

ASPECTS OF LOW-TEMPERATURE-INDUCED MEIOTIC NONDISJUNCTION IN *DROSOPHILA* FEMALES¹

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IT has been shown that aging females of *Drosophila melanogaster* at low temperature induces very high frequencies of meiotic primary nondisjunction of the X chromosomes (TOKUNAGA 1970). This paper reports the stage during meiosis at which this occurs; the finding that the nondisjunction is not restricted to the X chromosomes; and the finding of a positive correlation between number of retained mature eggs in treated females and frequency of nondisjunction.

MATERIALS AND METHODS

In the main experimental series, newly eclosed females were subjected to a pretreatment on minimal food at 25°C for 3 days (for composition of the minimal food see TOKUNAGA 1970). This pretreatment was followed by a treatment period of 1 or 2 weeks on minimal food at 10°C. After treatment, the females were placed on normal food at 25°C, and mated for 1 day to males that were at least 3 days old. These procedures were the same as those in some of the experiments in which the effect of low temperature with aging was first noticed (HILDRETH and ULRICH 1969). In the course of the present investigation, it was found that pretreatment with normal food leads to higher frequencies of nondisjunction than pretreatment with minimal food. At the time of this discovery most of the data had been obtained on flies pretreated with minimal food.

Beginning with the end of the treatment period, the females were transferred daily to new culture vials so that successive broods could be obtained. When only broods from the first 5 days were collected no second mating was initiated, but when later broods were collected a second mating was initiated on the sixth day after treatment. In some experimental series, the procedures just outlined were modified as will be indicated below.

In general, a single treated female was used for each culture vial. However, since the number of progeny in the first-day brood is very limited, this brood was mostly obtained from mass matings of 15 females and 30 males per vial. In some cases, later broods were collected from random samples of the treated females from which the first-day brood had been collected.

RESULTS

Low-temperature-induced nondisjunction occurring at the first meiotic division: According to earlier experiments (TOKUNAGA 1970), the first-day broods from low-temperature-treated females have been derived mostly from retained mature eggs which were subjected to the low temperature. This is the only brood among the first to 13th-day broods that shows an extremely high frequency of nondisjunction.

In the first series of experiments, treatment at 10°C followed 3 days of pre-

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treatment and lasted one week. In order to distinguish between nondisjunction at the first and second meiotic divisions, the following genetic procedures were employed. Treated females had one normal X chromosome marked by yellow (γ , 1-0.0) and one abnormal X chromosome marked by yellow² (γ^2) and by a short duplication of the tip of a γ^+ X chromosome on the right arm, very close to the kinetochore. These females were mated to Bar (B , 1-57.0) males. The normal X chromosome of the females also carried white (w , 1-1.5). Only B and the γ alleles will be considered further unless stated otherwise. In detail, the constitution of the abnormal X chromosome was $In(1LR)sc^{V1}, \gamma^2 sc car \cdot \gamma^+$ (LINDSLEY and GRELL 1968). It will be referred to as γ^+ duplication X .

The regular progeny of the cross consist of $B/+$ females and γ^+ and γ males. Nondisjunction at the first meiotic division would result in recognizable $\gamma^+ B^+$, XXY ♀ and $\gamma^+ B, X0$ ♂ exceptions. Nondisjunction at the second division would result in the same two classes of exceptions as well as γB^+ females and $\gamma^2 B^+$ females resulting from crossing over both in the long arm of the X chromosome and rarely to the right of the kinetochore. The X -chromosome constitution of each $\gamma^+ B^+$ exceptional female was tested by mating to γ males. If the female were the consequence of nondisjunction at the first division, her male progeny would be γ^+ and γ ; if from the second division, only γ^+ males would be found.

The progeny of the treated females of the first series of experiments are listed in Table 1. Here, the first-day brood was for the study of exceptions induced by low temperature, and the 6- to 13th-day broods were for the study of exceptions induced by aging (TOKUNAGA 1970). There were no γB^+ or $\gamma^2 B^+$ (XXY) daughters. A total of 212 $\gamma^+ B^+$ (XXY) females was recovered, of which 210 were from the first day and two from later broods. Each of these females was crossed to γ males. Nineteen females were either sterile (12 cases, including one from a 9th-day brood), or died (4 cases) or were lost before mating (3 cases). Each of the remaining 193 fertile females produced both γ^+ and γ sons, thus proving that they had one γ^+ duplication X and one normal X chromosome. Of the two exceptional daughters from later broods, one was sterile and the other proved to have resulted from nondisjunction at the first division.

TABLE 1

Progeny of crosses between B males and $In(1LR)sc^{V1}, \gamma^2 sc car \cdot \gamma^+/y w$ females

Brood (day)	Regular			Exceptional			Triploid intersexes		Total	X-chromosomal exceptions (percent)
	Females $\gamma^+ B$	Males $\gamma^+ B^+$	Males γ (or $\gamma^2 B^+$)	Females $\gamma^+ B^+$	Males γ (or $\gamma^2 B^+$)	Males $\gamma^+ B$	$\gamma^+ B^+$	$\gamma^+ B$		
1	1420	620	734	210	0	190	70	9	3253	14.44
6	4750	247	2546	0	0	0	0	0	9743	0.00
7	4919	2338	2688	0	0	1	0	0	9946	0.01
8	5132	2388	2883	1	0	0	0	0	10404	0.01
9	4162	2016	2384	1	0	1	0	0	8564	0.02
10-11	3525	1574	2090	0	0	1	0	0	7190	0.01

P_1 females low-temperature treated (10°C) for 1 week. Only the phenotypes dependent on the γ and B loci are listed. First-day brood is from 2085 treated females, and the remaining broods are from 142 females randomly selected among the 2085 treated females.

The results show that the low-temperature-induced nondisjunction occurred exclusively at the first meiotic division of the eggs retained by the P_1 females during treatment. This finding is similar to most cases of spontaneous nondisjunction (MERRIAM and FROST 1964) or nondisjunction induced by meiotic mutants (with two exceptions; see SANDLER *et al.* 1968).

The low-temperature-induced primary exceptions appear to be derived from a random sample of oocytes with respect to recombinational history. Forty-eight of the 210 $\gamma^+ B^+$ females (XXY) listed in Table 1, line 1, and 11 of the 70 $\gamma^+ B^+$ intersexes (XXY 3A) had white eyes ($59/280 = 21.1\%$). This frequency of homozygosis suggests that the exceptional oocytes have undergone normal recombination and is comparable to the homozygosis observed for *sc* by other authors: BEADLE and EMERSON (1935) recorded 21.3% *sc* homozygosis in attached-X; MERRIAM and FROST (1964) observed 6 *sc* and 15 *sc*⁺ homozygotes among 94 spontaneous primary exceptions; and DAVIS (1969) observed 48 homozygotes for either *sc* or *sc*⁺ among 132 *ca*nd-induced primary exceptions.

Low-temperature-induced nondisjunction of X chromosomes and autosomes: Table 1 shows that in addition to XXY and $X0$ exceptions, 79 flies, i.e., 2.4% of the total first day broods, were triploid intersexes. Seventy of these had $\gamma^+ B^+$ (XXY 3A) phenotype and nine were $\gamma^+ B$ (XX 3A). The recovery of the intersexes at this high frequency cannot be due to accidental presence of triploid females among the parents. If it were, then the intersexes should be found in various later broods instead of exclusively in the first brood.

The 70 XXY 3A intersexes apparently came from eggs which had two *X*, two second, and two third chromosomes, due to failure of reduction in meiosis before fertilization, and which were fertilized by *Y* sperm. The 9 XX 3A intersexes came from eggs in which the *X* chromosomes had disjoined normally but the long autosomes had failed to do so.

As to the short fourth chromosomes, the situation is not fully clear. Perhaps the failure of disjunction of the long autosomes was accompanied by nondisjunction of chromosome 4; perhaps chromosome 4 had disjoined normally. No decision is offered by the phenotype of the intersexes, since diplo-4 and triplo-4 intersexes cannot be distinguished readily by mere inspection. However, independent evidence for nondisjunction of chromosome 4 is shown below.

As pointed out above, 70 out of the total 79 triploid intersexes involved nondisjunction of the *X* chromosomes and fertilization by *Y* sperm. One would expect an equal number of $3X$ 3A triploids originating from $2X$ 2A eggs and *X* sperm. These triploids would have large slightly *B*-type eyes due to the *B* constitution of the *X* sperm. Prior to the recognition of triploid intersexes no search for triploid females had been made, so that such females, if occurring, were lost among the regular F_1 *B* females. If triploid female zygotes actually originated with the same frequency as the XXY 3A intersexes, then the frequency of nondisjunction of the *X* chromosomes would be considerably higher than judged by the value of 14.4% that is based on recovered exceptions only. However, this consideration implies more or less equal viability of triploid females and intersexes throughout development, an assumption which may not be valid.

TABLE 2

Progeny of crosses between B males and In(1LR)sc^{v1}, y² sc car · y⁺/y w females

Brood (day)	Regular			Exceptional					Total	X-chromo- somal ex- ceptions (%)	
	Females	Males	y(or y ²)B ⁺	Females	Males	Triploid females	Triploid intersexes				
	y ⁺ B	y ⁺ B ⁺		y ⁺ B ⁺	y(or y ²)B ⁺	y ⁺ B	y ⁺ B ⁺	y ⁺ B			
10°C series											
1	469	294	201	21	0	28	1	4	5	1023	5.28
2	2349	1372	1130	1	0	0	0	0	0	4852	0.02
3	3245	2059	1684	0	0	0	0	0	0	6988	0.00
25°C series											
1	1026	636	571	3	0	0	0	0	0	2236	0.13
2	3408	1950	1734	0	0	0	0	0	0	7092	0.00
3	2984	1708	1430	0	0	0	0	0	0	6122	0.00

P₁ females were low-temperature-treated (10°C) and control (25°C) temperature-treated for 1 week. Only the phenotypes dependent on the γ and B loci are listed. First-day brood is from 525 (10°C) and 600 (25°C) treated females, and the remaining broods are from 125 females randomly selected among the P₁ females from which the first-day brood was collected.

In order to ascertain whether the high frequency of autosomal nondisjunction is a typical feature of low-temperature-treated eggs, the experiment was repeated (Table 2). In this second series, broods of the first 3 days were collected and a control series added in which the females were aged on minimal food at 25°C. As in the first series, there was a high frequency of nondisjunctional exceptions of the X chromosomes in the first-day broods and only one exceptional female in the later broods. Other nondisjunctional exceptions, all arising in first-day broods, included one triploid female, nine intersexes, five haplo-4 females, and one male who was both $X0$ and haplo-4 (the haplo-4 flies are included but not listed separately in Table 2). Once again it is apparent that autosomal exceptions occurred exclusively in the first-day broods, which is evidence against accidental involvement of triploid and haplo-4 P₁ females. Additional evidence for this is the fact that no autosomal exceptions were found among the flies of the 25°C series listed in Table 2.

The total number of X -chromosome exceptions among the 1023 first-brood progeny of the second series includes 21 γ^+ B^+ females (XXY), 28 γ^+ B males ($X0$), the γ^+ B triploid female, and the 4 γ^+ B^+ out of a total of nine intersexes. They amounted to 5.3%. This value is significantly lower than that in the first series of low-temperature experiments (χ^2 $P < 0.001$), but is not significantly different from the earlier published value of 5.1% involving normal X chromosomes (TOKUNAGA 1970).

The experiments presented so far involve the presence of the γ^+ duplication X chromosome. In order to test for the possibility that the occurrence of autosomal nondisjunction was caused by the special X chromosome, a third low-temperature experiment was conducted simultaneously with the second; it differed from the second by involving only structurally normal X chromosomes in the P₁ females. Specifically the females were homozygous γw and the males $+$ $+$. A total of 1599 progeny from first-day broods from 1250 treated females included 23

yw females (*XXY*), 34 γ^+ males (*X0*), and 5 *yw* out of 10 intersexes (the other 5 intersexes were γ^+). The presence of 10 intersexes among the progeny is evidence that autosomal nondisjunction had been induced by the treatment independent of the structural type of *X* chromosomes in the P_1 females. The frequency of *X*-nondisjunctional exceptions was 3.9%, which is not statistically different from the 5.3% in the second series of experiments nor from the average 5.1% in the comparable experiments reported earlier ($\chi^2 P = 0.09-0.1$ in both comparisons).

In summary, the experiments demonstrate the occurrence of nondisjunction of a whole set of diploid chromosomes (or the failure of the meiotic first division), or of a set of second and third autosomes as well as of nondisjunction of the fourth chromosomes either alone or in combination with *X*-chromosome nondisjunction.

A lack of independence of nondisjunction of *X* chromosomes and autosomes is clear from the data of three experiments. In the first series of experiments, while *X* exceptions comprise 12.3% (210 + 190/3253) when chromosomes 2 and 3 disjoined, they are 88.6% (70/79) when both autosomes nondisjoined (Table 1, line 1). In the second series of experiments, while *X* exceptions are 4.8% (21 + 28/1023) when both autosomes disjoined, they are 50% (1 + 4/10) when both autosomes have nondisjoined (Table 2, line 1). In the third series of experiments, the former frequency is 3.6% (23 + 34/1599) and the latter 50% (5/10). A similar relation concerns the *X* and fourth chromosomes as seen in the second series of experiments: *X* exceptions are 16.7% (1/16) when chromosome 4 nondisjoined and 4.7% (21 + 28-1/1023) when chromosome 4 disjoined (excluding triploids and intersexes).

Factors influencing the frequency of induced nondisjunction: The striking difference between the percentages of exceptions in the low-temperature experiments 1 and 2 (14.4 vs. 5.3%) concerned flies of very similar constitution, including the possession of the γ^+ duplication *X* chromosome. Therefore the genetic differences between the females treated is not likely to be the sole cause of the variation of *X*-chromosomal exceptions.

A nongenetic factor known to influence the frequency of nondisjunction is the duration of the low-temperature treatment. The average frequency observed in earlier experiments with a 2-week treatment was significantly higher than after a 1-week treatment period. In the experiments reported so far, the duration of treatment was uniformly one week, so that this time factor could not account for the different frequencies of nondisjunction.

One possible source of variation in the frequency of nondisjunction could have been variations in the length of the interval from the last collection of newly eclosed females in the culture bottles to the next collection of females to serve as P_1 females in the crosses. Usually, this interval spanned only from 1 to 2 hr; in some cases it was longer, although always less than 4 hr. During longer intervals, females would have more opportunity to feed on normal food in the culture bottle before being placed on minimal food for treatment. Such slightly older females would have more mature eggs retained during pretreatment and treatment than the younger females.

In order to test the hypothesis that the frequency of nondisjunctional excep-

TABLE 3

The effect of different food supply on the number of Stage 14 eggs in the ovary and on the frequency of X-chromosome nondisjunction in 2 weeks low-temperature treated γw females

Food during pretreatment (3 days at 25°C)	Number of eggs after pretreatment		Food during treatment (2 weeks at 10°C)	Number of eggs after treatment		X exceptions in the first-day brood (percent)
	Average per ♀ (\pm SE)	Range		Average per ♀	Range	
Minimal	6.00 \pm 0.52	2-12	Minimal	7.4 \pm 0.47	4-12	8.1
Normal	40.68 \pm 2.33	23-67	Minimal	52.8 \pm 4.48	20-119	16.1
			Normal	60.2 \pm 3.05	41-93	20.0

The sample size for the egg count is 25 females in each series.

tions among the first-brood flies can be increased by increasing the number of retained mature eggs, a further experiment was made.

Females with normal X chromosomes of the homozygous genotype γw were low-temperature-treated for 2 weeks and then mated to wild-type males. In series A, the females were placed on normal food during the pretreatment period and on minimal food during treatment. In series B the females were kept only on normal food. In both series, some eggs were deposited during the pretreatment period, their estimated number averaging 5.4 per female (650 eggs from 120 females). After treatment 50 females in each series were separately mated to two wild-type males each and the first-day broods collected. In addition, at the end of the pretreatment period the ovaries of 25 females in each series were dissected out in order to obtain a count of the number of eggs. The ovaries of another 25 females were dissected out at the end of the treatment period (the ovaries were fixed, stained and mounted on slides for inspection; see KING, BURNETT and STALEY 1957).

The findings in this last series of experiments are given in Table 3, lines 2 (series A) and 3 (series B). For comparison, data from previous studies using minimal food throughout treatment and pretreatment are also presented (line 1). In series A, there were 51 out of 317 or 16.1% F_1 flies which were X-chromosomal exceptions; in series B, 41 out of 205 or 20.0% exceptions. These values are more than twice the value of 49 out of 604 or 8.1% exceptions obtained earlier after provision of minimal food throughout ($\chi^2 P < 0.001$). Concomitantly, the mean numbers of treated mature eggs in the females in both A and B series were much higher than in the experiments with minimal food only. In the latter there were 7.4 mature eggs per female in contrast to 52.8 in A and 60.2 in B series. Similarly, pretreatment with minimal food resulted in 6.0 eggs per female in contrast to 40.7 after pretreatment with normal food.

The results indicate that the greater the number of retained mature eggs in the treated females, the higher is the frequency of low-temperature-induced non-disjunctional exceptions among the first-day brood. This presupposes that all or most mature eggs are deposited during the first day after the end of treatment.

DISCUSSION

Our experiments have shown that low temperature applied to aged mature eggs induces nondisjunction of not only the *X* chromosomes but also the autosomes including whole chromosome sets. This is not the first instance of induction of polyploidy in *Drosophila*. MICKEY and BLOUNT (1951) and MICKEY (1958) were able by means of cold shock to produce mosaics for ploidy by affecting mitotic divisions during embryogenesis and larval stages. Whole triploid flies were obtained by BAUER (1946) after administering cold shocks of various intensities and durations to the polar cells of early embryonic stages. MICKEY and BLOUNT, however, failed to obtain the same results in an experiment with a different chromosomal constitution of the treated embryo.

In BAUER's experiment, the highest frequency of triploids was 0.75%. In our own series, the highest frequency of *XXY* triploid intersexes was 2.2% (Table 1), and the number of triploid females may be assumed to have been similar provided equal viability of the two genotypes. Actually, only a single such female was found; probably any other triploids were not recognized because their presence was not suspected. It seems likely that improvements in the technique of inducing triploidy by low-temperature treatment of mature eggs will make this a useful tool in the production of triploids.

It has been known since the work of MAVOR (1922–1929) that X rays increase the frequency of nondisjunction of the *X* chromosome. Later, PATTERSON, BREWSTER and WINCHESTER (1932) showed that X rays applied to retained maturing eggs increase nondisjunction. It is of interest to compare the effects of low temperature with those of X-ray treatment. Although both types of treatment result in increased nondisjunction, there are distinct differences. It was pointed out earlier (TOKUNAGA 1970) that the sex ratio of nondisjunctional exceptions (*XXY* : *X0*) induced by low temperature tends to be 1 : 1. This also holds true for all experiments reported in this paper. In contrast, the presence of many more *X0* males than *XXY* females is characteristic of both spontaneous and X-ray-induced cases. The excess of *X0* males over *XXY* females has been interpreted as due to meiotic loss of *X* chromosomes, a loss which is superimposed on nondisjunctional events (MORGAN, BRIDGES and STURTEVANT 1925). When this interpretation is applied to the X-ray-induced exceptions of PATTERSON, BREWSTER and WINCHESTER, a substantial number of *X0* males must be attributed to chromosome loss. This drastically reduces the frequency of true nondisjunction, in contrast to the very high frequency in low-temperature induction. Thus the three authors found 51 males and 5 females among 1262 offspring of X-rayed, aged mothers, i.e., 4.4% exceptions. Adjusted for equality of sex ratio, only 0.8% of the flies were nondisjunctional. Similarly, among 3997 control offspring of mothers aged without X raying, 5 males and 0 females were exceptional. The raw frequency of 0.1% exceptions decreases to no nondisjunctional exceptions observed. DAVIS (1969), however, argued from a detailed study of the claret-nondisjunctional (*cand*) gene in *D. melanogaster* that nondisjunction and chromosome loss probably have a common basis.

Another difference between X-ray- and low-temperature-induced nondisjunction is that the low-temperature effect is restricted to the retained mature eggs. The X-ray effect is similarly very high with regard to the retained mature eggs (first-day brood) but the subsequent broods also indicate higher frequencies of exceptions compared with the control (see Tables IV and V, PATTERSON, BREWSTER and WINCHESTER 1932). In contrast, MAVOR's studies covered the effect of X rays applied to females of ages ranging from pupal to adult stages without aging treatment (MAVOR 1924, 1929). In other words, the X-ray effect is not restricted to retained mature eggs but extends to the earlier egg stages.

The striking positive correlation between X-chromosomal and autosomal nondisjunction is not specific for the low-temperature effect. It has been observed in spontaneous nondisjunction (HALL 1970) and in some of the meiotic mutants (SANDLER *et al.* 1968). Nor is nondisjunction of exchange bivalents peculiar to low-temperature-induced nondisjunction; it has also been observed among spontaneous (MERRIAM and FROST 1964) and *cand*-induced (DAVIS 1969) primary exceptions. The bivalents that fail to disjoin in response to low temperature appear to constitute a representative sample with respect to exchange.

Abnormalities of the spindle involved in nondisjunction have been observed in eggs from homozygotes for the claret gene in *D. simulans* (WALD 1936). In analogy to the situation in *D. simulans*, DAVIS suggested that nondisjunction in a homozygote for the *cand* gene in *D. melanogaster* is also due to abnormal spindle formation.

The failure of the meiotic first division might occur rather frequently by the low-temperature treatment. This was strongly suggested by the excess of *XXY* triploid intersexes over the *XX* triploid intersexes in the first experimental series. Apparently this phenomenon serves as one of the factors which lead to the lack of independence of X chromosomes and autosomes in nondisjunction.

It remains to be seen whether the high susceptibility of retained mature eggs to low-temperature induction of nondisjunction is due to failure of cytokinesis, e.g., spindle defects or due to defects in the chromosomes, e.g., kinetochores.

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SUMMARY

Low-temperature-induced nondisjunction of the X chromosome in retained mature eggs occurred exclusively at the first meiotic division. In addition to X-chromosome exceptions, other exceptions occur as a consequence of nondisjunction of chromosome 4 and of chromosomes 2 and 3. Various combinations of exceptional types were found, including individuals nondisjunctive for chromosomes X, 2, 3, and possibly 4. The frequencies of the various types show that nondisjunction of the X chromosomes and autosomes is positively correlated.—

The frequency of exceptions induced among the first-day brood can be raised by increasing the number of mature eggs in the ovaries by feeding the females well, before the treatment.—The sex ratio of X-chromosome exceptions induced by low temperature is approximately 1:1.

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