A COLD-SENSITIVE ZYGOTIC LETHAL CAUSING HIGH FREQUENCIES OF NONDISJUNCTION DURING MEIOSIS I IN *DROSOPHILA MELANOGASTER* FEMALES[1]

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ABSTRACT

The *X*-linked, cold-sensitive zygotic lethal, *l(1)TW-6^{cs}*, both in homozygous and heterozygous females, induces nondisjunction of all four chromosomes at Meiosis I at both 25° and 17°. Nondisjunction frequencies approaching 0.5 for the *X* and fourth chromosomes have been observed at 16°–18°. The disjunction of the *X* chromosomes in males is not affected. The mutant causes mitotic irregularities in zygotes at both 25° and 17°. Mortality of *all* zygotes produced by the crosses *6^{cs}/6^{cs}* × *6^{cs}/B^{s}Y* and *FM7/6^{cs}* × *6^{cs}/B^{s}Y* is respectively 86% and 67–74% at 25° and 99.8–99.9% and 94% at 17°. The mortality of *6^{cs}* hemizygotes derived from females carrying no doses of *6^{cs}* (*C(1)DX,y f/Y* × *6^{cs}/B^{s}Y*) is 45–55% at 25° and 98% at 17°. The length of the temperature-sensitive period for *6^{cs}* homo- and hemizygotes is affected by the maternal dosage of *6^{cs}*; the shortest TSP is for zero and the longest is for two maternal doses. Mortality takes place primarily during embryogenesis with some larval and little pupal mortality. Analysis of sectioned embryos indicates that the large array of different patterns of damage observed could have arisen from abnormal cleavage divisions and the incomplete population of the blastoderm with nuclei.

IT is clear that the genetic dissection of meiosis is now well underway in *Drosophila melanogaster* (see forthcoming review by BAKER and HALL 1974). Until 1968 only five mutants that affect meiosis had been found. These were crossover suppressor in chromosome 3 of GOWEN (*c(3)G*) (GOWEN and GOWEN 1922), claret-nondisjunctional (*cand*) (LEWIS and GENCARELLA 1952), equation producer (*eq*) (SCHULTZ 1934), Segregation Distorter (*SD*) (SANDLER, HIRAIZUMI and SANDLER 1959), and Recovery Disrupter (*RD*) (NOVITSKI and HANKS 1961). In 1968 SANDLER *et al.* (1968) initiated a systematic search for autosomal mutants that affect meiosis in natural populations, recovering thirteen of them. Subsequently SANDLER (1971) obtained two autosomal meiotic mutants out of 35 ethyl methanesulfonate-treated, lethal-free second and third chromosome complements, and BAKER and CARPENTER (1972) have isolated 30 sex-linked meiotic mutants out of 209 EMS-treated *X* chromosomes. The influence of a number of the above mutants on crossing over and chromosome segregation has now

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been thoroughly examined. These include *ca*nd (D. G. DAVIS 1969), *c(3)*G (HALL 1972), *mei-S332* (B. K. DAVIS 1971), *mei-S51* (ROBBINS 1971), *nod* (CARPENTER 1973) and *mei-S282* (PARRY 1973).

Except for *mei-S332*, which produces nondisjunction at Meiosis II equally in both males and females, all of the above mutants are sex-specific, affecting meiosis only in females or in males. Also except for the dominance of *SD*, *RD* and the semi-dominance of *mei-S332*, all mutants are recessive. By examining the effects of the female meiotic mutants on recombination—including no effect or the uniform or non-uniform decrease of exchange along the chromosome—and by determining if the mutant also causes nondisjunction, whether nondisjunction occurs at Meiosis I or Meiosis II, whether specific chromosomes or all the chromosomes nondisjoin, whether nondisjunction is restricted to non-exchange chromosomes, whether there is preferential nondisjunction of non-homologous chromosomes, and whether there is meiotic and/or mitotic loss of chromosomes, it has been possible to infer that different female mutants affect different aspects of meiosis. Some of the steps of meiosis thought to be affected are the preconditions for exchange including synapsis, exchange itself, the distributive disjunction (see review by GRELL 1969) of non-exchange chromosomes at Meiosis I, the disjunction of both exchange and non-exchange chromosomes at Meiosis I and the disjunction of chromosomes at Meiosis II. Most of the mutants are rather restricted in their effects, but others appear to affect multiple aspects of meiosis. As yet none have been reported that have drastic effects on mitosis, although *ca*nd, *mei-S332* and *nod* do cause the formation of mosaic progeny through mitotic loss or nondisjunction. It has been thought that any mutant that did extensively alter both mitotic and meiotic nuclear divisions would be a zygotic lethal and therefore would not have been recovered in any of the above screens for meiotic mutants (BAKER and CARPENTER 1972).

This paper reports some of the properties of a cold-sensitive zygotic lethal, *l(1)TW-6*^{cs} (abbreviated to *6*^{cs} in this paper), which effects an extremely high level of meiotic nondisjunction in females both when homozygous and heterozygous. This effect is potentiated in the cold. Although crosses have yet to be done that are specifically designed to explore its effects on the disjunction of exchange *vs.* non-exchange chromosomes and on the preferential distributive disjunction of non-homologous chromosomes, sufficient data are presented to implicate it in postexchange disjunctional events of Meiosis I. Its effect on zygotic lethality including the stage distribution of mortality and temperature-sensitive period are examined in detail. Its dominant, intense disturbance of meiosis, along with its conditional zygotic lethality, make it a unique mutant and suggest that a survey of conditional zygotic lethals for possible effects on meiosis may be a profitable source of mutants that affect properties of nuclear divisions common to both meiosis and mitosis.

MATERIALS AND METHODS

1(1)TW-6^{cs}: The mutant *l(1)TW-6*^{cs} was recovered in a screen for ethyl methanesulfonate-induced, *X*-linked, cold-sensitive lethals, the details of which have been reported elsewhere

(WRIGHT 1973). All other mutations and special chromosomes used in this work are described by LINDSLEY and GRELL (1968) except for the multiply-inverted, *X*-chromosome balancer *FM7* (*In(1)FM7*, $y^{s1d}sc^{s}w^{a}sn^{x2}v^{0f}$ *B*) described by MERRIAM and DUFFY (1972).

Crosses: The parents for all the crosses reported here were raised at 25°, with a few exceptions. All crosses were made in half-pint milk bottles on standard cornmeal-oats-dextrose-agar food or in 85 × 23 mm shell vials on a medium including yeast, agar, dextrose, and salts (CARPENTER 1950). For crosses at 25°, parents were removed routinely on Day 7 and offspring were counted up to Day 18. For crosses at 16°–18°, parents were cleared on Day 18–21 and progeny were counted up to Day 45.

Stage distribution of mortalities and shift-up experiments: All eggs were collected and handled according to the method previously described (WRIGHT 1973). All eggs were laid at 25°. Except for the shift experiments reported in Figures 3 and 4, the egg collection periods (egg laying periods) were four hours long, which means that the eggs had developed at 25° for 2 hr ± 2 hr prior to being placed at the initial incubation temperature. For the shift experiments reported in Figures 3 and 4 a 2-hour laying period was used, and the eggs therefore were 1 hr ± 1 hr at 25° prior to placement at the initial incubation temperature. Since 6^{cs} causes so much very early embryonic lethality, it was not valid to assume that all unhatched eggs which did not turn brown or black were unfertilized eggs. Therefore all mortalities are calculated as a function of the total number of eggs picked. Mortalities for the different stages of development were calculated using the formulae previously published (WRIGHT 1973), except the word "fertilized" was omitted from the formulae. Percent mortality for any given stage of development indicates that percent of the total number of zygotes which die during that stage of development.

For the shift experiments reported in Figures 1 and 2 the predominant stage of development at the time of each shift was determined by the microscopic examination of an extra sample of 50 individuals at each shift time—i.e., they are very approximate developmental stagings for 6^{cs} homo- and hemizygotes at 25° in Figure 1 and at 17° in Figure 2. For the shift experiments reported in Figures 3 and 4 an extra sample of eggs from each cross at each shift time was *not* staged; instead live embryos from a wild-type stock were staged with the compound microscope after they had been collected and incubated at 17° and 25° for requisite lengths of time in a manner identical to that used in the actual shift experiments. See DOANE (1967) for the complete staging of Drosophila embryos at 25°.

Calculation of frequency of X *chromosome nondisjunction:* Since 6^{cs} causes zygotic lethality and since *FM4/Y* males have significantly reduced viability the presentation of percent exceptional offspring as a function of total number of adult progeny in many crosses is a meaningless measure of nondisjunction. Instead the formula $\frac{\text{no. } XX\text{-}O \text{ segregations}}{\text{no. } XX\text{-}O \text{ segregations} + \text{no. } X\text{-}X \text{ segregations}}$ was used to calculate the frequencies of *X* chromosome nondisjunction. In any cross the largest class of exceptional progeny, *X/O* or *XXY*, was used as an estimate of the number of *XX-O* segregations. Also the largest class of expected progeny, *XX* or *XY*, was used as an estimate of the number of *X-X* segregations. If the cross was such that only one type of *XX* female or *XY* male was produced, the number in the largest expected class was divided by two before use in the above formula. If two types of *XX* females or two types of *XY* males were produced, the number in the largest expected class was used directly in the above formula.

The above methods of calculation assume that the number of diplo-*X* ova and the number of nullo-*X* ova are equally frequent. Significant levels of *X* chromosome loss producing nullo-*X* ova would invalidate this assumption and cause overestimates of nondisjunction frequencies. This consideration emphasizes that the use of the above formula only permits a rough estimate of the frequency of nondisjunction to be made.

RESULTS AND DISCUSSION

Cold-sensitive zygote lethality: The data from Crosses 1 and 2 presented in Table 1 clearly indicate that 6^{cs} is a cold-sensitive zygotic lethal. In Cross 1 where

TABLE 1

Cold-sensitive zygotic lethality of 1(1)TW-6^{cs}

Cross 1. *FM4,*$y^{s1d}sc^{s}dm$ *B/*6^{cs} ♀ ♀ × $6^{cs}/Y$ ♂ ♂

Temp.	Phenotypes				Total	Percent of expected*
	B/+ ♀ ♀	*y B* ♂ ♂	+ ♀ ♀	+ ♂ ♂		
25°	280	122	215	310	927	93.8
16°	190	17	0	0	207	0

Cross 2. *C(1)DX,y f/Y* ♀ ♀ × $6^{cs}/Y$ ♂ ♂

Temp.	Phenotypes		Total	Percent of expected†
	y f ♀ ♀	+ ♂ ♂		
25°	401	457	859	114.0
16°	318	18	336	5.7

* Percent of expected $= \dfrac{+\,♀\,♀ \;+\; +\,♂\,♂}{2(B/+)\;♀\,♀} \times 100$

† Percent of expected $= \dfrac{+\;♂\,♂}{y\,f\;♀\,♀} \times 100$

Cross 1. For each temperature 10 vial cultures with 5 pairs of parents per vial.
Cross 2. For each temperature 2 half-pint bottle cultures with 10 pairs of parents per bottle.

the female parent is heterozygous for 6^{cs}, all $6^{cs}/6^{cs}$ female zygotes and $6^{cs}/Y$ male zygotes die at 16°, but significant numbers live at 25°. In Cross 2 most, but not quite all, of the $6^{cs}/Y$ zygotes die at 16°. Since in Cross 2 the female parent does not carry the 6^{cs} mutant gene at all, the increased mortality at 16° cannot be due to a maternal effect of 6^{cs}, but must be due to some 6^{cs}-produced defect in the zygote itself. In virtually all *C(1)DX*, $f/Y \times 6^{cs}/Y$ crosses performed at 16–18° to date a small percentage of $6^{cs}/Y$ zygotes escaped to eclose as adults.

Cold-sensitive female sterility: If female sterility is defined as the inability or drastically reduced capacity of females to produce adult progeny, then $6^{cs}/6^{cs}$ homozygous females are sterile at 16° and fertile at 25°. Fifty-five $6^{cs}/6^{cs}$ very young virgins mated at 16° to wild-type males produced no progeny at all. Fifty $6^{cs}/6^{cs}$ virgins aged for seven days at 25° and then mated to wild-type males at 16° produced only three adult progeny (WRIGHT 1973). These data, along with those presented in Table 2 for Crosses 3 and 4, indicate that $6^{cs}/6^{cs}$ females are almost but not quite completely sterile at 16°, and that it makes very little difference if oogenesis is permitted to proceed at 25° for a week prior to mating at 16°. Even if oogenesis proceeds at 16°, $6^{cs}/6^{cs}$ females lay significant numbers of eggs which have been fertilized and which undergo some development. Most of these zygotes die during embryogenesis (see below) although a few do die as larvae and pupae, and a very few eclose as adults with no obvious mutant phenotype.

Primary nondisjunction: When crosses involving either homozygous or heterozygous 6^{cs} females are made in such a way that patroclinous or matroclinous progeny can be recognized, a large number of exceptional progeny are found at

TABLE 2

Exceptional progeny produced by homozygous and heterozygous 1(1)TW-6^{cs} *females at 25° and 16°*

Cross 3. $6^{cs}/6^{cs}$ ♀♀ × $FM4,y^{s1d}sc^{s8}dm$ B/Y ♂♂

	Phenotypes				
	Expected progeny		Exceptional progeny		
Temp.	$B/+$♀♀	+♂♂	+♀♀	y B♂♂	Total
25°	256	290	56	7	609
16°	1	0	0	1	2

Cross 4. $6^{cs}/6^{cs}$ ♀♀ × w/Y ♂♂

	Phenotypes			
	Expected progeny		Exceptional progeny	
Temp.	+♀♀	+♂♂	w♂♂	Total
25°	254	266	21	541
16°	10	0	14	24

Cross 5. $FM4,y^{s1d}sc^{s8}dm$ $B/6^{cs}$ ♀♀ × w/Y ♂♂

	Phenotypes						
	Expected progeny				Exceptional progeny		
Temp.	$B/+$♀♀	+♀♀	y B♂♂	+♂♂	w♀♀	w♂♂	Total
25°	145	121	48	118	0	54	486
16°	194	104	20	0	2	132	452

Data pooled from 10 vial cultures with 5 pairs of parents for each cross at each temperature.

both 25° and 16° (see Table 2). In Cross 5 the number of exceptional w males found at 16° was much larger than the number found at 25°. The fertility of 78 of the patroclinous w males from Cross 5 was tested, and 74 or 95% were found to be sterile, and therefore are inferred to be X/O males.

Since 6^{cs}-induced primary nondisjunction of the X chromosomes during the first division of meiosis in the female parent could account for the appearance of almost all the exceptional progeny in both Cross 3 and Cross 5 (Table 2), the cross $FM/6^{cs} \times Oce/B^sY$ was designed to demonstrate that high frequencies of primary nondisjunction were actually taking place at both 25° and 16° (see Crosses, Table 3). To insure that in Cross 6 no secondary nondisjunction was involved due to the presence of XXY parental females, $FM4/6^{cs}$ virgins were collected from a stock in which the only Y chromosome present was the phenotypically recognizable B^sY. The data presented in Table 3 demonstrate that in Cross 6 significant numbers of XXY female and X/O male progeny are being produced indicating a high frequency of first division nondisjunction in the parental females.

Although the results of Cross 6 when compared to that of the control cross (Cross 7) clearly show that 6^{cs}-induced primary nondisjunction is taking place, not all the exceptional progeny recovered in Cross 5, Table 2 can be accounted for

TABLE 3

Results of crosses in which segregation of all three parental X *chromosomes and the* Y *chromosome can be scored*

Cross 6. *FM4,y^{s1d}sc^{s}dm B/6^{cs}* ♀♀ × *Oce/B^{s}Y* ♂♂

Temp.	Expected progeny*				Exceptional progeny		Total	Freq. *XX-O*†
	FM4/ Oce♀♀	*Oce/ 6^{cs}*♀♀	*FM4/ B^{s}Y*♂♂	*6^{cs}/ B^{s}Y*♂♂	*FM4/6^{cs}/ B^{s}Y*♀♀	*Oce/O*♂♂		
25°	159	133	53	112	77	67	601	.326
18°	17	13	1	1	3	31	66	.646

Cross 7. *FM4,y^{s1d}sc^{s}dm B/+* ♀♀ × *Oce/B^{s}Y* ♂♂

Temp.	Expected progeny*				Exceptional progeny		Total	Freq. *XX-O*†
	FM4/ Oce♀♀	*Oce/ +*♀♀	*FM4/ B^{s}Y*♂♂	*+/B^{s}Y*♂♂	*FM4/+/ B^{s}Y*♀♀	*Oce/O*♂♂		
25°	162	212	174	152	1	2	703	.009
18°	360	331	214	353	0	2	1260	.006

Pooled data from single female vial cultures. For Cross 6 eighteen single females at 25° and twelve at 18°. For Cross 7 eight single females at 25° and eleven at 18°.

* Ocellarless phenotype could not be scored at 18° in heterozygous *Oce* ♀♀.
† See MATERIALS AND METHODS for method of calculation.

by primary nondisjunction in the female parent. These include the four patroclinous *w* male progeny which were fertile when tested and the two white-eyed females. The former must be *XY* males and may have arisen as a result of secondary nondisjunction in *XXY* female parents (*FM4/6^{cs}/Y*)—an explanation made more likely by the fact that some *FM4/6^{cs}/Y* females were discovered in the process of constructing the *FM4/6^{cs}* × *6^{cs}/B^{s}Y* stock used to produce the female parents for Cross 6 (Table 3). The two *w* females could have arisen as a small cluster from a spontaneous mutation in a female parent, from second division nondisjunction in male parents, or as a result of very early mitotic nondisjunction of the *X* chromosome in *X/O* (*w/O*) zygotes (see below).

In Table 4 data are presented on the frequency of primary nondisjunction in homozygous *6^{cs}/6^{cs}* females, in females heterozygous for the multiply inverted balancer *FM4* (*FM4/6^{cs}*), and in females heterozygous for a normal X chromosome carrying the recessive visible marker mutations *ct*, *v*, and *g* (*ct v g/6^{cs}*). These females plus suitable control females were crossed at 25° and at either 16° or 18°.

It is apparent *6^{cs}* induces primary nondisjunction when heterozygous, i.e., the phenotype of meiotic dysfunction is dominant at both 25° and 16°. It is not clear whether heterozygosity with the multiply inverted chromosome *FM4* increases the frequency of nondisjunction relative to that obtained in heterozygotes with the uninverted chromosome *ct v g*. The results of Experiment 3 (Cross 6 *vs.* Cross 9) suggests that the former does give ten times as much nondisjunction. However, the frequency of *XX-O* segregations produced by *ct v g/6^{cs}* females in Experiment 1, Cross 8 in the cold is greater than that obtained from *FM4/6^{cs}* females in the cold in Experiment 2, Cross 5. It is possible that the difference be-

TABLE 4

Frequency of primary nondisjunction in heterozygous and homozygous 1(1)TW-6^{cs} *females*

Cross				25°				16° or 18°			
				Exceptionals			Freq.	Exceptionals			Freq.
No.	♀ parent	♂ parent	Exp. no.	*XX/Y*	*X/O*	*n*	*XX-O*	*XX/Y*	*X/O*	*n*	*XX-O*
5	$FM4/6^{cs}$	× *w/Y*	2	n.s.†	54	486	.271	n.s.	132	452	.405
6	$FM4/6^{cs}$	× Oce/B^sY	3	77	67	601	.326	3	31	66	—
7	*FM4/+*	× Oce/B^sY	3	1	2	703	.009	0	2	1260	.006
8	*ct v g/*6^{cs}	× *w/Y*	1	—	—	—	—	n.s.	336	1895	.493
9	*ct v g/*6^{cs}	× Oce/B^sY	3	5	16	1622	.031	18	55	944	.196
10	*ct v g/+*	× Oce/B^sY	3	0	3	2511	.004	0	0	1434	0
4	$6^{cs}/6^{cs}$	× *w/Y*	2	n.s.	21	541	.136	0	14	24	—
3	$6^{cs}/6^{cs}$	× *FM4/Y*	2	56	7	609	.279	0	1	2	—
11	$6^{cs}/6^{cs}$	× Oce/B^sY	3	28	76	561	.385	0	8	14	—
12	+/+	× Oce/B^sY	3	0	0	1166	—	0	3	3016	.004

* Experiments 1 and 2 at 16°; experiment 3 at 18°.
† n.s. = not scorable.
Four of the ten crosses at 16° or 18° produced insufficient progeny to permit valid calculations of the frequency of *XX-O* segregation.
Crosses 8, 9 and 10. Pooled data from 5 half-pint bottle cultures with 20 pairs of parents per bottle.
Cross 11. Pooled data from 18 single female vial cultures at 25° and 25 at 18°.
Cross 12. Pooled data from 25 single female vial cultures at both 25° and 18°.
Crosses 3 through 7. See previous tables.

tween the frequency of nondisjunction for *ct v g/*6^{cs} in Experiment 1, Cross 8 (.493) and Experiment 3, Cross 9 (.196) could be due to the 2° difference in temperature for the two experiments: Experiment 1 at 16° and Experiment 3 at 18°. However, that frequencies of nondisjunction obtained are quite variable is apparent from the results obtained with $6^{cs}/6^{cs}$ homozygous females at 25° in two crosses in Experiment 2 and one in Experiment 3: .136 *vs.* .279 *vs.* .385.

Particularly significant is the fact that in three of the crosses reported in Table 4 the frequency of *XX-O* segregations induced by 6^{cs} are so high (.405, .493, .385) as to approach random segregation of the *X* chromosomes at Anaphase I of meiosis (nondisjunction = .5). These nondisjunctional frequencies for the *X* chromosome are as high as or higher than those reported for any other meiotic mutant, e.g. ca^{nd}-.32 (D. G. DAVIS 1969), *c(3)G*-.32-.39 (HALL 1972), *mei-S332*-.29 (B. K. DAVIS 1971), *mei-9*-.28 and *mei-218*-.30 (BAKER and CARPENTER 1972), *mei-S51*-.11 (ROBBINS 1971), and *nod*-.018 (CARPENTER 1973). However, if *X* chromosome loss is high, the method of calculating *XX-O* segregation frequencies can lead to the calculation of spuriously high nondisjunction frequencies. Although the data for many crosses in Table 4 do show an excess of zygotes derived from nullo-*X* ova relative to diplo-*X* ova, this may not be due only to chromosome loss but may reflect in part reduced viability of the *XXY* zygotes which are homozygous and heterozygous for the conditional zygotic lethal, 6^{cs}.

Second division non-disjunction: In Crosses 1, 5, 6 and the $FM7/6^{cs} \times 6^{cs}/B^sY$ cross (Tables 1, 2, 3 and 10, respectively) no progeny diagnostic of second divi-

sion nondisjunction were found. These would have been *FM4/FM4/Y* females in Crosses 1 and 5, *FM4/FM4/B^{s}Y* females in Cross 6, and *FM7/FM7/B^{s}Y* females in the last cross. Although females of all three of these genotypes are poorly viable, it seems highly likely that, if produced by second division nondisjunction, some would have been recovered, particularly under the optimum culture conditions used for Cross 6 (Table 3) and for the *FM7/6^{cs}* × *6^{cs}/B^{s}Y* cross (Table 10).

Crosses 8 and 9 (Table 4) did produce some unusual female progeny which could have arisen from either first or second division nondisjunction. For Cross 8, *ct v g/6^{cs}* × *w/Y*, at 16° 34 unusual female progeny among 1895 total progeny were recovered. These were phenotypically: 16 *ct v g* ♀♀, 2 + *v g* ♀♀, 4 + + *g* ♀♀, 6 *ct* + + ♀♀, and 6 *ct v* + ♀♀. Only three unusual females among 994 total progeny were found for Cross 9 *ct v g/6^{cs}* × *Oce/B^{s}Y* at 18°. These were phenotypically: 1 *B^s ct v* + ♀, 1 *B^s ct* + + ♀, and 1 *B^s* + *v g* ♀. If only first division nondisjunction occurs, the *ct v g*, *ct v* + (*B^s ct v* +), and *ct* + + (*B^s ct* + +) females would have required a single crossover event and the + *v g* (*B^s* + *v g*) and + + *g* females a double crossover event prior to nondisjunction at Anaphase I. If second division nondisjunction occurs, then the *ct v g* females could have arisen without crossing over and the + *v g* (*B^s* + *v g*) and + + *g* females would have required only single crossover events prior to nondisjunction at Anaphase II. The possibility that all of these unusual females might arise through mitotic nondisjunction is considered below.

The data presented here do not definitively exclude the possibility that *6^{cs}* causes some second division nondisjunction, but if it does, the frequency must be low relative to first division nondisjunction, since only a total of 22 or 1.16% possible second division exceptional females were recovered in Cross 8, only 1 or 0.1% in Cross 9, and none in the other crosses considered above.

Meiosis in males: The mutant *6^{cs}* does not affect disjunction of the *X* chromosomes in males. In the cross *ct v g/ct v g* × *6^{cs}/B^s* the frequency of *XY-O* segregation was .007 at 25° and zero at 18°. Control crosses with +/*B^{s}Y* gave comparable frequencies of .002 at 25° and zero at 18°. These data indicate that *6^{cs}* does not alter first division disjunction in males.

If *6^{cs}* did affect meiosis in males, the cross *C(1)DX,y f/Y* × *6^{cs}/B^{s}Y* should yield exceptional *B^s* males (*6^{cs}/B^{s}Y/Y*) as a result of first division nondisjunction and exceptional wild-type females (*6^{cs}/6^{cs}/Y*) as a result of second division nondisjunction. (Exceptional *C(1)DX,y f/O* zygotes which result from nondisjunction at both divisions are lethal, presumably because of a lack of *bb*$^+$ genes; LINDSLEY and GRELL 1968.) No *B^s* males or wild-type females were found in this cross carried out continuously at 25° (Table 11) where approximately 50% of the homo- and hemizygous *6^{cs}* zygotes should survive, and no such exceptional progeny were found among 1397 *C(1)DX,y f/B^{s}Y* and 1189 *6^{cs}/Y* adult progeny which developed from the 16,028 eggs picked to carry out the temperature shift experiments reported in Figures 3 and 4 plus a duplicate temperature shift experiment not reported here. If *6^{cs}* increased second division nondisjunction to only 0.5–1.0% in the above experiments exceptional males and females would

have been recovered. It is clear that 6^{cs} does not effect *X* chromosome nondisjunction at either the first or second meiotic division in males.

Locus: The above data indicate that the EMS-treated *X* chromosome designated as *l(1)TW-*6^{cs} is responsible for three different phenotypes: cold-sensitive zygotic lethality, cold-sensitive female sterility, and cold-sensitive primary nondisjunction at Meiosis I in females. Although it is highly unlikely that more than one cold-sensitive mutation would be induced simultaneously into the same chromosome, the possibility does exist that more than one mutant gene is responsible for these phenotypes. Therefore, an attempt was made to map simultaneously the gene or genes responsible for two of the phenotypes—cold-sensitive zygotic lethality and nondisjunction. Initially, using the multiple marker chromosome *sc ec cv ct v g f*, zygotic lethality was localized to the region between *v* (1–33.0) and *g* (1–44.4). A subsequent cross at 16° using the marker chromosome *ct v g* localized the gene responsible for cold-sensitive zygotic lethality to 37.1 on the basis of 53 crossovers between *v* and *g*.

In order to map cold-sensitive zygotic lethality and nondisjunction simultaneously, *ct v g/*6^{cs} females were crossed to *ct v g/*$B^{s}Y$ males at 25°. Single virgin female progeny from all eight classes of crossover and non-crossover progeny were mated individually to *w/Y* males at 17°. These matings were then scored for primary nondisjunction and cold-sensitive zygotic lethality. Both of these phenotypes mapped between *v* (1–33.0) and *g* (1–44.4). A total of 63 females carrying crossovers between *v* and *g* were recovered. Of these, 51 were successfully scored for lethality, and all but two of these 51 females were also validly tested for primary nondisjunction. Cold-sensitive zygotic lethality mapped to a locus of 37.3 and primary nondisjunction to a locus of 37.2. Until more extensive recombination analyses positively show that the two phenotypes are controlled by elements separable by crossing over, all the mutant phenotypes are considered to be due to a single mutation.

Exchange frequencies and the nondisjunction of exchange and non-exchange bivalents: In the 6^{cs}*/sc ec cv ct v g f* × *FM4/Y* cross at 22° with only 143 scorable males, the following map distances were obtained (standard map distances from LINDSLEY and GRELL 1968 are given in brackets): *sc–ec* 3.7 (5.5), *ec–cv* 9.1 (8.2), *cv–ct* 3.3 (6.3), *ct–v* 11.1 (13.0), *v–g* 17.1 (11.4), and *g–f* 9.9 (12.7). These results do differ significantly from standard map distances, $p<.01$, which is not surprising considering the small numbers involved and viability problems with seven mutant markers. The data do show that there is not a polarized increase or decrease in crossing over from distal to the proximal ends of the *X* chromosome. In the cross 6^{cs}*/ct v g* × *w/Y* at 16°, on the basis of 341 scorable males, the map distance for *ct* to *v* was 15.8 (13.0) and *v* to *g* was 8.8 (11.4)—not significantly different from standard distances ($p>.2$). The cross 6^{cs}*/ct v g* × *ct v g/*$B^{s}Y$ at 25°, scoring 701 regular females only, gave map distances for *ct–v* of 13.0 (13.0) and for *v–g* 9.0 (11.4)—not significantly different ($p>.1$).

The above data indicate that relative frequency of scorable crossover chromosomes in the *ct–g* region to scorable non-crossover chromosomes in this region is not affected in females heterozygous for 6^{cs} at either the permissive or restrictive

temperatures. If, however, non-exchange chromosomes are preferentially involved in nondisjunction at Meiosis I, they would not be scorable since they would be in heterozygous *XXY* progeny (*ct v* + 6^{cs} *g*/+ + 6^{cs}+/*Y*). This means a 6^{cs}-produced reduction in crossing over might be masked. Further crosses specifically designed to explore this possibility must be done before a definitive conclusion can be reached.

Autosomal nondisjunction: In the crosses reported above, noticeable numbers of progeny were obtained which in addition to the expected phenotypes exhibited the Minute phenotype characteristic of haplo-*4* flies (LINDSLEY and GRELL 1968). Since triplo-*4*'s are virtually wild type, the appearance of these flies suggest that 6^{cs} induces nondisjunction of the fourth chromosome. The frequency of these Minute flies found in some of the crosses is reported in Table 5. It is apparent that at 18° more than three times as many of these Minutes were obtained than at 25°. If fourth chromosomes were to disjoin randomly, the expected percentage of Minute-like haplo-*4* flies would be 25%. In Cross 9, Table 5, the recovery of 226 Minute-like flies among 859 flies scored, or 26.3%, suggests that random disjunction of the fourth chromosome did occur in this cross. However, considering their poor viability the actual frequency of haplo-*4* zygotes may be significantly greater than the frequency observed, suggesting that something other than random nondisjunction is involved.

Nondisjunction of the second chromosome and third chromosome was tested by crossing 6^{cs} homozygous and heterozygous females to the males carrying the compound autosomes *C(2L)RM,dp;C(2R)RM,px* and *C(3L)RM,ri;C(3R)RM,sr*, respectively. The consequences of such a cross for the second chromosomes, for example, is that the only zygotes that are not unbalanced and can complete development successfully are those that arise from the fertilization of diplo-*2* eggs by nullo-*2* sperm or nullo-*2* eggs by diplo-*2* sperm. That is, nondisjunction of the second chromosome must occur during meiosis in the egg for the recovery of any progeny in these crosses with compound autosomes. The results of these crosses, presented in Table 6, clearly indicate that 6^{cs} induces nondisjunction of the second and third chromosomes in approximately equal frequencies. The

TABLE 5

Minute progeny from 1(1)TW-6^{cs} *and control crosses*

	Cross		25°			18°		
No.	♀ parent	♂ parent	Total*	*M*	Percent	Total*	*M*	Percent
6	*FM4/*6^{cs}	× *Oce/B*^{s}*Y*	601	26	4.3	66	9	13.6
7	*FM4/*+	× *Oce/B*^{s}*Y*	703	0	0	1260	0	0
9	*ct v g/*6^{cs}	× *Oce/B*^{s}*Y*	995	22	2.2	859	226	26.3
10	*ct v g/*+	× *Oce/B*^{s}*Y*	1541	0	0	1434	0	0
13†	*y ct*^{6}*v f car su-f·y*$^{+}$*/*6^{cs}	× *w/Y*	428	47	11.0	—	—	—

* Total = Total number of progeny examined for the Minute phenotype.
† Cross 13 at room temp. (22°). Female parent was *Dp(1;1)sc*^{v1} heterozygote.

TABLE 6

Nondisjunction of the second and third chromosomes induced by 6^{cs}

Cross			Progeny/25 ♀ ♀		
Exp. no.	Female parent	Male parent	Temp.	Diplo-*2* ova	Nullo-*2* ova	Total
1.	*FM6/6^{cs};+/+*	*+/Y;C(2L)RM,dp;C(2R)RM,px*	25°	6	2	8
			18°	7	2	9
			16°	0	1	1
1.	*6^{cs}/6^{cs};+/+*	*+/Y;C(2L)RM,dp;C(2R)RM,px*	25°	12	34	46
			18°	5	4	9
			16°	2	0	2
2.	*6^{cs}/6^{cs};+/+*	*+/Y;C(2L)RM,dp;C(2R)RM,px*	25°	39	54	93
			17°	1	5	6
2.	*+/+;+/+*	*+/Y;C(2L)RM,dp;C(2R)RM,px*	25°	0	0	0
			17°	0	0	0
				Diplo-*3* ova	Nullo-*3* ova	Total
1.	*FM4/6^{cs};+/+*	*+/Y;C(3L)RM,ri;C(3R)RM,sr*	25°	2	4	6
			18°	1	4	5
			16°	1	2	3
1.	*6^{cs}/6^{cs};+/+*	*+/Y;C(3L)RM,ri;C(3R)RM,sr*	25°	19	30	49
			18°	3	6	9
			16°	1	0	1

For each cross 5 vial cultures each with 5 pairs of parents were mated at the indicated temperature.

frequencies at 25° are comparable to those induced by *mei 218* of 257/100 ♀ ♀ for the second chromosome and 184/100 ♀ ♀ for the third chromosome, *mei 9* with 235/100 ♀ ♀ for the second and 173/100 ♀ ♀ for the third (BAKER and CARPENTER 1972), and *nod* with 10/100 ♀ ♀ and 6/100 ♀ ♀ for the second and third chromosomes, respectively (CARPENTER 1973). The overall excess of progeny arising from nullo-*2* and nullo-*3* ova (Table 6) indicates that 6^{cs} effects loss of autosomes as well as their nondisjunction.

In an attempt to measure more precisely the frequency of second chromosome nondisjunction, the number of adult progeny developing from randomly selected eggs from the cross $6^{cs}/6^{cs} \times C(2L)RM,dp;C(2R)RM,px$ were counted and expressed as a percent. The data, reported in Table 7, show that at 25° only 1.7% of the eggs picked were capable of completing development to the adult stage. Since in this cross only one-quarter of the zygotes arising from nondisjunction of the second chromosome at Meiosis I will be euploid and capable of survival, by multiplying by four one can calculate the actual percentage of diplo-*2* ova ($4 \times 0.5\% = 2.0\%$) and nullo-*2* ova ($4 \times 1.2\% = 4.8\%$) produced by the $6^{cs}/6^{cs}$ female parents.

The data in Table 6 indicate that at 25° homozygous females undergo five to

TABLE 7

Stage distribution of mortality and adult progeny recovered at 25° from randomly selected eggs laid by $6^{cs}/6^{cs}$ *or* $+/+$ *females mated to* C(2L)RM,dp;C(2R)RM,px *males*

		Mortality*				Adults			
♀ parent	Eggs picked	Embryonic	Larval	Pupal	Total	Diplo-2 ova	Nullo-2 ova	Total	Percent*
$6^{cs}/6^{cs}$	1953	83.6	14.0	0.7	98.3	10 0.5%*	24 1.2%*	34	1.7
$+/+$	1124	86.8	13.2	0	100	0	0	0	0

* Percent of eggs picked.

ten times as much autosomal nondisjunction as heterozygous *FM4/*6^{cs} females. The latter females show approximately equal frequencies of second and third chromosome nondisjunction at 25° and 18° but a reduced frequency at 16°. The number of progeny recovered from homozygous 6^{cs} females is drastically reduced at 18°, and is even further reduced at 16°. Considering the substantial increase in exceptional progeny recovered at 16° in Cross 5 (Tables 2 and 4), the decrease in apparent autosomal nondisjunction obtained at 18° and 16° for both homozygous and heterozygous 6^{cs} female parents is unexpected, particularly since only one-quarter of the progeny from heterozygous 6^{cs} females and only one-half of the progeny from homozygous 6^{cs} females should be homozygous or hemizygous for 6^{cs} and therefore subject to cold-sensitive zygotic lethality.

One possible explanation is that at reduced temperatures compound autosome males produce very few non-segregational sperm, a possibility that has not been checked by us. Another possibility is that at 16° nondisjunction and/or loss of unmonitored chromosomes increase so drastically as to reduce the possibility of recovering ova that show aberrant segregation for only the second or for only the third chromosome. A third possibility is that at 16°–18° $6^{cs}/6^{cs}$ females lay so few eggs that the reduced number of progeny/25 ♀ ♀ observed actually represents an increase in the number of progeny/egg. In an attempt to explore these possibilities $6^{cs}/6^{cs}$ females were mated at 25° and 17° to *y*^{2}*/Y;C(2L)RM,dp; C(2R)RM,px;C(3L)RM,h rs*^{2}*;C(3R)RM,+* males. Unfortunately, in two attempts to carry out this experiment females mated at 17° laid so few eggs that valid observations could not be made. The data for those females mated at 25° are reported in Table 8, and it is apparent that at 25° the simultaneous nondisjunction or loss of the second and third chromosome is not very frequent.

Zygotic lethality and mitotic nondisjunction or loss: As indicated previously, there is ample evidence that 6^{cs} is responsible for almost complete lethality of zygotes homozygous or hemizygous for 6^{cs} at 16°–18° (see Table 1 and Table 11). The attached-*X* crosses, Cross 2, Table 1 and Table 11, completely divorces this temperature-sensitive zygotic lethal effect from any possible maternal effect of 6^{cs}. It is not clear from the data available whether in addition to this recessive lethal phenotype 6^{cs} has a dominant zygotic lethal effect, i.e., reduced the viability of 6^{cs} heterozygotes. The difference in viability of *FM4/Oce* females (159 at 25°; 17 at 18°) in comparison with that of *Oce/*6^{cs} females (133 at 25°; 13 at

TABLE 8

Stage distribution of mortality and adult progeny recovered at 25° of eggs laid by $6^{cs}/6^{cs}$ *or* +/+ *females mated to* y^2/Y;C(2L)RM,dp;C(2R)RM,px;C(3L)RM,h rs^2;C(3R)RM, + *males*

Exp. no.	♀ parent	Eggs picked	Mortality*				Adults					
			Embryonic	Larval	Pupal	Total	Aneuploid		Euploid		Total	
							n	%*	*n*	%*	*n*	%*
1	+/+	1929	87.1	11.7	0.7	99.4	11	0.57	0	0	11	0.57
1	$6^{cs}/6^{cs}$	2000	87.2	12.3	0.1	99.6	6	0.30	2	0.1	8	0.40
2	$6^{cs}/6^{cs}$	2000	89.4	9.4	0.9	99.7	3	0.15	4	0.2	7	0.35

* Percent of eggs picked.

17°) found in the cross $FM4/6^{cs} \times Oce/B^sY$ (Cross 6, Table 3) is not convincing, and the significantly increased recovery of $B/+$ females (194) relative to + females (104) in the $FM4/6^{cs} \times w/Y$ cross at 16° (Cross 5, Table 2) probably can be accounted for by the inclusion of both $FM4/w$ females and $FM4/w/Y$ females in the former phenotypic class and only $6^{cs}/w$ females in the latter phenotypic class. The eventual definitive determination of whether or not 6^{cs} produces a dominant zygotic effect could impose important restrictions on the nature of the underlying mechanism responsible for the phenotypic effects of 6^{cs}.

One possible hypothesis is that the observed zygotic lethality might arise as a result of mitotic nondisjunction or loss. Some evidence has been found that mitotic irregularities do occur in zygotes carrying at least one dose of 6^{cs} or in zygotes that are progeny of females carrying at least one dose of 6^{cs}. These are incidental observations; no experiments having been specifically carried out to expose these phenomena.

The most extensive series of mosaics found are those that involve the Minute phenotype, presumably haplo-*4*/diplo-*4* mosaics. Most were bilateral mosaics occurring with a frequency of 0.64% at 18° and 0.86% at 25° in Cross 10, 1.6% at 22° in Cross 13 (Table 5), and 0.5% at 25° in Cross 6. These are probably minimum estimates, since no great care was taken to look for smaller mosaic spots. Two non-Minute mosaics were found in the progeny of the cross $FM4/6^{cs} \times Oce/B^sY$ and both are presumed to have arisen in *FM4/Oce* zygotes by the loss of the *FM4* chromosome to produce *FM4/Oce//Oce/O* mosaics.

Among the 428 progeny of Cross 13, $y\ ct\ v\ f\ car\ su\text{-}f\cdot y^+/6^{cs} \times w/Y$, 9 males with cut wings, non-forked bristles, and *v-car* eye color were recovered which were extensively mosaic for yellow (yellow spots on a wild-type background). One possible explanation is that in a $y\ ct\ v\ f\ car\ su\text{-}f\cdot y^+/Y$ zygote an abnormal mitotic event eliminated the *Y*, producing a $y\ ct\ v\ f\ car\ su\text{-}f\cdot y^+/O$ nucleus which then could populate various amounts of the embryo. With the *Y* eliminated in these regions the y^+ allele in the duplication on the right arm of the *X* chromosome would be free to variegate producing yellow spots in those regions where it is inactivated. That the extreme variegation is due to loss of the *Y* is supported by the observation that in a subsequent cross of $6^{cs}/6^{cs}$ females to $y\ ct\ v\ f\ car\ su\text{-}f\cdot y^+/Y$ males all eleven exceptional, cut, non-forked, *v-car* males recovered showed extensive variegation of yellow and proved to be sterile.

In addition to the mosaics described above in two crosses non-mosaic progeny were recovered with phenotypes which suggest that they arose through mitotic nondisjunction. In the cross $FM4/6^{cs} \times w/Y$ (Cross 5, Table 2) at 16° two white-eyed females were recovered. Although other explanations are possible (see above), the origin of these unusual females can be most reasonably explained by mitotic nondisjunction in a w/O zygote to give w/w nuclei which then populated most of the embryo. Unusual female progeny were also recovered in cross $ct\ v\ g/6^{cs} \times w/Y$ at 16° (Cross 8, Table 4). These were phenotypically: 16 $ct\ v\ g$, 2 $+\ v\ g$, 4 $+\ +\ g$, 6 $ct\ +\ +$, and 6 $ct\ v\ +$. The origin of all 34 of these females can be accounted for by customary crossing over in the female parent followed by mitotic nondisjunction of the X chromosome very early in the development of X/Y zygotes. The mitotic nondisjunctional event would produce $X/X/Y$ and O/Y daughter nuclei with the former populating the embryo. Alternative explanations of the origin of these 34 unusual females through crossing over and first or second division meiotic nondisjunction have been considered above. An attempt was made to repeat this experiment by crossing $ct\ v\ g/6^{cs}$ females to Oce/B^sY males at 25° and 18° along with the control cross of $ct\ v\ g/+ \times Oce/B^sY$ (Crosses 9 and 10 at 25° and 18°, Table 4). Only three of these unusual females were recovered in the $ct\ v\ g/6^{cs} \times Oce/B^sY$ cross at 18°, and all were carrying the B^sY, as expected of either mitotic nondisjunction in an X/Y zygote or meiotic nondisjunction. These three were phenotypically: 1 $B^s\ ct\ v\ +$, 1 $B^s\ ct\ v\ +\ +$, and 1 $B^s\ ct\ +\ v\ g$. The reduced recovery of unusual females may in part be ascribed to half as many progeny scored, a higher temperature (18° *vs.* 16°), and lowered expressivity of 6^{cs} with the frequency of XX-O segregations being 20% rather than the 49% for Cross 8. Currently the most conservative explanation would be that meiotic and not mitotic nondisjunction gives rise to these unusual females in Crosses 8 and 9.

Since all the female-specific mutants examined to date have exhibited some mitotic loss of maternally-derived chromosomes in the progeny (HALL, GELBART and KANKEL 1974), the mosaics reported above which arise from such loss may not be indicative of mitotic abnormalities extensive enough to account for the observed zygotic lethality. However, the loss of paternally-derived chromosomes for such mutants has not been reported, and therefore those results above that can be interpreted as misbehavior of paternally-derived chromosomes may be significant if the phenomenon can be unequivocally established. On the basis of the above genetic data alone one can not ascribe zygotic lethality to 6^{cs}-effected mitotic abnormalities; however cytological observations (see below) support this hypothesis.

Stage distribution of mortalities or effective lethal phase: Inspection of the stage distribution of mortalities in the cross $6^{cs}/6^{cs}$ females to $6^{cs}/B^sY$ males in Table 9 shows that although a majority ((1/2–3/4) of the lethality is embryonic, a not insignificant fraction takes place subsequently during the larval and pupal stages. This is true for development both at 17° and 25°. For eggs laid at 22° there appears to be a shift of some of the larval mortality to embryonic mortality in zygotes developing at 17° relative to those developing at 25°. On the other hand

TABLE 9

Stage distribution of mortalities and survival of zygotes from the cross, $6^{cs}/6^{cs}$ ♀ ♀ × $6^{cs}/B^{s}Y$ ♂ ♂

Temperature		Eggs picked	Mortality*				Adult progeny					
							Expected		Exceptional		Total	
Laid	Develop.		Egg	Larval	Pupal	Total	$6^{cs}/6^{cs}$	$6^{cs}/B^{s}Y$	$6^{cs}/6^{cs}/B^{s}Y$	$6^{cs}/0$	*n*	Percent*
22°	25°	500	70.0	22.8	3.0	95.8	5	8	2	6	21	4.2
25°	25°	1600	52.6	28.1	5.3	85.9	74	110	14	27	225	14.1
22°	17°	1397	90.1	8.9	0.9	99.9	0	0	0	1	1	0.07
25°	17°	1000	72.6	24.1	3.1	99.8	2	0	0	0	2	0.2

* Percent of eggs picked.

TABLE 10

Stage distribution of mortalities and survival of zygotes from the cross, $FM7/6^{cs}$ ♀ ♀ × $6^{cs}/B^{s}Y$ ♂ ♂

Temp. develop.	Eggs picked	Mortality*				Adult progeny							Freq. of *XX-0*†
		Egg	Larval	Pupal	Total	$FM7/6^{cs}$	$FM7/B^{s}Y$	$FM7/6^{cs}/B^{s}Y$	$6^{cs}/6^{cs}$	$6^{cs}/B^{s}Y$	$6^{cs}/0$	Total	
25°	500	36.2	30.8	7.4	74.4	25	14	23	14	25	27	128	.52
25°	500	43.0	16.0	8.0	67.0	21	19	17	29	39	40	165	.51
17°	500	66.6	23.6	3.6	93.8	11	10	6	0	3	1	31	.35

* Percent of eggs picked.
† See MATERIALS AND METHODS for method of calculation.
Eggs laid at 25°.

TABLE 11

Stage distribution of mortalities and survival of zygotes from the cross, C(1)DX,y f/Y♀♀ × 6^{cs}/B^{s}Y♂♂

Temp. develop.	Eggs picked	Mortality*				Adult progeny		
		Egg	Larval	Pupal	Total	*y f/B^{s}Y*♀♀	*6^{cs}/Y*♂♂	Total
25°	1500	65.3	9.6	5.9	80.9	121	166	287
17°	1500	74.3	15.3	4.1	93.7	82	12	94

* Percent of eggs picked.
Eggs laid at 25°.

among eggs laid at 25° the level of larval mortality appears to remain the same at 17° as that at 25°, but there is an overall increase in embryonic mortality at 17°.

In the *FM7/6^{cs}* × *6^{cs}/B^{s}Y* cross (Table 10) again between a half and two-thirds of the total mortality is embryonic, with a majority of the rest of the mortality being larval. Also as above the increase in total mortality at 17° is primarily due to an increase in embryonic mortality. In the *C(1)DX,y f/Y* × *6^{cs}/B^{s}Y* cross at 25° (Table 11) the highest level of embryonic mortality relative to total mortality is obtained. This is undoubtedly due in part to the fact that 25% of the zygotes produced by this cross are nullo-*X* zygotes and die during embryogenesis (POULSON 1945; SCRIBA 1964). The increase in total mortality at 17° in this cross occurs both during embryogenesis and during larval development.

In all three crosses (Table 9, 10 and 11) there is a definite increase in embryonic mortality at 17° relative to that found at 25°. Since for all three crosses parental females were maintained at 25° and the eggs were collected at 25° prior to incubation at 25° or 17°, this increase in embryonic mortality cannot be due to the effect of reduced temperature on the female parent but must be an effect of the reduced temperature directly on the developing zygote. It is clear from the data in Tables 9, 10 and 11 that there is an increase in embryonic mortality in *6^{cs}/6^{cs}* homozygotes and *6^{cs}/B^{s}Y* hemizygotes in the cold, but the data in Table 10 are not good enough to permit the same conclusion to be drawn for *FM7/6^{cs}*, *FM7/B^{s}Y*, and *FM7/6^{cs}/B^{s}Y* zygotes which are derived from *6^{cs}*-bearing mothers.

Is it valid to suggest that all the mortality that arises from nondisjunctionally-derived aneuploid zygotes is embryonic, and that none of the non-embryonic mortality is attributable to nondisjunction during Meiosis I? Observations made by SCRIBA (1967, 1969) on crossing individuals carrying compound autosomes *inter se* and those of HERSKOWITZ and SCHALET (1957) on zygotes hypoploid or hyperploid for either the left arm of the second chromosome or the right arm of the third chromosome (see discussion in WRIGHT 1970) do lead to the generalization that all zygotes aneuploid for major portions of the second or third chromosome should die during embryogenesis. However, data reported in Table 9 indicate that this generalization probably is not warranted, since in the cross of +/+

females to *C(2L)RM,dp;C(2R)RM,px* males all four types of zygotes produced are aneuploid (triplo-*2L*,haplo-*2R*;haplo-*2L*,triplo-*2R*; triplo-*2*; haplo-*2*), yet 13% do not die until the larval stage (probably the triplo-*2* zygotes). Therefore, the above suggestion probably is not valid, and in these crosses mortality during all three stages of development may be due to aneuploidy generated both by meiotic and mitotic abnormalities.

Embryonic abnormalities: If the observed embryonic lethality arises from the effects of aneuploidy, both meiotically and mitotically derived, one would not expect to find a single typical pattern of abnormalities among those embryos that fail to hatch, but rather one should find a spectrum of different lethal syndromes (see SCRIBA 1967, 1969). A survey of sectioned and stained serial sections of embryos that failed to hatch from the cross $6^{cs}/6^{cs} \times 6^{cs}/B^{s}Y$ at 18° confirmed this supposition. Embryos with many different patterns of damage were found and most of them could be inferred to have arisen from abnormal events very early in embryogenesis, i.e., abnormal cleavage divisions and the incomplete population of the syncytial blastoderm with nuclei. Stained serial sections of very young embryos (first-fifth cleavage division) laid at 18° by $6^{cs}/6^{cs}$ females mated to $6^{cs}/B^{s}Y$ males revealed the presence of abnormal mitotic figures, including multipolar spindles in a majority of the embryos inspected. It is not yet known whether these abnormal mitotic figures arise directly as a result of 6^{cs} activity or inactivity and are then responsible for mitotic loss and mitotic nondisjunction or whether the observed mitotic irregularities arise indirectly as the result of meiotically-derived aneuploidy. A detailed report of the cytological effects of 6^{cs} will be presented in a separate communication.

Temperature-sensitive period: The shift of different individuals down from 25° to 17° at progressively later stages in development makes it possible to determine how late in development individuals are susceptible to the restrictive temperature (17°)—i.e., a shift-down experiment for a cold-sensitive mutation should indicate the end of the temperature-sensitive period. Figure 1 presents the results on total mortality and on the stage distribution of mortalities for such a shift-down procedure on homo- and hemizygous 6^{cs} individuals. Since the overall difference between total mortality at 0 hr shift-down (100%) and no shift-down (84%) is only 16% and since there is a slow, gradual decline in total mortality from the 72 hr shift-down to the 216 hr shift-down, it is not possible to determine a precise end-point for the temperature-sensitive period. Whether some individuals as late in development as the third larval instar (120 hr) are still cold-sensitive is questionable. Certainly a significant percentage are still sensitive during the first larval instar (24 hr). A duplicate shift-down experiment gave very similar results.

The shift of different individuals up from 17° to 25° at progressively later stages in development makes it possible to determine how early in development individuals are sensitive to the restrictive temperature—i.e., a shift-up experiment for a cold-sensitive mutation indicates the beginning of the temperature-sensitive period. The data from such a shift-up experiment, presented in Figure 2, indicate that the cold-sensitive period for 6^{cs} homozygotes and hemizygotes

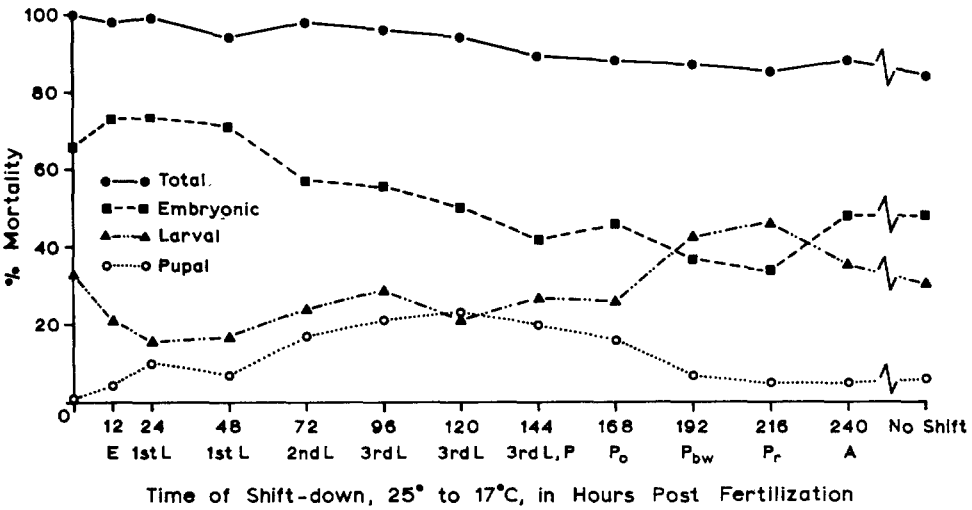

FIGURE 1.—Shift-down experiment. Total mortalities and stage distribution of mortalities as zygotes ± 2-hr old laid at 25° by $6^{cs}/6^{cs}$ females mated to $6^{cs}/B^sY$ were shifted down from 25° to 17° at progressively later stages in development. Mortalities are expressed as percentage of the number of zygotes (500 or 600) shifted at each time interval. Predominant stages of development at time of shift are: E = Embryonic; 1st L = 1st Larval; 2nd L = 2nd Larval; 3rd L = 3rd Larval; P = Pupal prior to disc eversion; P_o = Pupal—no eye pigment; P_{bw} = Pupal—brown eye pigment; P_r = Pupal—red eye pigment; A = Adult.

begins early in embryogenesis prior to 12 hrs of development at 17°. (See legend of Figure 3 for stage of embryogenesis.) This is demonstrated by the fact that of those embryos exposed to the cold for a 12-hr period at the beginning of embryogenesis, only a very small percentage (2.5%) were capable of completing development successfully when shifted up to 25°. One hundred percent of the zygotes die as a result of a 24-hr exposure to 17° prior to the shift-up. A duplicate experiment not presented here gave exactly the same results.

One can conclude from these shift experiments (Figures 1 and 2) that the temperature-sensitive period (TSP) for 6^{cs} homo- and hemizygotes derived from the cross $6^{cs}/6^{cs} \times 6^{cs}/B^sY$ begins early in embryogenesis prior to 12 hrs of development at 17° and extends at least into the first larval instar and perhaps longer into the second or third larval instars.

Temperature sensitivity of 6^{cs} homo- and hemizygotes early in embryogenesis is confirmed by the data presented in Figures 3 and 4 where shifts were made at 6-hr intervals during embryogenesis. The results of the experiments presented in Figure 3 indicate that of those zygotes that would have been capable of completing development successfully if raised continuously at 25°, some will die if exposed to the restrictive temperature for only 6 hours at the beginning of embryogenesis. The number increases substantially if the exposure time is lengthened to 12 hours, and an 18-hr exposure to 17° kills virtually all of them.

It is evident in the data that for those individuals raised continuously at 25° more 6^{cs} progeny of females carrying no doses of 6^{cs} $(C(1)DX,y\ f/Y)$ survive

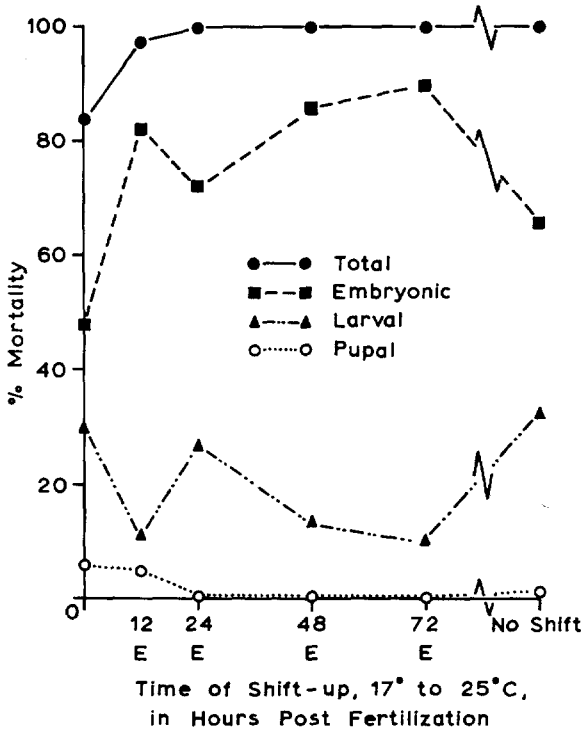

FIGURE 2.—Shift-up experiment 17° to 25°. See legend to Figure 1 for details. After 48 hr and 72 hr of development at 17° wild-type individuals are in the 1st Larval Instar, but most of the 6^{cs} individuals have died as embryos.

than those from females carrying one dose of 6^{cs} ($FM7/6^{cs}$), which in turn have more 6^{cs} progeny survive than females carrying two doses ($6^{cs}/6^{cs}$) (55% *vs.* 43% *vs.* 14%). The differences in survival probably are for the most part a reflection of the frequency of aneuploid zygotes which arise from different frequencies of meiotic nondisjunction and chromosome loss in females carrying different doses of 6^{cs}, but a maternal effect of 6^{cs} on the subsequent survival of 6^{cs} homo- and hemizygotes may also be involved (see below). In the shift-up experiments (Figure 3) the three curves are essentially a family of curves having the same overall shape but starting at the different levels of survival indicated above. By the 18-hr shift-up all three curves have reached the minimum levels of survival obtained. This indicates that regardless of their maternal origin most 6^{cs} homo- and hemizygotes exposed to 17° for 18 hours early in development will die.

In the shift-down experiments (Figure 4) the shape of the survival curve of $6^{cs}/Y$ hemizygotes derived from attached-*X* females ($C(1)DX,y\ f/Y$) is strikingly different from the curves obtained for the survival of 6^{cs} homo- and hemizygotes from $6^{cs}/6^{cs}$ and $FM7/6^{cs}$ female parents. The first curve reflects the effects of temperature shifts on the zygote itself, uncomplicated by any possible effect of 6^{cs} in the maternal genome. In this case, apparently the longer embryo-

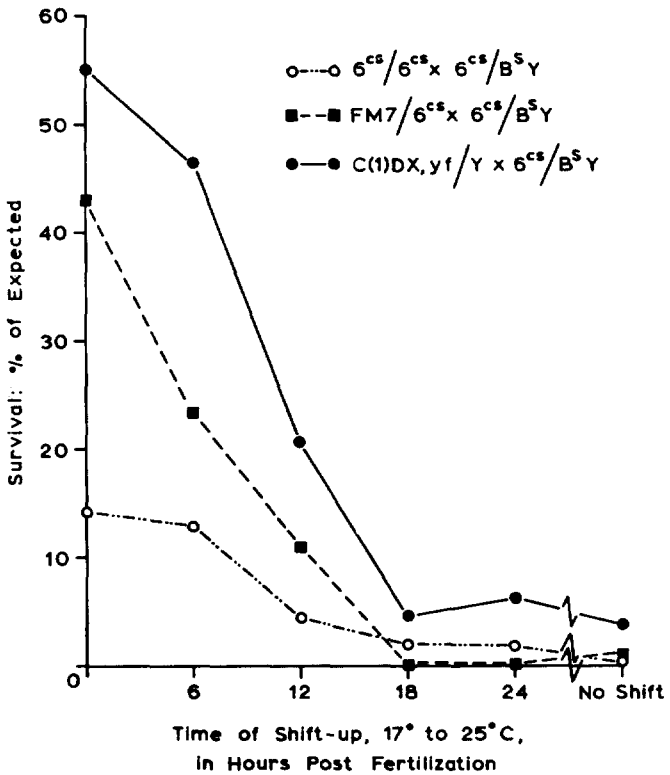

FIGURE 3.—Shift-up experiments during embryogenesis. Survival of homozygous and hemizygous 6^{cs} progeny as 500 eggs 1 hr ± 1 hr old laid at 25° by $6^{cs}/6^{cs}$ females, $FM7/6^{cs}$ females, and $C(1)DX,y\ f/Y$ females mated to $6^{cs}/B^sY$ males were shifted up from 17° to 25° at progressively later stages in development. Survival is expressed as "% of Expected". For the $6^{cs}/6^{cs} \times 6^{cs}/B^sY$ cross % of Expected for each point equals $\frac{6^{cs}/6^{cs} + 6^{cs}/B^sY + 6^{cs}/6^{cs}/B^sY + 6^{cs}/0\ adults}{500\ \text{eggs shifted}} \times 100$. For the $FM7/6^{cs} \times 6^{cs}/B^sY$ cross % of Expected for each point equals $\frac{6^{cs}/6^{cs} + 6^{cs}/B^sY + 6^{cs}/0\ adults}{\frac{1}{2}\ (500\ \text{eggs shifted})} \times 100$. For the $C(1)DX,y\ f/Y \times 6^{cs}/B^sY$ cross % of Expected for each point equals $\frac{6^{cs}/B^sY\ adults}{\frac{1}{4}\ (500\ \text{eggs shifted})} \times 100$.

Predominant embryonic stage at 17° of wild type at time of shift. 0 hr: Stage 1. Cleavage. 6 hr: Stage 6–11. Early gastrulation to extended germ band and beginning of stomodaeum and proctodaeum. 12 hr: Stages 13–14. Extended germ band with tracheal invaginations and segmentation of head trunk. 18 hr: Stages 15–16. Shortening begins and head involution and dorsal closure begin. 24 hr: Stage 18. Dorsal closure complete. Frontal sac deep. Midgut saclike, unconstricted

genesis proceeds at 25° prior to the shift-down to the restrictive temperature, the higher will be the percentage of $6^{cs}/Y$ hemizygotes capable of completing development successfully. This is not true for those 6^{cs} progeny derived from female parents with one or two doses of 6^{cs}. Embryonic development at 25° for 6-, 12- and 18-hour periods prior to shift-down to the restrictive temperature does not increase the percentage of 6^{cs} progeny that survive. Although a 24-hour period

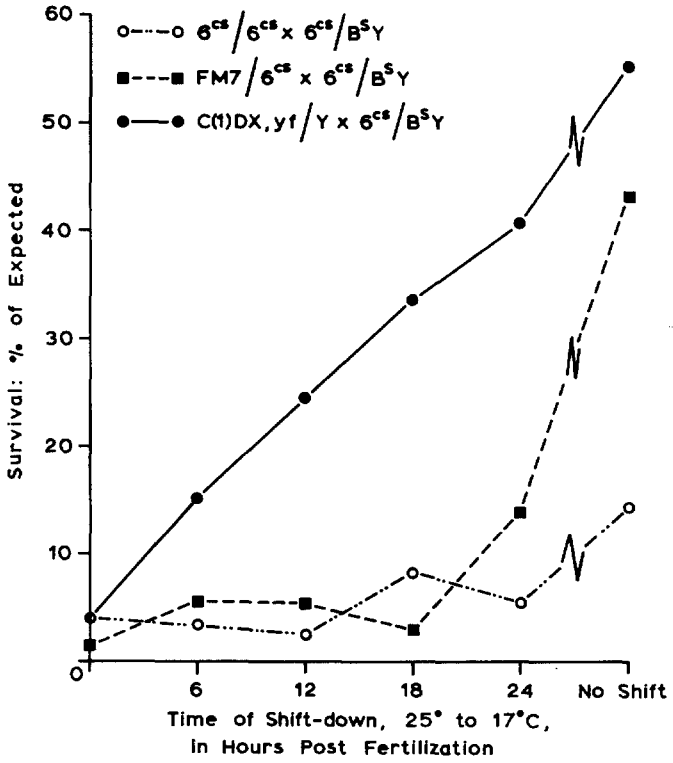

FIGURE 4.—Shift-down experiments during embryogenesis. See legend for Figure 4 for details of procedure. Predominant embryonic stage at 25° of wild type at time of shift. 0 hr: Stage 1. Cleavage. 6 hr: Stage 11–13. Extended germ band with beginning of stomodaeum and proctodaeum to tracheal invagination. 12 hr: Stages 18–19. Dorsal closure complete. Midgut saclike, unconstricted to midgut constricted. 18 hr: Late Stage 20. Larval differentiation complete, active muscular movements. 24 hr: Larvae hatched—Early first larval instar.

at 25° does increase the survival of 6^{cs} zygotes derived from $FM7/6^{cs}$ females, it has little effect on 6^{cs} zygotes derived from $6^{cs}/6^{cs}$ females. These data indicate that in addition to affecting the segregation of chromosomes during meiosis, the presence of one or more dose of 6^{cs} in the maternal genome prolongs the temperature-sensitive period of 6^{cs} homo- and hemizygotes, i.e., a maternal effect.

It appears that in these shift experiments the number of zygotes capable of surviving is dependent on the number of maternal doses of 6^{cs} (or $+^{6^{cs}}$), the duration of exposure to the restrictive temperature at the beginning of development, and the genotype of the zygote (survival of $FM7/6^{cs}$, $FM7/B^{s}Y$, $FM7/6^{cs}/B^{s}Y$ and $C(1)DX,y\ f/B^{s}Y$ zygotes in these shift experiments has been ommitted to avoid utter confusion).

SUMMARY AND PROPOSED MODEL

Any model that is proposed to explain the effects of the mutation $l(1)TW\text{-}6^{cs}$ must account for the following observations:

1. That meiotic nondisjunction of all four chromosomes occurs in females homozygous or heterozygous for 6^{cs}, that the frequency of first division nondisjunction of the X and fourth chromosomes is increased significantly at 16°–18° relative to 25° and can approach 0.5, and that if 6^{cs} induces second division nondisjunction of the X chromosomes at all, it does so infrequently, i.e., less than 2%.

2. That 6^{cs} does not increase either first or second division meiotic nondisjunction in males hemizygous for 6^{cs} at either 16°–18° or at 25°.

3. That some progeny of females homo- or hemizygous for 6^{cs} exhibit mitotic loss or mitotic nondisjunction of the X, Y, and fourth chromosomes at both °25 and 18° and that abnormal mitotic figures occur with high frequency in young embryos produced by the cross $6^{cs}/6^{cs} \times 6^{cs}/B^sY$ at 18°.

4. That at 25° 45–55% and at 17° 98% of the 6^{cs} hemizygotes derived from female parents carrying no doses of 6^{cs} die—i.e., zygotic lethality uncomplicated by any maternal influence of 6^{cs}.

5. At 25° 86% and at 17° 99.8–99.9% of the zygotes produced by $6^{cs}/6^{cs}$ females mated to $6^{cs}/B^sY$ males die, and at 25° 67–74% and at 17° 94% of all the zygotes produced by $FM7/6^{cs}$ females mated to $6^{cs}/B^sY$ males die.

6. That when $6^{cs}/6^{cs}$ homozygous females are mated at 16°–18° to males of any genotype virtually all progeny, whether homozygous or heterozygous for 6^{cs}, die, making $6^{cs}/6^{cs}$ females essentially sterile at 16°–18°.

7. That when matings of heterozygous 6^{cs} females are made at 16°–18° almost all progeny heterozygous for 6^{cs} and almost all progeny homozygous or hemizygous for the wild-type allele ($+^{6^{cs}}$) may die.

8. That at both 25° and 18° 6^{cs}-produced mortality is primarily embryonic with some larval but very little pupal mortality, and that at 17° there is an increase in embryonic mortality relative to that at 25°.

9. That the temperature-sensitive period of 6^{cs} homo- and hemizygotes derived from $6^{cs}/6^{cs}$ females begins early in embryogenesis prior to gastrulation and does not end until the second larval instar or perhaps even later during the third larval instar.

10. That regardless of maternal origin 95–100% of all 6^{cs} homo- and hemizygotes raised for the first 18 hours of development at 17° die even if at 18 hours or subsequently they are shifted up to 25°.

11. That when 6^{cs} hemizygotes derived from female parents carrying *no* doses of 6^{cs} develop for progressively longer periods of time at 25° prior to being shifted down to 17°, the proportion of these 6^{cs} hemizygotes that survive immediately increases, progressively foreshortening the TSP significantly relative to the TSP found for 6^{cs} homo- and hemizygotes derived from females carrying one and two doses of 6^{cs} (see Observations 9, 12 and 13).

12. That when 6^{cs} homo- and hemizygotes derived from female parents carrying *one* dose of 6^{cs} develop for progressively longer periods of time at 25° prior to being shifted down to 17° the proportion of these zygotes that survive does not immediately increase. Apparently embryogenesis must be completed at 25° prior to the shift-down before any increase of survival occurs.

13. That when 6^{cs} homo- and hemizygotes derived from female parents carrying *two* doses of 6^{cs} develop for progressively longer periods of time at 25° prior to being shifted down to 17° the proportion of these zygotes surviving does not increase until either the second or third larval instar.

It is probably sufficient to ascribe all of the observed mortalities to the effects of aneuploidy of the first, second and third chromosomes, along with some to aneuploidy of the fourth chromosome. Very high frequencies of meiotic nondisjunction along with mitotic nondisjunction or mitotic loss could probably account for the levels of aneuploidy necessary. The data presented here indicate that sufficiently high levels of meiotic nondisjunction do occur, but the data are as yet insufficient to implicate mitotic loss or nondisjunction as the sole cause of lethality in zygotes derived from females with no doses of 6^{cs} (Observation 4). The data of SCRIBA (1967, 1969) and of HERSKOWITZ and SCHALET (1957) indicate that meiotically-derived aneuploidy of the second and third chromosomes results only in embryonic lethality. Other data reported here (Table 7) show that although mortality is primarily embryonic, some meiotically-derived aneuploidy does not result in death until the larval stages. Since nothing direct is known about the stage distribution of mortalities which result from mitotic aneuploidy arising at different stages of development, one can accept that the stage distribution of mortalities caused by 6^{cs} could be due to both meiotically and mitotically-derived aneuploidy.

A possible molecular model that would account for most of the rest of the observations listed above is that the mutant allele 6^{cs} is responsible for the synthesis of a defective protein both during oogenesis and during development of the zygote (at least during embryogenesis). A variable percentage (from individual to individual) of these mutant protein molecules assumes an abnormal conformation at 25°. This percentage is increased at lower temperatures. If sufficient numbers of these molecules in the abnormal conformation are present, aberrant nuclear divisions ensue which can result in meiotic nondisjunction at Meiosis I, and mitotic nondisjunction or mitotic loss. This effect of abnormally-conformed mutant molecules occurs even in the presence of normally-conformed wild-type molecules. Females carrying different doses of 6^{cs} put proportionally different levels of mutant molecules in eggs. The final survival percentages and stage distribution of mortalities observed result from the complex interactions of a number of parameters superimposed on the inherent background of individual variability. These parameters are: the number of mutant molecules incorporated in the egg during oogenesis, the synthesis of additional mutant molecules during embryogenesis in those zygotes carrying 6^{cs} alleles, the dilution of maternally-derived mutant molecules by wild-type molecules as embryogenesis proceeds in those zygotes carrying the wild-type allele ($+^{6^{cs}}$), and the temperature regime to which the developing zygote is subjected.

For instance, in Observation 11 the immediate, progressive increase in the percent survival of 6^{cs} hemizygotes carrying no maternally-derived mutant molecules when shifted down at progressively later stages in development is probably due to the fact that as more and more of the crucial mitoses early in development

are successfully completed at 25° prior to shift-down in the presence of subthreshold numbers of abnormally-conformed, zygotically-produced mutant molecules, the higher the probability becomes that zygotes will be able to complete development successfully without undergoing fatal mitoses after the shift-down to 17°. However, that 6^{cs} homo- and hemizygotes carrying maternally-derived mutant molecules do not show this immediate, progressive increase in percent survival (Observations 12 and 13) may be due to the fact even though some embryos may have successfully completed crucial mitoses early in development at 25° prior to shift-down, after shift-down the level of maternally-contributed mutant molecules is still so high as to make it very unlikely that zygotes can successfully complete development at 17° without undergoing fatal mitotic divisions. Similar permutations of the parameters presented by the model can also adequately explain Observations 4 through 10.

Unaccounted for by this model are Observations 1 and 2, that 6^{cs} induces high frequencies of nondisjunction at Meiosis I but little or none at Meiosis II, and that 6^{cs}-induced meiotic nondisjunction occurs in females and not in males. Speculations on the molecular mechanisms of just how 6^{cs} affects certain nuclear divisions and not others should await definitive answers to a number of specific questions, such as: Does 6^{cs} effect high frequencies of mitotic nondisjunction or loss in zygotes derived from females carrying no doses of 6^{cs}; i.e., does it produce mitotic irregularities uncomplicated by meiotically generated aneuploidy? Is the apparent unaltered frequency of recombination on the *X* chromosome real, and is this also true for the autosomes? Does 6^{cs} preferentially cause the nondisjunction of non-exchange chromosomes *vs.* exchange chromosomes, and does it in any way alter the distributive disjunction of non-homologous chromosomes? The sophisticated crosses needed to expose these parameters of *X*-linked meiotic mutants have already been used by BAKER and CARPENTER (1972) and CARPENTER (1973), and their use with 6^{cs} awaits the incorporation of 6^{cs} into the necessary stocks.

Finally one should note that the locus of 36 on the *X* chromosome of one of the extensively analyzed meiotic mutants, *nod* (CARPENTER 1973), raises the possibility that *l(1)TW-*6^{cs}—located at 37.1—is an allele of it. This is not very far considering the problems involved in mapping meiotic mutants. CARPENTER (1973) reports that *nod*, no distributive disjunction (see synonym *mei-254*^{a} in BAKER and CARPENTER 1972), a recessive mutant with slight dominant effects under some conditions, causes the nondisjunction of all four chromosomes almost exclusively at Meiosis I, with frequencies of 1.8% for the *X* chromosome, 10.1/100 ♀ ♀ for the second chromosome, 5.8/100 ♀ ♀ for the third chromosome, and 86% for the fourth chromosome. Except for the fourth chromosome these frequencies are more than an order of magnitude less than those reported here for 6^{cs}. Like 6^{cs}, *nod* has no effect on meiosis in males but does effect some mitotic irregularities. It does not alter recombination frequencies in the *X* and second chromosomes. Virtually all of the nondisjunction in *nod* involves non-exchange chromosomes. This preferential nondisjuction of non-exchange chromosomes and the absence of evidence of nonhomologous disjunction suggested to CARPENTER

(1973) that distributive disjunction is inoperative in *nod*. Unfortunately, due to the magnitude of the dominant effects of 6^{cs}, it will probably be difficult to establish allelism between *nod* and *l(1)TW-*6^{cs}.

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