NON-RANDOM ASSORTMENT IN *SD* HETEROZYGOUS XYY MALES OF *DROSOPHILA MELANOGASTER*¹⁾

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A male of *Drosophila melanogaster* heterozygous for Segregation Distorter (*SD*) produces a high frequency of dysfunctional *SD*⁺-carrying sperms; *SD* is thus transmitted to the progeny in a great excess over its theoretical frequency of 50% (Hartl, Hiraizumi and Crow 1967).

In 1967, Hiraizumi and Nakazima reported that when males heterozygous for *SD* were mated to normal *SD*⁺ females, relatively more males were recovered among the *SD*-bearing progeny, while more females were found among the *SD*⁺-bearing progeny. They explained this by suggesting that there was some homology between a part of the X chromosome and the *SD* region such that in a fraction of cells the *SD* and the X chromosome would partially pair in meiosis and hence would tend to separate from one another. Recently, Denell, Judd and Richardson (in press) proposed another model to explain this phenomenon, *i.e.*, at the first meiotic division the *SD* and the sex chromosome bivalents separate at random from each other, but the fraction of the *SD*⁺-bearing sperms which are rendered dysfunctional is slightly greater in Y-bearing than in X-bearing gametes.

The present experiment shows that *SD* also tends to assort preferentially with the Y chromosomes when *SD* males are XYY. This result supports the hypothesis of Hiraizumi and Nakazima.

MATERIALS

Strains of *Drosophila melanogaster* used in this study were as follows.

- 1) *R(SD^{cn})*-14. A second chromosome line carrying *SD*, Activator of *SD* [*Ac(SD)*], and a recessive marker *cn* (cinnabar eye color, 2R: 57.5). This line will be abbreviated as *SD^{cn}*.
- 2) *In(2LR) Cy cn²*. A homozygous lethal second chromosome line carrying large inversions in both arms, a dominant marker *Cy* (Curly wing), and a recessive marker *cn²*. This line will be abbreviated as *Cy*.
- 3) *In(2LR) Pm²*. A homozygous lethal second chromosome line carrying inversions in

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both arms, and a *Plum* eye color variegation. In the presence of an extra Y-chromosome, the variegation is almost completely suppressed and such flies are phenotypically almost wild type in eye color. This stock will be abbreviated as Pm^2 . Pm^2 was found to suppress the action of SD^{cn} almost completely.

4) *cn bw*. A standard second chromosome line carrying two recessive markers *cn* and *bw* (brown eye color, 2R: 104.5). This strain has been the standard for most of the previous *SD* studies.

5) SD^+ . A wild type second chromosome line which was isolated from a stock carrying a recessive sex linked marker for yellow body. This stock will be abbreviated as SD^+ .

METHODS

Before the present experiments were performed, all of the second chromosome lines had been backcrossed to the standard *cn bw* females for a sufficient number of generations that the chromosome pairs other than the second were replaced by those from the *cn bw* stock.

A *Tokyo* second chromosome line was first used as a source of wild type, SD^+ , since this line had been used for many previous *SD* studies. It was soon found, however, that the *Tokyo* chromosome was not suitable for the present study because the *k* values of $SD^{cn}/Tokyo$ males (k =the segregation frequency of SD^{cn}) were too high, the average *k* value being practically 1. Several wild type laboratory stocks were tested for their sensitivity to *SD*, and the second chromosome from a *yellow* stock was found suitable.

Fig. 1 Figure showing the mating scheme (see text for full information).

* The presence of an extra-Y chromosome was determined by a suppressed Pm^2 phenotype.

** Reciprocal matings were made.

A *Cy/Pm*² stock was synthesized by combining the *Cy* and the *Pm*² lines, and a *Cy/Pm*² strain with an extra Y-chromosome was established from the occasional non-disjunctional females found in the normal *Cy/Pm*² stock. The mating scheme is shown in Fig. 1.

Briefly, *Cy/Pm*²; *XXY* females were mated with *SD*^{cn}/*SD*⁺ males. From this mating four progeny types were selected: (1) *Cy/SD*^{cn}; *XX*(Y?), (2) *SD*^{cn}/*Pm*²; *XY*Y, (3) *SD*⁺/*Pm*²; *XY*Y, and (4) *Cy/SD*⁺; *XY*(Y?). The genotypes (2) and (3) are not phenotypically distinguishable but they can be distinguished by progeny tests.

The genotype (1) females were individually mated with *SD*⁺/*cn bw* males and the *F*₁ *SD*^{cn}/*SD*⁺ males were mated individually with *Cy/Pm*² females. The presence of an extra Y-chromosome in the *F*₁ males could be determined by the presence of flies with a suppressed *Pm*² phenotype in the *F*₂ progeny.

The *Cy/SD*^{cn} and *Cy/SD*⁺ progeny of the mating *SD*^{cn}/*SD*⁺; *XY*Y ♂ × *Cy/Pm*² ♀♀, whose second chromosome constitution is distinguishable because of *cn*² in the *Cy* chromosome, were mated with *Cy/Pm*² to test for the presence of an extra Y-chromosome, and the *Pm*²/*SD*^{cn} and *Pm*²/*SD*⁺ progeny of the same mating, whose sex chromosome constitution can be determined from whether *Pm*² is variegated or not, were mated with *cn bw* to determine whether the second chromosome transmitted from the original male parent was *SD*^{cn} or *SD*⁺. No bias is introduced by the progeny testing because practically all of these matings were fertile.

The males of genotype (2) [or (3)] were mated with *Cy/Pm*² females. All of the non-*Cy* *F*₂ flies are *SD*^{cn}/*Pm*² (or *SD*⁺/*Pm*²), and therefore the presence of an extra Y-chromosome could be determined phenotypically. The sex chromosome constitution of the *Cy/Pm*² progeny of the males of genotype (2) could also be determined by whether or not *Pm*² was suppressed.

The genotype (4) males were individually mated with *Cy/Pm*² females and the *Pm*²/*SD*⁺ *F*₁ progeny were scored. As before, the presence of an extra Y-chromosome in the parental male was inferred from suppressed *Pm*² flies among his progeny. Based on the genotypes of progeny flies in each mating, the constitutions of the second and the sex chromosomes in the sperms transmitted from their parental males could be determined. For simplicity these will be called as sperm types in the following discussions.

RESULTS

The observed numbers and the relative frequencies of the different sperm types produced by males in each mating are presented in Table 1.

Recovery rate of the X and the Y chromosome. The numbers of the X and the Y chromosomes recovered among *F*₂ progeny flies are presented in Table 2.

There was no significant heterogeneity among matings ($\chi^2=0.56$, d.f.=3, $0.95>p>0.90$), and the goodness of fit to the theoretical segregation ratio of 1 X : 2 Y was excellent ($\chi^2=0.08$, d.f.=1, $0.90>p>0.70$).

If, however, the two segregating autosomal classes are examined separately, there is a highly significant heterogeneity among classes ($\chi^2=21.46$, d.f.=5, $p<0.001$). The

Table 1. Observed numbers and the relative frequencies of sperm types produced by the males shown

Genotype of parental XYY male							
<i>SD^{cn}/SD⁺</i>		<i>SD^{cn}/Pm²</i>		<i>SD⁺/Pm²</i>		<i>SD⁺/Cy</i>	
<i>SD^{cn}+</i>	X	<i>SD^{cn}+</i>	X	<i>SD⁺+</i>	X	<i>SD⁺+</i>	X
	77 (0.111)		157 (0.079)		246 (0.079)		8 (0.090)
	YY		YY		YY		YY
	71 (0.103)		128 (0.064)		224 (0.072)		7 (0.079)
	XY		XY		XY		XY
	217 (0.314)		800 (0.400)		1,288 (0.415)		32 (0.352)
	Y		Y		Y		Y
	326 (0.472)		915 (0.458)		1,344 (0.433)		42 (0.472)
Total	691		2,000		3,102		89
<i>SD⁺+</i>	X	<i>Pm²+</i>	X				
	42 (0.131)		136 (0.083)				
	YY		YY				
	46 (0.143)		97 (0.059)				
	XY		XY				
	183 (0.570)		714 (0.432)				
	Y		Y				
	50 (0.156)		701 (0.425)				
Total	321		1,648				

Table 2. Observed numbers and relative frequencies of the sex chromosomes produced by the males shown

Sex chromosome				
Genotype of male	Second chromosome	X	Y	Total
<i>SD^{cn}/SD⁺</i>	<i>SD^{cn}</i>	294 (0.300)	685 (0.700)	979
	<i>SD⁺</i>	225 (0.409)	325 (0.591)	550
	Total	519 (0.339)	1,010 (0.661)	1,529
<i>SD^{cn}/Pm²</i>	<i>SD^{cn}</i>	957 (0.327)	1,971 (0.673)	2,928
	<i>Pm²</i>	850 (0.345)	1,609 (0.655)	2,459
	Total	1,807 (0.336)	3,580 (0.664)	5,387
<i>SD⁺/Pm²</i>	<i>SD⁺</i>	1,534 (0.332)	3,080 (0.668)	4,614
<i>SD⁺/Cy</i>	<i>SD⁺</i>	40 (0.312)	88 (0.688)	128

heterogeneity is mostly due to the SD^{en}/SD^+ males. If this mating is deleted, then the remaining classes become very homogeneous ($\chi^2=2.50$, d.f.=3, $0.50>p>0.25$). As is seen in Table 2, SD^{en} tends to be recovered with the Y chromosome, while the SD^+ tends to be recovered with the X chromosome.

Frequency of each sperm type. The frequencies of sperm types were highly significantly heterogeneous among matings ($\chi^2=57.79$, d.f.=9, $p<0.001$), and this was also due to mainly to the SD^{en}/SD^+ males. If we eliminate these matings, then the rest again become homogeneous ($\chi^2=4.37$, d.f.=6, $0.75>p>0.50$).

As was also seen in Table 1, there is a marked decrease in the frequency of the SD^+-Y , and a marked increase in the frequency of the SD^+-XY sperm types. Note that, as compared with other mating types, the frequency of the $SD^{en}-Y$ sperm type seemed to increase, while that of the $SD^{en}-XY$ sperm type decreased.

Table 3. Numbers and relative frequencies of sperm types arranged according to the k values of parental SD^{en}/SD^+ ; XY males

k	No of progeny	SD^{en} class				SD^+ class			
		X	YY	XY	Y	X	YY	XY	Y
0.95	59	5 0.089	3 0.054	12 0.214	36 0.643	0 0.000	0 0.000	3 1.000	0 0.000
0.93	29	4 0.148	1 0.037	9 0.333	13 0.481	0 0.000	0 0.000	2 1.000	0 0.000
0.90	51	5 0.109	2 0.043	18 0.391	21 0.457	0 0.000	2 0.400	2 0.400	1 0.200
0.88	40	2 0.057	2 0.057	9 0.257	22 0.629	0 0.000	1 0.200	4 0.800	0 0.000
0.84	58	7 0.143	5 0.102	9 0.184	28 0.571	1 0.111	2 0.222	6 0.667	0 0.000
0.83	40	4 0.121	4 0.121	9 0.273	16 0.485	0 0.000	2 0.286	5 0.714	0 0.000
0.81	42	1 0.029	2 0.059	8 0.235	23 0.676	0 0.000	0 0.000	7 0.875	1 0.125
0.74	54	5 0.125	6 0.150	17 0.425	12 0.300	0 0.000	2 0.143	9 0.643	3 0.214
0.70	113	12 0.152	9 0.114	33 0.418	25 0.316	5 0.147	0 0.000	25 0.735	4 0.118
0.66	60	4 0.111	2 0.056	11 0.306	19 0.528	4 0.167	4 0.167	10 0.417	6 0.250
0.65	124	10 0.123	8 0.099	29 0.358	34 0.420	11 0.256	5 0.116	25 0.581	2 0.047
0.61	56	7 0.206	5 0.147	9 0.265	13 0.382	1 0.045	9 0.409	11 0.500	1 0.045
0.56	95	4 0.075	10 0.189	21 0.396	18 0.340	6 0.143	4 0.095	20 0.476	12 0.286
0.47	107	6 0.120	7 0.140	15 0.300	22 0.440	7 0.123	8 0.140	28 0.491	14 0.246
0.45	84	1 0.026	5 0.132	8 0.211	24 0.632	7 0.152	7 0.152	26 0.565	6 0.130
Regression		+0.018	-0.291	-0.106	+0.258	-0.136	-0.046	+0.313	-0.229

Relation between the segregation ratio and the amount of non-random association. Thus far, all of the comparisons were based on the pooled data. Further analyses were made of the SD^{en}/SD^+ male mating to see whether the non-random assortment described above was dependent on the k values. The k values, the observed number of each sperm type, and the relative frequency in each segregating class is shown in Table 3 for each male genotype.

The regression coefficients of the Y chromosomes recovered among the F_1 progeny on k , after an arcsin transformation with correction, were computed for the two segregating classes, *i.e.*, +0.0070 for SD^{en} and -0.0404 for SD^+ class; neither of them was statistically significant. A χ^2 test revealed that the frequencies of the Y chromosomes were homogeneous among the 15 tested males ($\chi^2=13.95$, d.f.=14, $p\div 0.50$ for SD^{en} , and $\chi^2=9.22$, d.f.=14, $0.90>p>0.75$ for SD^+). Thus, the frequency of the Y chromosomes in each class was independent of the k values.

The regression coefficients of the frequency of each sperm type in each class on k were then computed. There were two significant, or almost significant regressions. In the SD^{en} progeny class, the frequency of YY-sperm type increased with decreasing k value ($t=3.93$, d.f.=13, $p<0.01$), and in the SD^+ class the frequency of the XY sperm type decreased as k decreased, although with only border line significance ($t=1.91$, d.f.=13, $p\div 0.05$).

It is interesting to note that, in the SD^{en} class, the frequency of Y-sperm type decreased as k decreased, although not significantly so ($t=1.55$, d.f.=13, $0.20>p>0.10$). Comparing the regression coefficients listed at the bottom of Table 3, this suggests that the increase in the frequency of YY-sperm type is compensated by the decrease in the frequency of the Y-sperm type. In the SD^+ class, both Y- and X-sperm types tended to increase in frequency when k decreased, although again not significantly so. This may suggest that the decrease in the frequency of XY-sperm type is compensated by the increase in the X- and the Y-sperm types.

DISCUSSIONS

The present results clearly support the hypothesis that SD^{en} tends to assort more frequently with the Y-chromosome and SD^+ with the X-chromosome: the frequencies of the Y-chromosomes recovered in the above two F_2 classes were highly significantly different ($\chi^2=18.28$, d.f.=1, $p<0.001$). Since the frequency of the Y chromosome, when the two segregating classes are pooled together, was close to the theoretical value of $2/3$, any selective mechanisms (sperm competition, differential viability among genotypes, etc.) can hardly explain the above phenomenon.

Since this did not occur in the SD^{en}/Pm^2 male (the frequency of the Y-chromosome recovery was the same in the SD^{en} and Pm^2 progenies), the above phenomenon must be due to certain *conditions* which involve the segregation distortion of SD^{en} but which are not due to the structure of the SD^{en} chromosome itself.

The frequency of Y-chromosome recovery was independent of the k value of the parental male, but the relative frequencies of some of the sperm types were changed with changing k . Since the frequencies of the sperm types will depend upon the pairing

condition of the sex chromosomes, this may suggest that the pairing condition in the sex chromosomes is somehow related to the degree of distortion. A simple explanation of the relationship is not possible at this time; it must wait for future studies.

It may be that there is an intracellular state determined by *SD*⁺ which permits *SD*^{en} to operate, and this intracellular state also modifies the pairing condition in the sex chromosomes. It may be, of course, that the condition determined by *SD*⁺ could directly be the pairing of the sex chromosomes. In any event, the second chromosome must somehow be involved in the sex chromosome pairing which results in the non-random assortment of these two chromosome pairs.

SUMMARY

Segregation Distorter (*SD*) was found to assort more frequently with the Y-chromosome in *SD*^{en}/*SD*⁺; *XY* males of *Drosophila melanogaster*. Because gametic and zygotic selection can be eliminated as possible explanations of this non-random assortment, it is suggested that the pairing pattern in the sex chromosomes is changed when segregation distortion occurs. The hypothesis of homology between the *SD* region and part of the X-chromosome suggested by Hiraizumi and Nakazima (1967) could be one possible explanation.

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