9
					916

* Data obtained from a subsequent count in the same cross; the flies were classified only as Pin or Lobe.

sion distal to the centromeric heterochromatin of the SD^{Ra} chromosome, *In(2L)*-32A–C; 35B–C. Second, with respect to the fertility test used for mapping the sterility factor(s), this was more stable and precise in females than in males. It was sufficient, as a matter of fact, in the F_1 females to monitor whether or not they had laid eggs to establish whether they were homozygous for the sterility factor(s). From the results for the females, it can be established: (a) recombinants that have, either entirely or in part, the region of the left arm of the SD^{Ra} chromosome are completely fertile; (b) that the crossovers carrying the right arm of SD^{Ra} are entirely sterile; and (c) that of the crossovers between *L* and *Pin*, some were sterile and some fertile, indicating that the female sterility factor is to be found between these two markers. This factor has therefore been mapped on the basis of the fertility data of crossovers in the *L Pin* region (Table 4). Of 257 recombinants, 129 (73 *Pin* fertile + 56 *Lobe* sterile) are crossovers in the region between *L* and the sterility factor (referred to as $fs(2)^{TLM}$) and 128 (75 *Pin* sterile and 53 *Lobe* fertile) in the region between $fs(2)^{TLM}$ and *Pin*. In that the distance between *L* and *Pin* is 35.3 map units, these data make it possible to localize the recessive factor responsible for female sterility at 17.7 units from *L* (129:257) = x:35.3). Hence, the $fs(2)^{TLM}$ maps at 89.7.

As can be seen from Table 3 the male fertility data do not agree with those of the female, suggesting that the factor responsible for male sterility is different from that for female sterility. In any case, it must be mentioned that the males have been classified as fertile or sterile on the basis of the fertility of the females

TABLE 4

Mapping of the fs(2)^{TLM} *recessive factor on the basis of the fertility of recombinant chromosome/*SD^{Ra} *females from the cross* ♀ ♀ Sp Bl L Pin/SD^{Ra} × ♂ ♂ SD^{Ra}/SM5

	72.0 *L*	89.7 +	107.3 *Pin*		
	+	*fs(2)^{TLM}*	+		
Crossover				Fertile	Sterile
L Pin+				53	56
L+ *Pin*				73	75

crossed with them. This criterion is obviously less sensitive than that used for the females since causes other than his actual sterility could come into play.

The results for the recombinants in the *L-Pin* region (38 *Sp Bl L* fertile males and 59 *Pin* sterile males) and the recombinants in the *Bl-L* region (47 *Sp Bl* fertile males and 56 *L Pin* sterile males) are not in agreement with those obtained for the females, which would exclude the possibility that the male sterility factor is localized in one of these two regions. Evidence that this factor is localized in the *Sp-Bl* region is given by the fact that the *Bl* recombinants are generally fertile and *Bl*+ recombinants generally sterile and, conclusively, by the one fertile *Sp* male of the 26 *Sp* males examined.

These results situate the male sterility factor to the left of *Bl*, near *Sd^{Ra}*, unless, as seems more likely, it is *Sd^{Ra}* itself.

Even more revealing with respect to the male situation is dissection and examination of the morphology of the testes of some recombinant males, the results of which are given in Table 5. The *L Pin* and *Sp L Pin* recombinants, in fact, while all sterile, have perfectly normal testes, while those of the fertile *Sp Bl L* and *Sp Bl* males are malformed. In particular, the dissection of 13 *Sp Bl/SD^{Ra}* males showed that all 13 had malformed testes. Nevertheless, the sperm of all 13 males were numerous and motile. More interesting, of the sterile *Pin* recombinants, about half showed normal testes and the remainder malformed testes.

Therefore, male sterility is not due to homozygosis of *fs(2)^{TLM}*, but seems to depend on homozygosis of the *Sd^{Ra}* factor or another gene closely associated with

TABLE 5

*Fertility and morphology of testes of recombinant chromosome/*SD^{Ra} *males from the cross* ♀ ♀ SD^{Ra}/Sp Bl L Pin × SD^{Ra}/SM5 ♂ ♂

Crossover	No. fertile	Morphology of testes	
		Normal	Malformed
Sp Bl	13	0	13
L Pin	0	18	0
Sp Bl L	15	0	15
Pin	0	24	22
Sp L Pin	0	6	0

Sd^{Ra}. The $fs(2)^{TLM}$ factor appears to act in males by causing malformation of the testes, without altering spermatogenesis, even if the overall fertility of males homozygous for the $fs(2)^{TLM}$ gene is less than that of the control males (TRIPPA, LOVERRE and CICCHETTI, submitted).

A further experiment showed that the interpretation of the mapping data was correct. *L Pin/SM5* recombinant males, which were, therefore, Sd^{Ra} *L* $fs(2)^{TLM}$+ *Pin/SM5*, in the cross with SD^{Ra}/*SM5* females, gave Sd^{Ra} *L* $fs(2)^{TLM}$+*Pin*/Sd^{Ra} $fs(2)^{TLM}$ individuals, homozygous for the Sd^{Ra} factor and heterozygous for the $fs(2)^{TLM}$ gene. All the males (17) were sterile with normal testes, and all the females (16) fertile with perfectly developed ovaries.

The presence of Sd^{Ra} and of any stabilizer of *Sd*, *St* (*Sd*) was previously ascertained in 15 recombinant *L Pin/bw-5* males and 31 recombinant *Pin/bw-5* males. These all showed k values between 0.68 and 0.76, indicating both the absence of an *Sd* stabilizer on the SD^{Ra} chromosome and the recovery in all crossovers of the gene Sd^{Ra}. The latter result was the most probable, on the other hand, in the crossovers *Sp*+ Sd^{Ra}+ *Bl*+ *L Pin* and *Sp*+ Sd^{Ra}+ *Bl*+ *L*+ *Pin* could be produced only by three crossovers, one being between the *Sd* locus (52.9; TANZARELLA *et al.* 1972) and *Bl* (54.8).

CONCLUSIONS

On the basis of the results reported here, the genetic structure of the SD^{Ra} chromosome can be defined as consisting of: (1) a small paracentric inversion in *2L*; (2) Sd^{Ra}, responsible for a moderate degree of segregation distortion ($k = 0.72$) when heterozygous with the *bw-5* chromosome *2*; (3) an Rsp^{ins} allele, like all *SD* chromosomes so far examined, the presence of which can be deduced from the fact that the SD^{Ra} chromosomes was never involved in suicide events and that, in combination with chromosomes sensitive to *Sd*, it did not show any sterility (HARTL 1977); (4) the recessive $fs(2)^{TLM}$ gene, located at 89.7, responsible for the modifications of the morphology and structure of the testes and ovaries and consequently for female sterility and a decrease in the total fecundity of the males (unpublished data); and (5) a recessive mutation at the *bw* locus, bw^{Ra}, which when heterozygous with *bw* exhibits a dark brown eye-color phenotype and in the bw^{Ra}/*bw*; *st*/*st* genotype shows a pale-yellow eye color. A complete description of the SD^{Ra} chromosome is, therefore, represented by: *In(2L)*32 A-C; 35B-C SD^{Ra}, Sd^{Ra}, $fs(2)^{TLM}$, bw^{Ra}.

In common with other *SD* chromosomes detected, the SD^{Ra} chromosome confers a heavy genetic load on both heterozygotes and homozygotes. In heterozygotes, this is manifested only in the male and is due to dysfunction by *Sd* of half of the sperm. In the homozygous condition it is present as male sterility and, unlike in all *SD* chromosomes studied to date, also as female sterility, with two different modalities: in males due to homozygosity for either the Sd^{Ra} gene itself or some element closely linked to it and in females as a consequence of the homozygosis of the $fs(2)^{TLM}$ recessive mutation.

It must be noted, however, that the $fs(2)^{TLM}$ mutation maps quite far from the *SD* region. Its association with Sd^{Ra} is therefore most likely due to chance and the association transient since there are no inversions to maintain linkage disequilibrium. Its presence together with an *Sd* factor is, in any case, just as interesting because of possible implications in the population genetics of *SD*, obviously depending on the gene frequencies of the mutations $fs(2)^{TLM}$ and *Sd* in natural populations. Furthermore, unlike most *SD* chromosomes, but similar to the already known Italian SD^{R-1} chromosome (NICOLETTI and TRIPPA 1967), SD^{Ra} has neither recessive lethals associated with Sd^{Ra} nor stabilizing factors of *Sd*, *St* (*Sd*) on the right arm. The latter statement is based on the fact that several recombinant Sd^{Ra}-bearing males, including *Pin* and *L Pin* recombinant males, are stable, *i.e.*, exhibit $\bar{k}$ values that are not statistically different from those of $SD^{Ra}/bw\text{-}5$ males.

As regards the fertility of homozygous *SD* males, SD^{Ra}/SD^{Ra} individuals are sterile and SD^{Ra}/SD^{R-1} and $SD^{Ra}/SD\text{-}72$ individuals are fertile. This result is in agreement with the observations of HARTL (1973) on the fertility of male "homozygous" for different *SD* chromosomes only if both fertile "homozygotes" are considered to be equivalent to complementing *SDi/SDj* combinations. Most of the 55 combinations tested by HARTL (1973) were, in fact, sterile, and only 16 were fertile.

Another feature of the present case of segregation distortion is that from this same male a third chromosome, named III^{Ra}, was isolated, this being a carrier of a dominant gene localized at 49.7 ± 0.8 that increases the level of distortion of the *SD* chromosomes from seven natural populations from southern Italy, but decreases the level of distortion by the SD^{R-1} chromosome from a natural population near Rome (TRIPPA and LOVERRE 1975). The frequency of chromosomes with an effect similar to that of the III^{Ra} chromosome is about 0.70 in the southern Italian populations tested. It is also to be noted that the SD^{+} chromosomes of the Ranna population are virtually all insensitive to the Sd^{Ra} factor and that, when the III^{Ra} chromosome is present in the genotype of a male carrier of SD^{Ra}, the distorted segregation presents the intermediate values of SD^{Ra} ($k = 0.68$).

These results, together with the fact that the SD^{Ra} chromosome complements SD chromosomes extracted from different populations, suggest that these are different *Sd* genes, or at least that the various cases of segregation distortion are complex genetic systems that have selected their own modes of action and control (TRIPPA and LOVERRE 1972; CHARLESWORTH and HARTL 1978). Each population containing *SD* may, in fact, have evolved its own peculiar genetic structure with its own modifiers and typical *Sd* gene. The *SD* system would thus be the first case of *Drosophila melanogaster* of a complex genetic system in which such a remarkable genetic heterogeneity of the components has been described. The various co-adapted *SD* complexes would therefore seem to be examples of evolutionary convergence in a way similar to the thalassemias and the G6PD deficiencies in man, which are genotypically extremely heterogeneous (WEATHERALL and CLEGG 1972; LUZZATTO and TESTA 1979). As in these conditions, different *SD* systems can also develop the same *SD* "phenotype" as the

result of different genetic events. The simultaneous presence in the natural populations of different co-adapted gene complexes would be positively selected, these being efficient in heterozygotes and at the same time minimizing damage in homozygotes. Thus, the Ranna population represents an extremely interesting example of interacting chromosomes that maintain the frequency of *SD* chromosomes at a value of 0.04 (TRIPPA *et al.* 1972), a frequency lying between the extreme values of 0.01–0.10 so far detected in the United States, Italy and Japan (reviewed in HARTL 1975). The value of 0.04 is very close to the equilibrium value claimed by CHARLESWORTH and HARTL (1978) and by CROW (1979) for an *Sd* Rsp^{ins} chromosome. According to one computer run by CROW, this value would be reached by an ideal population about 1000 generations after the appearance of an *Sd* Rsp^{ins} chromosome. In this ideal population, *Sd* Rsp^{ins} chromosomes interact with $Sd^{+}Rsp^{ins}$ and $Sd^{+}Rsp^{sens}$ chromosomes, the two other types of second chromosome that can be considered important in the dynamics of the frequency of *SD* chromosomes in natural populations of *D. melanogaster*.

This does not mean that the Ranna population is at equilibrium as far as these three chromosomes are concerned. Still, in relation to the computerized data of CROW it could, in fact, be situated at any one the points with a value of 0.04 for *Sd* Rsp^{ins} chromosomes on the spiral described by the frequencies of the *Sd* Rsp^{ins} chromosome before reaching the final equilibrium value.

This seems to be the case of the Ranna population. We have examined a sample of non-*SD* chromosomes from this population, and of 210 chromosomes tested for sensitivity to the Sd^{R-1}*cn L Pin* chromosome (a marked recombinant of the original SD^{R-1}), only 20 (0.09) have shown to be sensitive to distortion by Sd^{R-1}. The remainder were all insensitive with segregation ratios lower than 0.60 (unpublished data). However, whether these insensitive chromosomes were $Sd^{+}Rsp^{sens}$ suppressor-bearing chromosomes or whether they are insensitive to distortion because they are genetically $Sd^{+}Rsp^{ins}$ remains unascertained. These frequencies are quite different from those so far observed. Indeed, for the Madison population the estimate of insensitive second chromosomes is 0.45 (HARTL 1970), which is fully in agreement with the estimate of 0.48 obtained in a population from North Carolina (HARTL and HARTUNG 1975). The frequency of insensitive second chromosomes in Japan is much lower, 0.03 (KATAOKA 1967). If CROW's assumptions are valid for the Ranna population, the above results would be roughly comparable to those reached after about 100 generations by the ideal population. Therefore, the first *SD* chromosome would have appeared in the Ranna population about five years before the detection of the SD^{Ra} chromosome.

It can be concluded that in natural populations there are contrasting forces that counterbalance each other, some of which have the tendency to spread the *SD* chromosomes (distorting capacity of the *Sd* factors—*SD* effect in the true sense, frequency of SD^{+} chromosomes sensitive to the *Sd* factors, enhancers and inversions) and others having the tendency to eliminate them from the populations (various modifying genes that lower the degree of distortion, SD^{+} chromosomes insensitive to *Sd*, recessive lethal genes associated with *Sd*, genes respon-

sible for male and/or female sterility and, finally, the semisterility of heterozygous *SD* males and the sterility of homozygous *SD* males).

Each natural population, therefore, seems to be characterized by different frequencies of the various genetic components of the *SD* system, and its genetic structure is probably the outcome of modes of action that are similar but not identical.

The authors express their thanks to L. SANDLER for critical reading of the manuscript and A. MICHELI for his excellent technical assistance.

LITERATURE CITED

CHARLESWORTH, B. and D. L. HARTL, 1978 Population dynamics of the segregation distorter polymorphism of *Drosophila melanogaster*. Genetics **89**: 171–192.

CROW, J. F., 1979 Genes that violate Mendel's rules. Sci. Amer. **240**(2): 104–113.

DENELL, R. E. and B. H. JUDD, 1968 Segregation-distortion in *Drosophila melanogaster*: the location of stabilizer of SD. Drosoph. Inform. Serv. **43**: 119–120.

GANETZKY, B., 1977 On the components of segregation distortion in *Drosophila melanogaster*. Genetics **86**: 321–355.

HARTL, D. L., 1969 Dysfunctional sperm production in *Drosophila melanogaster* males homozygous for the segregation distorter elements. Proc. Natl. Acad. Sci. U.S. **63**: 782–789. ——, 1970 Meiotic drive in natural populations of *Drosophila melanogaster*. IX. Suppressors of *segregation distorter* in wild populations. Can. J. Genet. Cytol. **12**: 594–600. ——, 1973 Complementation analysis of male fertility among the segregation distorter chromosomes, in *Drosophila melanogaster*. Genetics **73**: 613–629. ——, 1975 Segregation distortion in natural and artificial populations of *Drosophila melanogaster*. pp. 83–91. In: *Gamete competition in Plants and Animals*. Edited by D. L. MULCAHY. North-Holland, Amsterdam. ——, 1977 Mechanism of a case of genetic coadaptation in populations of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **74**: 324–328.

HARTL, D. L. and N. HARTUNG, 1975 High frequency of one element of Segregation Distorter in natural populations of *Drosophila melanogaster*. Evolution **29**: 512–518.

HARTL, D. L. and Y. HIRAIZUMI, 1976 Segregation distortion after fifteen years. pp. 615–666. In: *The Genetics and Biology of Drosophila*, Vol. 1b. Edited by E. NOVITSKI and M. ASHBURNER. Academic Press, New York and London.

HARTL, D. L., Y. HIRAIZUMI and J. F. CROW, 1967 Evidence for sperm dysfunction as the mechanism of segregation distortion in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **58**: 2240–2245.

KATAOKA, Y., 1967 A genetic system modifying segregation distortion in a natural population of *Drosophila melanogaster* in Japan. Japan J. Genetics **42**: 327–337.

LEWIS, E. B., 1962 Salivary gland chromosome analysis of segregation distorter lines. Drosoph. Inform. Serv. **36**: 87.

LINDSLEY, D. L. and E. H. GRELL, 1968 *Genetic variations of* Drosophila melanogaster. Carnegie Inst. Wash. Publ. **627**.

LUZZATTO, L. and U. TESTA, 1979 Human erythrocyte glucose-6-phosphate dehydrogenase: Structure and function in normal and mutant subjects. Curr. Topics Hematol. **1**: 1–70.

NICOLETTI, B., 1968 Il controllo genetico della meiosi. Atti Ass. Genet. Ital. **13**: 1–71.

NICOLETTI, B. and G. TRIPPA, 1967 Osservazioni citologiche su di un nuovo caso di "*Segregation Distortion*" (*SD*) in una popolazione naturale di *Drosophila melanogaster*. Atti. Ass. Genet. Ital. **12**: 361–365.

NICOLETTI, B., G. TRIPPA and A. DE MARCO, 1967 Reduced fertility in SD males and its bearing on segregation distortion in *Drosophila melanogaster*. Atti Acad. Naz. Lincei **43**: 383–392.

SANDLER, L., 1977 Evidence for a set of closely linked autosomal genes that interact with sex-chromosome heterochromatin in *Drosophila melanogaster*. Genetics **86**: 567–582.

SANDLER, L. and Y. HIRAIZUMI, 1960 Meiotic drive in natural populations of *Drosophila melanogaster*. V. On the nature of the *SD* region. Genetics **45**: 1671–1689.

SANDLER, L., Y. HIRAIZUMI and I. SANDLER, 1959 Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion. Genetics **44**: 232–250.

SANDLER, L., D. L. LINDSLEY, B. NICOLETTI and G. TRIPPA, 1968 Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics **60**: 525–558.

TANZARELLA, C., V. SPANO', A. MICHELI and B. NICOLETTI, 1972 Localizzazione genetica e caratteristiche funzionali del fattore SD^{R-1} in *Drosophila melanogaster*. Atti Ass. Genet. Ital. **17**: 108–109.

TOKUYASU, K. T., W. J. PEACOCK and R. W. HARDY, 1972a Dynamics of spermiogenesis in *Drosophila melanogaster*. I. Individualization process. Z. Zellforsh. **124**: 479–506. ——, 1972b Dynamics of spermiogenesis in *Drosophila melanogaster*. II. Coiling process. Z. Zellforsh. **127**: 492–525. ——, 1977 Dynamics of spermiogenesis in *Drosophila melanogaster*. VII. Effects of *Segregation Distorter* (*SD*) chromosome. J. Ultrastruct. Res. **58**: 96–107.

TRIPPA, G. and A. LOVERRE, 1972 Analysis of second chromosomes sensitive to the action of the segregation distorter chromosome (SD^{R-1}) in *Drosophila melanogaster*. Atti Ass. Genet. Ital. **17**: 114–117.

TRIPPA, G. and A. LOVERRE, 1975 A factor on a wild third chromosome that modifies the Segregation Distortion phenomenon in *Drosophila melanogaster*. Genet. Res. **26**: 113–125.

TRIPPA, G., A. LOVERRE, A. MICHELI and I. MIOLA, 1972 Frequencies of SD chromosomes in natural populations of *Drosophila melanogaster*. Drosoph. Inform. Serv. **49**: 81.

WEATHERALL, D. J. and J. B. CLEGG, 1972 *The Thalassaemia Syndromes*, 3rd Ed. Blackwell Scientific Publications, Oxford-London-Edinburgh-Melbourne.

ZIMMERING, S., L. SANDLER and B. NICOLETTI, 1970 Mechanisms of meiotic drive. Ann. Rev. Genetics **4**: 409–436.

Corresponding editor: L. SANDLER