SEGREGATION-DISTORTION AND REGULARLY NONFUNCTIONAL PRODUCTS OF SPERMATOGENESIS IN DROSOPHILA MELANOGASTER[1]

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THE meiotic process generally ensures that in any diploid heterozygote the two alternative homologous elements will be recovered in functional gametes in equal frequencies. Exceptions have been described in a number of organisms and where the inequality is shown to be a consequence of the meiotic divisions, *meiotic drive* may be said to be operative (SANDLER and NOVITSKI 1957).

Segregation-distortion is a striking case of meiotic drive in spermatogenesis of *Drosophila melanogaster* (SANDLER, HIRAIZUMI and I. SANDLER 1959). In males heterozygous for Segregation-Distorter (*SD*), a locus proximally located on the right arm of chromosome 2, the *SD*-bearing chromosome is recovered considerably more frequently than its homologue. Many stocks now consistently yield a k value of 0.99, k being the proportion of *SD*-bearing flies in the total recovered progeny. SANDLER *et al.* showed that the failure of recovery of the SD^+ chromosome was not associated with zygote lethality. It was established that synapsis was a requirement for *SD* action and that the phenomenon was not evident in oogenesis; subsequently, a number of other properties have been described (cf. SANDLER and ROSENFELD 1962). Hypotheses of meiotic loss and extra replication (the latter being analogous to that reported by STURTEVANT and DOBZHANSKY (1936) for "sex-ratio" in *Drosophila pseudoobscura*), were negated by experimental analyses. An explanation in terms of meiotic drive was clearly demanded, but despite the magnitude of the effect of the locus its mode of action has not been understood. SANDLER *et al.* (1959) presented a formal cytogenetic model and offered some preliminary cytological observations in support of the hypothesis that the *SD* locus caused a break or misreplication in its synapsed homologue and that ensuing sister chromatid reunion generated a dicentric chromatid and an acentric fragment. The ultimate result would be death of the SD^+ products of the second meiotic division.

This paper is concerned with the description of experiments and observations designed to elucidate the nature of the SD phenomenon. It has been shown that although the genetic consequences of *SD* are clear and unambiguous, *there is no counterpart in meiosis which could account for these effects*, in fact, meiosis ap-

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pears to be absolutely normal! Because of this paradox it has been necessary to extend observations into later stages of sperm development and behavior, and this has led to the generalization that one half of the products of spermatogenesis in *D. melanogaster* are regularly nonfunctional: (somewhat similar to the scheme suggested by NOVITSKI and I. SANDLER (1957) in their brilliant but unappreciated analysis of segregation in a translocation heterozygote). More specifically, it has been found that all products of meiosis proceed through spermiogenesis to yield motile sperm, all of which are capable of entering the sperm storage organs of the female. One half of these sperm, however, appear to be incapable of successfully fertilizing an egg. Since an identical situation is found in controls, it has been inferred that the *SD* locus does not specifically cause malfunction of certain meiotic products, but rather that it is involved in the distribution of chromosomes at meiosis. It is further concluded that the products of spermatogenesis which are regularly nonfunctional are determined as early as the first meiotic division, and that *SD* influences the orientation of the chromosome 2 bivalent at metaphase I of meiosis such that the *SD*-bearing chromosomes are preferentially segregated to the potentially functional cells.

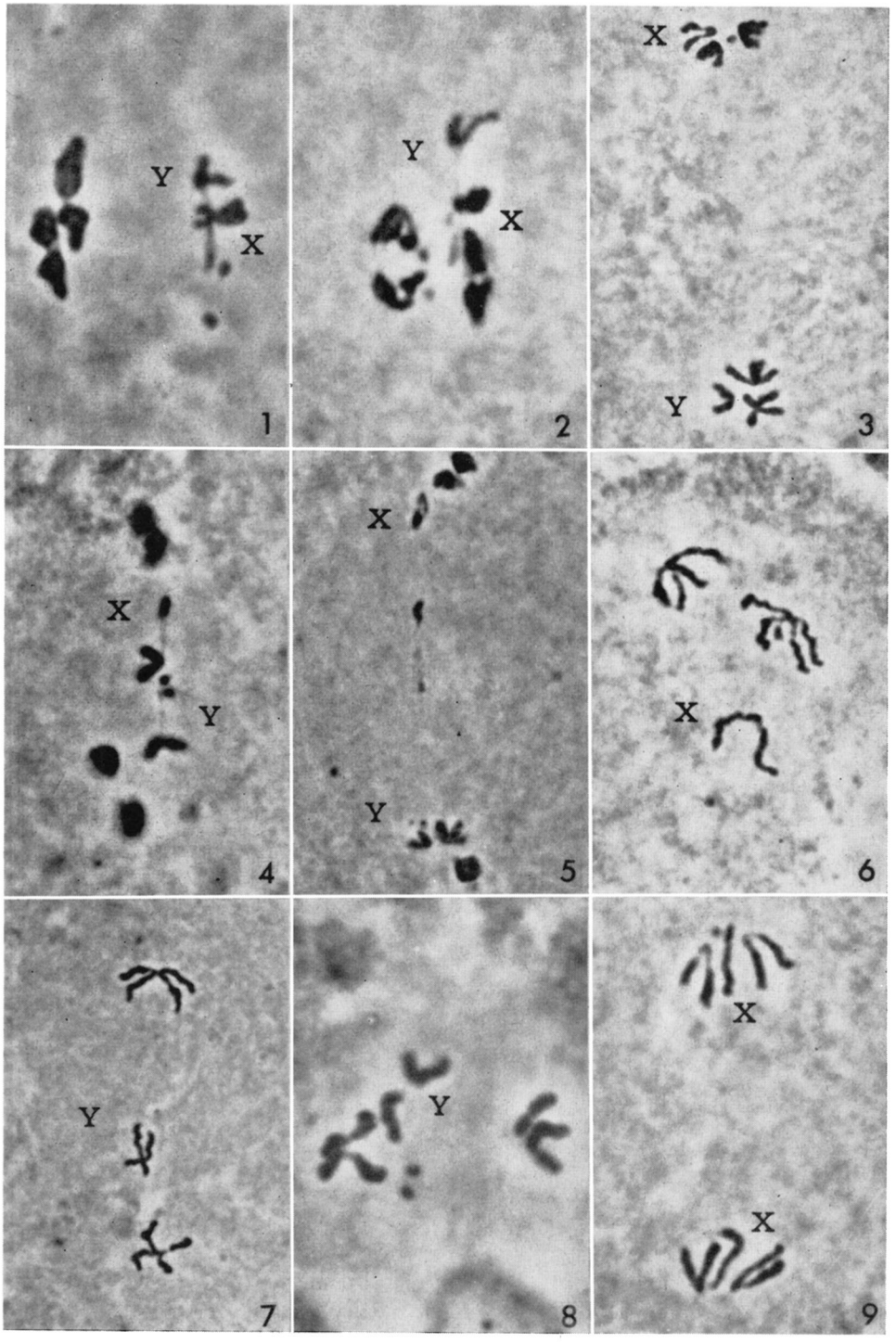
Cytological observations on spermatogenesis:

Testes were dissected in BEADLE-EPHRUSSI saline, and transferred to acetic-orcein (2 percent orcein in 60 percent acetic acid) for squashing. Slides were made permanent in Euparal, the cover slips being removed either in 1:3 acetic alcohol or by the dry ice method. Observations on both temporary and permanent preparations were made with phase optics. Dissections were made on all stages from young pupae to mature males, with newly emerged males the most convenient stage. A large part of the cytological work was on SD-72 males, but another line, SD-5, was also examined. We are indebted to DR. L. SANDLER for supplying the stocks and much associated information.

As SANDLER *et al.* (1959) pointed out in their report, the formal model presented for *SD* action has characteristic cytological expectations. An acentric fragment should be found in first anaphase figures, sometimes lagging on the spindle, or occasionally included in the polar chromosome groups. Either case would lead to second division cells in which the fragment would be visible. If the metaphase II chromosomes were clearly observable then a dicentric chromatid loop should be visible in about 50 percent of the cells. Most strikingly, dicentric bridges should appear at anaphase II.

The material proved to be quite suitable for cytological observation, particularly in those stages which are critical to an evaluation of the above points. Examination of several hundreds of cells at all stages of meiosis, taken from many individuals, failed to substantiate any of the expectations. The course of meiosis in an *SD* heterozygote closely follows descriptions given for wild-type males by COOPER (1949, 1950), and was similar to observations on normal laboratory stocks (*cn bw;* Oregon-R) made as controls in the current study. The normality of the meiotic divisions is illustrated in Figures 1 to 9.

The autosomes at metaphase I appear to have terminal association between homologues, but in an earlier diplotene-like stage chromomere patterns demonstrate a homologous pairing similar to that expected in normal meiosis. None of



FIGURES 1 and 2.—Metaphase I showing X and Y pairing configurations. FIGURE 3.—Anaphase I with regular disjunction of all chromosomes. FIGURE 4.—Anaphase I having a lagging X/Y separation. The proximal and distal segments of the X are clearly shown. FIGURE 5.—Anaphase I with a bridge involving the X and Y. FIGURE 6.—Metaphase II in an X cell. FIGURE 7.—Metaphase II in a Y cell. FIGURE 8.—Early anaphase II in a Y cell. FIGURE 9.—Anaphase II in an X cell.

the associations observed seemed to be equivalent to the chiasmata reported by COOPER (1949), but divergence of opinion on this point may rest on semantic rather than observational differences. The X and Y association is seen in a variety of MI configurations, (Figures 1,2) but in all cases pairing involves an interstitial region of the X and the short arms of the Y. At anaphase I the X frequently takes on a characteristic appearance (Figures 3,4), a conspicuous constriction separating the proximal heterochromatic region from the major euchromatic segment.

Nondisjunction of autosomes and of sex chromosomes has been observed, although rarely, in all stocks examined. Dicentric bridges, resulting from either chromatid or half-chromatid exchange, are another infrequent AI aberration (Figure 5). Anaphase II and metaphase II are particularly suitable for observation (Figures 6 to 9): all chromosomes including the fourth chromosomes are usually clearly resolvable. These stages are conspicuously free of irregularities. Often in SD males sister X and Y second division cells are discernible, indicating that all four products of each primary spermatocyte are formed without chromosome damage of any kind.

In order to confirm that SD^+-bearing cells actually do proceed through meiosis normally, an SD^+-bearing chromosome which was cytologically recognizable was used. A translocation between the second and fourth chromosomes, T(2;4)d was found to be suitable in that it was 95 percent sensitive to *SD* in genetic tests. This element segregated normally at AI, and was found in as many second division cells as was the nontranslocated, *SD*-bearing, chromosome 2.

Spermiogenesis: Since no evidence of *SD* action was seen at meiosis, observations were made on certain aspects of the maturation of sperm in the male. In *D. melanogaster* the last four spermatogonial divisions take place within a cyst, so that groups of 16 primary spermatocytes proceed through meiosis together, yielding 64 postmeiotic cells. In many preparations it was possible to identify all 16 cells in the first division, and the expected 32 in second-division cysts. Following meiosis the 64 cells proceed through spermiogenesis in a discrete "sperm bundle" (Figure 10). If failure of the SD^+ cells took place during this stage, then some difference might be expected in the number of sperm heads per bundle, in SD and controls. The results of counts for various stocks are shown in Table 1. This included counts from a *cn bw* male which escaped *SD* action. No significant

TABLE 1

Sperm bundle counts

Type of male	Mean number of sperm per bundle	Number of bundles counted
SD	59.8 ± 0.8	35
cn bw	59.2 ± 1.1	23
cn bw (ex SD)	59.5 ± 1.6	10
SD/T(2;4)d	60.0 ± 1.1	44
Oregon-R	60.3 ± 2.3	25

difference exists between SD and the controls: the SD^+ cells differentiate into sperm not visibly distinct from those sperm bearing *SD*. Observations extending the equivalence of these cells derive from the final stage of maturation of sperm in the male. As described by LEFEVRE and JONSSON (1962) mature sperm bundles become circinate at the base of the testis, and then sperm swim individually into the seminal vesicle. In most males, the first sperm to enter the vesicle do so 6 to 10 hours after eclosion. Dissection of testes at this time have shown that all 64 sperm from a bundle normally enter the vesicle—both *SD* and SD^+ sperm are motile. Motility of the sperm was confirmed by dissections in saline solution.

Sperm storage and usage: Since the studies on meiosis and spermiogenesis did not indicate the mode of operation of the *SD* locus, an analysis of sperm transfer and subsequent usage in fertilization was undertaken. KAUFMANN and DEMEREC (1942) reported that males transmitted 3,000 to 4,000 sperm at a single mating, their counts being made on the sperm mass squashed from the vagina. Recent reports place the storage capacity of the female at around 700 sperm (KAPLAN, TINDERHOLT and GUGLER 1962; LEFEVRE and JONSSON 1962). The discrepancy between the numbers of stored sperm and transferred sperm suggested that the failure of SD^+ gametes might occur at this stage, with these sperm lacking the ability to gain entry to the storage organs. Since KAUFMANN and DEMEREC utilized six-day-old virgin males in their work, younger males were used here in the hope that inseminations would be smaller and hence permit greater accuracy in counting.

Counts made on the sperm mass in the vagina-uterus of females dissected immediately following insemination showed that males aged from 24 to 48 hours usually transferred 300 to 400 sperm. Few or no sperm were present in the storage organs at this time, but after one to two hours, storage was virtually complete and only rarely were sperm still found in the vagina even where an egg had not been laid by the female. The counts of stored sperm compared closely to the vaginal count, clearly showing that all sperm, both *SD* and SD^+, were able to gain entry into the storage organs. Zygotic death was ruled out by SANDLER *et al.* and observations made in this current work confirmed their results. The time of operation of the SD effect must then lie between sperm storage and fertilization. In the following experiments direct counts were made to compare the number of sperm stored, with the number of progeny produced by similarly inseminated females.

All males were collected within an hour of emergence, stored without females and isolated individually for 12 hours preceding the experiment. Each male was placed with a *y* (yellow body) virgin female aged three to four days, in a shell vial containing food. Time and duration of all matings were recorded. Following a mating the male was transferred to a vial with another virgin female. All transfers of flies were completed without etherization. Since the time of the first mating varied over a range of 3 hours, mated females were designated alternately (over time) for dissection or progeny counting. In succeeding matings the same groups were maintained, thus the mates of each male were either all dissected

or all kept for progeny counting. The latter females were transferred to fresh vials daily for five days, then on alternate days for five more transfers; additional transfers were made for those still fertile.

Dissections were made between 2 and 6 hours following mating, the technique being similar to that used for males. Temporary orcein preparations were satisfactory for several days when stored at sub-zero temperatures. The linear nature of the ventral receptacle, the major storage organ in *D. melanogaster*, enabled accurate counting of sperm heads to be made with phase microscopy (Figures 11 to 14). The chitinous wall of the spermathecae did not lead to difficulty because of the low number of sperm usually present in these organs (Figure 15). In coded preparations counts by the two authors indicated that the experimental

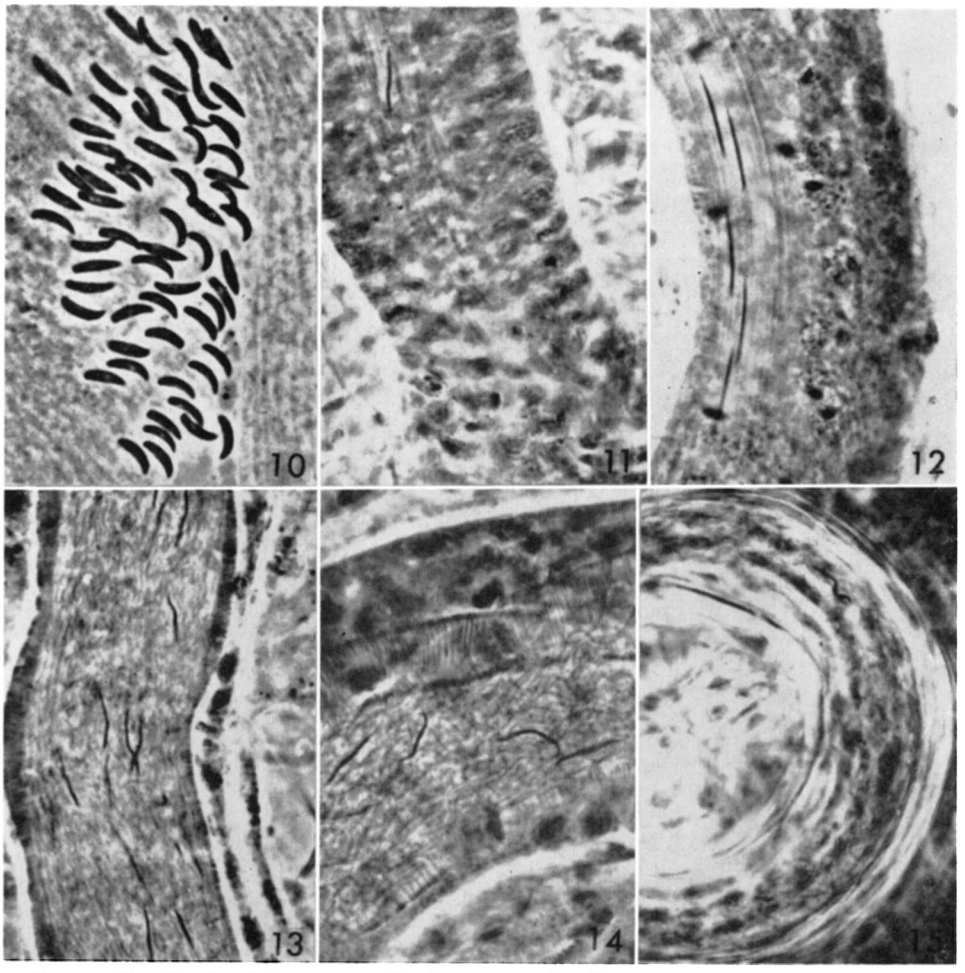


FIGURE 10.—Heads of the spermatids of a "sperm bundle." FIGURE 11.—Opening of receptacle into uterus with a single sperm head visible. FIGURE 12.—Sperm heads in proximal region of receptacle. FIGURE 13 and 14.—Sperm in distal region of receptacle. FIGURE 15.—A single sperm head in a spermatheca.

TABLE 2

Comparison of sperm and progeny counts

	Mating No.	Stored sperm		Progeny		
		Mean No. of sperm	No. of females in sample	Mean No. of progeny	No. of parent females in sample	Progeny per sperm
SD 18 hr	1	58.10	31	45.28	28	.779
	4 (terminal)	106.68	31	51.07	30	.478
SD 66 hr	1	384.26	35	160.70	37	.418
	2	194.35	37	81.05	38	.417
	3	42.65	20	23.03	34	.540
Ore-R 66 hr	1	311.55	42	123.38	40	.396
	2	233.93	44	99.60	40	.426
	3	134.52	33	72.32	25	.538

error was within one to 2 percent, although in very heavy inseminations it may have been as high as 5 to 10 percent. Following the observed experimental matings, routine k value checks were made with all SD males. In all cases the efficiency of the *SD* locus approached 100 percent.

SD males of two ages were used (18 hours and 66 hours after eclosion): 18 hours was found to be the earliest time at which males would mate readily. The 66-hour-old males were used in order to provide another level of insemination and as a safeguard against any effect which might be peculiar to the first sperm produced. Control experiments using Oregon-R males of similar ages were carried out.

In the 18-hour-old SD male series, three matings were observed for each male, the male then being placed with another female for a further eight hours. The latter females (Mating No. 4-terminal) could possibly have been inseminated more than once. In this experiment, although the second and third matings were of normal duration (16 to 20 minutes) no sperm were transferred, and hence no results are reported for these matings. In the other experiments, all matings were observed. The control experiment using 18-hour-old Oregon-R males was abandoned since these males did not mate readily.

The results of the experiments are shown in Table 2 and graphically in Figures 16 to 18. The graphs represent the distribution of individual observations, ranked in order of magnitude; the slopes illustrate the large variation between counts for individual males. In most matings the mean sperm count is of the order of twice the size of the corresponding progeny value (see Table 2: Progeny-Sperm ratio). In general, the approximate 2:1 relationship holds consistently along each graph, for both SD and Oregon-R males at all levels of insemination. Noticeable deviations from this ratio occur in the low values of the ranked series in the first mating of 18-hour-old SD and in the third mating of 66-hour-old SD and Oregon-R. Here, the progeny value approaches the number of stored sperm. In these same experiments the 2:1 ratio holds for the higher values. Although recovery of sperm has been cited as being 50 percent, in point of fact, in most experiments

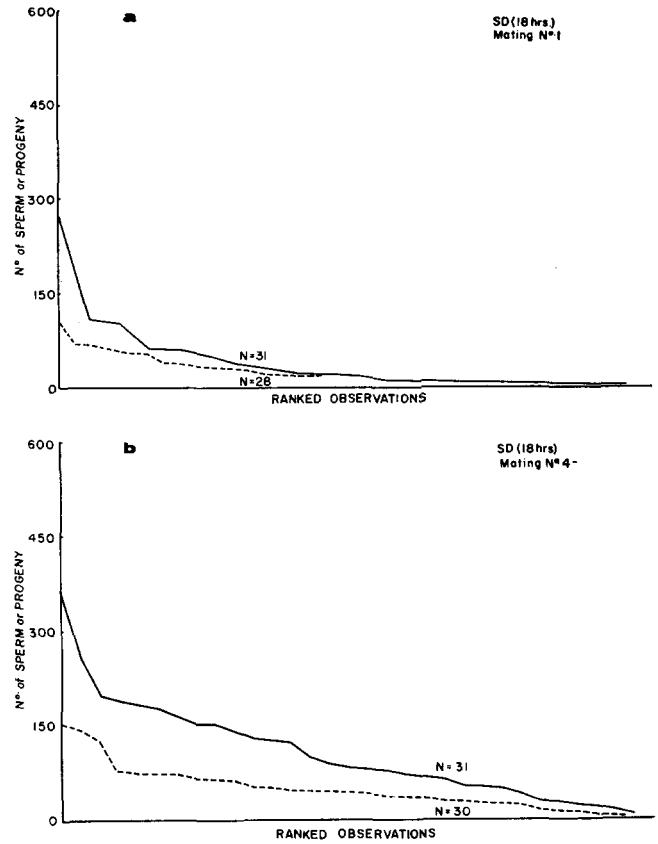


FIGURE 16.—Distributions of sperm and progeny counts from γ females inseminated by 18-hour old SD males. (a) first mating. (b) fourth and terminal matings. (— Sperm. ---- Progeny).

it falls close to 42 percent. A pertinent observation stems from a small experiment with heavily inseminated γ females in which some 90 percent of eggs yielded adults. These results are considered in the discussion.

Dissections of females at the end of their fertile period showed the storage organs to be empty of sperm—or nearly so. The fate of the nonrecovered sperm is not known, but in females which were dissected before the end of their productive period, the number of sperm were consistent with the assumption that both recovered and nonrecovered gametes were being discharged at similar rates. There was no indication of any significant amount of sperm degeneration within the storage organs: this is in accord with the observations of YANDERS (1964) on X-ray damaged sperm.

Recovery of total progeny: Since the 2:1 ratio of sperm to progeny applies to control as well as to SD males, it might be predicted that both types of males would yield approximately the same number of progeny over any given period. On the original model (SANDLER *et al.*), SD would be expected to have one half the productivity of controls, providing the recovery of progeny reflects the pro-

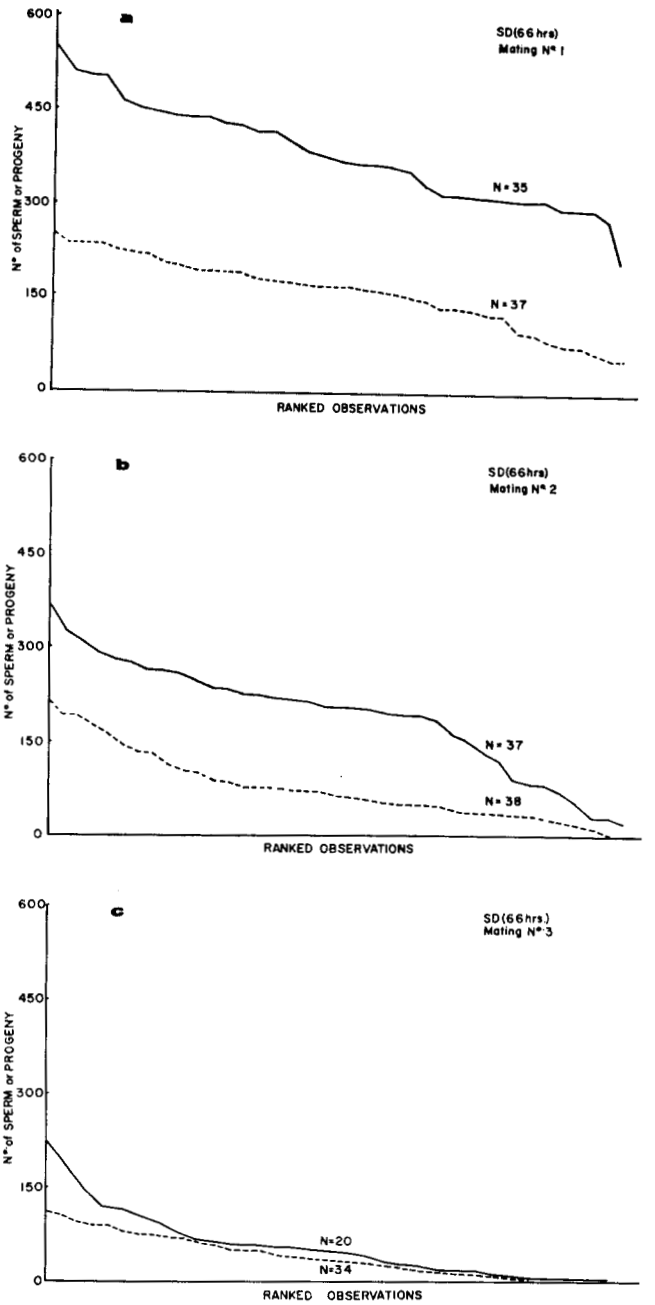


FIGURE 17.—Distributions of sperm and progeny counts from y females inseminated by 66-hr old SD males. (a) first mating. (b) fourth and terminal matings. (— Sperm. ---- Progeny).

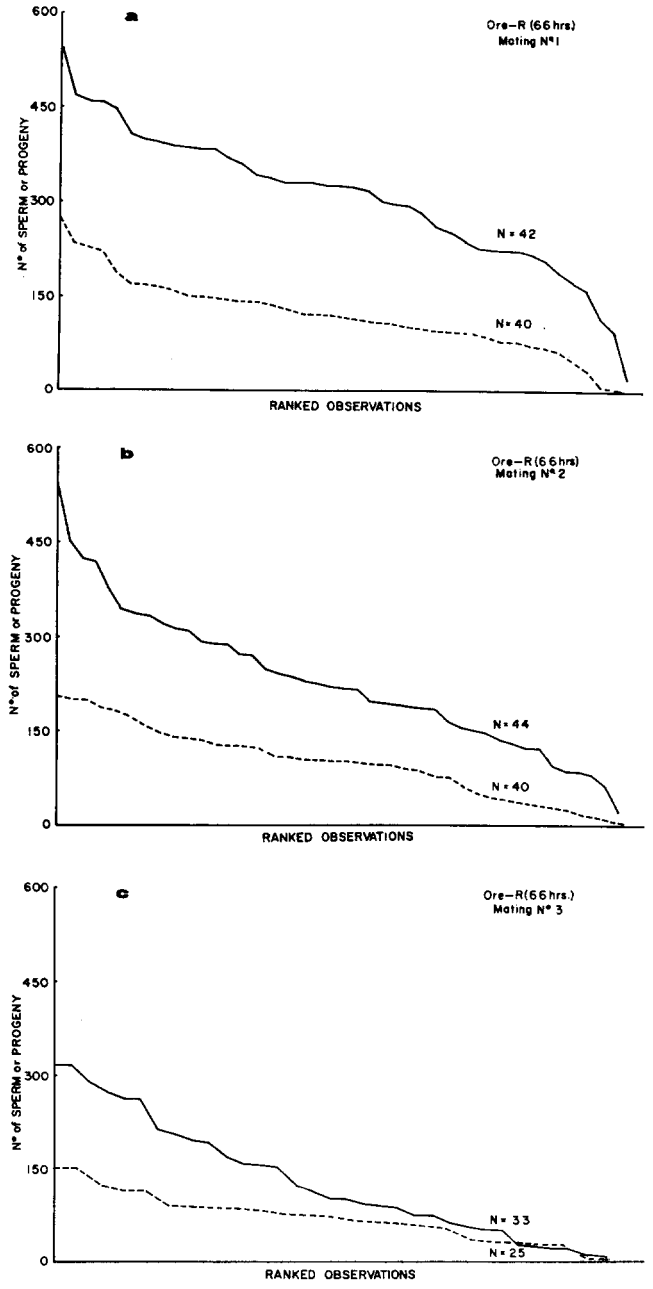


FIGURE 18.—Distributions of sperm and progeny counts from γ females inseminated by 66-hour old Oregon-R males. (a) first mating. (b) second mating. (c) third mating. (— Sperm, ---- Progeny).

duction of sperm. SANDLER and HIRAIZUMI (1961) have cautioned that a 50 percent reduction in sperm production might still provide enough gametes to ensure complete fertility. However, LEFEVRE and PARKER (1963) have shown that all available gametes are utilized if a male is provided with five virgins each day. Accordingly, in the present experiment, individual males were placed with five virgins in each of several successive broods, brood length being one, two and four days, respectively, in each of the three experiments. The control males were taken from the *cn bw* stock used in regular maintenance of the SD cultures. These have the same genetic constitution as SD males, except for the one second chromosome. Results of the experiments are given in Table 3.

The differences in daily productivity evident between these experiments relate

TABLE 3

Total progeny production for SD and cn bw *males (mean number of offspring per male)*

	Days per brood					
	1 day		2 days		4 days	
Brood	SD	*cn bw*	SD	*cn bw*	SD	*cn bw*
1	50.4	47.6	245.4	49.8	461.6	339.9
SE	±18.0	±16.4	±17.5	±11.8	±20.0	±11.2
Fert. ♂ ♂	5	5	5	4	15	15
2	265.6	347.2	569.8	470.8	255.0	264.2
SE	±20.5	±13.3	±16.7	±18.0	±13.6	±15.2
Fert. ♂ ♂	5	5	5	4	14	15
3	348.2	263.0	619.8	396.5	288.4	387.4
SE	±21.2	±19.5	±8.3	±6.1	±31.7	±19.3
Fert. ♂ ♂	5	5	5	4	14	11
4	435.4	425.2	363.0	478.8	373.7	524.4
SE	±11.6	±25.4	±18.1	±24.4	±92.7	±48.3
Fert. ♂ ♂	5	5	5	4	7	11
5	388.0	436.6	50.4	272.8	516.5	532.3
SE	±9.1	±11.7	±8.2	±10.9	±24.5	±49.3
Fert. ♂ ♂	5	5	5	4	2	10
6	323.0	280.8	....	....	....	426.4
SE	±12.9	±26.2	....	....	....	±45.1
Fert. ♂ ♂	5	5	....	....	0	10
7	234.6	228.6	....	....	....	185.4
SE	±13.9	±27.0	....	....	....	±100.8
Fert. ♂ ♂	5	5	....	....	....	5
8	183.0	392.2	....	....	....	10.0
SE	±12.7	±14.4	....	....	....	±8.0
Fert. ♂ ♂	5	5	....	....	....	2
9	254.8	381.8	....	....	....	....
SE	±24.9	±8.5	....	....	....	....
Fert. ♂ ♂	5	5	....	....	....	0
Total number of progeny	12145	14265	9242	8467	17277	29626
Mean number of progeny per male per day	269.89	317.00	184.84	211.68	83.06	93.75

directly to the number of days per brood, but since both control and SD males respond similarly to the experimental conditions, a measure of confidence is given to the sampling procedures.

A brood by brood comparison shows a striking similarity between SD and *cn bw* male productivity, but in the four-day brood experiment, which was conducted until all males became sterile, SD males had a noticeably shorter period of fertility. Since the mating system of these experiments ensured utilization of sperm as they became available, the extended period of fertility of the control males presumably reflects an extended period of gamete production, rather than an accumulation from earlier production. The difference in the duration of fertility could well be determined by the genetic difference between the lines; other differences have been noted, e.g. testis morphology, time of first mating.

These experiments offer support to the notion that SD and control males produce the same proportion of functional sperm from any primary meiotic cell.

DISCUSSION

Mechanism of SD *action:* On the basis of the cytological information, it is clear that the formal model of breakage and sister chromatid reunion cannot be regarded as the actual mechanism of *SD* action. An extensive cytological examination of males heterozygous for the *SD* element did not reveal any meiotic irregularities. Meiosis appeared to be normal in all respects, and the use of cytologically distinct *SD* and SD^+ chromosomes established that this statement pertains to both second chromosomes of an *SD* heterozygote. No support can be given for the preliminary cytological observations of SANDLER *et al.*, in which they reported an acentric fragment consistently at second metaphase, a dicentric loop at second metaphase, and frequently a bridge in early second anaphase cells.

The observations on sperm bundles, sperm maturation, transfer and storage did not reveal any differences between *SD* and SD^+ gametes, nor was there any disparity evident in these stages between *SD* heterozygotes and control males. A distinction between *SD* and SD^+ gametes is finally realized at the time of fertilization: sperm containing SD^+ were found not to fertilize eggs. Of the sperm stored by a female from a mating with an SD male, just one half are recoverable an acentric fragment consistently at second metaphase, a dicentric loop at second metaphase, and frequently a bridge in early second anaphase cells.

The observations and data of the present study are entirely consistent with the conclusion that one half of the sperm produced by a *D. melanogaster* male are regularly nonfunctional with respect to the ability to fertilize an egg. Such a possibility has already been anticipated by NOVITSKI and I. SANDLER (1957) in their account of gamete recovery from certain translocation-bearing [$T(1:4)B^s$] males. In order to explain inequalities in recovery of the various components of this system, they invoked the concept of a regular class of nonfunctional gametes with the further supposition that particular chromosomes had certain probabilities of being included in the functional sperm. This abstraction provided a simple

explanation for a set of data which were otherwise incomprehensible. In their uncanny prediction, these authors foresaw both the possibility that this mechanism might well be of general applicability, and that it must be related to the geometry of the meiotic divisions. The data for Oregon-R in the present work support the notion of generality in that it implies that the production of two classes of gamete—functional and nonfunctional—is a regular aspect of the developmental pattern in Drosophila males.

The unique properties of the SD system, in particular its high efficiency, permit the conclusion that the two classes of sperm produced are indeed intimately related to the planes of the meiotic divisions. Since *SD* always separates reductionally from SD^+ at the first division, and since all functional gametes contain the *SD*-bearing second chromosome, the inference is inescapable that one pole of the first anaphase spindle ultimately yields two functional sperm, the other the two nonfunctional sperm. Even though the failure of the SD^+ gametes does not occur until the spermatozoon stage, SD still must be regarded as a valid case of meiotic drive. It is proposed that the definitive event is an orientation of the second chromosome bivalent at first metaphase, such that the *SD*-bearing chromosome proceeds toward the "functional" pole at anaphase in the majority of primary spermatocytes. In short, the meiotic drive exhibited by the *SD* locus is seen to depend upon a specific mode of segregation on a spindle which regularly possesses a polar differentiation. The mechanism of the orientation of the chromosomes is unknown.

A possible indicator of inequality between the poles of the primary spermatocyte, of the sort discussed above, is seen in the observation of polarized distribution of certain cytoplasmic inclusions at the first meiotic division (PEACOCK and ERICKSON 1964).

Efficiency of sperm use: Inspection of the experimental data (Table 2) reveals that the 50 percent recovery of sperm, discussed above, is never fully realized. In most experiments there is about a 42 percent recovery of stored sperm as progeny. At least a part of this depression in recovery is readily explicable on the basis of hatchability of eggs. For the particular stock from which the females were obtained it was found that some 10 percent of eggs fail to yield adult flies: if the recovery ratio is corrected by this factor it approaches the expected figure of 50 percent. This close agreement implies that the female uses sperm in fertilization of eggs with a high degree of efficiency. Although nothing is known of the manner in which the female controls utilization of stored sperm, it may be worth recording that, in many dissections of storage organs, a single sperm was seen near the opening of the ventral receptacle, even though a large number may have been present more distally (see Figure 11).

The data on sperm recovery can be explained only with an inference of monospermy. This is, of course, in strong contrast to the "classical" theory of polyspermy in Drosophila, a notion which has already been disproven by the recent work of HILDRETH and LUCCHESI (1963), in which they found monospermy to be the general rule. These authors did note a small proportion (about 4 percent)

of dispermic eggs, and if this is taken into account, the sperm-progeny ratio conforms even more closely to the expected figure.

A conclusion of high efficiency of sperm use was also reached by LEFEVRE and JONSSON (1962), although they observed a ratio of sperm to progeny approaching 1:1. At first sight these two results seem contradictory, and the experiments of LEFEVRE and JONSSON appear to discount the notion of two distinct classes of sperm. If the storage capacity of a female is taken into account, the discrepancy may be resolved. In their experiments, LEFEVRE and JONSSON used three-day-old virgin males, and it is conceivable that the insemination level in the first matings was heavier than any in the current work. In support of this view are their observations that in the first two matings the female storage organs are generally filled, whereas in the present experiments all inseminations were at a level lower than the limitations set by storage capacity. For the third and fourth matings, in which the stored sperm level is more comparable to the data reported here, LEFEVRE (personal communication) found that the recovered progeny numbered about one half the count of stored sperm. The differences between the sperm-recovery figures discussed above are thought to reflect the level of insemination. It has been shown that in inseminations where the number of sperm transferred to the vagina is less than the storage capacity of the female, all sperm are stored. For heavier inseminations, which exceed the capacity of the storage organs, it is proposed that the nonfunctional class of sperm is competitively excluded by the functional sperm.

If the number of sperm transferred to a female is sufficiently great, the storage organs will be filled to capacity, and the majority of the stored sperm will be of the functional class. This would yield a progeny count suggestive of 100 percent recovery of sperm. Where as many as 530 progeny are obtained from a once-inseminated female (LEFEVRE and JONSSON) then it must be assumed that nonfunctional sperm were largely excluded from storage. The concept of competition for storage between the two types of sperm is rendered more plausible by the report of the analogous phenomenon of sperm displacement (LEFEVRE and JONSSON). Where a second mating to a male carrying different markers was made, sperm from the second male displaced many of the previously stored sperm. LEFEVRE and JONSSON have interpreted displacement in terms of the observed circulation of sperm within and between the storage organs, and have shown that sperm from different males may have differing abilities in this respect.

Another departure from a strict 50 percent recovery appears in certain of the sperm-progeny series (Figures 16a, 17c, 18c). In the first mating of some young males, and in the last of several successive matings of older males, a 1:1 ratio of sperm to progeny exists. For the most part this relationship holds only where the insemination was of the order of 60 sperm or less. Apparently, where a male is able to transmit only a small quantity of sperm (for reasons at present unknown), competition between the two sperm types is operative. Analogy to the competitive situation visualized above is obvious, but here, the site of action would be in the male. Functional and nonfunctional classes of sperm are ordinarily indistinguishable except at the time of fertilization, but the two situations discussed here—

high and low insemination—presumably provide conditions such that other differences between the sperm types may be manifested.

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SUMMARY

A cytological analysis was made of the mode of action of Segregation-Distorter, a second-chromosome locus which demonstrates an unusually high frequency of recovery from heterozygous males. Meiosis and sperm development were found to be without any visible abnormalities, and it was shown that the SD^+-bearing chromosome was included in 50 percent of motile sperm. When the number of sperm transferred in an insemination was less than the storage capacity of the female (ca. 700) all sperm entered the storage organs. Counts of stored sperm and of progeny recovered from comparable females showed that only one half of the sperm are able to fertilize eggs; the nonfunctional sperm all carry the SD^+-bearing chromosome. Where the number of sperm transferred is greater than the storage capacity or where the numbers are very small, competitive inclusion results in a departure from the 2:1 ratio of stored sperm to progeny. These same relationships are found with control wild-type males. The conclusion was made that *D. melanogaster* regularly forms two functional and two nonfunctional sperm from each primary spermatocyte. The determination of these two classes of sperm occurs at the first meiotic division, where an inequality of the two spindle poles is proposed. The mode of operation of *SD* is regarded to be a specific orientation at metaphase I relative to this polarity, such that the *SD*-bearing homologue of the second chromosome bivalent moves to that pole, leading to the production of two functional gametes.

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