TEMPERATURE SENSITIVITY OF SEGREGATION-DISTORTION IN *DROSOPHILA MELANOGASTER*[1]

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SEGREGATION -distortion, a meiotic drive phenomenon occurring in *Drosophila melanogaster*, is caused by a locus designated *SD*. From previous studies (SANDLER, HIRAIZUMI and SANDLER 1959; SANDLER and HIRAIZUMI 1960a, b, c), it is known that the following properties characterize *SD*: (1) with only rare exceptions, a male heterozygous for *SD* and a structurally normal SD^+ chromosome produces progeny among which almost all possess the *SD* chromosome. The k value is the proportion of *SD*-bearing (among successful) sperm. From an ordinary heterozygote it is expected to be 0.50; male *SD* heterozygotes, however, usually show k values of 0.90–1.00. (2) Heterozygous females show a normal 1:1 segregation ratio, but their heterozygous sons will (with certain exceptions) again exhibit abnormal k values. (3) When the *SD* chromosome is heterozygous with inversions having one breakpoint near the centromere, such as *In(2LR)Cy*, segregation-distortation is inhibited. Thus close pairing is apparently necessary for *SD* to act. (4) An *SD* chromosome is insensitive to the distorting action of another *SD* chromosome. (5) The *SD* locus, which is complex in nature, lies in the centromere region of chromosome 2, between the markers purple and cinnabar. It is postulated that *SD* operates by nonrandomly orienting toward the "functional pole" during Meiosis I, thus being included in most of the functional sperm (PEACOCK and ERICKSON 1965). Segregation-distortion is widespread in nature (MANGE 1961; GREENBERG 1962; HIRAIZUMI and NAKAZIMA 1965). Almost all *SD* chromosomes recovered from nature possess inversions on the right arm of chromosome 2 which effectively suppress crossing over between *SD* and *St(SD)*; the latter, a modifier on the right tip of chromosome 2, maintains stably high k values (SANDLER and HIRAIZUMI 1960b).

Since several drive mechanisms were known to be temperature sensitive, it seemed desirable to examine the behavior of *SD* at different temperatures. Results of these experiments show that certain *SD* lines, when treated at 30°C or at 19°C, exhibit k values up to 25 percent lower than control males raised at room temperature. The greatest reduction in k values is observed 8–9 days following treatment, indicating that the most sensitive period occurs during or just prior to early meiosis.

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MATERIALS AND METHODS

The experiments to be described were done during the period 1961–1965, in two different places (University of Wisconsin and Western Reserve University), under similar but not identical conditions. The following stocks were used: *cn bw*, a standard laboratory stock marked by cinnabar and brown on chromosome 2; the phenotype is white eyes. *cn bw; e; ey*, carrying cinabar and brown on chromosome 2, ebony body color on chromosome 3, and eyeless on chromosome 4. This stock was used to check for possible inequality in recovery of chromosomes 3 and 4. *SD-72*, a stable (i.e. showing k values of about 0.9–1.0) *SD* chromosome collected in Madison, Wisconsin; *SD-205*, a stable *SD* chromosome collected in Baja California; *R-207*, a stable *SD* recombinant carrying brown; *R-206*, an unstable (i.e. showing k values from about 0.7–1.0) *SD* recombinant carrying brown.

Males heterozygous for the *cn bw* chromosome and one of the four *SD* chromosomes were treated at different temperatures (30°, 28–29° and 19°C), for varying lengths of time (1–12 days), at various stages of development (larva—adult). Treated males were mated individually to homozygous *cn bw* virgin females (in most cases obtaining several broods for each male), and k values were determined for each male by progeny counts. Details of methods will be given as specific experiments are described.

The nutrient medium at University of Wisconsin had a cornmeal-molasses-agar base, while that at Western Reserve University was cornmeal-Karo syrup-agar. Experiments done at Western Reserve involved *R-207* at 30° for 48- and 24-hour periods, and *R-207* at 28–29° for varying lengths of time. The remaining experiments were carried out at the University of Wisconsin.

Where statistical tests of significance were necessary, a 2×2 contingency table was used to obtain the chi-square value.

RESULTS

Preliminary Experiments with R-207 *and* SD-205

A preliminary test for temperature sensitivity consisted simply of raising *R-207/cn bw* males through eclosion in a 30°C incubator, and then checking their k values. The setup was as follows: pair matings of *R-207/ cn bw* males to *cn bw* females were made at room temperature (about 25°). Two days later, parents were discarded and the vials were put into a 30° incubator. Upon eclosion, F_1 *R-207/ cn bw* males were collected and individually mated to two virgin *cn bw* females at room temperature (brood 1). After three days, each male was transferred to two virgin *cn bw* females (brood 2). Parents were discarded three days later.

Not surprisingly, the prolonged heat treatment adversely affected development and fertility of the F_1. Only about 10% of the parental matings produced viable F_1; of these 447 males tested, 60% were sterile in brood 1, and 29% in brood 2. As shown in Table 1, the reduction of k values in fertile heat-treated males was striking: less than 3% of the treated males in either brood exhibited k values of 0.95 or higher, as compared to over 98% in the untreated males. Mean k values for the two broods of treated males are essentially the same (0.76 and 0.77).

To test heritability of the temperature effect, 275 F_2 *R-207/cn bw* males (the sons of 31 F_1 males which exhibited k values ranging from 0.55 to 1.0, averaging 0.74) were individually mated to *cn bw* females. The k values of the F_2 sons ranged only from 0.95 to 1.0, with a mean of 0.99. So the temperature effect is not heritable.

The preliminary experiment did not, of course, indicate which developmental

stage(s) are sensitive to the heat effect. By administering heat for short periods at various stages of development, sampling sperm in a series of broods, and using information about the chronology of sperm development, it is possible to identify the sensitive period. Several studies indicate that meiosis is first observed in the prepupal stage of development. KHISHIN (1955), from cytological observations of spermatogenesis, found that primary spermatocytes are first seen dividing at the onset of prepupation. Bundles of early spermatids are seen at pupation proper, while bundles of fully formed spermatozoa are first observed in older pupae. The testis of a newly emerged male contains all stages of spermatogenesis from spermatogonia to fully formed spermatozoa. AUERBACH (1954) irradiated young males, then sampled their sperm in 3-day broods. She noted that, in most cases, treated spermatocytes (identified by first occurrence of radiation-induced crossing over) became available for insemination in the third brood, i.e. 6–9 days following treatment. The first brood was derived from treated mature sperm, the second brood mostly from cells treated during spermiogenesis, and the fourth brood from cells treated as spermatogonia.

R-207 males at 30°C with 48-hour periods: Parental matings (one *R-207/cn bw*; *e*/+ male with two *cn bw*; *e*; *ey* females) were made and left at room temperature. Parents were discarded after 24 hours, and the vials containing developing F_1 were divided into four groups to be treated in a 30° incubator as follows:

group DE: during days 4 and 5 of development
group EF: during days 5 and 6 of development
group FG: during days 6 and 7 of development
group GH: during days 7 and 8 of development

Except for the above-mentioned 48-hour periods at 30°, development took place at room temperature. F_1 *R-207/cn bw* males (20–30 per group) were collected within 24 hours of eclosion and individually mated to *cn bw*; *e*; *ey* females. Every three days, each male was transferred to 2–3 virgin *cn bw*; *e*; *ey* females until four 3-day broods were obtained.

Results, summarized in Figure 1, show a striking reduction in k values about 8–10 days following treatment. The greatest reduction probably occurs on the 8th day following treatment, as this is the one day common to the four groups showing the lowest mean k values.

R-207 *males at 30°C for 24-hour periods:* This experiment was done at the same time as the one above, and utilized the same mating scheme. Seven groups of developing F_1 males were treated in a 30° incubator as follows:

group D: during day 4 of development
group E: during day 5 of development
group F: during day 6 of development
group G: during day 7 of development
group H: during day 8 of development
group I: during day 9 of development
group J: during day 10 of development

Otherwise, development proceeded at room temperature. As above, four 3-day

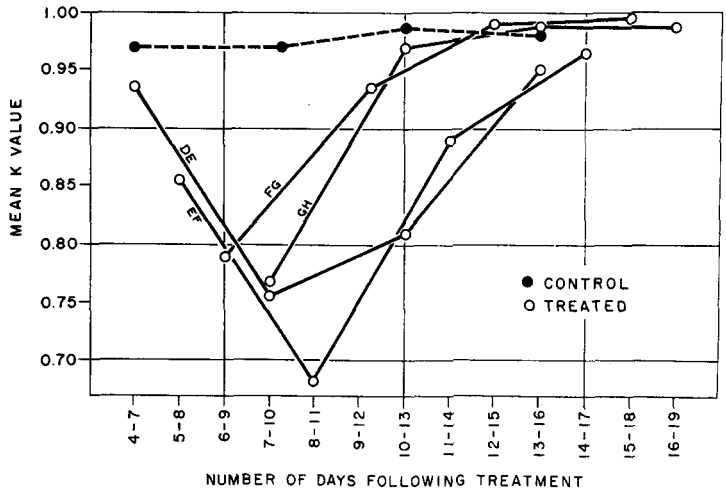

FIGURE 1.—Mean k values (proportion of *SD* progeny) of *R-207/cn bw* males following a 48-hour period at 30°C. The lines join consecutive broods from a given group of males. The abscissa is the number of days which elapsed between treatment and insemination.

broods (designated by numbers 1–4) were obtained for each male. Table 2 and Figure 2 summarize the results of this experiment; where several broods (such as D-1, G-2 and J-3) represent the same number of days following treatment, their k values are pooled to arrive at a single grand mean k value. Note that k values are most depressed in the interval of 8–11 days following heat treatment: mean k values of less than 0.90 occurred for the periods of 7–9, 8–10 and 9–11 days following treatment. Here it appears that the greatest effect is seen 9 days following treatment. The fact that the mean k value for the interval 6–8 days following treatment is moderately depressed (0.909), while that for 5–7 days

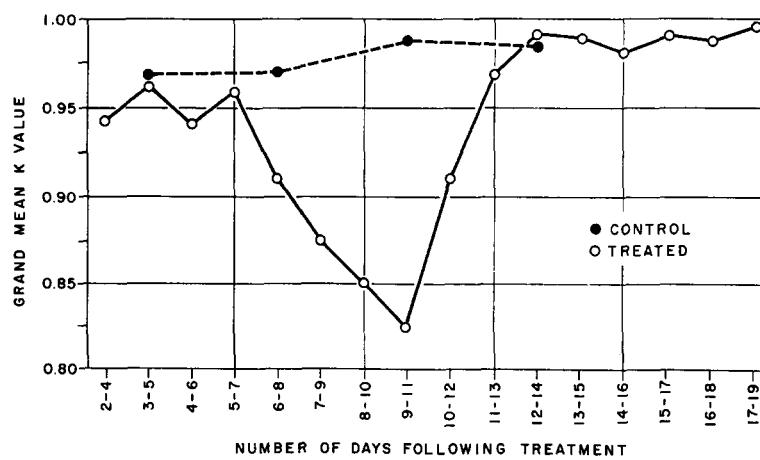

FIGURE 2.—Mean k values (proportion of *SD* progeny) of *R-207/cn bw* males following a 24-hour period at 30°C.

TABLE 1

Distribution of k *values (proportion of SD progeny) for* R-207/cn bw *males raised through eclosion at* 30°C, *as compared to control males raised at room temperature*

	Percent males		
k value	Brood 1	Brood 2	Controls
0.98–1.00	2.3	0.3	96.4
0.95–0.97	0.6	2.0	2.1
0.92–0.94	2.3	4.3	1.1
0.89–0.91	6.3	4.6	0.4
0.86–0.88	6.4	6.9	..
0.83–0.85	10.9	10.8	..
0.80–0.82	11.4	9.8	..
0.77–0.79	8.6	8.9	..
0.74–0.76	14.9	14.4	..
0.71–0.73	5.1	13.8	..
0.68–0.70	12.5	9.6	..
0.65–0.67	5.7	7.5	..
0.62–0.64	4.0	3.7	..
0.59–0.61	4.0	1.7	..
0.56–0.58	2.3	1.4	..
0.53–0.55	1.7	0.6	..
0.50–0.52	..	..	..
0.47–0.49	0.6	..	..
0.44–0.46	..	..	..
0.41–0.43	..	..	..
0.38–0.40	0.6	..	..
Mean *k* value	0.761	0.770	0.995
No. of males tested	175	305	281
Total counts	14,211	40,682	28,907

following treatment is very high (0.958), indicates that the 8th day following treatment is also affected. Likewise, some influence is seen on the 10th day following treatment.

R-207 *males at about 28°C for varying lengths of time:* In these experiments a 28–29°C incubator (more often 28° than 29°) was used. Parental pair matings (*R-207/cn bw*; *e*/+ male × *cn bw*; *e*; *ey* females) were left at room temperature for 48 hours; then males were discarded while the females were transferred to fresh vials and allowed to lay eggs for 24 hours before being discarded. The original set of vials was used as groups B, C . . . . . I, receiving 24-hour heat treatments. The second set of vials (comprising groups A, AB, AC . . . . . AH) was immediately placed in the incubator, one group being removed each day thereafter for eight days. The treated *R-207/cn bw*; *e*/+ sons were collected within 24 hours of eclosion and mated to *cn bw*; *e*; *ey* females (obtaining three 3-day broods for each male) to check their *k* values.

Results show very little effect of 28–29°C treatment. Nearly all the treated males exhibited *k* values above 0.97. Eight broods had mean *k* values ranging

TABLE 2

Summary of results from treatment of R-207/cn bw *males at* 30°C *for 24-hour periods*

No. of days after treatment	Mean k values of the broods designated in parentheses	Weighted grand mean k value	No. of males tested
2–4	0.942 (J-1)	0.942	34
3–5	0.961 (I-1)	0.961	20
4–6	0.941 (H-1)	0.941	39
5–7	0.961 (G-1) 0.955 (J-2)	0.958	64
6–8	0.913 (I-2) 0.906 (F-1)	0.909	43
7–9	0.899 (H-2) 0.849 (E-1)	0.874	78
8–10	0.963 (D-1) 0.804 (G-2) 0.841 (J-3)	0.851	68
9–11	0.896 (F-2) 0.740 (I-3)	0.824	43
10–12	0.967 (E-2) 0.852 (H-3)	0.910	76
11–13	0.992 (D-2) 0.957 (G-3)	0.968	43
12–14	0.990 (F-3)	0.990	22
13–15	0.991 (H-4) 0.985 (E-3)	0.988	68
14–16	0.987 (D-3) 0.978 (G-4)	0.981	41
15–17	0.990 (F-4)	0.990	17
16–18	0.986 (E-4)	0.986	33
17–19	0.994 (D-4)	0.994	11

from 0.91–0.96. Only two broods showed sizeable reductions in k values about 9–10 days following treatment: AH-3 (treated for 8 days) with a mean k value of 0.83, and AG-3 (treated for 7 days) with a mean k value of 0.88. These data are interpreted to mean that the threshhold for the heat effect on segregation distortion occurs just above 28°.

R-207 *males raised at 19°C through eclosion:* A group of 34 *R-207/cn bw* males, raised through eclosion in a 19° cold room and mated individually to *cn bw*; *e*; *ey* females, showed a small (though highly significant) drop in k values. As seen in Table 3 these males had a mean k value of 0.93, and a distribution that

TABLE 3

Distribution of k *values for* R-207/cn bw *males raised through eclosion at 19°C*

	Percent males	
k value	19°C	Controls
0.98–1.00	23.5	96.4
0.95–0.97	26.5	2.1
0.92–0.94	23.5	1.1
0.89–0.91	5.9	0.4
0.86–0.88	5.9	. .
0.83–0.85	11.8	. .
0.80–0.82	. .	. .
0.77–0.79	2.9	. .
Mean k value	0.930	0.995
No. of males tested	34	281
Total counts	1,658	28,907

TABLE 4

Distribution of k *values for* SD-205 *males raised through eclosion at 19°C, as compared to control males raised at room temperature*

	Percent males	
k value	19°C	Controls
0.98–1.00	55.3	92.4
0.95–0.97	21.4	4.7
0.92–0.94	9.6	1.9
0.89–0.91	8.2	0.9
0.86–0.88	3.5	. .
0.83–0.85	0.7	. .
0.80–0.82	. .	. .
0.77–0.79	0.7	. .
0.74–0.76	. .	. .
0.71–0.73	0.7	. .
Mean *k* values	0.962	0.993
No. of males	145	106
Total counts	22,721	10,044

differed considerably from that of the controls. (N.B. This experiment was done at the same time as the first one, and therefore the same controls are used).

SD-205 *males raised at 19°C through eclosion:* The non-recombinant chromosome *SD-205* was also tested for sensitivity to cold treatment; results, summarized in Table 4, show a small but highly significant drop in *k* values. These 145 males had a mean *k* value of 0.962, and a distribution of *k* values somewhat different from that of control males.

Experiments with Lines R-206 *and* SD-72

It has been established that a stable *SD* recombinant (*R-207*), when exposed to 30°C for 24 hours or longer, exhibits substantially lower *k* values in sperm sampled about eight or nine days following treatment. In addition, this line and line *SD-205* show somewhat reduced *k* values when raised at 19°C through eclosion.

The question arises whether temperature shock might under some conditions *enhance*, rather than decrease, *k* values. Such an effect is undetectable in a stable *SD* chromosome, of course, since *k* values are about as high as they can be. But with an unstable or semistable recombinant, an increase (as well as a decrease) in *k* values would be noticed. Furthermore, such a line might be more sensitive to environmental modification.

With this in mind, as well as the desire to (1) refine the test for the sensitive period, and (2) observe the effect of 19°C treatment in 24-hour doses, the following series of experiments was undertaken. A semistable *SD* recombinant (*R-206*) and a stable non-recombinant (*SD-72*) were each tested for sensitivity to 24-hour doses of 30°C and 19°C. The stages treated ranged from larvae to late pupae,

and sperm were sampled in one-day broods including collectively days 5–14 following treatment.

The experimental design was as follows: males of the constitution *SD-72/cn bw* and *R-206/cn bw* were individually mated to *cn bw* females and left at room temperature (26°C) until pupae were seen on the sides of the vials. Then parents were discarded, and the developing F_1 were treated as follows: (a) some vials were put in a 30°C incubator for 24 hours, and then returned to 26°C; (b) some vials were put in an 19°C cold room for 72 hours, and then returned to 26°C; (c) the remaining vials were kept at 26°C as controls. When the F_1 flies began hatching (five or six days after treatment), groups of usually about 45 males (designated W, X, Y and Z) were collected on each of four successive days from each experimental set. Each male was mated (transferring without etherization) to two or three *cn bw* virgins every day for six successive days (broods 1–6), so that k values could be determined for sperm sampled in six non-overlapping 24-hour periods. In general, the F_1 males comprising groups W were treated as pupae (being the first to hatch, they were also the most advanced stages treated), while Z group males were prepupae or larvae when treated. In all, about 150–170 males (each brooded up to six times) were tested for each of the four experimental groups. About 100 males (each brooded six times) comprised the control groups.

Of the four experimental sets (heat-treated *SD-72* and *R-206*, cold-treated *SD-72* and *R-206*), all except the heat-treated *SD-72* chromosome exhibited a striking reduction in k values eight or nine days following treatment. The data are summarized in Tables 5–8 and in Figure 3. The latter consists of four graphs representing the pooled results of each experiment. Each graph compares mean control k values with the weighted means of k values for each day (following

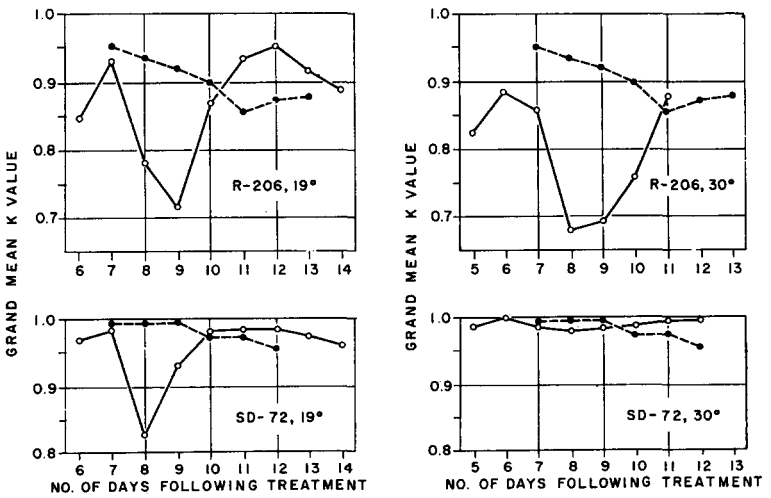

FIGURE 3.—Mean k values (proportion of *SD* progeny) of *R-206/cn bw* and *SD-72/cn bw* males following a 24-hour period at 30°C or 19°C. Hollow circles are treated; solid circles are controls.

TABLE 5

Summary of results from treatment of R-206/cn bw *males at* 30°C *for 24 hours*

No. of days following treatment	Mean k values of groups: W	X	Y	Z	Grand mean k value	No. of males tested	Total counts
5	0.848	0.799	....	....	0.823	83	7,485
6	0.825	0.870	0.954	....	0.884	117	11,999
7	0.918	0.905	0.806	0.800	0.857	154	20,677
8	0.725	0.662	0.652	0.690	0.681	144	17,031
9	0.681	0.661	0.712	0.723	0.694	137	17,660
10	0.690	0.751	0.784	0.797	0.758	131	12,740
11	....	....	0.874	0.886	0.880	66	6,952
12	....	....	....	0.873	0.873	35	4,854

Within each group column, brood mean k values are listed in order from top to bottom. Grand mean k values are calculated for each day by summing all k values for a given day and then dividing by the total number of males tested for that day.

treatment) of the experimental groups. Inspection of the tables is helpful; note that k values for, say, brood 4 of group W plus brood 3 of group X plus brood 2 of group Y plus brood 1 of group Z in Table 6 are summed, and then divided by the total number of males to arrive at a final grand mean k value for day 9. This seems justified, since all evidence indicates that the number of days following treatment, rather than the stage of development of the individual flies (excluding larvae which contain no meiotic cells) during which treatment occurred, is the important factor.

R-206 *males at 30°C for 24 hours:* Note, in Table 5 and Figure 3, that consistently low mean k values (0.65–0.73) are observed in all broods on both the 8th and 9th days following treatment; the grand means for these days are 0.68 and 0.69 respectively. On the 10th day following treatment a definite reduction (grand mean of 0.76) occurs, but the range among the four broods is somewhat greater (0.68–0.79). By the 11th day following treatment, all broods exhibit control k values.

The difference between the grand mean k value for day 7 and the corresponding control mean (0.857 *vs.* 0.953) may indicate some effect at this stage. However, the day-to-day variability of control k values was so great (0.95–0.85) that I would prefer not to make any conclusive statements about experimental k values that fall within this range. Note too that of the four broods providing information about day 7, two (W-4 and X-3) have mean k values of about 0.90, and two (Y-2 and Z-1) have mean k values of about 0.80; thus the difference between experimental and control is not clearcut.

Rather puzzling are the low mean k values for days 5 and 6 (0.823 and 0.884 respectively). This does not coincide with results from previous experiments—none of which, to be sure, involved a semistable *SD* chromosome, but a similar low mean k value was found for day 6 of cold-treated *R-206.* Since there are no control values covering those days for direct comparison, only a comparison with the range of control values can be made; even this is not too helpful. There may

be a rather bizarre partial explanation, however. It was noted, the first day on which progeny counts were to be made, that some newly emerged flies had escaped from their vials due to insufficiently compact cotton plugs. Brood W-1 of *R-206* males treated at 30° was thrown out and all other vials were immediately replugged; but there was no way, of course, to tally the escapees from the first few batches of matings—which are precisely the broods in question. (Since large numbers of progeny remained in these vials, it had been decided not to discard them.) If brown-eyed flies emerge slightly sooner than white-eyed (i.e. *cn bw*) flies—which from my experience appears to be the case—it could happen that k values are slightly reduced by the loss of, say, the first 20 flies from each vial. For example, the mean k value for the brood W-2, consisting of 40 males, is changed from 0.84 (i.e. 3058/3637) to 0.87 (i.e. 3858/4437) when corrected for the loss of 800 brown-eyed flies. Similarly, the mean k value of brood X-1, consisting of 43 males, is changed from 0.80 (i.e. 3089/3848) to 0.84 (i.e. 3949/4708) when corrected for the loss of 860 brown-eyed flies. The grand mean k value for day 5 then would have been 0.85 rather than 0.82. These are not drastic shifts, but it seems possible that the k values observed in these two broods are somewhat lower than they might have been if no flies had escaped.

R-206 *males at 19°C for 72 hours:* Note in Table 6 and Figure 3 that cold treatment profoundly affects k values in this *SD* line. The greatest reduction occurs nine days following treatment, although brood Z-1 was unaffected (due probably to treatment of premeiotic stages). A grand mean k value computed from only the three affected broods, excluding Z-1, becomes 0.655 (rather than 0.716). On the 8th day a considerable effect is also seen. While brood W-5 exhibits lower k values on day 10, the other three groups fall within the control range, as does the grand mean k value of 0.869.

Since some newly emerged flies escaped from brood W-1 vials, the low k value seen on day 6 may be explained as above.

TABLE 6

Summary of results from treatment of R-206/cn bw *males at* 19°C *for 72 hours*

No. of days following treatment	Mean k values of groups: W	X	Y	Z	Grand mean k value	No. of males tested	Total counts
6	0.846	....	....	....	0.846	39	4,111
7	0.903	0.953	....	....	0.929	85	11,391
8	0.744	0.760	0.837	....	0.779	118	10,901
9	0.636	0.590	0.752	0.889	0.716*	154	15,424
10	0.776	0.860	0.888	0.948	0.869	152	13,674
11	0.892	0.938	0.945	0.957	0.933	149	15,361
12	....	0.932	0.963	0.962	0.952	108	10,874
13	....	....	0.920	0.915	0.917	70	6,273
14	....	....	....	0.889	0.889	38	5,540

Within each group column, brood mean k values are listed in order from top to bottom. Grand mean k values are calculated for each row by summing all k values for a given day and then dividing by the total number of males tested for that day.

* If brood Z-1 is omitted from the calculation, the grand mean k value for day 9 becomes 0.655.

TABLE 7

Summary of results from treatment of SD-72/cn bw *males at* 30°C *for 24 hours*

No. of days following treatment	Mean k values of groups: W	X	Y	Grand mean k value	No. of males tested	Total counts
5	0.987	....	....	0.987	28	1,758
6	0.998	0.999	....	0.999	68	5,998
7	0.984	0.986	0.998	0.991	109	11,857
8	0.967	0.975	0.992	0.981	103	10,085
9	0.981	0.975	0.990	0.983	91	10,510
10	0.986	0.980	0.992	0.987	94	6,642
11	....	0.992	0.997	0.995	70	5,549
12	....	....	0.997	0.997	37	3,862

Within each group column, brood mean k values are listed in order from top to bottom. Grand mean k values are calculated for each row by summing all k values for a given day and then dividing by the total number of males tested for that day.

Experimental mean k values are higher than control mean k values for days 11 (0.933 *vs.* 0.856) and 12 (0.952 *vs.* 0.873). It might appear that cold treatment enhances k values slightly when applied to spermatogonia. But again, these elevated experimental k values are no higher than those observed in controls on day 7; thus it is not at all clear that the difference in this case has any biological significance.

SD-72 *males at 30°C for 24 hours:* As seen in Table 7 and Figure 3, this *SD* line is clearly unaffected by 30°C treatment, at least in 24-hour doses. It is not known whether heat treatment of longer duration would cause a reduction in mean k values.

SD-72 *males at 19°C for 72 hours:* Note in Table 8 and Figure 3 that a conspicuous drop in k values is observed 8 days following treatment (grand mean k

TABLE 8

Summary of results from treatment of SD-72/cn bw *males at* 19°C *for 24 hours*

No. of days following treatment	Mean k values of groups: W	X	Y	Z	Grand mean k value	No. of males tested	Total counts
6	0.971	....	....	....	0.971	18	2,017
7	0.985	0.984	....	....	0.985	63	7,007
8	0.792	0.841	....	....	0.827	63	5,151
9	0.862	0.951	0.938	....	0.930	90	6,004
10	0.974	0.988	0.992	0.989	0.988	122	9,882
11	0.993	0.983	0.997	0.998	0.993	122	11,193
12	....	0.994	0.996	0.998	0.996	103	11,212
13	....	....	0.995	0.987	0.992	63	5,035
14	....	....	0.971	0.987	0.979	62	7,162

Within each group column, brood mean k values are listed in order from top to bottom. Grand mean k values are calculated for each row by summing all k values for a given day and then dividing by the total number of males tested for that day.

value of 0.827), with almost complete recovery by the ninth day following treatment (grand mean k value of 0.930).

DISCUSSION

Results of the above experiments are roughly summarized as follows:

Chromosome	Degree of sensitivity to 24-hour periods at:		
	30°C	28–29°C	19°C
R-206	great		great
R-207	great	none	slight
SD-72	none		great
SD-205			slight

Clearly, segregation-distortion is influenced by at least one environmental factor, temperature.

In general, sensitivity to temperature shock is dependent on the stage of meiosis which a particular sperm is undergoing rather than the developmental stage of the individual fly. That is, a considerable depression in k values is observed about 8–9 days following treatment, regardless of whether it was administered to prepupae, pupae or adults.

These findings provide further evidence that segregation-distortion is indeed a meiotic phenomenon.

Occasionally a small reduction in k values was observed 11 days following treatment; this was noted in 3-day broods, but never when males were remated daily—suggesting that the effect is due to lack of sperm depletion from earlier inseminations, so that some "early brood" sperm were included in later broods.

The magnitude of the greatest temperature effect observed is about the same in all sensitive *SD* lines: that is, a reduction in k values of approximately 15–25%. Thus, in stable *SD* lines, control k values are about 1.0 and experimental k values average about 0.75–0.85—whereas with the semistable recombinant *R-206*, control k values average about 0.90 and experimental k values about 0.68-0.80. In no case was the mean k value reduced to 0.50, although rarely individual males would show almost complete suppression of segregation-distortion following treatment.

The effect of heat treatment is often exaggerated when flies are exposed for two or more days, as compared to one day, during the sensitive period—but not in a strictly quantitative way. This is no doubt because a larger proportion of spermatocytes are affected in a period of two or more days.

Several meiotic drive mechanisms in *Drosophila melanogaster* are temperature sensitive. In all of these, the sensitive period occurs about the time of early meiosis, and in all cases the effect is to make segregation more nearly normal. ERICKSON and HANKS (1961) report that the *RD* effect (high recovery rate of the X-chromosome) is greatly reduced by 18°C treatment. Males developing at this temperature from early pupae until the second day of adult life showed the

greatest reduction in sex ratio 7–8 days following treatment, when the proportion of females was 0.534 as compared to 0.648 in the controls.

ZIMMERING and PERLMAN (1962) tested the influence of 18°C on segregation in A- type B^s translocation males. Segregation ratios of both X^D:IV and X^P:Y were markedly changed in groups treated as prepupae, which contain a high proportion of spermatocytes. ZIMMERING (1963) also examined the effect of cold treatment on segregation in males carrying X and Y chromosomes with greatly reduced homology. He found that males heterozygous for the sc^4-sc^8 X chromosome and the sc^8·Y chromosome, when raised at 18°C, exhibit much more normal transmission of the Y chromosome than when raised at 26°C—i.e. about 42% Y sperm and 3.5% XY sperm *versus* only 24% Y sperm and 2% XY sperm. A modified univalent Y chromosome behaves almost identically at 18°C. Thus the increased recovery of the Y from XY males cannot be attributed to an increase in pairing (and therefore regular disjunction) between the X and Y at lower temperatures, since a Y chromosome without a homologue gives the same result. ZIMMERING and GREEN (1965) studied the behavior of a univalent X chromosome at a lower temperature, by comparing the relative frequencies of XY *versus* Y sperm transmitted by sc^4-sc^8/Y/Y males. In such males the Y chromosomes almost invariably form a bivalent while the X acts as a univalent. Rather than the expected equality of XY and Y sperm, only about 33% of the former type are produced by males raised at 26°C. Males raised at 18°C, however, produce 43% XY sperm. This shift toward more normal segregation at 18°C does not seem to depend on an increased frequency of pairing between X and Y.

A temperature effect has also been described in *Drosophila pseudoobscura* for the *sr* (sex ratio) gene, a sex-linked factor which causes male carriers to produce almost 100% females. DARLINGTON and DOBZHANSKY (1942) found that at 25°C the progenies of *sr* males consist of 93.8% females, while at 22°C there were 96.3% females and at 16°C, 98.9% females. Here the lower temperatures produce *less* normal segregation ratios, as opposed to the situations (described above) which prevail in *Drosophila melanogaster*. WALLACE (1948) studied the effect of temperature on experimental populations of *Drosophila pseudoobscura* containing varying initial frequencies of *sr*. In all populations the frequency of *sr* decreased with time; at 25°C it disappeared entirely within 210 days, and at 16½°C reached a stable equilibrium frequency of 6–10% within 420 days. It would be interesting to do this sort of study with experimental populations of *SD*.

The exact mode of operation of the temperature effects, in *SD* and other drive mechanisms, is unknown. How these findings apply to the behavior of segregation-distortion in nature is not clear either. The *SD* gene is common in natural populations, many of which must be exposed for considerable periods of time to temperatures as high as 30°C or as low as 19°C. Since the survival of *SD* in natural populations depends on the maintainance of high k values (HIRAIZUMI, SANDLER and CROW 1960), perhaps temperature is an important factor in reducing the efficacy of segregation-distortion in nature. The insensitivity of the *SD-72* chromosome to heat treatment (at least in 24-hour doses) may be due to the

evolution of linked modifiers associated with the *SD* complex. Note that the two chromosomes sensitive to heat treatment were both recombinants; it would have been interesting to test these recombinants, made heterozygous with complementary recombinant strands, to see whether the sensitivity disappears.

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SUMMARY

Behavior of the second-chromosome gene for segregation-distortion (*SD*), which at room temperatures (25°C) causes grossly aberrant segregation ratios, can be altered by treatment at higher and lower temperatures. In these experiments, males heterozygous for the *cn bw* chromosome and one of four different *SD* chromosomes were treated at various temperatures (30°, 28–29°, and 19°C) for varying lengths of time (1 to 12 days) at various stages of development (larva—adult). It was concluded that: (1) not all *SD* chromosomes are equally sensitive to heat or to cold; also, the same chromosome may be sensitive to one and not to the other. (2) In all temperature-sensitive *SD* lines, the greatest reduction in k values (proportion of *SD* progeny) was 15–25%. (3) The greatest reduction in k values is seen 8–9 days following treatment, indicating that the most sensitive period occurs around the time of early meiosis. (4) Temperature sensitivity of *SD* depends on the stage of meiosis which a particular sperm is undergoing, rather than the developmental stage of the individual fly. No effect is found following treatment of larvae, which contain no meiotic cells. (5) These findings provide further evidence that segregation-distortion is indeed a meiotic phenomenon. (6) The threshold for the heat effect is about 28°; the threshold for the cold effect was not determined. (7) The temperature effect is not heritable.

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