SEX CHROMOSOME MEIOTIC DRIVE SYSTEMS IN *DROSOPHILA MELANOGASTER* I. ABNORMAL SPERMATID DEVELOPMENT IN MALES WITH A HETEROCHROMATIN-DEFICIENT *X* CHROMOSOME (sc^4sc^8)

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ABSTRACT

The meiotic drive characteristics of the $In(1)sc^{4L}sc^{8R}/Y$ system have been examined by genetic analysis and by light and electron microscopy. sc^4sc^8/Y males show a direct correlation between nondisjunction frequency and meiotic drive. Temperature-shift experiments reveal that the temperature-sensitive period for nondisjunction is at meiosis, whereas that for meiotic drive has both meiotic and post-meiotic components. Cytological analyses in the light and electron microscopes reveal failures in spermiogenesis in the testes of sc^4sc^8 males. The extent of abnormal spermatid development increases as nondisjunction becomes more extreme.

A number of meiotic drive systems involving the sex chromosomes have been described in the *Drosophila melanogaster* male. In each of these, complementary products of meiosis are recovered unequally among the progeny. Among these sex chromosome systems (see ZIMMERING, SANDLER and NICOLETTI 1970; PEACOCK and MIKLOS 1973), the $In(1)sc^{4L}sc^{8R}/Y$ genotype has been most fully described. This *X* chromosome (denoted here as sc^4sc^8), is a crossover product of the two inverted sequence chromosomes, $In(1)sc^4$ and $In(1)sc^8$ and is deficient for a major portion of the basal heterochromatin. GERSHENSON (1933), in first examining the meiotic behavior of this chromosome in males, found a high frequency of nondisjunctional exceptions in progeny analysis. Among these exceptions he noted an excess of those derived from sperm carrying no sex chromosomes (*nullo* gametes (*O*)) over those from sperm carrying both the *X* and the *Y* (*XY* gametes). He argued that the sc^4sc^8 and *Y* chromosomes frequently did not pair and were consequently lost at meiosis.

GERSHENSON formulated expectations for the gamete classes (*X*, *Y*, *O* and *XY*) on the assumption that unpaired chromosomes were randomly distributed to

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anaphase poles of the first meiotic division. These formulations were modified by SANDLER and BRAVER (1954) in a re-examination of the sc^4sc^8 system; they interpreted the gamete inequalities to reflect a higher probability of *Y* than of *X* chromosome loss. In their study they avoided viability complications which had been present in GERSHENSON's earlier analysis and found that among regular progeny carrying a parental *X* or *Y* chromosome the *X* was recovered in a higher frequency. They confirmed GERSHENSON's findings of a high level of primary nondisjunction of the sex chromosomes and an excess recovery of the *O* sperm class over the *XY* sperm class.

The *Meiotic Loss* explanation of the sc^4sc^8 meiotic drive system was shown to be invalid by cytological analyses which demonstrated that there was no chromosomal loss in either division of meiosis (COOPER 1964; PEACOCK 1965). PEACOCK (1965) found that in the second meiotic division the frequency of *X* cells equaled that of *Y* cells, and the frequency of *O* equaled that of *XY*. Further, the univalent chromosome exhibited nonrandom segregation on the first division spindle, both usually moving together to an anaphase pole, and the frequencies of *O* and *XY* cells at meiosis II were in close agreement with the frequency of metaphase I cells in which the sex chromosomes were not associated in a bivalent.

The cytological frequency of nondisjunction was in close agreement with the nondisjunction frequency derived from genetic analyses. The correspondence between these genetic and cytological estimates set limitations to the possible explanations of the sc^4sc^8 system, since in the cytological estimate the reciprocal gamete types were in equal frequencies, whereas in the genetic estimate the complementary products were in grossly unequal frequencies. In an attempt to resolve this paradox, PEACOCK (1965) suggested that the nonrandom movement of univalents on the anaphase I spindle may have been in accord with a preexisting inequality of the two spindle poles in the primary spermatocyte. Such an inequality was compatible with the notion put forward by NOVITSKI and SANDLER (1957) that the Drosophila male regularly produced a class of nonfunctional sperm as well as normal sperm. This concept was applied by PEACOCK and ERICKSON (1965) in their *Functional Pole* explanation for the Segregation Distorter (*SD*) system of meiotic drive. However, recent electron microscope studies (NICOLETTI 1968; TOKUYASU, PEACOCK and HARDY 1972*a*,*b*; PEACOCK, TOKUYASU and HARDY 1972) have provided strong evidence that the *Functional Pole* hypothesis does not apply in the *SD* system and that the failure of recovery of the SD^+ sperm is due to an *SD*-induced aberration in their development. These data support the *Sperm Dysfunction* interpretation for *SD* applied by HARTL, HIRAIZUMI and CROW (1967).

Since there is no direct evidence in support of the suggestion that Drosophila males regularly produce a class of nonfunctional sperm, the *Functional Pole* explanation of sc^4sc^8 is inappropriate. We have reanalyzed the sc^4sc^8 system and have concluded that a direct relation exists among meiotic drive, nondisjunction, and abnormal spermatid development.

THE TIME OF THE sc^4sc^8 EFFECT

According to the *Meiotic Loss* and *Functional Pole* explanations of the sc^4sc^8 results, the gamete inequalities originate in meiosis. Zygotic inviability has been shown not to be a significant factor by two classes of evidence; crosses to free and attached-*X* females (SANDLER and BRAVER 1954) yielded similar recoveries of the four gamete types, indicating that differential viabilities were unimportant; and studies of egg hatchability (PEACOCK 1965) showed that zygotic viability in sc^4sc^8 crosses was comparable to the high level of controls. Although it was clear that the unusual genetic results did not originate from viability complications, there was no direct evidence identifying the meiotic divisions as being the source of the sc^4sc^8 effect.

ZIMMERING (1963) found that when sc^4sc^8/Y males were raised at 18° rather than at 26°, the complementary gamete classes approached equality. We have utilized this observation to determine the time of the sc^4sc^8 effect by using temperature-shift experiments to delimit the critical period.

> $In(1)sc^{4L}sc^{8R}$, *y* males with either y^+Y (Experiment 1) or B^sY (Experiment 2) were raised at 18°, 25° or 27°. These males were shifted to 25° and used in brooding experiments. Individual males (6–30 hr after eclosion) were mated with four *y* virgin females (2–3 days after eclosion). Every two days the male was transferred to a new set of virgins. Females were transferred to new food every three days until sterile. All transfers of flies were performed without anesthetization.

The results of these experiments are summarized in Table 1, and their analysis is presented graphically in Figure 1. The data from the y^+Y males raised at 25° show a positive correlation between the frequency of nondisjunction and parental age, and regression analysis shows this line to be of significant slope ($Y = 0.214 + 0.012\,X$). An age effect may also occur in the B^sY males but it is not significant ($Y = 0.164 + 0.006\,X$).

In both experiments the initial nondisjunction levels at 18°, 25°, and 27° increase with increasing temperature. In subsequent broods of the males raised at 18° and 27°, nondisjunction frequencies at first remain at their characteristic levels, with an age effect in the y^+Y data, and then show a transition to the 25° level. The midpoints of the transition periods in both y^+Y and B^sY males are at graphically by CHANDLEY and BATEMAN (1962), and from our assumption that, ively. The initial lag period extends for 7 and 9 days respectively for the 27° and 18° males. We infer from the duration of the initial lag that the period of temperature sensitivity for nondisjunction is at *meiosis*. This conclusion derives both from the 25° developmental timetable of spermatogenesis derived autoradiographically by CHANDLEY and BATEMAN (1962), and from our assumption that, following the shift to 25°, a longer period of time is necessary to reestablish 25° cellular development in the males raised at 18° than in the 27° males. Efficiency of sperm sampling is important in these experiments. We attempted to maximize

TABLE 1

Gamete recoveries from $sc^4sc^8/y+Y$ *(Experiment 1) and* sc^4sc^8/B^sY *(Experiment 2) males raised at 18°, 25° or 27° and crossed to y/y females*

Brood		Gamete classes *X*	*Y*	*O*	*XY*	Total	Brood		Gamete classes *X*	*Y*	*O*	*XY*	Total
Experiment 1							Experiment 2						
18°	1	2989	2626	137	117	5869	18°	1	1809	1547	103	56	3515
	2	3444	3081	204	125	6854		2	1620	1602	84	70	3376
	3	3331	3209	258	143	6941		3	1461	1444	126	70	3101
	4	3486	2662	550	142	6838		4	1842	1638	159	57	3696
	5	2633	1827	352	90	4902		5	1280	1019	153	55	2507
	6	1405	786	537	42	2770		6	661	317	202	7	1187
	7	1121	467	615	17	2220		7	396	149	198	10	753
25°	1	2171	1126	579	69	3945	25°	1	3485	2354	636	152	6627
	2	2481	1304	735	66	4586		2	3365	2162	669	166	6362
	3	954	397	402	16	1769		3	2490	1582	716	91	4879
	4	535	292	288	16	1131		4	1997	1072	635	78	3782
	5	434	261	190	18	903		5	1829	1280	437	92	3638
	6	128	92	74	9	303		6	943	635	269	49	1896
	7	287	201	151	11	650		7	1179	663	365	55	2262
27°	1	342	169	218	11	740	27°	1	190	149	78	8	425
	2	618	345	439	35	1437		2	1044	730	336	72	2182
	3	382	159	331	11	883		3	617	302	334	28	1281
	4	321	187	251	21	779		4	716	376	401	35	1528
	5	698	412	320	32	1462		5	1308	909	411	74	2702
	6	1085	572	533	33	2223		6	1853	1257	484	98	3691
	7	919	453	497	37	1906		7	1077	837	377	83	2374

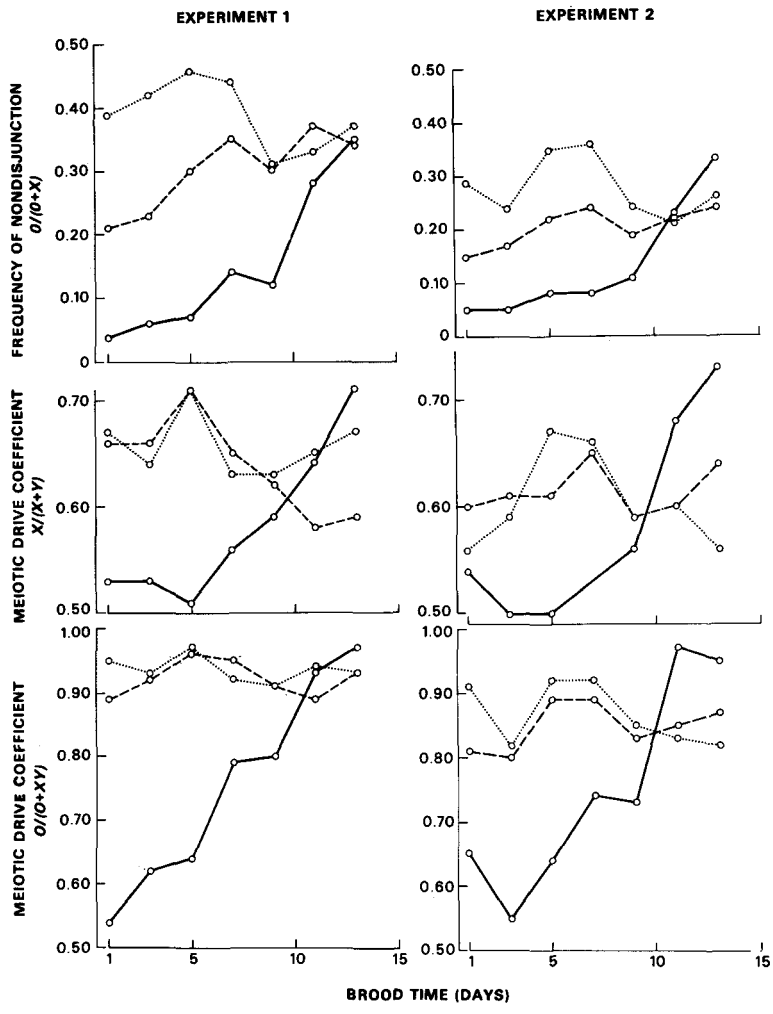


FIGURE 1.—Graphs of the data of Table 1 showing the changes with time in nondisjunction frequency $[O/(O+X)]$ and meiotic drive coefficients $[X/(X+Y)$ and $O/(O+XY)]$ for $sc^4sc^8/y+\ Y$ and sc^4sc^8/B^SY males raised until eclosion at 18° (——), 25° (.....) and 27° (. . . .), and mated and maintained at 25°. Each data point is at the mid-point of a brood.

the regular sequential usage of sperm by providing excess mating partners at regular intervals throughout the experiment.

The duration of the initial lag period in the nondisjunction data showed that there was no temperature effect on nondisjunction during post-meiotic development. This does not appear to be the case for meiotic drive; inspection of the curves reveals an initial lag period of only 5 days for the disjunctional drive coefficient $(X/(X+Y))$ and little or no lag period for the nondisjunctional drive coefficient $(O/(O+XY))$. Thus recovery ratios may be modified in the immediate post-meiotic period as well as during meiosis itself. The meiotic drive coefficients

at 27° do not differ significantly from those at 25° and hence show no temperature response. Replicates of these experiments using mass matings (4–7 males with 20 virgin females) have yielded comparable data for both nondisjunction and meiotic drive coefficients. These replicates did not show the overshoot demonstrated by the 18° males in experiment 2 (Figure 1); this effect may therefore be due to the low progeny numbers in brood 7.

In conclusion, nondisjunction levels appear to be temperature-sensitive solely at meiosis, whereas meiotic drive coefficients may have both meiotic and postmeiotic components.

THE NATURE OF THE TEMPERATURE EFFECT

Cytological analysis of meiosis: PEACOCK (1965) showed that at 25° unpaired sex chromosomes moved together to a pole of the anaphase I spindle, and indicated, from preliminary data, that univalent movement is randomized at 18°. Since the temperature-sensitive period for nondisjunction is at meiosis, we extended the cytological analysis of univalent movement. Testis squashes were prepared from newly emerged sib males raised at either 18° or 25° (Table 2).

These results show two temperature effects:

1) Movement of univalents is temperature-sensitive. At 25° in all three experiments the close agreement between the proportion of nondisjunctional cells and the proportion of cells having unpaired sex chromosomes at metaphase I (last two columns of table) corroborates the nonrandom univalent movement described previously. At 18°, the frequency of nondisjunction is significantly lower than the proportion of cells having univalent sex chromosomes at metaphase I, the approximate 1:2 relation indicating that the unpaired X and Y chromosomes move essentially at random. At neither temperature is there evidence of loss of either the X or Y chromosome. Furthermore, irrespective of the frequency of nondisjunction, the complementary types are found in approximately equal proportions, $X = Y, O = XY$.

2) The second temperature response, which is not constant between experiments, is the effect on the probability of bivalent formation at metaphase I (second last colum, Table 2). For example, in experiment 4 there is no significant temperature effect, whereas in experiment 5 the probability of failure of metaphase I pairing was decreased threefold at 18° relative to the 25° figure. Presumably this difference can be attributed to those aspects of the genotype which were not controlled in these experiments.

The same temperature effect can be inferred from the genetic data in Table 1, since a doubling of the 18° nondisjunction frequency, to allow for random chromosome distribution, does not fully account for the observed differences between the 18° and 25° values. A temperature effect on X-Y bivalent formation can also be seen in a comparison of the 27° and 25° data in these experiments.

Further analysis of the temperature effect: The temperature-shift experiments which delimited the time of the sc^4sc^8 effect (Table 1) showed that 18° culture temperature (rather than 25°) resulted in changes in the relative recoveries of

TABLE 2

Cytological distribution of sex chromosomes in spermatocytes from sc^4sc^8/Y *males cultured at 18° or 25°*

Y chromosome used	Temperature	Metaphase I		Anaphase I		Metaphase II or Anaphase II				Proportion of M1 cells having unpaired chromosomes	Proportion of nondisjunctional cells (AI, MII, AII)
		Paired	Unpaired	Disjunctional	Nondisjunctional	*X*	*Y*	*O*	*XY*		
Expt. 3 B^8Y	18°	122	47	55	16	139	131	23	23	0.28	0.16
	25°	173	103	62	39	68	87	45	57	0.37	0.39
Expt. 4 y^+Y	18°	160	74	51	9	100	85	25	25	0.32	0.20
	25°	218	105	82	27	138	155	70	59	0.33	0.29
Expt. 5* y^+Y	18°	82	14	59	12	107	102	11	3	0.15	0.09
	25°	72	54	89	65	143	138	93	97	0.43	0.41

* This experiment was carried out at a different time to experiment 4.

TABLE 3

Gamete recoveries from $sc^4sc^8/y+Y$ *(Experiment 6) and* sc^4sc^8/B^SY *(Experiment 7) males raised at 18°, 20°, 22°, 23° or 27° and crossed to y/y females*

	Experiment 6 $sc^4sc^8/y+Y$ Gamete classes					Experiment 7 sc^4sc^8/B^SY Gamete classes				
Temperature	*X*	*Y*	*O*	*XY*	Total	*X*	*Y*	*O*	*XY*	Total
18°	2844	2618	121	100	5683	3596	3142	139	74	6951
20°	4229	4053	311	200	8793	4156	3730	282	134	8302
22°	3247	2677	372	141	6437	3710	2610	464	116	6900
23°	3728	2621	850	139	7338	4113	2249	954	92	7408
27°	2352	1651	645	91	4379	1392	779	450	50	2671

complementary gametes as well as reducing the frequency of nondisjunction. We have examined these effects in more detail in a further set of experiments using several different culture temperatures.

Individual males, 6–30 hr old, ($sc^4sc^8/y+Y$ in experiment 6, sc^4sc^8/B^SY in experiment 7), cultured at 18°, 20°, 22°, 23° or 27° were mated to four *y* virgin females (2–4 days old) at 25°. Males and females were shaken over to new food vials three times at two-day intervals.

The results of these experiments are shown in Table 3 and Figure 2. The frequency of nondisjunction has a temperature response showing unequal rates of change over the range of culture temperatures. The largest change occurs between 22° and 23°. Doubling of the 18° nondisjunction frequency (to allow

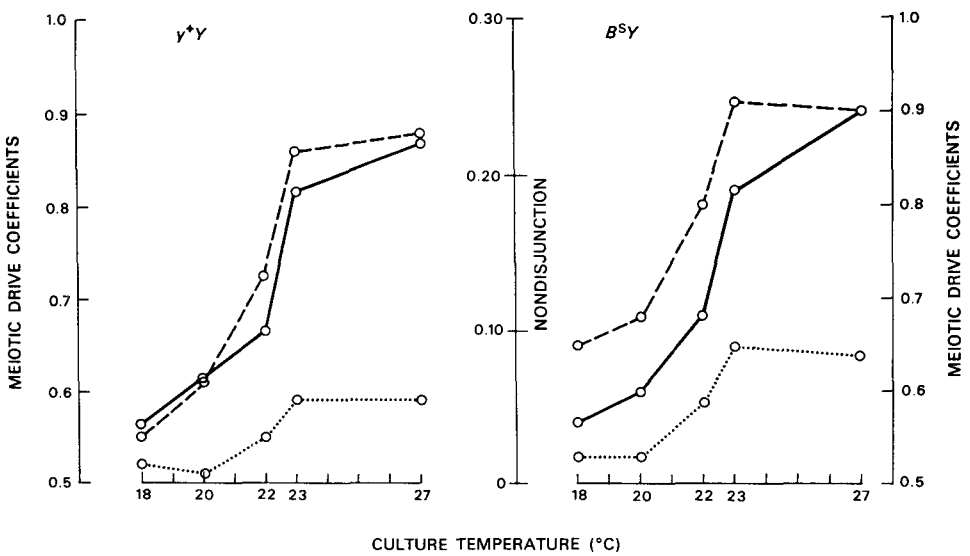


FIGURE 2.—Changes in meiotic drive coefficients [$X/(X+Y)$ ·····, $O/(O+XY)$ ——] and nondisjunction frequency [$O/(O+X)$ ——] of $sc^4sc^8/y+Y$ and sc^4sc^8/B^SY males raised at different temperatures.

for the random movement of univalents) does not yield a nondisjunction level sufficient to account for that observed at 23° and 27°, showing that in these experiments there is a temperature effect on the probability of metaphase I *X-Y* bivalent formation. Without cytological data at each temperature point we cannot partition the relative contributions of univalent movement and pairing probability to the observed nondisjunction levels; however, the values do suggest a qualitative change in univalent movement around 22°. Another feature of Figure 2 is the strong correlation between the temperature effect on nondisjunction and on the meiotic drive coefficients. This correlation may reflect a relationship between the extent of pairing and the magnitude of meiotic drive.

In summary, the genetic and cytological results from sc^4sc^8 males raised at different culture temperatures show that (1) nondisjunction increases nonlinearly in the range between 18° and 27°, and (2) meiotic drive coefficients increase similarly over the 18°–25° range, whereas (3) bivalent formation sometimes decreases with increasing temperature and (4) univalent movement is near random at 18° and becomes increasingly nonrandom with increasing temperature.

NONDISJUNCTION AND MEIOTIC DRIVE

The parallel responses to temperature changes of nondisjunction and meiotic drive coefficients may reflect a causal relationship between these parameters. We have analyzed this relation in data collected at a single culture temperature (25°) to avoid the complexities evident at different temperatures. We have chosen 25° since the bulk of our data has been collected at this temperature, and since the meiotic behavior of the sex chromosomes at this temperature ensures that disjunctional and nondisjunctional gametes are derived from spermatocytes having paired and unpaired sex chromosomes respectively.

Independently derived sc^4sc^8 stocks have considerable variability in the extent of nondisjunction; variability in nondisjunction levels also exists between males within a stock. We have examined levels of meiotic drive in males having different levels of nondisjunction; the data are from experiments 1, 2, 8, 9, 10 and experiment 1C of MIKLOS, YANDERS and PEACOCK (1972). Individual male progenies were ranked into nondisjunction intervals and the meiotic drive coefficients for these intervals computed (Table 4).

These results show a relation between nondisjunction and meiotic drive that parallels the correlation observed in the temperature-response experiments. Differential recoveries of complementary gamete types, both disjunctional and nondisjunctional, increase with increasing nondisjunction. This can readily be seen in the change of the *O* gamete recovery compared to the almost constant recovery of the *XY*. Analysis of the regression of nondisjunction frequency on meiotic drive coefficients yields the equations $Y = 0.52 + 0.49\,X - 0.20\,X^2$ for the disjunctional gametes, and $Y = 0.70 + 0.76\,X - 0.51\,X^2$ for the nondisjunctional gametes (a graphical representation of these data is given in MIKLOS, YANDERS and PEACOCK 1972).

The relation we have observed in these experiments between meiotic drive and

TABLE 4

Progeny from $sc^4sc^8/y+Y$ *and* sc^4sc^8/B^SY *(Experiments 1, 2, 8, 9, 10 and*) males arranged in intervals of increasing frequency of nondisjunction*

Nondisjunction frequency $[O/(O+X)]$ class interval mean	Meiotic drive coefficients $\frac{X}{X+Y}$	$\frac{O}{O+XY}$	Gamete types X	Y	O	XY	Total progeny	No. of tested males
0.05	0.56	0.74	0.54	0.42	0.03	0.01	3222	20
0.11	0.55	0.76	0.51	0.41	0.06	0.02	23439	122
0.16	0.58	0.80	0.51	0.37	0.10	0.02	36141	123
0.23	0.61	0.86	0.51	0.32	0.15	0.02	22153	62
0.27	0.66	0.88	0.51	0.27	0.19	0.03	8044	28
0.32	0.68	0.93	0.50	0.24	0.24	0.02	1781	7
0.39	0.67	0.92	0.46	0.22	0.29	0.03	2523	15
0.43	0.68	0.90	0.43	0.20	0.33	0.04	721	5
0.56	0.70	0.95	0.36	0.16	0.46	0.02	1055	8
0.60	0.78	0.97	0.37	0.10	0.51	0.02	705	7
0.64	0.74	0.97	0.31	0.11	0.56	0.02	1767	17
0.69	0.73	0.97	0.27	0.10	0.61	0.02	1405	13
0.71	0.82	0.99	0.27	0.06	0.66	0.01	583	5
0.77	0.76	0.99	0.21	0.07	0.71	0.01	783	8

* Experiment 1C, MIKLOS, YANDERS and PEACOCK 1972.

nondisjunction appears to be a general one since comparable correlations are found in previously published data (Table 5). Meiotic drive coefficients predicted by the regression equations closely conform to the observed coefficients over a wide range of nondisjunction frequencies.

Since meiotic drive is present among the products of disjunctional meioses (X or Y) as well as among products of nondisjunctional meioses (O or XY) it is clear that nondisjunction, *per se*, is not a prerequisite for meiotic drive. Further-

TABLE 5

Nondisjunction and meiotic drive coefficients from previously published data

Genotype of male	Nondisjunction frequency $[O/(O+X)]$	Meiotic drive coefficients $\frac{X}{(X+Y)}$	$\frac{O}{(O+XY)}$	Reference
$sc^4sc^8/y+Y$	0.33	0.63 (0.66)*	0.90 (0.89)*	SANDLER and BRAVER (1954)
	0.31	0.70 (0.65)	0.89 (0.88)	SANDLER and BRAVER (1954)
	0.34	0.68 (0.67)	0.94 (0.90)	ZIMMERING (1963)
	0.34	0.67 (0.67)	0.92 (0.90)	ZIMMERING (1963)
sc^4sc^8/B^SY	0.46	0.71 (0.70)	0.96 (0.94)	PEACOCK (1965)
	0.47	0.73 (0.70)	0.95 (0.94)	PEACOCK (1965)
	0.18	0.68 (0.60)	0.93 (0.82)	PEACOCK (1965)
	0.33	0.71 (0.66)	0.94 (0.89)	PEACOCK (1965)
	0.34	0.70 (0.67)	0.94 (0.90)	PEACOCK (1965)
$sc^4sc^8/y+Y$	0.14	0.57 (0.58)	0.73 (0.80)	RAMEL (1968)

* The values in parentheses are those calculated from the regression equations derived from the data in Table 4.

more since both meiotic drive coefficients are similarly correlated with the frequency of nondisjunction, we conclude that nondisjunction is an indicator (consequence) of an underlying variable which also affects the recovery of gametes. We propose, on the basis of COOPER's (1964) conclusion that there are multiple pairing sites in both the *X* and *Y* chromosomes, that the underlying variable is the probability of pairing. Thus in males with a high probability of pairing of the sex chromosomes we expect a low frequency of nondisjunction and low meiotic drive coefficients in both disjunctional and nondisjunctional gametes; in contrast, in males having a low probability of pairing we expect a high frequency of nondisjunction and high meiotic drive coefficients. At any level of pairing the meiotic drive coefficient for nondisjunctional progeny will be expected to be higher than that for disjunctional progeny. In another paper we have developed the postulate that pairing of sex chromosomes is of importance in determining the recovery of meiotic products (PEACOCK and MIKLOS 1973; also BAKER and CARPENTER 1972).

TESTS FOR ABNORMAL SPERMATID DEVELOPMENT IN THE sc^4sc^8 SYSTEM

The genetic and cytological results have given no indication of the mechanism by which the differential recoveries arise. The findings that there are precise equalities of complementary gamete types at the end of meiosis, yet gross inequalities in reciprocal classes of progeny unaccompanied by zygotic lethality, restrict gamete loss to the period between the end of meiosis and the time of fertilization. Since the *SD* meiotic drive system is associated with a failure of development of a significant fraction of spermatids (PEACOCK, TOKUYASU and HARDY 1972), we have examined this possibility for sc^4sc^8 males at three levels: (1) progeny production, (2) light microscopy of the testis and (3) electron microscopy of individual cysts.

Progeny production analysis: HARTL, HIRAIZUMI and CROW (1967) have shown, in some mating regimes, a reduction in the number of progeny produced by SD/SD^+ males relative to SD^+/SD^+ controls. They inferred from these results that SD^+ sperm undergo dysfunction in SD/SD^+ males. If this is true for sc^4sc^8 we would expect those males with high nondisjunction levels to have fewer progeny than males with lower nondisjunction levels. We have not sampled sperm from sc^4sc^8 males over their entire fertile period, but we have some data of maximum sampling for shorter periods. In the continuous 25° treatment in experiments 1 and 2 males have been grouped into nondisjunction intervals. The data are summarized in Table 6. The major point arising from this table is that an inverse correlation exists between progeny number and the extent of meiotic drive. This relationship is consistent with a sperm dysfunction mechanism for the sc^4sc^8 system. In an additional experiment (8) in which nondisjunction frequencies showed a clear-cut bimodal distribution, probably due to a segregating dominant autosomal modifier, the same phenomenon was observed (Table 6).

Progeny numbers need not accurately reflect sperm production even under

TABLE 6

Comparisons of the productivities of sc^4sc^8 *males having different nondisjunction frequencies*

	Sperm sampling period (days)	Y chromosome used	Progeny/male	Number of males tested	Meiotic drive X/(X+Y)	Meiotic drive O/(O+XY)	Nondisjunction frequency O/(O+X) Interval	Nondisjunction frequency O/(O+X) Mean
Experiment 1	15		448	17	0.64	0.91	0.15–0.20	0.17
(25°)	15	y^+Y	328	13	0.68	0.93	0.20–0.25	0.22
	15		235	6	0.72	0.96	0.25–0.35	0.30
Experiment 2	15		699	20	0.59	0.82	0.10–0.15	0.12
(25°)	15	B^SY	635	17	0.62	0.86	0.15–0.20	0.17
	15		576	4	0.67	0.92	0.20–0.25	0.22
Experiment 8	3	y^+Y	209	176	0.55	0.78	0.05–0.25	0.10
(25°)	3		113	71	0.73	0.97	0.35–0.80	0.54

conditions of maximized sampling procedures. This is shown by the following experiments (9 and 10) where transferred sperm and recovered progeny were simultaneously monitored.

Males 24 hr old were placed individually with a single y virgin female (2–3 days old) and observed. Following a mating the male was removed and placed with another y virgin female. Approximately half of the mated females were dissected after 2 hr and the number of sperm stored in the ventral receptacle and spermathecae determined with the aid of phase optics (see PEACOCK and ERICKSON 1965). For progeny analysis, the remaining females were regularly subcultured every second day until they became sterile.

The results of these experiments (Table 7) shows that for males; raised at 18°, where meiotic drive is much reduced, the recovery of stored sperm is twice as

TABLE 7

Comparisons of stored sperm and recovered progeny from inseminations of y/y *females* at 25° by* sc^4sc^8/y+Y *males raised at 18° and 25°*

	Mean number stored sperm	Mean number progeny	Ratio of recovered sperm (progeny)/stored sperm	Meiotic drive X/(X+Y)	Meiotic drive O/(O+XY)	Nondisjunction frequency O/(O+X)	Total progeny
Experiment 9							
Mating 1, 25° ♂	388(32)	160(36)	0.41	0.60	0.83	0.11	5756
Mating 2, 25° ♂	181(27)	79(24)	0.44	0.58	0.79	0.11	1901
Mating 1, 18° ♂	404(36)	344(18)	0.85	0.50	0.56	0.04	6187
Mating 2, 18° ♂	174(30)	162(32)	0.93	0.50	0.53	0.03	5197
Experiment 10							
Mating 1, 25° ♂	324(19)	84(44)	0.26	0.59	0.84	0.16	3683
Mating 2, 25° ♂	200(19)	75(47)	0.38	0.61	0.86	0.19	3522
Mating 1, 18° ♂	404(37)	237(63)	0.59	0.51	0.55	0.04	15208
Mating 2, 18° ♂	174(29)	210(42)	1.21†	0.52	0.60	0.06	8545

* The number of females dissected or used for genetic analyses is shown in parentheses.
† Not significantly different from 1.00.

efficient as for males raised at the higher temperature. Thus it is difficult to compare progeny production between males having temperature-induced differences in meiotic drive. A difficulty is also evident within temperatures. If the first matings of the 18° males of the two experiments are compared, it can be seen that although the mean numbers of stored sperm per insemination are identical, the mean numbers of recovered progeny are grossly different. Furthermore the progeny:sperm recovery ratios of the two matings of 18° males in experiment 10 differ greatly. It should be noted that even where the efficiency of sperm recovery varies in successive matings (Expt. 10, 25° and 18°) the genetic results do not differ significantly. Thus differences in the efficiency of sperm recovery are unrelated to the inequalities in recovery of complementary gamete types.

Another aspect of progeny production investigated by HARTL, HIRAIZUMI and CROW (1967) was the correlation between the extent of *SD* activity and the early onset of male sterility. This same correlation is evident in sc^4sc^8 males. In the experiments reported in Table 1, the proportion of fertile males at 25° decreases more rapidly with time than at 18° (Figure 3). The difference between the half-lives of fertility of the high meiotic drive males (25°) and the low meiotic drive males (18°) must be underestimated here, since the 18° males were in fact mated at 25° and in their later broods have the higher degree of drive characteristic of that temperature (see Figure 1). It could be expected that continuous 18° culture would have further extended the fertile period. These results complement the progeny production data and are consistent with a sperm dysfunction mechanism for the sc^4 sc^8 meiotic drive system.

Light microscope analysis: In the case of segregation distorted, the improper

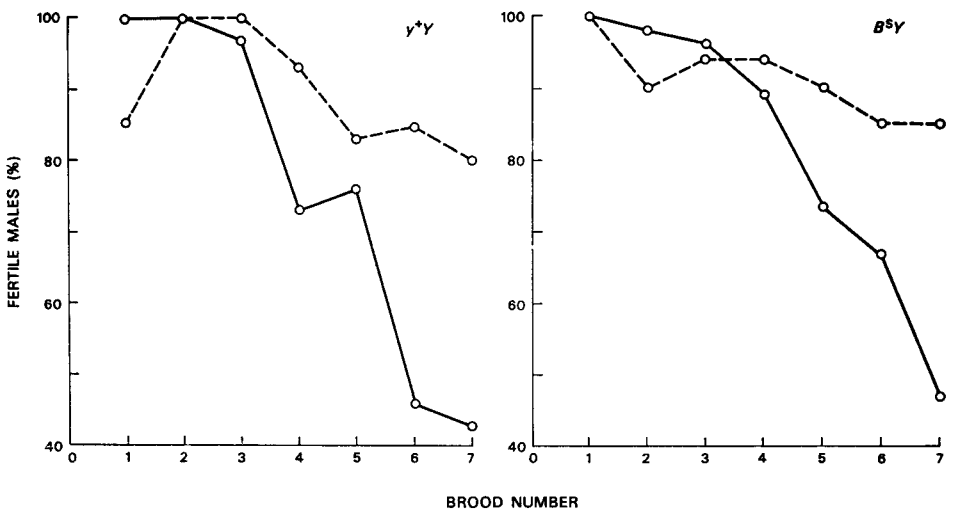


FIGURE 3.—Fertility periods of $sc^4sc^8/y+Y$ and sc^4sc^8/B^SY males raised until eclosion at 25° (——) or 18° (– – –), then mated and brooded at 25°. The data are from the experiments detailed in Table 1. In Experiment 1 ($sc^4sc^8/y+Y$) the number of males used in the 18° and 25° experiments were 40 and 37 respectively. In Experiment 2 (sc^4sc^8/B^SY) the number of males used in the 18° and 25° experiments were 20 and 45 respectively.

development of SD^+ spermatids, first analyzed in the electron microscope, was also detectable with the light microscope (PEACOCK, TOKUYASU and HARDY 1972). In contrast to control males, SD/SD^+ males possessed refractile bodies in the base of the testis that represented the syncytial spermatids which had been separated from the normally developed spermatids in the coiling phase of spermiogenesis. We examined testes of sc^1sc^8 males to determine if such structures were present.

Males (0–24 hr old) were mated with y virgin females (2–3 days old) for two days, at the end of which males were removed, the testes dissected into 0.7% sodium chloride, immediately transferred into freshly mixed glacial acetic acid and ethanol (1:3) and stored at −10° until genetic data were available from the females. Selected testes were

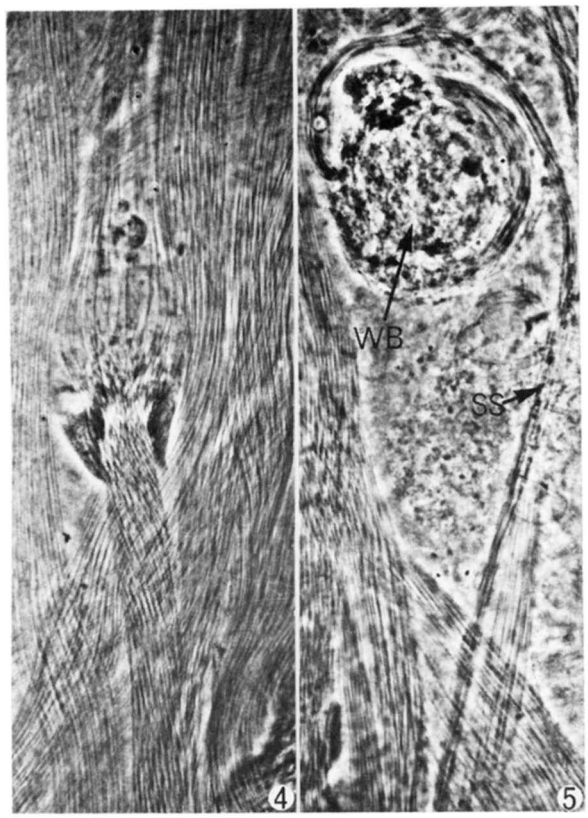
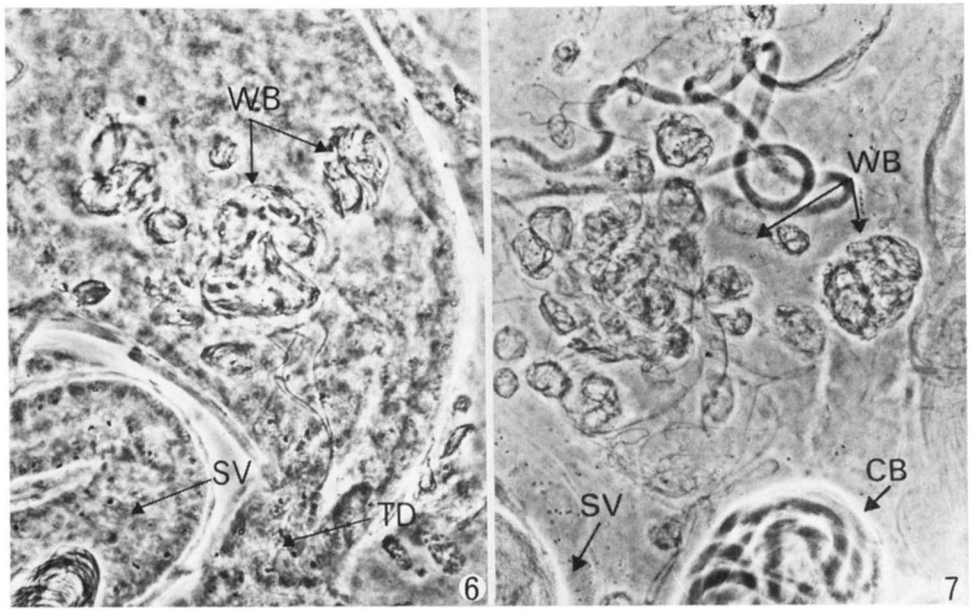


FIGURE 4.—Photomicrographs of portion of the mid-region of the testis of a $sc^1sc^8/y+Y$ male showing an individualization bulge in a spermatid cyst. The spermatids below the bulge have been individualized, whereas those above it are still in a syncytial state. Discarded nucleoplasm and cytoplasm are contained within the budge (×510).

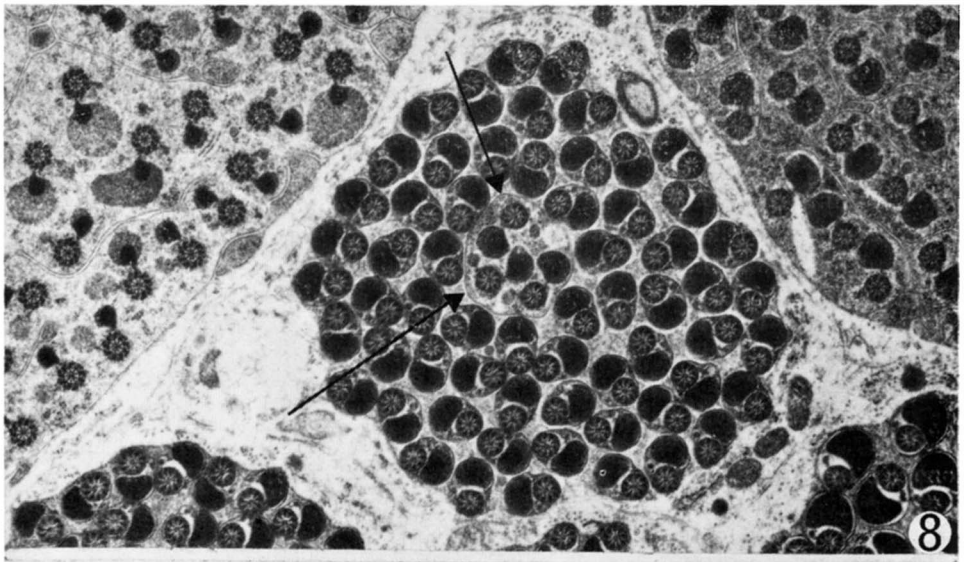
FIGURE 5.—In this photomicrograph, syncytial spermatids of a cyst have been partially included into a waste bag (WB). This inclusion will progress as the cyst continues to coil and the waste bag is further drawn to the base of the testis. The syncytial spermatids (SS) have a darkened and granular appearance (×510).

treated with 1 N HCl at 60° for five minutes and stained in Feulgen for 30 minutes. The stained testes were transferred to a microscope slide in a drop of 45% acetic acid. Preparations were examined and photographed with phase optics.

In these preparations it was possible to identify the major features of spermiogenesis previously described by electron microscopy (TOKUYASU, PEACOCK and HARDY 1972*a*,*b*). Individualization bulges Figure 4) were readily identifiable and at the apex of the testis waste bags were clearly seen. It was also possible to detect waste bags which were in the process of being drawn down to the base of the testis as the spermatids in a cyst were coiled. In a majority of these waste bags abnormal spermatid tails were visible (Figure 5). Abnormal tails were also seen along the linear spermatid bundle following individualization; they were differentiated from the normally individualized spermatids by virtue of their refractivity and granular appearance. Invariably at the base of the testis waste bags containing refractile abnormal spermatid tails were found (Figures 6 and 7). Wild-type controls had only normal waste bags without any included spermatid tails. There were no easily quantifiable differences between males exhibiting high and low values of nondisjunction except that males showing high values tended to have larger waste bags at the base of the testis than did sibs with lower nondisjunction values. In general there were approximately ten waste bags containing abnormal spermatids at the base of each testis. This number probably



FIGURES 6 and 7.—Photomicrographs of the basal regions of testes from $sc^{1}sc^{8}/Y+Y$ males. CB—coiled bundle of spermatids in the base of the testis; WB—waste bags predominantly of degenerating spermatids; SV—seminal vesicle and TD—testicular duct. (Figure 6, ×450; Figure 7, approx. ×500).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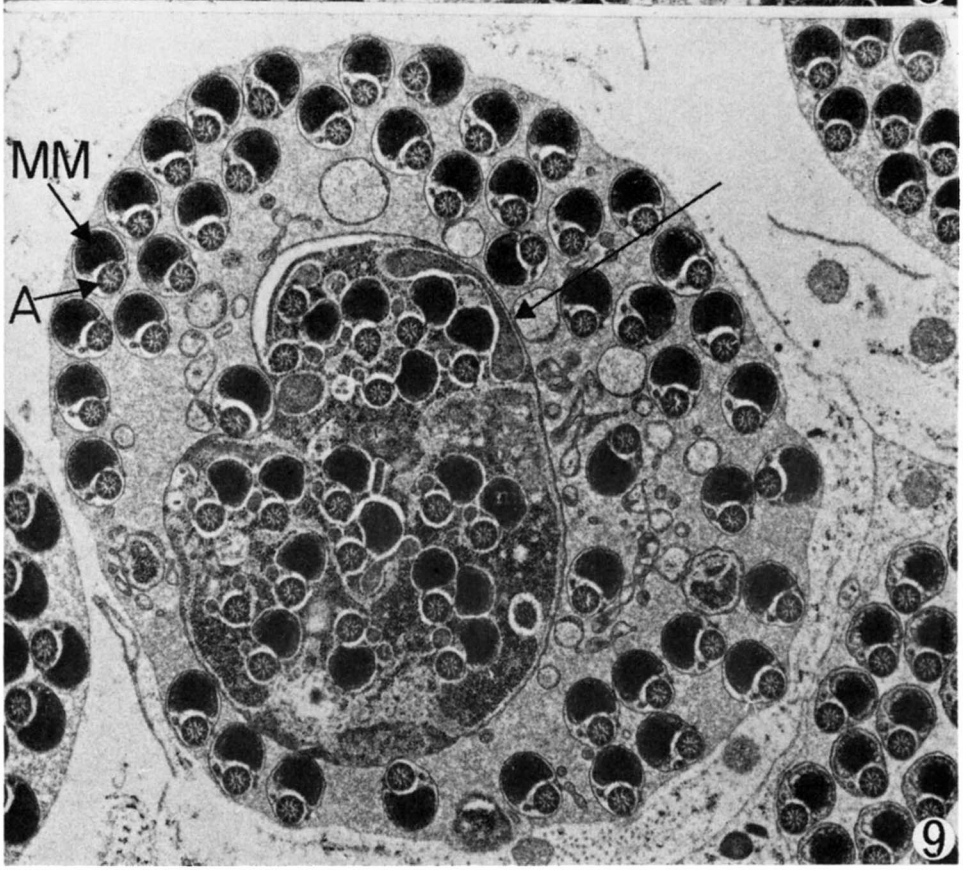
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reflects a situation in which waste bags are retracted to the base and resorbed by the cells of the terminal epithelium. These observations suggest a considerable amount of spermatid breakdown analogous to that seen in the *SD* system where non-individualized members of cysts are included in the waste bags.

Electron microscope analysis: The light microscope analysis showed evidence of spermatid breakdown comparable to that in *SD* heterozygotes. We have examined testes of sc^4sc^8 males in the electron microscope to determine if there is abnormal spermatid development similar to the *SD* pattern, and if so, to determine whether the number of aberrant spermatids per cyst is correlated with the frequency of nondisjunction and hence with meiotic drive.

Experiment 11: Males (0–24 hr old) were mated with 3 y virgin females (2–3 days old) for two days. The females were transferred to fresh food every third day for three broods; meiotic drive and nondisjunction were assayed among the progeny of each male. Following the two-day mating period testes were dissected in 0.7% sodium chloride and immediately transferred to ice-cold 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for two hours. After three washes in 0.1 M buffer, the testes were post-fixed in 2% osmium tetroxide in 0.1 M sodium phosphate buffer for two hours in the cold. They were then washed three times in buffer, taken through a graded series of ethyl alcohol, left overnight in 70% alcohol, and finally brought to room temperature in 100% ethanol. Further dehydration in propylene oxide was followed by a graded series into an Epon-Araldite mixture (MOLLENHAUER 1964) and vacuum embedding and polymerizing was performed at 85° for 24 hr. Thick sections (0.2–1.0 μ) were cut and inspected under phase optics in the light microscope. Silver sections were cut (Reichert OmU2 ultramicrotome), stained in uranyl acetate and lead citrate (REYNOLDS 1963) and examined in a Philips EM200 electron microscope. All scoring was carried out on photographs magnified ×19600.

Experiment 12: This experiment differed from Experiment 11 in that the initial mating period extended for four days and the testes, after fixation in glutaraldehyde and post-fixation in osmium tetroxide, were stored in phosphate buffer at 4° for three weeks until progeny analyses revealed the meiotic drive characteristics of the males. Testes were then embedded, sectioned and examined as before. No detectable ultrastructural deterioration was noted as a result of the extended storage in cold buffer.

Since preliminary observations of bundles of spermatid heads revealed no obvious developmental abnormalities, the analysis was restricted to the spermatid

FIGURE 8.—Transverse section of a cyst from a $sc^4sc^8/y+Y$ male showing 61 individualized spermatid tails and 3 spermatid tails remaining within the syncytial membrane (arrow). (×15,600).

FIGURE 9.—Transverse section of a cyst from a $sc^4sc^8/y+Y$ male showing 46 individualized spermatid tails and 18 spermatid tails remaining within the syncytium (arrow). (×15,600) A—axoneme; MM—major mitochondrial element.

TABLE 8

Electron microscope and genetic data from $sc^4sc^8/y+Y$ *males crossed to* y/y *females*
(Genetic data were obtained from those males used for electron microscopy)

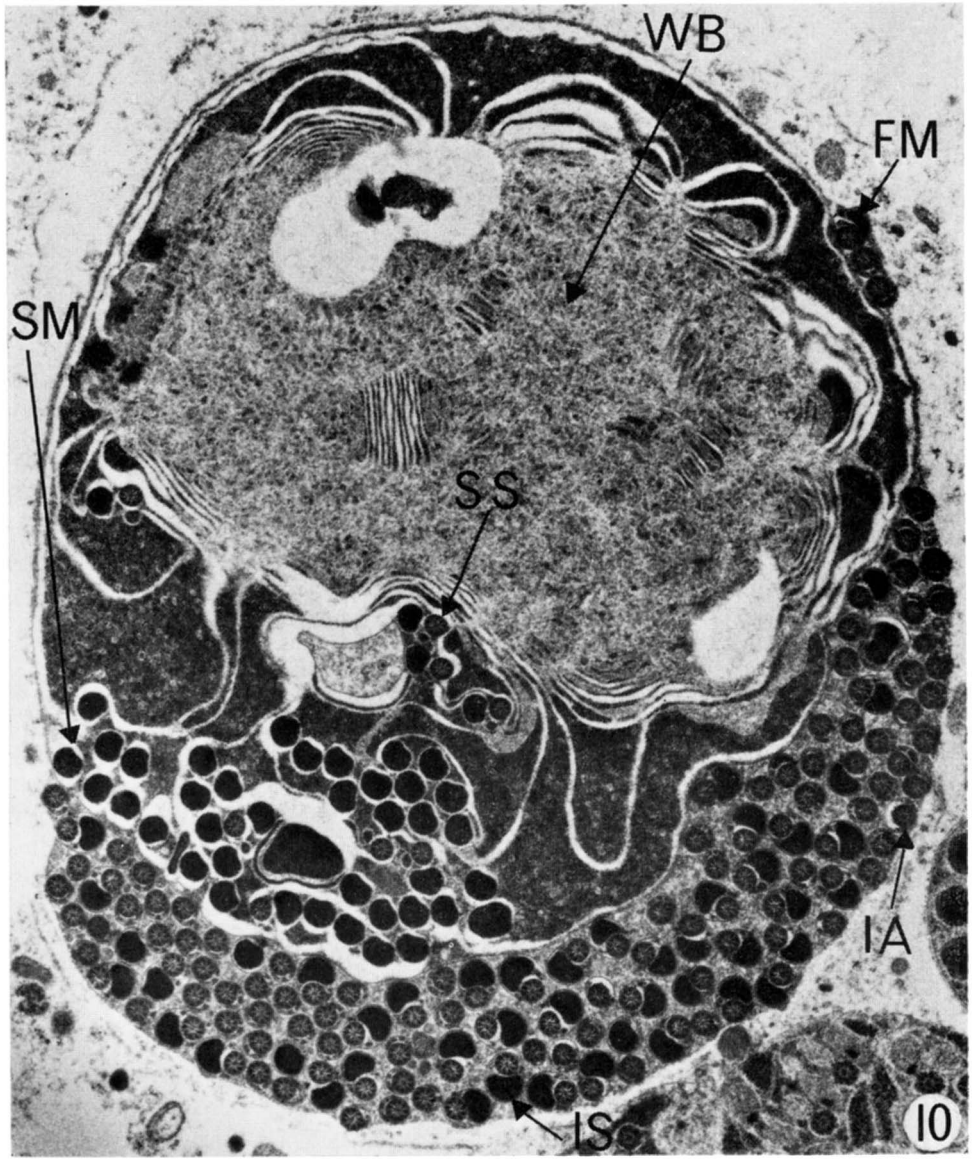
		Mean no. of syncytial spermatids/bundle	Mean no. of bundles examined	No. of males examined	Mean no. of progeny/male
Disjunctional meiotic drive coefficient $[X/(X+Y)]$					
Interval	Mean				
0.50–0.55	0.52	6.7	14.0	7	266
0.55–0.60	0.59	4.7	9.2	5	288
0.60–0.65	0.63	11.5	4.8	4	374
0.65–0.70	0.68	6.9	9.4	10	268
0.70–0.75	0.72	10.7	7.7	3	137
Nondisjunctional $[O/(O+XY)]$ meiotic drive coefficient					
Interval	Mean				
0.60–0.70	0.66	6.0	13.2	4	290
0.70–0.80	0.75	6.3	12.4	7	276
0.80–0.90	0.85	7.3	7.2	11	317
0.90–1.00	0.95	10.0	8.6	7	186
Nondisjunction frequency [O/(O+XY]					
Interval	Mean				
0.10–0.15	0.13	3.6	5.2	5	347
0.15–0.20	0.19	9.4	9.3	4	244
0.20–0.25	0.23	7.9	9.6	5	373
0.25–0.30	0.28	5.7	14.0	7	265
0.30–0.35	0.32	8.0	6.8	4	203
0.35–0.40	0.37	13.0	13.0	3	183
0.50	0.50	12.0	5.0	1	97

tails of post-individualization cysts both in the precoiled and coiled states. Sections across post-individualization cysts which had not been coiled revealed both individualized and syncytial (non-individualized) spermatids (Figures 8 and 9). As in the case of *SD* heterozygotes (PEACOCK, TOKUYASU and HARDY 1972), the syncytial spermatids were separated from the individualized ones during coiling, were included in the waste bag, and were broken down in the basal region of the testis.

The numbers of individualized and syncytial spermatids were scored in all possible cysts in a testis. In order to avoid scoring any cyst twice, only one transverse section in the mid-region of the testis was used for analysis. In Experiment 11, data were obtained from both testes in nearly all males; males were 3 days old at dissection, and there was an average of ten scorable cysts/testis. In Experi-

FIGURE 10.—Electron micrograph of a transverse section of a cyst in the lower region of an individualization bulge of a $sc^4sc^8/y+Y$ male. Of the 128 spermatids in the cyst, 42 have been individualized normally (*IS*), 8 have remained in the syncytium (*SS*), and the remaining 78 spermatids show a separation of the axonemal and mitochondrial elements of the tails. The axonemal elements appear to have been individualized (*IA*) and some mitochondrial elements have been retained in the syncytium (*SM*). In addition, some spermatids show failure of full development of the mitochondrial elements (*FM*). The figure includes a section across a lobe of a waste bag (*WB*) formed by the process of individualization. (×12,600).

ment 12, only a single testis was examined from each male (5–6 days old at dissection) and only an average of five cysts/testis were scorable. Since analysis of the regression of syncytial sperm *versus* nondisjunction showed the two experiments to be homogeneous, the data were pooled. Individual males were ranked either by the meiotic drive coefficients or by the frequency of nondisjunction. A summary of these data is given in Table 8. Regression analyses using individual male data revealed that the mean number of syncytial sperm showed a positive correlation to the magnitude of both meiotic drive coefficients and to increasing nondisjunction frequency (*disjunctional meiotic drive coefficient*, $Y = 13.2\,X + 0.7$; *nondisjunctional meiotic drive coefficient*, $Y = 12.7\,X + 3.0$;



nondisjunctional frequency, $Y = 23.1\ X + 1.6$). These positive correlations indicate that, as in the case of *SD*, abnormal spermatid development underlies the meiotic drive system.

If the difference in recovery of the complementary products is attributed to loss of the *Y* and the *XY* gametes, then at the mean level of nondisjunction observed in these experiments, we have calculated from the relationships in Table 4 that there should be 15 syncytial spermatids per cyst. The observed number was 9 per cyst. Since in some stocks of *SD* heterozygotes the observed frequency of abnormal tail development underestimates the actual level of spermatid failure (which can be more precisely estimated by analysis of spermatid heads), it is conceivable that this same underestimate occurs in sc^4sc^8 males. In two males (nondisjunction frequencies of 0.39 and 0.50) bundles were seen in which the number of syncytial spermatids exceeded 32. This indicates that at the highest levels of nondisjunction, spermatids other than the *Y*- and *XY*-containing ones may fail to develop normally.

Abnormalities other than failure of individualization were occasionally observed. Cysts were seen in which the mitochondrial and axonemal elements of some spermatids were completely separated (Figure 10). Another aberration was the incidence of bundles having 96 or 128 spermatids rather than 64, presumably reflecting the occurrence of extra gonial divisions. The two aberrations did not occur sufficiently frequently to permit a test for any correlation to nondisjunction frequency.

CONCLUSIONS

The sc^4sc^8 *meiotic drive system:* These experiments have provided evidence that the sc^4sc^8 meiotic drive system is associated with abnormal spermatid development. Furthermore, a positive correlation exists between the number of abnormally developed spermatids in a cyst and the extent of inequality in recovery of complementary gamete classes. The meiotic, genetic and spermiogenic data are consistent with the conclusion that the abnormal syncytial spermatids are generally those carrying either a *Y* chromosome or both the *X* and *Y* chromosomes. Since the cytological results have shown that in disjunctional meiotic products there are equal numbers of *X* and *Y* cells, and that there are equal numbers of *O* and *XY* cells in nondisjunctional products, it follows that if the deficiencies in recovery of *Y* and *XY* gametes are explained by improper development of only *Y* and *XY* spermatid then, (1) irrespective of the frequency of nondisjunction, there should be at least 32 normal spermatids in each cyst, and (2) nondisjunction as measured genetically by the ratio $O/(O+X)$ should correspond closely to the cytological measure. Both of these conditions are substantiated by the data. The genetic and cytological frequencies of nondisjunction are in excellent agreement over a wide range of nondisjunction (0.07–0.46, Table 4, PEACOCK and MIKLOS 1973), and in only two cysts in males with high meiotic drive levels have fewer than 32 normal spermatids been detected.

The temperature-shift experiments have established that the initial determination of aberrant spermatid development occurs at meiosis and have shown that an induced change in the probability of *X*-*Y* bivalent formation is accompanied

by changes in meiotic drive coefficients. The positive correlation between the frequency of nondisjunction and the magnitude of the meiotic drive coefficients suggests that the conditions which promote increased nondisjunction may also determine the probability of abnormal spermatid development. These conditions, since they affect the frequency of nondisjunction, must apply at or before metaphase I. An obvious determinant of nondisjunction of the sex chromosomes is their probability of pairing. Pairing of homologs at meiosis seems removed from the process of spermiogenesis; however we feel that several lines of evidence support the proposal that pairing is of major importance in determining the magnitude of meiotic drive (PEACOCK and MIKLOS 1973; BAKER and CARPENTER 1972).

The observation that the unequal gamete recoveries are correlated with abnormal spermatid development is consistent with the finding in the temperature-shift experiments that meiotic drive, in contrast to nondisjunction, has post-meiotic as well as meiotic components. Thus the magnitude of meiotic drive may be primarily determined at the time of chromosome pairing, but may be varied to some extent during the process of spermiogenesis.

Comparison to the SD *meiotic drive system:* Two features common to both the *SD* and sc^4sc^8 systems are that high meiotic drive males usually produce fewer progeny than males with lower meiotic drive levels, and that the average period of male fertility is inversely correlated with the magnitude of the meiotic drive system. Our data on the efficiency of sperm storage and usage have indicated that progeny production should be regarded with caution since similar insemination levels in females of the same genotype can result in very different numbers of progeny (Table 7). Therefore, if progeny number data are to be useful in analyses of the mechanisms of meiotic drive systems, they must be supplemented with direct investigations of sperm development, transfer and usage.

In neither system is there any explanation for the correlation between drive and the onset of sterility. In both types of male, sterility is not due to cessation of meiotic activity, since we have observed meiotic cysts after the onset of sterility. One possibility is that the period of fertility may be influenced by the extent of spermatid degeneration in the basal region of the testis; on this basis males with high levels of meiotic drive, and hence large numbers of degenerating spermatids, would be expected to have a shorter period of fertility than males with lower levels of meiotic drive.

The most obvious similarity between the *SD* and sc^4sc^8 systems is that both are associated with abnormal spermatid development. However this does not necessarily imply a common mechanism of action since different drive systems could have similar post-individualization cytology. Many lesions could cause abnormal spermatid development, and if the various aberrations resulted in failure of individualization of some spermatids in a cyst, then the processes of removal and degeneration of the abnormal spermatids would proceed in the manner described in our studies. In neither the *SD* nor sc^4sc^8 system is there any direct evidence on the nature of the primary lesion other than that the time of action is known to be at meiosis.

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