THE EFFECTS OF LOW TEMPERATURE AND AGING ON NONDISJUNCTION IN DROSOPHILA[1]

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NONDISJUNCTION of the *X* chromosomes in Drosophila was first studied by BRIDGES (1913, 1914, 1916). He determined the frequencies of nondisjunction under standard conditions. Recently HILDRETH and ULRICHS (1969) encountered variations in the frequency of nondisjunction when they subjected females to "high" (25°C) or "low" (10°C) temperatures during prolonged periods of aging preceding egg deposition. The experiments presented here were designed to obtain detailed data on the effects of temperature and aging on nondisjunction of the *X* chromosomes in females of *Drosophila melanogaster*, including data on successive broods of females treated in various ways.

MATERIALS AND METHODS

Nondisjunction was studied among the F_1 of the cross $y\ w$ ♀ × + ♂ (Samarkand wild-type). The $y\ w$ P_1 females were collected from stock cultures of the type $y\ w$ ♀ × $y\ w/y^+\ Y$ ♂. The $y^+\ Y$ chromosome is a *Y* chromosome to which a small section of an *X* chromosome carrying the normal allele of *y* has been translocated. Any *XXY* females in the stock would be recognizable by their y^+ phenotype. Thus the use of $y\ w$ parental females in the experimental cross assured that all were *XX*.

In the main groups of experiments, the parental females were newly eclosed virgins "pretreated" by being placed on "minimal food"[2] at 25°C for 3 days which was the procedure used by HILDRETH and ULRICHS. After this common pretreatment period, the females were "treated" by being placed at 10°C or 25°C on minimal food for 0 day (control series "C-0"), or 3 days or 1, 2 or 3 weeks.[3] During the aging periods of from 1 to 3 weeks the females were transferred from 1 to 3 times into new vials. After the respective "treatment"–i.e., the temperature and aging periods exclusive of the 3 days of pretreatment—each female was placed on normal food (standard culture medium—cornmeal, agar, molasses, yeast) and kept at 25°C. Each female was given the opportunity to mate with two wild-type males[4], at least 3 days old, on the first and again the sixth and seventh day after treatment. Each female was kept in a separate culture vial and transferred to new vials daily until the fifth day, and then every second day until the 13th day. In the control C-0 series, daily brood collections were made during the entire 13 day period.

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[2] For the purpose of this paper, the term "minimal food" stands for a food mixture which reduces the ability of females to oviposit. A modified version of BURDICK's medium (1954) was used. It consisted of 20 g agar, 10 g brewer's yeast, 43 g sugar, 4.3 g sodium nitrate, 1.4 g dipotassium phosphate, 0.7 g potassium chloride, 0.014 g ferrous sulfate, 0.7 g magnesium sulfate, 0.14 g tegosept in 12 ml of 95% ethanol, and water added to make a final volume of 1000 ml (HILDRETH and ULRICHS 1969).

[3] The term "treatment" was defined as the period of aging under minimal food conditions that followed 3 days of pretreatment. Therefore the treatment periods of 0, or 3 days or 1, 2, or 3 weeks at 10°C or 25°C correspond to actual aging periods of 3, 6, 10, 17 or 24 days, respectively.

[4] In the following pages, the matings of single females with two males are somewhat inaccurately called "pair matings."

The number of eggs deposited in the treatment series during the first day after termination of treatment was less than in the succeeding broods. In order to obtain sufficient data on first-day broods the experiment with single females was supplemented by mass matings using the same experimental procedures as in the main series except that only first-day broods were collected. To avoid overcrowding, a maximum of 15 females and 30 males per vial for the 10°C series and a minimum of 5 females and 10 males per vial for the 25°C series was used for the first-day brood collection.

In addition to the control series C-0, in which the females were pretreated for 3 days, another control group, C, was reared which did not undergo pretreatment. This control is discussed later, as are also two special types of experimental series involved in the analysis of the first-day broods.

It is of importance to know how many eggs are laid by virgin females during the pretreatment and treatment periods. During the pretreatment period 225 females of the C-0 control series together laid 95 eggs, a mean of 0.42 eggs per female. For the experimental series, data are available for the number of eggs laid jointly over the pretreatment and early treatment periods or for later treatment periods alone. For each period, the number of eggs laid per female was estimated by counting the approximate numbers of eggs in several randomly selected vials of the experimental series, each of which contained 30 females during the treatment. The estimated number of eggs deposited before mating is about five per female at the highest and less than one at the lowest among the various series.

Normal segregation of the X chromosome in the cross $y\ w$ ♀ × + ♂ leads to wild-type females and $y\ w$ males. Nondisjunction in the egg yields $y+\ w+$ triple-X metafemales, $y\ w$ females (XXY), + males ($X0$) and $0Y$ zygotes which result in embryonic death. The triple-X metafemales have a very low viability, and hardly any develop to imagoes. If they occurred at all in our cultures they were not distinguished from regular + females.

Although some of the $X0$ males are probably the result of X chromosome loss rather than nondisjunction, the $y\ w$ and + exceptions were scored and discussed as if they were products of nondisjunction. No adjustment of the theoretical nondisjunctional frequency based on the actual count of $y\ w$ exceptions was done.

Among the total of 833 exceptional + males obtained from the series of experiments performed, 823 were sterile and therefore scored as $X0$, which includes 28 + males that died before the fertility test. Ten males were fertile. If nondisjunction of the XY pair in males is as frequent as nondisjunction of the X chromosome in females, the expected frequency of + XY males by coincidence of nondisjunction in both parents would have been 1.5 (based on the data on males) instead of the observed 10. Unless all or some of the 10 fertile + males were contaminants, it seems that the frequency of XY nondisjunction is several times that of XX nondisjunction. It seems conservative to regard the 10 males as contaminants and not include them in the data. No attempt was made to distinguish between $y\ w\ XXY$ and $y\ w\ XX$ exceptions.

Statistical analyses were based on the chi-square test, using Yates' correction when, given one degree of freedom, an expected class was less than five. The level of significance used is $P = 0.01$ unless noted otherwise. On account of the usually low frequency of nondisjunctional exceptions, it was at times necessary to pool the data of some broods.

RESULTS

The main body of data from the two control series and the eight experimental series is given in Tables 1 through 3. Numbers without parentheses include data on both female and male exceptions. They constitute the material to be analyzed in the following pages. In addition, the data of F_1 males only are listed within parentheses. (Theoretically, the nondisjunction frequencies are about twice the listed values of exceptions in these tables and Figures 1 and 2 as explained in MATERIALS AND METHODS.)

TABLE 1

Nondisjunction data: control series, C-0 and C

Broods (days)	Control series			
	C-O		C	
	Nd/Total	% Nd	Nd/Total	% Nd
1	2/ 3404	0.058	0/ 27	0.
	(2/ 1686)	(0.118)	(0/ 17)	(0.)
2	6/ 14672	0.040	4/ 12236	0.032
	(2/ 7393)	(0.027)	(3/ 6259)	(0.047)
3	9/ 21581	0.041	6/ 18135	0.033
	(8/ 10705)	(0.074)	(6/ 9069)	(0.066)
4	8/ 24 104	0.033	5/ 19755	0.025
	(4/ 11 892)	(0.033)	(5/ 9933)	(0.050)
5	8/ 26104	0.030	1/ 6001	0.016
	(7/ 12967)	(0.053)	(0/ 2905)	(0.)
6	4/ 22527	0.017	6/ 4783	0.125
	(2/ 11186)	(0.017)	(5/ 2350)	(0.212)
7	8/ 21706	0.036	2/ 4239	0.047
	(7/ 10752)	(0.065)	(1/ 2069)	(0.048)
8	9/ 19640	0.045	4/ 3191	0.125
	(7/ 9732)	(0.071)	(3/ 1519)	(0.197)
9	14/ 20668	0.067	8/ 5364	0.149
	(10/ 10335)	(0.096)	(7/ 2680)	(0.261)
10	13/ 17237	0.075	3/ 6060	0.049
	(9/ 8410)	(0.107)	(1/ 2998)	(0.033)
11	10/ 13872	0.072	0/ 3299	0.
	(7/ 6927)	(0.101)	(0/ 1623)	(0.)
12	8/ 10213	0.078	2/ 2486	0.080
	(5/ 4985)	(0.100)	(2/ 1220)	(0.163)
13	2/ 6311	0.031	6/ 3089	0.194
	(1/ 3068)	(0.032)	(1/ 1486)	(0.067)
14			2/ 3126	0.063
			(1/ 1456)	(0.068)
15			3/ 2539	0.118
			(1/ 1234)	(0.081)
16			0/ 2085	0.
			(0/ 1015)	(0.)
Totals	101/222 039	0.045	52/ 96 415	0.053
	(71/110 038)	(0.064)	(36/ 47823)	(0.075)

Nd: Nondisjunctional exceptions. Numbers without parentheses: both sexes jointly; numbers in parentheses: males only.

TABLE 2

Nondisjunction data: high-temperature series (25°C)

Broods (days)	Treatment series: 3 days Nd/Total	3 days % Nd	1 week Nd/Total	1 week % Nd	2 weeks Nd/Total	2 weeks % Nd	3 weeks Nd/Total	3 weeks % Nd	Totals Nd/Total	Totals % Nd
1	1/ 2891 (1/ 1353)	0.034 (0.073)	2/ 1513 (2/ 739)	0.132 (0.270)	2/ 1118 (1/ 537)	0.178 (0.186)	2/ 2321 (2/ 1150)	0.086 (0.173)	7/ 7843 (6/ 3779)	0.089 (0.158)
2	6/ 9730 (5/ 4891)	0.061 (0.102)	(4/ 6594 (2/ 3350)	0.060 (0.059)	4/ 6113 (4/ 3082)	0.065 (0.129)	1/ 8307 (0/ 4175)	0.012 (0.)	15/ 30744 (11/ 15498)	0.048 (0.070)
3	5/11015 (4/ 5371)	0.045 (0.074)	5/ 9293 (4/ 4683)	0.053 (0.085)	1/ 6783 (0/ 3335)	0.014 (0.)	4/14079 (3/ 6914	0.028 (0.043)	15/ 41170 (11/ 20303)	0.036 (0.054)
4	4/ 9267 (3/ 4548)	0.043 (0.065)	2/ 8027 (1/ 3945)	0.024 (0.025)	2/ 6225 (2/ 3099)	0.032 (0.064)	2/ 6700 (2/ 3294)	0.029 (0.060)	10/ 30219 (8/ 14886)	0.033 (0.053)
5	6/ 6761 (5/ 3286)	0.088 (0.152)	5/ 7418 (5/ 3714)	0.067 (0.134)	8/ 6723 (8/ 3255)	0.118 (0.245)	9/ 5001 (8/ 2498)	0.179 (0.320)	28/ 25903 (26/ 12753)	0.108 (0.203)
6-7	15/15910 (13/ 7628)	0.094 (0.170)	13/13447 (12/ 6428)	0.096 (0.186)	16/11047 (15/ 5184)	0.144 (0.289)	30/ 9609 (23/ 4584)	0.312 (0.501)	74/ 50013 (63/ 23824)	0.147 (0.264)
8-9	11/10702 (5/ 5045)	0.102 (0.099)	29/12511 (22/ 5887)	0.231 (0.373)	32/11871 (19/ 5753)	0.269 (0.330)	33/ 9074 (18/ 4354)	0.363 (0.413)	105/ 44158 64/ 21039	0.237 (0.304)
10-11	5/ 4705 (3/ 2284)	0.106 (0.131)	15/ 6906 (8/ 3367)	0.217 (0.237)	11/ 5187 (6/ 2449)	0.212 (0.244)	20/ 4790 (12/ 2335)	0.417 (0.513)	51/ 21588 (29/ 10435)	0.236 (0.277)
12-13	1/ 1163 (1/ 586)	0.085 (0.170)	3/ 1553 (2/ 760)	0.193 (0.263)	5/ 1021 (3/ 510)	0.489 (0.588)	3/ 1410 (2/ 702)	0.212 (0.284)	12/ 5147 (8/ 2558)	0.233 (0.312)
Totals	54/72144 (40/34992)	0.074 (0.114)	78/67262 (58/32873	0.115 (0.176)	81/56088 (58/27204)	0.144 (0.213)	104/61291 (70/30006)	0.169 (0.233)	317/256785 (226/125075)	0.123 (0.180)

Nd: nondisjunctional exceptions. Numbers without parentheses: both sexes jointly; numbers in parentheses: males only.

A. *The effect of aging females at high temperature (25°C) on frequency of nondisjunctional exceptions*

Each of the four experimental series shows a trend toward higher frequency of nondisjunctional exceptions with increased aging (Table 2, last line). A comparison of the totals of the four experimental groups with that of the control C-0 (Table 1) shows an increase of exceptions in each experimental group, the differences being significant at the 0.2% or lower level. In each experimental series, the frequency of exceptions is higher in the later than in the earlier broods (Table 2, Figure 1). The borderline for this phenomenon seems to be represented by the fifth-day brood. There are three apparent exceptions to this age effect. These are the first-day broods of the 1-, 2-, and 3-week treatment series, which give higher frequencies than the next three broods. Statistical analysis shows, however, that the relatively high frequencies in the first-day broods of these series are not significantly different from the sum of the second- to fourth-day broods of the same series, and the first-day brood of the control C-0 does not differ significantly from the sum of the first-day broods ($P = 0.5–0.7$).

On the other hand, the pooled data of the 1-, 2-, and 3-week series show a

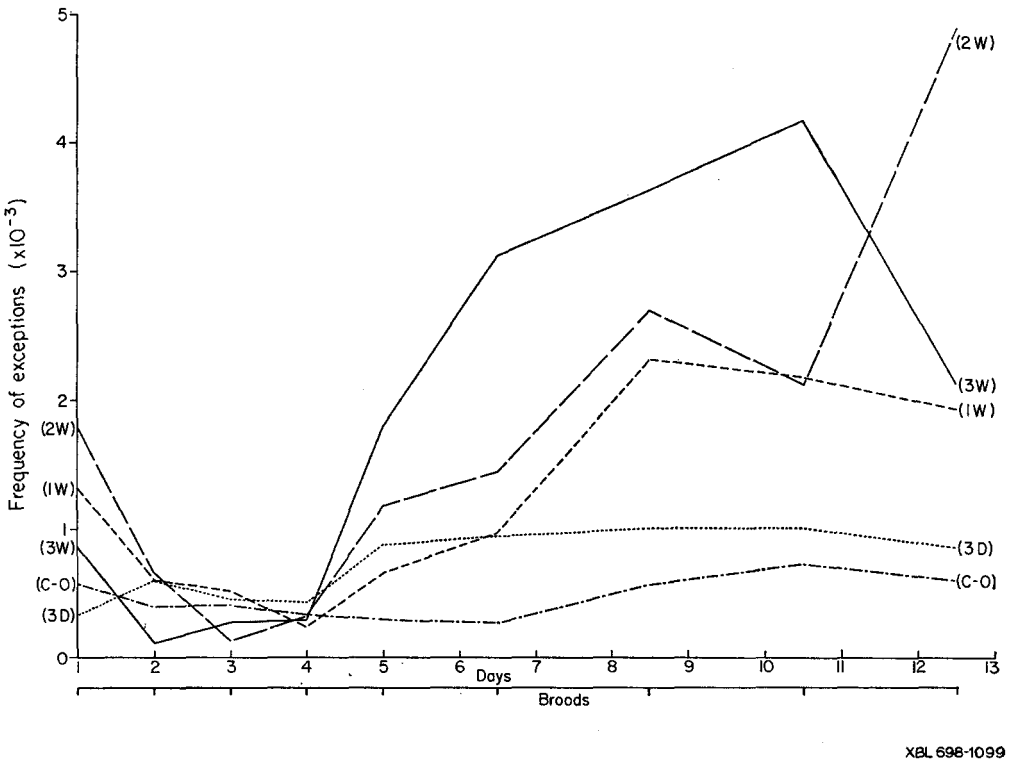

FIGURE 1.—The frequencies of nondisjunctional exceptions in each brood of the control C-0 series and the treatment series at 25°C. 3D, 1W, 2W, and 3W: 3-day, 1-, 2-, or 3-week periods of aging.

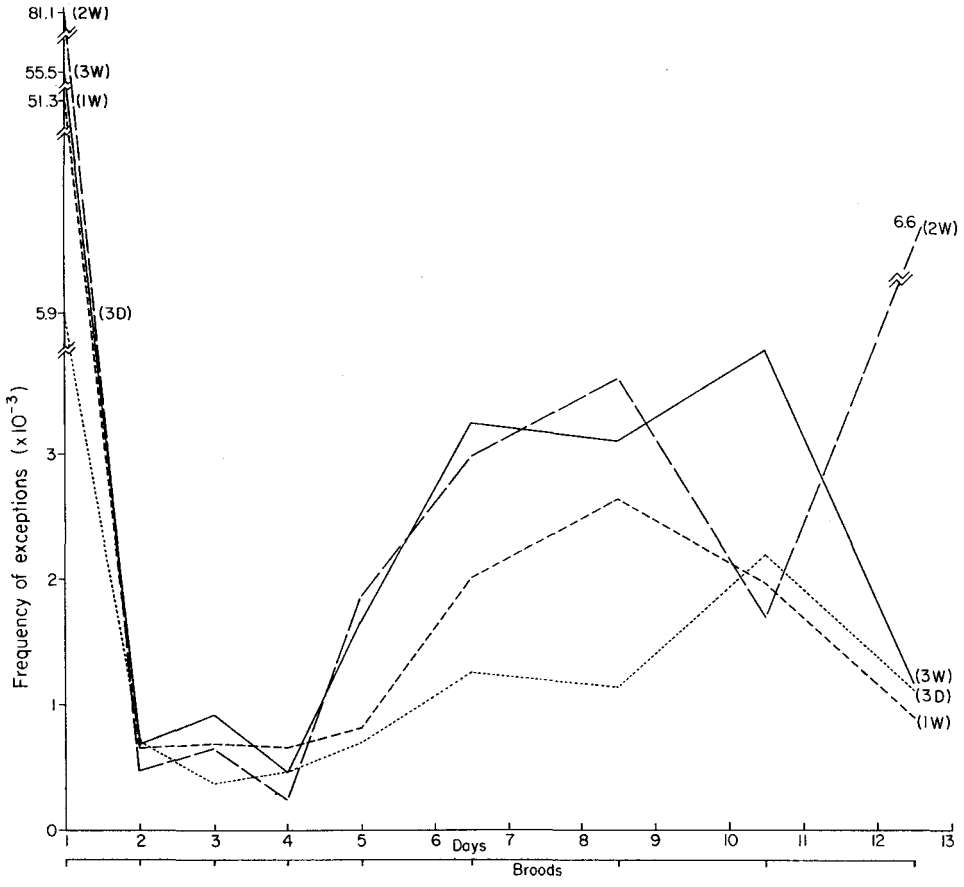

FIGURE 2.—The frequencies of nondisjunctional exceptions in each brood of the treatment series at 10°C.

significant difference in frequency of nondisjunctional exceptions between the sum of the first broods and the sum of the second- to fourth-day broods ($P = 0.01$). A similar comparison involving pooled data from the 3-day and 1-, 2-, and 3-week series is insignificant at the 7–8% level, and a comparison involving pooled data of the control C-0 and the four experimental series yields borderline significance at the 5–7% level.

As is shown later, the second- to fourth-day broods in each experimental series at 25°C do not show any age effect. In contrast the preceding analysis indicates that the first-day brood aged at 25°C shows a tendency to increased frequency of exceptions with increased period of aging, from 1 to 2 or 3 weeks. This tendency, if real, is of a minor nature, and more data are required to establish its reality.

The differences between the early and late broods were analyzed further by subdividing the broods into two subgroups, comparing the first- to fourth-day broods with the fifth to the last day broods. Chi-square tests between the two subgroups show no difference in the control C-0 ($P = 0.3$–0.5), a difference

significant at the 3% level in the 3-day treated series, and differences significant below the 0.1% level within the 1-, 2-, and 3-week treated groups. When the two subgroups were composed of the first- to fifth-day and the sixth- to last-day broods, very similar results were obtained. There is thus a significant difference between the early and late broods of all four experimental series but not in the control C-0.

Further tests relate to the question whether there are differences between the early and late subgroups of the control *vs* experimental series. No significant differences were found between the first- to fourth-day broods, whereas the fifth- to last-day broods differed at less than 0.1% probability. Homogeneity tests for the early subgroups showed insignificant differences ($x^2 = 2.40$, df = 4, P = 0.5–0.7), whereas tests of the late subgroups showed highly significant inhomogeneity ($x^2 = 200.7$, df = 4, $P < 0.001$).

Additional comparisons between different early broods within the control and experimental series, showed no aging effect during the first- to fourth-day brood period. On account of the small numbers of nondisjunctional exceptions it was necessary to group the four broods in sets of two: first- plus second-day broods *vs* third- plus fourth-day broods. All comparisons show statistical homogeneity. In addition, comparisons between the sums of the second- to fourth-day broods for the control C-0 and for the four experimental series also show homogeneity in contrast to the highly significant differences between the frequencies of exceptions in the fifth- to thirteenth-day broods of the control C-0 and the various aged series.

In order to analyze the aging effect between the individual fifth- to thirteenth-day broods, each brood of the various experimental series was compared with the corresponding group of the control C-0 series. Since the two 10– 11th- and 12–13th-day broods represented somewhat small samples, the 10–13th-day broods were treated as a single group. The only significant difference between the control C-0 and the 3-day-treated series was that between the 6–7th-day broods ($P < 0.001$). Between the 1-week-treated series and the control there was a significant difference between the 6–7th-day, 8–9th-day and 10–13th-day broods; the only insignificant difference related to the 5th-day brood (P = 0.2–0.3). Comparisons between the control C-0 and the 2- and 3-weeks series showed significant differences for all brood groups. It thus appears that the 6–7th-day brood is most sensitive to the aging effect of 25°C under the minimal food condition. The 8–13th-day broods are next in sensitivity, with the 5th-day brood being least sensitive.

In summary, aging at 25°C increases the frequency of nondisjunctional exceptions in the later broods. In the earlier broods there is no effect of aging except for the first brood in pooled data of the 1-, 2-, and 3-week treatment series, which give higher frequencies of exceptions in first-day than in the 2–4th-day broods.

B. *The effect of aging females at low temperature (10°C)*

1. *Comparisons with C-0 controls:* Table 3 and Figure 2 provide the data for

the various series of females aged at 10°C. On the whole, the pattern of effects of aging is similar at 10°C and 25°C. There is, however, a striking difference between the effects of the two temperature treatments on the frequency of exceptions in the first broods.

Comparisons between the 25°C control C-0 and the various low-temperature series show significant increases in nondisjunctional exceptions of the sums of all broods in all four treated series. The same is true for the sum of all broods except the first, and for the sums of the 5–13th-day broods. For sums of the 2–4th-day broods, only the 3-week treated series differs significantly from the control C-0 ($P = 0.01$).

Comparisons between the low temperature treated and control C-0 series show no significant differences for each of the 2nd-, 3rd-, and 4th-day broods and the 3-day and 1-week treated 5th-day broods. The exception is the 3rd-day brood of the 3-week treatment series, discussed later ($P = 0.03$–0.04). After treatments of 2 and 3 weeks, but not for shorter periods, the 5th-day broods have significantly higher rates of exceptions than the control C-0 ($P = 0.002$). The same holds true for most of the comparisons for 6–7th-, 8–9th-, and 10–13th-day broods, with only borderline significance reached by the 3-day treated series for the last two brood groups ($P = 0.05$ and 0.02). The lack of effect of treatment on the early broods except the first and the effects on the later broods correspond to the findings from high-temperature treatments, whereas there is a contrast between the two temperature effects.

The greatest difference concerns the first-day broods. In contrast to the 25°C series, for which there was no clear-cut difference between first-day treated and C-0 broods, all 10°C treated series—3 days, 1, 2, or 3 weeks—produce first-day broods with very highly increased frequencies of exceptional flies (Table 3, line 1). The first-day broods are analyzed below in detail.

2. *Comparisons with high-temperature aged series:* In the preceding section the frequency of nondisjunctional exceptions in low-temperature-aged females was compared with that in high-temperature control C-0. The treated series thus is different from the controls in two attributes, temperature and age. In order to separate the influences of these factors, the results of the various low-temperature-aged series were compared with those of the high-temperature series of equally aged females. (It is likely that the general physiological effects of aging at the two temperatures are not identical. However, no adjustment for such differences has been made. On the whole this did not seem necessary).

Comparisons between the totals of all broods at high and low temperatures show no significant difference in the 3-day aged series ($P = 0.1$–0.2) but highly significant differences in the 1- and 2-week series, and a difference at the 5–6% level for the 3-week series. These differences are primarily due to the exceedingly high frequency of exceptions in the first-day broods of the low-temperature series. This is shown by comparisons between the first-day brood of low-temperature-treated series and the sum of all others, and between the first-day brood and the sum of the 2–4th-day broods ($P < 0.001$ in each comparison). Similarly, the first-day brood of all low-temperature series differs significantly from those of

the corresponding high-temperature series. These differences in the first-day broods between the high- and low-temperature series show that the main factor responsible for the difference is the temperature and not the aging treatment.

In order to test for differences between the high- and low-temperature series independently of the first-day brood, the sum of the 2–13th-day broods was used for comparison. Three of the four comparisons yielded insignificant differences (more than 10% level) and the fourth, relating to the 2-week treatment, was significant at about the 2% level. Thus, there seems to be only a minor temperature effect, if any, for the sum of 2–13th-day broods.

Further analysis showed some minor temperature effects on certain broods of specific aging series. One of these concerns the third-day brood, which does not show a temperature effect in any but the 3-week aging series ($P = 0.02$–0.03). This is similar to the situation in which the sum of the 2–4th-day broods are compared with the control C-0. Three of the latter comparisons show no significant differences, but the fourth, involving the 3-week series, does reach significance. Specifically, the difference between the 2–4th-day broods of the high- and low-temperature 3-week series may be assigned to the 3rd-day brood, but only at a 3% level of significance.

The comparisons between the later broods of the low- and high- temperature series indicate no significant differences except two of the four comparisons of the 6–7th-day broods. Those for the 1- and 2-week treated series are significant at about the 2% level.

In summary, low-temperature treatment profoundly increases the frequency of exceptional flies in the first-day brood. Later broods are mostly not, or in two cases only slightly, affected. One of these, the 3rd-day brood, is affected only by the longest treatment period (3 weeks), the other, the 6–7th-day brood, shows an effect after 1- and 2-week treatment.

C. *Analysis of the first-day broods*

For the 3-day low-temperature series, the frequency of nondisjunctional exceptions in the first brood is nearly 0.6%, and for the 1-, 2-, and 3-week treated series it is 5.14, 8.11, and 5.55%, respectively (Table 3, line 1). These frequencies are from 10 to 140 times those of the first-day broods of the control C-0 and from 17 to 65 times those of the corresponding high-temperature-treated series. The following analysis is intended to determine the stage in oogenesis that is responsible for the unusually high frequencies of nondisjunctional exceptions.

Each of the first-day broods came from eggs deposited within 24 hours after the treated virgin females were given the opportunity to mate. This accounts for the rather small number of offspring obtained in comparison with subsequent brood collections. The sizes of first-day-brood sibships from pair-mated parents, control C-0, or treated with high or low temperatures, are given in Table 4 together with the frequencies of nondisjunctional exceptions among them. It is seen that the majority of treated females produced only a few offspring during the first-day brood collection. The highest number was 25, from a 3-day treated female. The

TABLE 3

Nondisjunction data: low-temperature series (10°C)

Broods (days)	Treatment series								Totals	
	3 days		1 week		2 weeks		3 weeks			
	Nd/Total	% Nd	Nd/Total	% Nd	Nd/Total	% Nd	Nd/Total	% Nd	Nd/Total	%Nd
1	9/ 1525 (4/ 728)	0.590 (0.549)	64/ 1246 (42/ 627)	5.136 (6.698)	49/ 604 (24/ 297)	8.112 (8.080)	8/ 144 (2/ 68)	5.555 (2.941)	130/ 3519 (72/ 1720)	3.694 (4.186)
2	6/ 8489 (5/ 4168)	0.070 (0.119)	3/ 4563 (2/ 2246)	0.065 (0.089)	2/ 4121 (2/ 2074)	0.048 (0.096)	5/ 7197 (4/ 3565)	0.069 (0.112)	16/ 24370 (13/ 12053)	0.065 (0.107)
3	5/13286 (4/ 6563)	0.037 (0.060)	7/10180 (5/ 5097)	0.068 (0.098)	7/10655 (7/ 5309)	0.065 (0.131)	20/21507 (20/10657)	0.092 (0.187)	39/ 55628 (36/ 27626)	0.070 (0.130)
4	4/ 8523 (3/ 4177)	0.046 (0.071)	6/ 8956 (6/ 4427)	0.066 (0.135)	2/ 8129 (1/ 3990)	0.024 (0.025)	(4/ 8869 (4/ 4483)	0.045 (0.089)	16/ 34477 (14/ 17077)	0.046 (0.081)
5	4/ 5651 (4/ 2753)	0.070 (0.145)	5/ 6121 (4/ 3003)	0.081 (0.133)	5/ 2688 (3/ 1317)	0.186 (0.227)	6/ 3609 (5/ 1834)	0.166 (0.272)	20/ 18069 (16/ 8907)	0.110 (0.179)
6-7	19/15143 (14/ 7144)	0.125 (0.195)	24/11787 (16/ 5583)	0.203 (0.286)	37/12379 (24/ 5857)	0.298 (0.409)	57/17632 (39. 8607)	0.323 (0.453)	137/ 56941 (93/ 27191)	0.240 (0.342)
8-9	8/ 6463* (3/ 2970)	0.123 (0.101)	31/11750 (14/ 5678)	0.263 (0.246)	50/13919 (29/ 6705)	0.359 (0.432)	54/17456 (38/ 8581)	0.309 (0.442)	143/ 49588 (84/ 23934)	0.288 (0.350)
10-11	7/ 3201 (4/ 1583)	0.218 (0.252)	11/ 5587 (9/ 2735)	0.196 (0.329)	7/ 4141 (5/ 2011)	0.169 (0.248)	27/ 7085 (16/ 3423)	0.381 (0.467)	52/ 20014 (34/ 9752)	0.259 (0.348)
12-13	1/ 908 (0/ 472)	0.110 (0.)	1/ 1123 (0/ 560)	0.089 (0.)	6/ 908 (5/ 460)	0.660 (1.086)	2/ 1723 (1/ 853)	0.116 (0.117)	10/ 4662 (6/ 2345)	0.214 (0.255)
Totals	63/63189 (41/30558)	0.099 (0.134)	152/61313 (98/29956)	0.247 (0.327)	165/57544 (100/28020)	0.286 (0.356)	183/85222 (129/42071)	0.214 (0.306)	563/267268 (368/130605)	0.210 (0.281)

* In this group there was accidental failure to count all offspring.

Nd: nondisjunctional exceptions. Numbers without parenthesis: both sexes jointly; numbers in parenthesis: males only.

highest number of siblings among which exceptional flies appeared was 10 (two cases), from the 3-day low series. All other exceptions came from sibships of 8 or less. These facts strongly suggest that the nondisjunctional offspring of the first-day broods came from the first 10 fertilized and viable eggs deposited by a treated female after the first mating.

Under normal conditions the number of functional ovarioles in *Drosophila melanogaster* has been estimated as between 10 and 30 per ovary (KING 1957; KOCH and KING 1966). If the most mature oocyte at the distal end of each ovariole is inseminated and develops successfully before the rest of the oocytes, one can expect 20 to 60 offspring per female from eggs that were mature at the time of the first mating. The fact that the first-day brood from the low-temperature-treated female shows the striking effect on the frequency of exceptions among sibships of less than 10 leads to the conclusion that the effect is on the mature eggs. However, since all females were pretreated for 3 days at 25°C on minimal food in the present series, one cannot specify the actual number of mature eggs in the ovaries at the end of the treatment without checking directly.

In order to obtain information on the state of the ovaries, egg counts in the ovarioles of the females were made immediately after the 2-week high- and low-temperature treatment. The ovaries were dissected out, fixed, stained by the Feulgen method, mounted on a slide, and the number of Stage 14 eggs was determined under the microscope (KING, BURNETT and STALEN 1957; CUMMINGS and KING 1969). Some of the females had an egg in the uterus, that was excluded from the count.

In each series, 25 females (50 ovaries)were studied. The females with low-temperature treatment showed an average of 7.4 ± 0.47 (SE), and a range of from 4 to 12 eggs at Stage 14 per female (3.7 ± 0.19; 1 to 7 per ovary). The females with high-temperature treatment showed an average of 68.84 ± 2.76, and a range of from 37 to 92 eggs per female (34.42 ± 1.21; 14 to 55 per ovary). No eggs between Stage 11 to 13 were observed except one Stage 13 egg in the low series.

These data compared with the number of F_1 from the females treated in the above manner and shown in Table 4, columns 7 and 8 indicate the following: In the low-temperature series, the highest number of F_1 per female was 8 (average 1.4) and the highest number of mature eggs per female was 12 (average 7.4), whereas in the high-temperature series, 17 (average 2.6) and 92 (average 68.8) were the comparable numbers, respectively. There were thus more mature eggs present in the ovaries than corresponding numbers of F_1 individuals of the first-day brood. This may be due to the presence of unfertilized or disintegrated mature eggs as a result of the treatment, a situation reported by PATTERSON, BREWSTER and WINCHESTER (1932) in their studies of the joint effect of aging and of X radiation on the frequency of nondisjunction. Furthermore, lethality during development may also be a contributing factor to the difference in numbers of mature eggs and F_1.

The presence of unfertilized eggs was suggested by the study of 2-week low-temperature treated series. Those vials intended for first-day brood egg

TABLE 4

Ananlysis of the first-day broods from pair matings in the control C-0 and in each experimental series. Number of parental (p) females classified according to the number of their offspring (see first column)

Number of F_1 per P ♀	Control C-O	Treatment series							
		3 days		1 week		2 weeks		3 weeks	
	25°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C
0	39	6	6	36	36	53	79	152	194
1	25	4	9	27	27 (1)	30	38 (3)	55	34 (2)
2	34	3	19	31 (1)	31 (1)	19	36 (2)	16	11 (4)
3	57 (1)	10	9	28 (1)	23 (7)	8	22 (5)*	7	3
4	54	10	15	11	15 (2)†	8 (1)	11	4	1
5	50	13	25 (1)	13	16 (4)‡	7	5 (1)	3	2
6	48	15	14 (3)	4	8 (2)	11	3 (2)§	1	1
7	28	10	17	7	6 (2)	3	1		
8	25	14	12 (3)	7	2 (1)	3	1		
9	22	15	10	4	1	3			
10	10	13	7 (2)	1		2		1	
11	7	9	5			1			
12	15	7	3	2		1			
13	11	7	1	2		2			
14	1	4	2						
15	4	1				1			
16	8	2		1					
17	1					1			
18		3							
19		1							
20	1								
21		2							
22		2							
25		1							
Total P ♀	440	152	154	174	165	153	196	239	246
Total F_1	2351	1243	850	523	413	398	278	155	85
Average F_1 per ♀	5.3	8.1	5.5	3.0	2.5	2.6	1.4	0.6	0.3
Total exceptions	1	0	9	2	20	1	13	0	6
% exceptions	0.042	0.	1.058	0.382	4.842	0.251	4.676	0.	7.058

collections which failed to produce any viable offspring were checked for the presence of eggs laid. From 165 pair matings, a total of 17 vials did not produce offspring but contained a maximum estimated number of 10 eggs per female. In 11 of the 17 vials, second day brood collections produced a total of 236 offspring, none of which was a nondisjunctional exception.

While counting the mature eggs in the 2-week low-temperature treated female, however, severe disintegration was not observed. Only occasional accumulation of a small amount of black pigment in the egg, and in very rare cases abnormal appearance of the cytoplasm, was noticed. In the low-temperature treated ovary, where few mature eggs were observed, 10 or more ovarioles were present in most of the cases. Most of them contained only very early stages of the ova. It seems likely that the oocyte, rather than having disintegrated, did not noticeably develop as judged from the 2-week treatment series. This assumption is well supported by the study of ovaries at the end of the 3 days pretreatment on minimal food at 25°C. From the 25 females (50 ovaries), the average of 6.0 ± 0.52, and a range of from 2 to 12 eggs at Stage 14 per female (3 ± 0.21; 0 to 7 per ovary), was observed. Therefore, during the following 2-week low-temperature treatment, these Stage 14 eggs are retained in the ovary and consequently show an extremely high nondisjunction frequency at meiosis. In the high-temperature series, the presence of a large number of eggs is apparently due to the presence of retained eggs.

The above conclusion that the low temperature effect is on the mature egg is supported indirectly by the outcome of a special experiment in which newly eclosed females, instead of being pretreated at 25°C for 3 days on minimal food, were placed immediately on minimal food at 10°C for 3 days and then transferred to normal food at 25°C and given the opportunity to mate. (All subsequent handling, including brood collections, was the same as for the control C-0). In the newly eclosed females the most advanced eggs are in Stage 7 (KING 1957). It is estimated that under normal conditions it takes about 19 hours to proceed from the beginning of Stage 8 to Stage 14. It then takes an estimated 1.98 hours in Stage 14 to reach full maturity. This estimation varies according to the condition of the female (DAVID and MERLE 1968). Thus at least 21 hours are required by newly eclosed females to produce mature eggs ready to be fertilized. If the first-day-brood effect of low-temperature-treated females on nondisjunction is exerted on mature eggs, no increase of exceptions over other than low-temperature-treated flies was to be expected from the females without pretreatment. The first day brood collection from 420 females (120 pair mated, 300 mass mated), yielded only a single normal segregant male. The second-day brood consisted of a total of 9493, including four male exceptions (0.04%). Based on males only, 4 out of 4741 were exceptions. This second-day brood, obviously, is not derived from treated mature eggs but from earlier stages, which do not show a low-temperature effect although

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Number of nondisjunctional exceptions is given in parentheses. Each exceptional fly came from a different mother except in the following four cases:

* Two out of the listed five from one mother.

† Two from one mother.

‡ Three out of the listed four from one mother.

§ Two from one mother.

they had been exposed to 10°C for 3 days. A x^2 test applied to the sum of the first two broods from nonpretreated 3-day low-temperature-treated females compared with the first-day brood of the control C-0 showed complete homogeneity ($P = 1$), but a comparison of the first two broods from nonpretreated 3-day low-temperature-treated females with the first-day brood of the pretreated 3-day low-temperature series yielded significant differences ($P = 0.001$).

A special experiment was performed to test whether the low-temperature effect on the mature egg is still effective after removal of the treatment. The low-temperature experiments of 1 and 2 weeks' treatment time were repeated in mass matings and, at the end of treatment the females were transferred to minimal food at 25°C for 24 hours. They were then placed on normal food and mated, and first-day broods were collected. These broods did show increased exceptions. During the 24 hours at 25°C before mating, a total of 550 females which were treated for 1 week at 10°C deposited 12 eggs (= 0.02 eggs per female), and a total of 1350 females treated for 2 weeks at 10°C deposited 164 eggs (= 0.02 eggs per female). In the 1-week-treated series exceptions occurred in 3.27% (62 out of 1892 flies; males only: 29/927), and for the 2-week-treated series, in 6.66% (135 out of 2027 flies; males only: 67/1024). Compared with the 1-week low-temperature series (Table 3, line 1), which gave 5.13% exceptions, the 3.27 percentage is significantly different, while the 6.66 percentage is not significantly different from 8.11% of the 2-week low series (line 1). However, the two percentages, 3.27 and 6.66, are so much higher than all but first-day brood frequencies that there is no doubt that the low-temperature effect persists beyond 24 hours.

It has long been known that the male exceptions are much more frequent than female exceptions. This preponderance of male exceptions has been attributed not to a process of nondisjunction but to single *X* chromosome loss at meiosis (MORGAN, BRIDGES and STURTEVANT 1925). This is borne out by these experiments, including control C-0 and eight series of high- and low-temperature treatment, which yielded 318 female and 667 male exceptions. It is noteworthy that the hypothesis of an *X* chromosome loss does not seem to apply to all broods of the C-0 and high- and low-temperature series. The first-day broods of the low-temperature series seem to show a rather substantial tendency toward equality of the sex ratio of exceptions seen in the last two columns of Table 3, line 1. Here the ratio is 58♀♀ : 72♂♂, $P = 0.1$–0.2 for deviation from equality. The clarification of this interesting problem will depend on future study.

In summary, the high frequency of exceptions is caused by the low temperature treatment of the mature eggs retained in the ovary during the treatment period. This effect persisted beyond 24 hours after the termination of the 2 weeks low temperature treatment.

D. Analysis of control series

The foregoing results involved females who had been kept on minimal food. In each series, aging effects were apparent in the later halves of the broods. This section deals with tests made with females cultured under normal conditions.

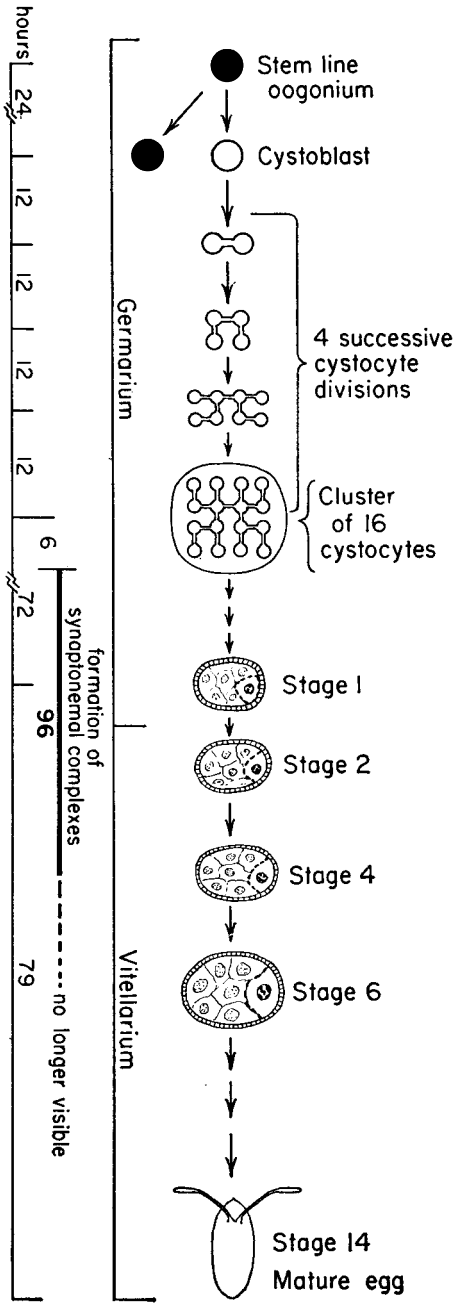
Newly eclosed females were placed at 25°C with males (at least 3 days old)

on normal food. Daily brood collections were made from the day of eclosion until the 16th day. During this period males were present from the first to the fourth day and again during the ninth and tenth days. In this control "C" series, 120 pair matings were used, supplemented for the first 4 days by mass matings involving 300 females.

The frequencies of exceptions in each brood varied (Table 1). More data will be required for a fully valid analysis. It is, however, already possible to draw certain conclusions if one groups broods in a manner used in earlier discussions. If one divides the C series into four successive groups, the following facts appear: The first group, consisting of broods 1–4, shows 0.029% exceptions (15/50153); the second group, consisting of broods 5–8, shows 0.071% (13/18214); the third group, consisting of broods 9–12, shows 0.075% (13/17209); and the last group, consisting of broods 13–16, shows 0.101% (11/10839). A χ^2 test shows the four groups to be heterogeneous ($P = 0.007$), suggesting that the later broods tend to have an increased frequency of exceptions. There is also heterogeneity when the broods are divided into 2 groups only, 1–8 *vs* 9–16 ($P = 0.007$).

As seen earlier, the first- to fourth-day broods of the various experimental series at high-temperature treatment as well as the control C-0 were very similar despite their differences in aging treatment. In the control C series, the corresponding broods are those of the first to the fifth days as judged by the very small number of eggs laid on the first day. This small number is accounted for by the procedure which led to brood collection immediately after eclosion, whereas a minimum of 21 hours is required to develop eggs ready for insemination and deposition. For the same reason the 13th day of the experimental and C-0 series is represented by the 14th day of the C series. If one tests for homogeneity between the 1–5th-day *vs* the 6th–14th-days grouped broods, one finds a significant increase of exceptions in the latter group ($P < 0.001$). If a test is made using the female's age as a dividing criterion instead of the brood number, one must consider that the females of the C-0 series went through 3 days' pretreatment, so that the first- to fourth-day broods of the C-0 controls come from females corresponding to the fourth to seventh days age of the C controls without pretreatment. With these ages used as dividing markers, the 4–7th-day and the 8–16th-day broods of the C series also are significantly different ($P = 0.01$–0.02). In the C-0 series, on the contrary, a comparison between the 1–4th-day *vs* 5–13th-day grouped broods does not yield a significant difference ($P = 0.3$–0.5). Such differences do appear, however, when the C-0 broods are grouped in smaller units, e.g., 1–4th-, 5–9th-, and 10–13th- day broods or 1–3rd-, 4–5th-, 6–7th-, 8–9th-, and 10–13th-day broods: for comparisons involving three groups, $P = 0.02$–0.03; for comparisons involving five groups, $P = 0.01$–0.02. Similarly, when the early 1–4th-day broods of the C-0 series are compared with the last (10–13th-day) broods, the latter are higher in frequency of exceptions than the former ($P = 0.02$–0.04). Corresponding comparisons in the C series give highly significant differences: 1–4th-day group *vs* 11–14th-day group, $P = 0.009$; 1–5th-day group *vs* 13–16th-day group, $P < 0.001$.

Thus both the C-0 controls with 3 days' aging pretreatment and the C controls


hours
24
12
12
12
12
6
72
96
79
formation of synaptonemal complexes
no longer visible
Germarium
Vitellarium
Stem line oogonium
Cystoblast
4 successive cystocyte divisions
Cluster of 16 cystocytes
Stage 1
Stage 2
Stage 4
Stage 6
Stage 14
Mature egg

without such pretreatment show a tendency to increase frequency of exceptions in the later part of life of the females during the 13 or 16 days, respectively, of their egg laying. Whether the relative difference between the two control series in the intensity of the aging effect is caused by the difference in pretreatment of the two control series (3 days *vs.* none) or is due to statistical variability remains unknown.

DISCUSSION

An association between maternal age and nondisjunction of chromosomes in humans has long been known, particularly for chromosome 21 (PENROSE and SMITH 1966) and the sex chromosome (LENZ, NOWAKOWSKI, PRADER and SCHIRREN 1959). In Drosophila, no proof for such an association has been furnished (KELSALL 1963) except when aging is combined with X-ray treatment (PATTERSON, BREWSTER and WINCHESTER 1932; UCHIDA 1962). The latter combination is also effective in humans (UCHIDA, HOLUNGA and LAWLER 1968).

This paper reports on aging and low temperature effects on *X* chromosomal nondisjunction in Drosophila based on counts of the viable nondisjunctional exceptions including all the *X*0 males, some of which actually are likely to be the result of *X* chromosome loss.

Aging effects at 25°C are found in the later broods of 6th- to 13th day and even the least sensitive 5th-day brood can be affected by longer periods of aging. A similar pattern of aging effect was observed at 10°C, which perhaps indicates that the speed of oogenesis after the removal of treatment does not differ greatly between the two different temperature treatments. It has been estimated that oogenesis requires about six days, at maximum efficiency, from oogonia to Stage 1 oocytes (KOCH and KING 1966). Stage 1 to full maturity at Stage 14, has been estimated to be 79 hours (DAVID and MERLE 1968). Thus, the whole cycle requires approximately 10 days (Figure 3). Accordingly, the 5th-day broods represent 16-cell cyst stages at the end of treatment and the 13th-day broods represent oogonia. However, this does not take into consideration a possible delay resulting from the aging on minimal food.

The aging effect on nondisjunction occurred not only in the experimental series with storage on minimal food, but also in untreated control females. In the C control, where daily brood collections under normal conditions were carried out for 16 days, there was a clear trend toward increasing frequency of exceptions in the later broods.

The drastic low-temperature effect found in the first-day brood in the experimental series requires a minimum duration of treatment period. It is not the result of the temperature shock experienced upon the initial change from 25°C to 10°C and the terminal change back to 25°C. It is restricted to mature eggs, and

FIGURE 3.—Schematic representation of the estimated timing of egg development in an ovariole operating at maximum efficiency at 25°C (after the studies of KING 1957, KOCH and KING 1966, KOCH, SMITH and KING 1967 and DAVID and MERLE 1968). The intervals on the time scale are not proportional to one another. Also, the dimensions of the different stages are not proportional to one another.

is maintained beyond 24 hours after cessation of the low-temperature treatment in cases of 1- and 2-week treatments. The tendency toward an equal sex ratio of exceptions found in the drastic low-temperature effect case suggests that the exceptions in this case do not include many cases of chromosome loss.

Another effect on nondisjunction demands further work. It is the weak effect in the 3-week low-temperature series on the 3rd-day brood. The 3-week effect may have been involved in the earlier experiments of HILDRETH and ULRICHS (1969), in which the low-temperature treatment was also 3 weeks. Their data, which were not divided into daily broods, cover offspring corresponding to the first-, second-, and in part the third-day brood. Thus the effect on nondisjunction observed by them must have been caused mainly by the effect on the first-day brood and, to a minor degree, on the third-day brood.

Studies on amphibians and mammals have led to various assumptions regarding the cause of nondisjunction. One of these assumptions suggests postovulatory overripeness of normally mature primary oocytes (WITSCHI and LAGUENS 1963; BUTCHER and FUGO 1967; MIKAMO 1968). In humans, it has usually been assumed that aging affects the eggs prior to ovulation, but GERMAN (1968) has suggested that the aging effect is exerted after ovulation. This suggestion, however, has been shown to account for, at most, only a small fraction of cases (PENROSE and BERG 1968; CANNINGS and CANNINGS 1968; JAMES 1968; GOODHART 1968; MATSUNAGA and MARUYAMA 1969).

In *D. melanogaster*, the eggs involved in producing the first-day brood in the present experimental series of high- and low-temperature treatment correspond to vertebrate eggs exposed to overripeness in the studies just cited. The effects of aging apparent in later broods are based on influences exerted on younger egg stages, which correspond to those in the majority of human cases relating to chromosome 21.

The high series did not show a clear-cut effect on nondisjunction in the first-day brood, although a tendency toward increase with increasing length of aging existed. More data are needed to substantiate the possible effect of overripeness independent of temperature in Drosophila, but the effect, if any, would be very minor in comparison with the effect of combining overripeness and low temperature.

It is hoped that the drastic low-temperature effect on the first-day brood will become a useful tool for clarification of the nature of the process of nondisjunction.

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SUMMARY

Females of *Drosophila melanogaster* were subjected, during a premating treatment on minimal food, to various periods of aging from 3 days up to 3 weeks at

25°C and 10°C. The effects of these treatments were measured as increases in the frequency of nondisjunctional exceptions of the *X* chromosomes.—No aging effect at 25°C was found among the first- to at least fourth-day broods although there was a possibility of the presence of a minor effect on the first-day brood when aging was longer than one week. The effects found in later broods roughly correspond to the 16-cell cyst stages up to oogonia at the end of treatment.—An age effect in the females on nondisjunctional exceptions is also apparent in flies raised under continuously normal conditions. In a study of 16 successive daily broods, the later broods had increased frequencies of exceptions.—An exceedingly strong increase of exceptions occurs in the first-day broods after 1 or more weeks of treatment of the females at low temperature (10°C). The frequency of exceptions is as high as 140 times that found in controls and experimental series not involving low temperature. Even low-temperature treatment of only 3 days' duration seems to be effective. It is not the temperature shock involved, but a certain minimal length of exposure to low temperature that is responsible for the striking increase of exceptions. The effect is almost exclusively based on influences on the retained mature eggs in the treated female and extends over at least 24 hours after removal from treatment.—A weak low-temperature effect has also been observed in the 6–7th-day broods of the 1- and 2-week-treated series as well as in the third-day brood of 3-week treated females.

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