*EVIDENCE FOR SPERM DYSFUNCTION AS THE MECHANISM OF SEGREGATION DISTORTION IN DROSOPHILA MELANOGASTER**

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Drosophila melanogaster males heterozygous for the *segregation distorter* (*SD*) second chromosome produce a gross excess of *SD*-bearing progeny, the percentage of *SD* progeny in some stocks regularly exceeding 0.99. Sandler, Hiraizumi, and Sandler[1] have shown the *SD* phenomenon to be prezygotic; more recent data have shown it to be temperature-sensitive in or near meiosis I.[2] Evidently some critical event occurs at about that time.

An early model for the mechanism of *SD* action involves a fracture of the *SD* homologue and formation of a reversed sister-chromatid reunion.[1] Such a breakage hypothesis received some support when a higher frequency of X-ray-induced meiotic crossing over was found in *SD* males than in comparable controls.[3] The model was frustrated, however, by the failure to observe such cytological abnormalities in meiosis.

An alternative model, the functional pole hypothesis of Peacock and Erickson,[4] proposes first that the primary spermatocyte of normal *Drosophila* males is polarized, with one of the Anaphase I dyads forming two normal functional sperms, the other dyad forming two morphologically normal but nonfunctional sperms; and secondly that the *SD* homologue is preferentially directed to the nonfunctional pole. Our subsequent discussion concerns only this latter part of the hypothesis, and not at all whether the spermatocyte is, in fact, normally polarized. Peacock and Erickson have developed an argument for the hypothesis by comparing the number of sperms stored in females with the number of progeny obtained from comparable females inseminated by *SD* or non-*SD* males. In both *SD* and controls only one half of the stored sperms appeared to be capable of fertilization.

There are thus two general hypotheses: (1) the sperms receiving the non-*SD* chromosome are somehow rendered unable to follow the normal course leading ultimately to fertilization, and (2) the sperms receiving the non-*SD* chromosome are normally destined to be nonfunctional; the primary event here is a preferential chromosome orientation at Metaphase I. We shall call these the *dysfunctional sperm* and the *functional pole* hypotheses. The dysfunction hypothesis includes a break as one possibility, but is intended to include any mechanism that interferes with normal sperm development or function. The two hypotheses are not necessarily exclusive: It could be, for example, that only one half of the sperms are normally functional but that *SD* makes half of these dysfunctional.

In this paper we report a negative correlation between the degree of distortion and the number of offspring produced by an *SD* male under conditions where sperm number appears to be the limiting factor in progeny production. This result argues for the dysfunction hypothesis.

Materials.—The original *SD* chromosomes from nature carry an inversion-linked complex of three separable genetic elements: *SD* itself and an "activator" (*Ac*(*SD*)) both located near the centromere of chromosome II, and a "stabilizer" (*St*(*SD*))

TABLE 1

LIST of CHROMOSOMES USED*

SD chromosome	Composition	*k* value[8]	Origin
SD^{NH}-2	*SDAc(SD)St(SD)*	0.99	*SD^{NH}-2* (Japan)
SD-72	*SDAc(SD)St(SD)*	0.99	*SD-72* (Madison)
R(SD-36)-1	*SDAc(SD)*	0.87	*SD-36* (Madison)
R(cn)-14	*SDAc(SD)*	0.86	*SD-36* (Madison)
R(cn)-2	*SD*	0.63	*SD-36* (Madison)
R(cn)-5	*SD*	0.60	*SD-36* (Madison)
R(pr)-3	*Ac(SD)*	0.57	*SD-36* (Madison)
R(pr)-7	*Ac(SD)*	0.54	*SD-36* (Madison)
R(SD^{NH}-1)-2	*St(SD)*	0.53	*SD^{NH}-1* (Japan)
R(SD-72)-103	*St(SD)*	0.53	*SD-72* (Madison)

Control chromosomes	Comments
cn bw	Recessives *cn* (*cinnabar eyes*) and *bw* (*brown eyes*). Together they produce white eyes. Standard stock, egg hatchability about 50%.[1]
cn	Recessive *cn*, carried as *cn/cn bw*.
Tokyo	Second chromosome from wild-type *Tokyo* stock, carried as *Tokyo/cn bw*.
Cy bw	Dominant *Cy* (*Curly wings*) with recessive *bw*. Complex inversions, insensitive to *SD* action. Carried as *Cy bw/cn bw*.

* The *k* value is the proportion of *SD* progeny from the cross *SD/cn bw* × *cn bw/cw bw*.

located near the tip of the right arm.[1, 5–7] Each combination of these genes gives a chromosome which can be uniquely characterized by its degree of distortion, its sensitivity to other *SD*'s, and its effect on the sex ratio.[8] Table 1 presents the *SD*'s used in this study, their composition, degree of distortion, and origin. All other chromosomes are from laboratory stocks which have been backcrossed to the standard *cn bw* strain for a sufficient number of generations (usually more than 100) to obtain virtual isogenicity for all but the tested second chromosome.

Methods.—The "early sperm" experiments were performed by placing individual males less than four hours old with single *cn bw* females for eight hours. Each male was provided with another *cn bw* female for the next 12 hours, and with a third for an additional 24 hours. The fertilized females were transferred to fresh medium every three or four days and two pairs of a mutant stock (*Curly* wings and *Plum* eyes, or *vestigial* wings) were placed in the vacated vial to produce larvae which tunnel and aerate the food. Since over 80 per cent of the males are sterile in the first eight-hour period, the data from the first and second broods are pooled.

The "fertile period" experiments utilized single males less than 24 hours old. These were mated with two or three *cn bw* females for three days, then with another two to three *cn bw* females for four days, again mated for three days, then four days, alternating thus until all the males became sterile. The fertilized females were allowed to deposit eggs for a total of seven days. Unless otherwise noted, all males were heterozygous: one second chromosome was non-*cn bw* (*SD* or control), the other *cn bw*.

Results and Interpretation.—On the functional pole hypothesis, functional and nonfunctional sperms normally exist and the non-*SD*-bearing sperms cannot participate in fertilization because they arise from the nonfunctional pole. The dysfunction hypothesis, on the other hand, is indifferent to the existence of a polarized meiocyte. If no such polarity exists, then there will be two kinds of sperm produced: functional (carrying *SD*) and dysfunctional (carrying *SD*$^+$). If such polarity does exist, then there will be four kinds of sperm: functional

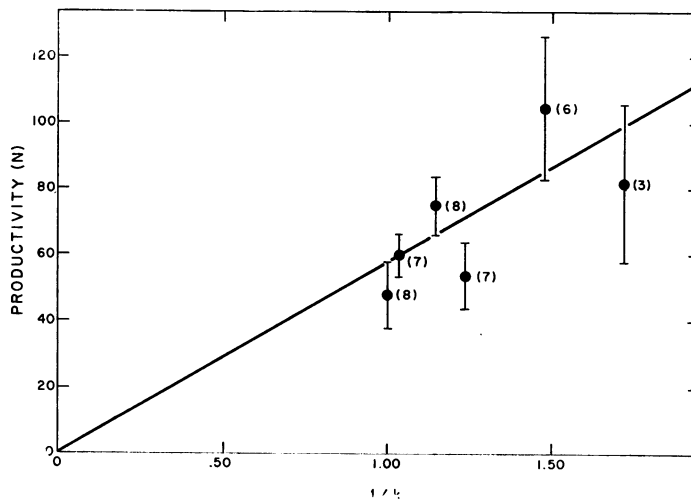

FIG. 1.—Number of progeny (N) and proportion of SD progeny (k) of $R(cn)$-14/cn bw males. The vertical line shows the standard error of the progeny number and the number in parentheses shows the number of parental males. Line drawn freehand to pass through the origin.

(carrying SD^+), functional (with SD), nonfunctional (with SD^+), and nonfunctional (with SD). The first class is dysfunctional since it carries the non-SD chromosome, and the latter two function in neither case, so only one fourth of the sperms are capable of fertilization.

Hence, the functional pole model predicts that the fraction of sperms available for fertilization will be one half in both SD and non-SD males, while the dysfunction hypothesis implies that SD males will produce one half as many functional sperms as non-SD males.

Actually the distortion is not always complete. The k value (proportion of SD progeny in a backcross) can range from approximately 0.5 to nearly 1.0 depending on the strain and the experimental conditions. The functional pole hypothesis implies no necessary correlation between offspring production and k. The dysfunction hypothesis, on the contrary, predicts that productivity will be proportional to $1/k$, for if with probability p a non-SD-bearing sperm functions normally, then $k = 1/(1 + p)$. But when sperm number limits progeny produced, then productivity is proportional to the fraction of functional sperm, namely, $(1/2) + (p/2) = 1/2k$. This amounts to saying that the number of SD progeny (but not the total number) will be constant for strains with different k values.

Table 2 shows the result of an early sperm experiment in which the total numbers

TABLE 2

PRODUCTIVITY AND DEGREE OF SEGREGATION DISTORTION OF YOUNG MALES*

	SD^{NH}-2	$R(cn)$-14	*Tokyo*
Number of males tested	39	12	29
Total progeny produced and standard error	103.9 ± 8.0	133.0 ± 19.1	235.8 ± 12.1
Proportion of non-*cn bw* progeny (k)	1.00	0.882	0.569
Number of non-*cn bw* progeny and standard error	103.9 ± 8.0	117.3 ± 16.5	134.2 ± 7.1

* Pooled results of 0–12-, 12–24-, and 24–48-hr broods.

TABLE 3

SUPPRESSION OF SEGREGATION DISTORTION BY *Curly* INVERSIONS ALSO SUPPRESSES PRODUCTIVITY EFFECT IN BROODS FROM YOUNG MALES*

Genotype of male	*R(cn)-14/Cy bw*	*cn/Cy bw*	*Tokyo/Cy bw*
Number of males tested	14	7	11
Total progeny produced and standard error	243.6 ± 25.4	267.7 ± 17.4	234.8 ± 15.7
Proportion of non-*Cy bw* progeny (k)	0.484	0.482	0.492

* Pooled results of 12–24- and 24–48-hr broods.

of progeny are greatly different and negatively correlated with k. Moreover, the numbers of non-*cn bw* progeny are roughly equal, as expected on the dysfunction hypothesis.

There is, however, the possibility that these are simply strain differences in fertility. The fertility differences must then reside in the tested chromosome, since all other chromosomes have been made the same through repeated backcrossing. To rule out this possibility, we did an early sperm experiment with *R(cn)-14*, which has a variable k value,[9] and correlated the number of progeny with the extent of distortion within the same *SD* strain. The pooled data from the 0–12- and 12–24-hour broods are shown graphically in Figure 1, where the number of progeny (N) is plotted against $1/k$. The expected proportionality is evident. The correlation between N and $1/k$ in the raw data is 0.408 and highly significant; for the points in Figure 1, which are group means, it is 0.7.

The females fertilized by males 24-48 hours old were permitted to deposit eggs for seven days in a single vial. Under these conditions, as expected if the carrying capacity of the vial and not the number of sperms now limits productivity, the differences disappear, indicating no differential survival of progeny from the non-*SD* males. *R(cn)-14* produced an average of 103.0 flies; a Tokyo control produced an average of 103.7.

A repetition of this experiment was performed with *R(SD-36)-1* and a correlation of 0.108 ($d.f. = 48$, $p < 0.15$) was obtained. The variance of $1/k$ in this experiment, however, was not sufficiently large to provide a reliable estimate of the correlation but, despite its lack of significance, its being positive is reinforcing.

TABLE 4

LIFETIME PRODUCTIVITY AND DEGREE OF DISTORTION OF *SD* MALES AND CONTROLS

Genotype of male	Chromosomes employed	Number of males tested	Mean number of progeny per fertile male per week	Mean number of progeny per male	Mean number of non-*cn bw* progeny per male	Mean k	Mean fertile period (days)
SDAc(SD)St(SD)	SD^{NH}-*2* *SD-72*	25	159.2	375.8	369.8	0.984	13.3
SDAc(SD)	*R(SD-36)-1* *R(cn)-14*	24	172.7	511.0	401.8	0.799	17.5
SD	*R(cn)-2* *R(cn)-5*	24	166.9	611.8	341.0	0.557	22.4
Ac(SD)	*R(pr)-3* *R(pr)-7*	24	169.2	689.0	363.6	0.528	25.2
St(SD)	$R(SD^{NH}$-*1)-2* *R(SD-72)-103*	23	170.0	694.7	367.7	0.529	25.2
Control	*cn bw/cn bw*	37	162.2	639.8	(319.9)	(0.500)	24.5

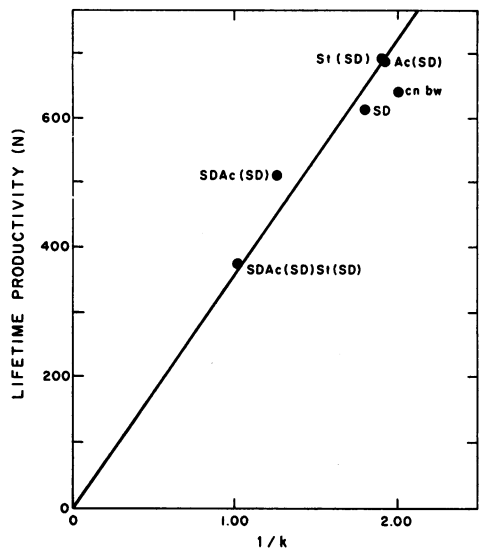

FIG. 2.—Lifetime progeny production (N) and k value, showing graphically some of the data in Table 4. The correlation is 0.95.

Given the proportionality between N and $1/k$, it might be argued that *SD* has an effect on fertility which coincidentally correlates with the k value, but which arises from a mechanism different from the distortion itself. This seems unlikely from the intrastrain correlations. It is made still less plausible by the finding that young *R(cn)-14/Cy bw* males ($k = 0.5$) show the same productivity as *Tokyo/Cy bw* males (Table 3), a result which would require the *Cy* chromosome to suppress the fertility effects of *SD* as well as the distortion. The table also shows that the *Tokyo* chromsome is not "superfertile" since its fertility is comparable to that of the *cn* chromosome.

The results of the fertile period experiments (Table 4 and Fig. 2) are wholly in agreement with the early sperm experiments. Indeed, the two approaches seem to be different experimental ways of examining the same phenomenon. The lifetime productivity is again proportional to $1/k$. Most of the correlation can be accounted for by length of the fertile period. Thus, while the number of progeny per fertile male per day is equal in the *SD*'s and controls (since here the sperm supply is not limiting), the number of progeny for the entire fertile period reveals the proportionality. This suggests that the total number of sperms is already present in fairly young flies, and that the supply is exhausted sooner in the *SD*'s. Perhaps the dysfunctional sperms are never ejaculated. Nicoletti has recently reported morphological abnormalities in the testes of *SD* males.[10]

Summary and Conclusions.—There is a significant negative correlation between the number of offspring of a male with a distorted segregation ratio (*SD*) and the degree of distortion, either when sperms are exhaustively sampled from very young males or when sperms are sampled throughout the entire fertile period. *SD* chromosomes whose distortion is suppressed show no such effect. The reduction in fertility is in quantitative agreement with the expectation if the non-*SD* sperms are rendered dysfunctional by the distorting mechanism.

Our observations provide no information on the hypothesis that one half of the

sperms from a single meiosis are normally nonfunctional. They do argue that if there is such a normal polarity, then the mechanism of *SD* is independent of it.

The detailed mechanism of *SD* is still unknown. The temperature sensitivity[2] near meiosis I may indicate when *SD* first acts. Our observations show that the final effect of *SD* is to produce dysfunctional sperms. All of this is consistent with the chromosome breakage hypothesis, but that is not supported by direct cytological observations. The implication that the dysfunctional sperms are not ejaculated, along with Nicoletti's observations of certain degenerating elements in the testes of *SD* males,[10] leads obviously to the hypothesis that the degenerating elements are the non-*SD*-bearing sperms becoming dysfunctional.

Note.—While preparing this paper we were pleased to learn that Dr. Nicoletti and colleagues in Rome have independently found the 2:1 ratio of lifetime progeny production of non-*SD* and high-*k* *SD* males, using an original Italian *SD* strain, SD^{ROMA}-*1*.[11]

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