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THE EFFECTS OF TEMPERATURE AND AGING ON NONDISJUNCTION IN MALES OF *DROSOPHILA MELANOGASTER**

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SUMMARY

Drosophila males of the constitution y/y^+ Y were aged for 10 days at 25°C and 10°C and then mated daily for 13 days at 25°C to virgin y w females. The frequencies of exceptional XXY and XO offspring in the 25°C pre-aged group, to which the father contributed either (a) both an X and a Y chromosome or (b) neither of them ("paternal exceptions") were compared with those in the 10°C pre-aged groups. Likewise, the frequencies in the two pre-aged groups were compared with those in a 25°C not pre-aged group. In the latter a slight increase of paternal exceptions in later broods represents a chronological aging effect. The increase of total frequency of exceptions in the 25°C pre-aged series over that of the not pre-aged series is interpreted as an effect of pre-aging, chronological aging, or both. The total frequencies of paternal exceptions were not significantly different in the 25°C and 10°C pre-aged series, but a highly significantly greater number of exceptions in the seventh- and eighth-day broods was found in the 10°C pre-aged series than in the 25°C pre-aged series. In all experiments the frequency of paternal XO exceptions was greatly in excess of that of XXY exceptions. The data on brood patterns suggest that the stages most sensitive to the production of paternal exceptions by pre-aging at 10°C are those of the primary spermatocytes.

INTRODUCTION

In D. melanogaster, exposure of females before mating to 10°C for extensive periods leads to an extremely high frequency of meiotic nondisjunction in mature eggs retained during the exposure. A second, weaker effect on nondisjunction occurs when earlier stages of oogenesis--including oogonial to 16-cystocyte stages at the time of mating--are aged before mating (Tokunaga[12]). In the following pages such pre-mating treatment is called pre-aging, in contrast to the chronological aging which occurs in flies after mating. This study presents the effects of pre-aging at different temperature on meiotic nondisjunction in males. Unless specifically noted otherwise the term "nondisjunction in males" as used hereafter encompasses all events resulting in the production of paternal exceptional offspring which obtain from their father either an X and a Y chromosome or neither. "Nondisjunction in females" signifies the production of maternal exceptional offspring which obtain from their mother either two X chromosomes or none.

MATERIALS AND METHODS

The frequencies of exceptions, of the types XXY females and XO males, were studied among the progeny of the cross $y\,w/y\,w$ ♀ × $y/y^{+}Y$ ♂. The symbol $y^{+}Y$ stands for a Y chromosome to which a small section of an X chromosome carrying the normal allele of y has been translocated. Among the progeny of this cross, the regular segregants are y ♀ and w ♂. Paternal exceptions can be identified as + ♀ (XXY) and y w ♂ (XO), and maternal exceptions as w ♀ (XXY) and y ♂ (XO).

Three groups of 50 parent males, 5 to 6 hours old, were mated individually at 25°C to three 1-day-old y w females, either immediately or after pre-aging for 10 days on standard food at 25°C and 10°C. On each of a total of 13 days, the males were transferred to new culture vials containing 3 virgin 1-day-old y w females. The progeny from the females of each day's mating were collected over a period of 11 days; on each of the first 6 days, and finally on the eighth day, the inseminated females were transferred to fresh vials. The sum of the offspring of the females mated on one day constituted that day's brood.

The three series of cultures from 25°C not pre-aged, and from 25°C and 10°C pre-aged males constitute the three main experiments. In addition, the 10°C pre-aged treatment series was repeated twice. In these repeat experiments mass matings instead of single matings were performed after the pre-aging treatment. In one series, 240 treated males were initially mated each day to virgin females for the successive collection of the first- to the fourth-day broods (15 males and 45 females per culture bottle). For the fifth-day brood 150 of the males which were the fathers of the broods during the first 4 days were selected at random and used for the collection of brood numbers 5 to 10 (three males and nine females per vial). In another series, only the fifth- to ninth-day broods produced by 335 treated males were collected (15 males and 45 females per culture bottle, one vial with 5 males and 15 females).

The data were analyzed by χ^2 tests using Yates' correction when, with one degree of freedom allowed for an expected class was less than five.

RESULTS

A. Frequencies of XXY and XO exceptions in the main experiments (Table I)

1. Paternal exceptions

Heterogeneity tests for the 13 broods of each of the three experimental series show that the exceptions are not distributed at random over the different broods. In each series the P value for homogeneity is less than 0.001 (df = 12). The heterogeneity follows specific patterns. In the not pre-aged series, later broods tend to show higher frequencies of exceptions than the earlier ones (Fig. 1a). This may be seen also when the successive 13 broods are divided into four groups consisting of the first- to third-, fourth- to sixth-, seventh- to ninth-, and tenth- to 13th-day broods. The exceptions in the first group have a frequency of 0.05%, in the second, 0.09%, in the third, 0.10%, and in the last, 0.18%. The higher frequencies of exceptions in the later groups are statistically significant in all comparisons except that between the second and the third groups (P = 0.7).

Comparisons of the total data between the three series indicate that the frequency of exceptions is significantly higher in the 25°C and the 10°C pre-aged series than in the not pre-aged series ($P < 0.001$) whereas the two pre-aged series show no significant difference between each other (P = 0.6).

In the main experiments the two highest frequencies of exceptions occurred on the seventh and eighth days in the 10°C pre-aged series: 0.54 and 1.14% respectively (Fig. 1a). The third highest percentage, 0.31, occurred on the eighth day in the 25°C pre-aged series. Since the broods of each series are heterogeneous, the effect of pre-aging on the frequency of exceptions that was demonstrated in com-

parisons of the total numbers of exceptions was also studied in comparisons brood by brood. A significant effect of pre-aging at 10°C compared with pre-aging at 25°C on the frequency of exceptions is found exclusively in the seventh- and eighth-day broods. All other broods, where the χ^2 test is applicable, did not deviate significantly between the two series. Clearly, the differences between the seventh- and eighth-day broods of the two series are due to the effects of low temperature during a low-temperature-sensitive period of spermatogenesis which results 7 to 8 days later in increased production of exceptions.

It is interesting that the temperature-sensitive period for production of paternal exceptions coincides with a temperature-sensitive period for male fertility. It is known that the size of the daily brood from individual males is variable, usually declining in the later broods (Hannah-Alava and Puro[6], Slizynski[11]). In the data presented here, the general tendency toward a reduction of the brood size is obvious in the later broods of the not pre-aged and the 25°C pre-aged series. In the 10°C pre-aged series, the sizes of some specific broods, especially those from the fifth till the eighth day, are particularly reduced. However, here the brood size starts to increase again from the ninth day on (Table I). Such temporarily reduced fertility has been known before from studies of X-rayed males, where it served to identify specific developmental stages of spermatogenesis which were unusually sensitive to X-rays (Hannah-Alava[5], Auerbach[1], Friesen[4] and Welshons[14]). In the 10°C pre-aged case the pattern of low fertility is induced by low temperature.

2. Maternal exceptions

Since the P females of each brood listed in Table I were all treated alike, maternal exceptions are expected to be of similar frequency in all broods of all series studied. Analysis of the frequency of maternal exceptions in the total data between the three series indicates that they are indeed homogeneous ($P = 0.7$, $df = 2$). In the 25°C not pre-aged and pre-aged series, the distribution of maternal exceptions over each of the 13 days' broods also show that they are, as expected, homogeneous ($df = 12$; $P = 0.6$ in the not pre-aged, 0.7 in the pre-aged series). However, contrary to expectation, the 10°C pre-aged series show a highly heterogeneous brood distribution.

The detailed record shows that all 10 maternal exceptions in this series (6 XXY and 4 XO among a total of 1843 progeny) occurred in the seventh-day brood of a single male and its three females. This brood consisted of 186 individuals. It is not likely that the low-temperature preaging treatment of the male parent was responsible for the high frequency of maternal exceptions, since none of the other 49 equally treated males and none of the more than 450 similarly treated males in later experiments led to a high frequency of maternal exceptions. It is therefore likely that one of the three mates of the male was responsible for the unusual finding.

B. Frequencies of XXY and XO exceptions in additional experiments (Table II)

The 10°C preaging experiments were repeated twice and the results are given in Table II. The data on the frequencies of paternal exceptions in the repeat experiments confirmed the existence of the low-temperature effects found in the main experiments. As seen in Fig. 1b, the seventh- and eighth-day broods of the two repeat 10°C

pre-aged series show the highest frequencies of exceptions among the broods studied, as had been originally found in the main series (Fig. 1a). When the sum of the repeat data for the seventh- and eight-day 10°C pre-aged broods are compared with the seventh- and eighth-day data of the 25°C not pre-aged and pre-aged series, highly significant differences in the frequency of exceptions are revealed ($P < 0.001$ in each of the two comparisons). This confirms the earlier conclusion that it is the low temperature during pre-aging which is responsible for the increased frequency of exceptions. The rest of the broods compared either do not differ from the two 25°C main series (in the first- to fifth- and in the ninth-day broods) or show a significant increase over the 25°C not pre-aged series, but were not significantly different from the 25°C pre-aged series (sixth- and tenth-day broods).

It will be noticed that the low-temperature effect is much greater in the repeat experiments than in the main one. The causes of the variations of this effect are not yet known.

DISCUSSION

Comparison of the total frequency of paternal exceptions in the 25°C not pre-aged series with that in the 25°C pre-aged series showed that the frequency in the latter series was significantly increased. An interpretation of this finding depends on the fate of the sperm produced during the pre-aging period. According to Lüning[7], such sperm is not retained during the pre-aging period but is lost or absorbed. If one accepts this view and assumes further that pre-aging does not change the course of spermatogenesis, it follows that the increased frequency of paternal exceptions in the pre-aged series was due to the increased chronological age of the pre-aged males. On the other hand, Mossige[9]

found that mature sperm is retained in pre-aged males at least for a few days. In this case, an effect of pre-aging on spermatogenesis would result in an increase of exceptions independent of the effect of chronological age. Perhaps the increase of exceptions in the 25°C pre-aged series is due to a combination of the effects of increased chronological age and the pre-aging process. In the future, data on the offspring of not pre-aged males in broods beyond the 13 days studied in this paper might contribute to an understanding of the effect of chronological age.

The three 10°C pre-aged series all yielded their high frequencies of paternal exceptions in the seventh- and eighth-day broods. In the same broods the lowest fertility of the 10°C pre-aged males was observed. Apparently the temperature-sensitive stages for the induction of paternal exceptions as well as for low fertility correspond to the seventh- and eighth-day broods. According to the timetable of spermatogenesis by Chandley and Bateman[2], which is based on their radioactive tracer studies, the seventh- and eighth-day broods were derived from sperm which was at the primary spermatocyte stage at the end of the 10°C pre-aging period. This estimate presupposes that the rate of spermatogenesis returns to normal soon after the end of exposure to 10°C. It also presupposes that the treatments used for estimating timing of spermatogenic stages do not alter the rate of spermatogenesis. The evidence presented by Chandley and Bateman indicates that the latter assumption may not be fully valid. They found that spermatogenesis proceeds more rapidly after X-ray irradiation than after exposure to radioactive tracers. Other factors are also known which make if difficult to relate precisely the broad pattern of offspring to the temporal pattern of spermatogenesis/(Hannah-Alava[5]). It may be

added that the period of temperature sensitivity of a meiotic drive genotype also involves the primary spermatocyte stage (Erickson and Hanks[3]). Here, however, the sensitivity persists through the meiotic divisions.

Pre-aging at 10°C has different effects in oogenesis and spermatogenesis. In females, the temperature-sensitive stage for exceptions involves mature eggs retained by the females during pre-aging, whereas in males the sensitive stage involves the primary spermatocytes. Quantitatively the results also differ. In females, up to 20% of sex chromosome exceptions were produced, whereas in males the highest frequency observed was less than 2%. Moreover, 10°C pre-aged femaes produced a considerable number of autosomal exceptions consisting of triploid females and interseces (Tokunaga[13]), whereas neither 10°C nor 25°C pre-aged males produced any autosomal exceptions. Finally, at the temperature-sensitive stage the ratio of XXY to XO exceptions from 10°C pre-aged females was close to unity, whereas the same ratio in all series of males was greatly shifted toward an excess of XO exceptions. The equality in frequencies of XXY and XO maternal exceptions is compatible with their origin as a result of nondisjunction in its narrower sense, whereas the excess of XO paternal exceptions suggests the participation of such meiotic abnormalities as chromosome loss (Morgan, Bridges and Sturtevant[8], Peacock[10]).

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FOOTNOTE AND REFERENCES

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Table I. Frequencies of XXY and XO exceptions among the progeny of $\underline{y}\ \underline{w}/\underline{y}\ \underline{w}$ ♀ × $\underline{y}/\underline{y}^{+}Y$ ♂. The table lists the total offspring of each of three groups of 50 P males separately subjected to the following treatments: (a) 25°C not pre-aged; (b) 25°C pre-aged; and (c) 10°C pre-aged. All exceptional males were tested for fertility and proved to be sterile.

Experimental series	Broods (days)	Totals	Exceptions: Paternal + (XXY)	Paternal yw (XO)	Paternal %	Maternal w (XXY)	Maternal y (XO)	Maternal %
25°C not pre-aged	1	17960	2	1	0.02	1	10	0.06
	2	21222	4	8	0.06	4	10	0.07
	3	18318	3	9	0.07	3	5	0.04
	4	20835	7	16	0.11	6	3	0.04
	5	21861	1	18	0.09	4	11	0.07
	6	21803	2	15	0.08	4	7	0.05
	7	16869	5*	12	0.10	2	7	0.05
	8	13944	2	11	0.09	5	1	0.04
	9	12195	3	9	0.10	7	6	0.11
	10	9936	2	9	0.11	2	5	0.07
	11	10259	11	17	0.27	1	2	0.03
	12	9739	5	17	0.23	2	5	0.07
	13	9990	2	9	0.11	1	4	0.05
	Totals	204931	49	151	0.10	42	76	0.06
25°C pre-aged	1	21632	4	19	0.11	4	9	0.06
	2	24560	4	10	0.06	4	8	0.05
	3	20827	13	21	0.16	5	10	0.07
	4	19578	6	20	0.13	4	10	0.07
	5	14571	7	18	0.17	0	9	0.06
	6	12103	4	13	0.14	0	5	0.04
	7	11857	6	19	0.21	3	8	0.09
	8	8754	14	13	0.31	1	2	0.03
	9	8610	4	11	0.17	1	5	0.07
	10	9469	4	8	0.13	2	3	0.05
	11	10214	2	12	0.14	2	4	0.06
	12	10801	4	9	0.12	3	6	0.08
	13	10456	1	7	0.08	2	1	0.03
	Totals	188432	73	180	0.14	31	80	0.06
10°C pre-aged	1	18615	5	12	0.09	6	4	0.05
	2	8317	3	4	0.08	1	3	0.05
	3	10547	5	15	0.19	1	0	0.01
	4	7315	4	7	0.15	3	2	0.07
	5	1297	0	0	0.00	0	1	0.08
	6	2936	0	5	0.17	0	0	0.00
	7	1843	1	9	0.54	6	4	0.54
	8	1486	5	12	1.14	0	0	0.00
	9	4339	2	2	0.09	2	2	0.09
	10	12702	9	17	0.20	1	6	0.06
	11	12235	5	12	0.13	1	5	0.05
	12	9680	1	9	0.10	0	7	0.07
	13	7808	3	3	0.08	5	4	0.12
	Totals	99120	43	107	0.15	26	38	0.06

*One individual was a triploid intersex of XXY constitution.

Table II. Repeated experimental data of 10°C pre-aged series. The two series of experimental data are separately listed without and with parentheses.

Broods (days)	Totals	Exceptions					
		Paternal			Maternal		
		+(XXY)	yw(XO)	%	w(XXY)	y(XO)	%
1	37455	14	17	0.08	6	10	0.04
2	10630	1	4	0.05	3	3	0.06
3	8796	2	6	0.09	0	3	0.03
4	3739	1	0	0.03	0	1	0.03
5	1213 (4151)	0 (0)	1 (3)	0.08 (0.07)	0 (0)	1 (3)	0.08 (0.07)
6	61 (6608)	0 (3)	0 (14)	0.00 (0.26)	0 (0)	0 (1)	0.00 (0.02)
7	886 (3281)	5 (3)	8 (31)	1.47 (1.04)	0 (0)	0 (1)	0.00 (0.03)
8	87 (3932)	0 (9)	1 (62)	1.15 (1.80)	0 (1)	0 (1)	0.00 (0.05)
9	908 (6091)	0 (3)	0 (8)	0.00 (0.18)	0 (0)	0 (2)	0.00 (0.03)
10	7396	5	16	0.28	2	3	0.07

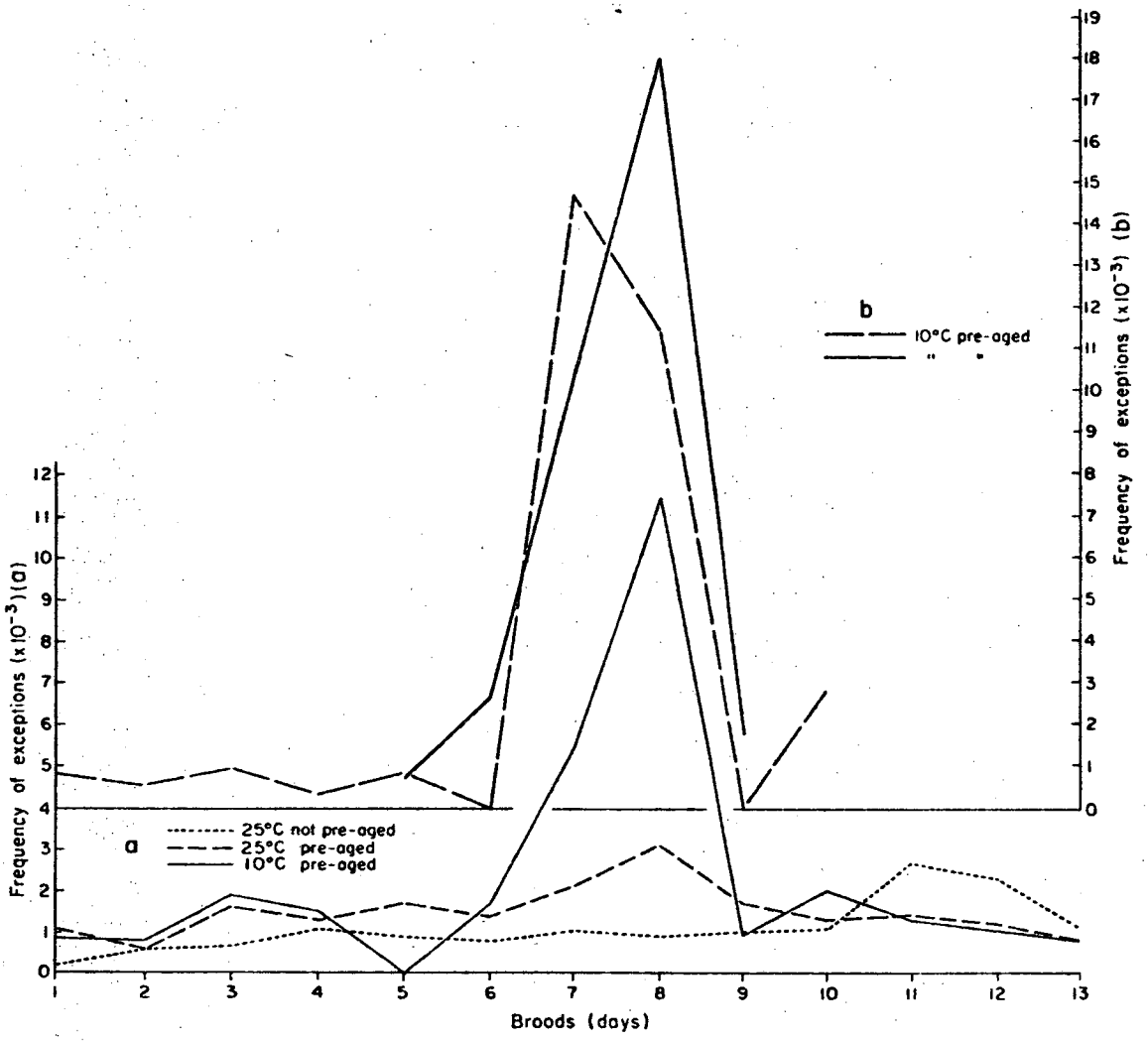

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Fig. 1. Frequencies of paternal exceptions.
a. Main experiments.
b. Repeat experiments.
(See text for details.)