

## Non-disjunction and maternal age in *D. melanogaster*

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### 1. INTRODUCTION

Evidence of a maternal age effect in man has long been available in the case of mongolism (Penrose, 1933, 1957). More recent work with chromatin-positive cases of Klinefelter's syndrome indicates that here too parental age may exert an important influence (Lenz, Nowackowski, Prader & Schirren, 1959). Both mongolism and chromatin-positive Klinefelter's syndrome have been shown to be results of trisomy. There is some evidence that other trisomic syndromes may occur more frequently with advancing maternal age (Smith, Patau, Therman & Inhorn, 1962)

There is a widely held view that this situation is also found in *Drosophila*. This appears to have arisen from a paper by Mottram on the effect of CO<sub>2</sub> treatment upon the frequency of non-disjunction (Mottram, 1930). In it Mottram wrote, 'Newly hatched red-eyed females were "aged" for one week before being subjected to CO<sub>2</sub>. "Ageing" increases the number of exceptional males due to non-disjunction. "Ageing" consists of keeping the virgin females crowded in a small flask without males.' He gives no references on this point and does not himself elucidate further, with the exception of the following figures:

Table 1. *A comparison of XO exceptional ♂♂ obtained with or without CO<sub>2</sub> treatment before and after ageing 1 week (after Mottram)*

	Regular		Exceptional
	♂♂	♀♀	♂♂ (XO)
Pure CO <sub>2</sub> for 4½ hr	2931	3003	3
Controls	2445	2397	—
Pure CO <sub>2</sub> for 4½ hr. after ageing 1 week	2323	2244	6
Controls	1934	1868	2

Whereas this may indicate some increased sensitivity to CO<sub>2</sub> upon ageing, or an increasing proportion of oöcytes in a sensitive stage, there is little evidence here for any age effect in the controls. In the non-aged controls no exceptions were found amongst 4842 regular flies, whilst in the controls of the aged series 2 exceptional XO males were found amongst 3802 regular flies. Obviously these numbers are far too small for the difference to be significant. It therefore seemed important to repeat

this experiment using larger numbers. Hence it is the purpose of this paper to report, (a) the results of this repetition, and (b) results obtained from a more comprehensive study of maternal age and frequency of non-disjunction, using a scheme first described a year ago (Kelsall, 1961).

## 2. MATERIALS AND METHODS

In the repeat of Mottram's ageing experiment white, singed (*w, sn*) males were crossed to Oregon K (*OrK*) females in vials, one pair in each vial, and kept at 25°C. The exceptions here are patroclinous *w, sn*, XO males.

For the more comprehensive study, the scheme used allows the detection of XO and XYY males, as well as XXY females, in the same progeny. An inversion brown variegated (*bwV<sup>A</sup>*), originally obtained by H. J. Muller, and located on the second chromosome, gives a dominant mottling of the eyes apparently owing to the proximity of neighbouring heterochromatin. This mottling is suppressed by one extra Y chromosome in excess of the normal complement. On a wild-type background *bwV<sup>A</sup>* gives brown eyes mottled red, whilst the extra Y individuals have wild-type eyes. This phenotypic expression of the extra Y is greatly amplified on a homozygous vermilion or scarlet background. Here the eye colour is changed from a yellowish base flecked with bright red to a colour indistinguishable from vermilion or scarlet. Suitable markers allow the three different X chromosomes to be identified and hence the origin of the X's in any individual can be decided.

In this investigation

$$\frac{+}{m}; \frac{bwV^A}{BlL}; \frac{st}{st} \text{ females were crossed to } y/Y; \frac{bwV^A}{BlL}; \frac{st}{st} \text{ males}$$

Sixty bottles with fifteen pairs in each were made up and kept at 25°C. The flies were transferred to fresh bottles every third day and the offspring were counted in 3-day broods. The regular progeny would then be

$$+ \text{ or } m/Y; \frac{bwV^A}{BlL}; \frac{st}{st} \text{ males and } \frac{+ \text{ or } m}{y}; \frac{bwV^A}{BlL}; \frac{st}{st} \text{ females,}$$

since both the *bwV<sup>A</sup>* and the *BlL* chromosomes are lethal when homozygous.

Exceptional individuals may arise from non-disjunction at the first or second meiotic divisions in either sex, with the following results:

(1) *First meiotic division in the male.* XY sperm would give XXY individuals (scarlet-eyed, +/y or m/y). O sperm would give XO sterile males phenotypically indistinguishable from the regular progeny.

(2) *Second meiotic division in the male.* YY sperm would give XYY males (scarlet, + or m). XX sperm would give XXX inviable females.

(3) *First meiotic division in the female.* XX eggs would give XXY (scarlet, +/m) or XXX (inviable) females. O eggs would give XO (patroclinous yellow) or YO (lethal) males.

(4) *Second meiotic division in the female.* Again, XX eggs would give XXY (this time scarlet,  $m/m$  or  $+/+$ ) or XXX (inviable) females.

It can be seen that for various reasons this scheme is capable of detecting only half of the zygotes arising from non-disjunctive gametes in each class.

Individuals arising from XY sperm in class (1), and XX eggs in class (3) were phenotypically indistinguishable. They were classified by further crosses. The presence of an extra Y in classes (1), (2) and (3) was shown by the presence of patrocinous males, when exceptional virgin daughters of the original exceptions were crossed to suitably marked males.

Infrequent though regular crossing-over between the  $bwV^4$  and  $BlL$  chromosomes resulted in individuals without  $bwV^4$  which simulated the extra-Y exceptions. These were eliminated during the test-crosses.

### 3. RESULTS

Table 2 presents the results of a repeat of Mottram's work in which the exceptional XO males in the progeny of 'Aged' and 'Non-aged' females are recorded. In this repeat I obtained 7 exceptions in 24,279 in the non-aged group and 3 exceptions in 15,450 in the aged series. Overall frequencies are low, but there is obviously no indication here of any parental age effect.

Table 2. *A comparison of exceptional XO ♂♂ in the progeny of 'aged' and 'non-aged' females*

	Regular	Exceptional XO ♂♂	Exceptions (%)
Before 'ageing'	24,279	7	0.029
'Aged' 1 week	15,450	3	0.019

Table 3 gives the results of the larger investigation, where several types of non-disjunctive individuals were scored. The figures are given in successive 3-day broods. Statistical analysis of the figures in Table 3 shows no significant trend.

Table 3. *The numbers and percentages of non-disjunctive individuals in successive 3-day broods in D. Melanogaster*

Age of parents in days	Total number of flies	Total number of exceptions	Exceptions (%)
0-4	17,000	17	0.10
3-7	30,500	21	0.067
6-10	26,000	21	0.081
9-13	17,500	15	0.086
12-16	13,000	19	0.146
15-19	10,000	8	0.080
18-22	6,000	7	0.117
21-25	1,100	1	0.090
24-28	1,000	0	0.00
Total	123,000	109	Mean = 0.089

Figures for individual types of non-disjunction also show random distribution throughout the age group. The frequencies of the exceptional gametes based on these 123,000 flies are as follows:

- (1) Flies originating from XY sperm: 13 individuals, i.e. 1 in 9400.
- (2) Flies originating from YY sperm: 5 individuals, i.e. gamete frequency of 1:24,600.
- (3) (a) Flies originating from XX eggs: 27 individuals, i.e. gamete frequency of 1:2300, since half these eggs will be fertilized by an X sperm, giving XXX inviable females.  
(b) Flies originating from O eggs: 64 individuals, i.e. gamete frequency of 1:960, since half the O eggs would result in YO lethals.
- (4) This class was represented by one fly. This was an XXY exception which on crossing to a brother gave 63+ and no *m* males. Because of its infrequency other explanations are possible.

#### 4. DISCUSSION

It will be useful at this point to reiterate briefly the different classes of non-disjunction. They are:

- (1) Non-disjunction at the first meiotic division in males, giving XY and O sperm.
- (2) Non-disjunction at the second meiotic division in males, giving YY and XX sperm.
- (3) Non-disjunction at the first meiotic division in females, giving XX and O eggs.
- (4) Non-disjunction at the second meiotic division in females, giving XX eggs.

The frequencies of non-disjunction in class (3) approximate to those found by Bridges (1913), although the exceptional females (XX eggs) occur a little more commonly than in his experiments. This is probably due to frequency variations known to occur between stocks (Safir, 1920). Types (1) and (2) which are the classes resulting from non-disjunction at spermatogenesis are considerably less frequent than their counterparts in oögenesis. The sole representative of class (4) is of little importance due to its extreme rarity and therefore the many possible explanations of its origin. In any event, if this exception represents a non-disjunctional event at the second meiotic division of oögenesis, it is at best an extremely rare event having a gamete frequency of around 1 in 60,000.

For none of the non-disjunctional events in my experiments was there any indication of an age effect. This included the repetition of Mottram's work (Table 2), the original source of the belief in an age effect on non-disjunction in *Drosophila*. Clearly, these results are in complete agreement with Anderson's findings (Anderson, 1927), which show no significant difference between the non-disjunctional frequencies in the first and second broods obtained from flies of a high non-disjunction line produced by X-rays.

Hence, Lamy's (Lamy, 1949) observations of patroclinous daughters of attached X females remains the only evidence which might be construed as an influence of

age on non-disjunction in *Drosophila*. Lamy crossed *w* males to *ywf* females and changed the flies to fresh food two or three times weekly. Amongst the progeny she found a proportion of *w* females, which, when progeny-tested, were found to have two free X chromosomes, each carrying *w*, and a Y chromosome. Her figures were as follows:—32 white females were found amongst 14,313 offspring, of which 7587 were regular F<sub>1</sub> males, so that the overall frequency was 0.42% exceptions. The percentages tabulated according to paternal age were: 1–14 days 0.06%, 14–21 days 0.34%, 21–28 days 0.52%, 28–35 days 0.83%, 35–38 days 1.6%. These figures show a significant upward trend, indicating that the female exceptions increase with paternal age. However, present evidence, to be reported elsewhere, suggests that this phenomenon is unrelated to other non-disjunctional events.

The results presented here show that the maternal age-effect on non-disjunction which is well established in man is not found in *Drosophila*. This is not surprising when one considers that gametogenesis, in particular oögenesis, follows widely different courses in the two species. In mammals the number of germ cells in the female does not increase after birth although spermatogenesis is a continuous process. In *Drosophila* both sexes show continuous gametogenesis. Oögenesis in *Drosophila* lacks the long dictyotene stage which in mammalian females lasts from early embryonic life until immediately prior to ovulation, and according to a suggestion by Slizynski (1960) this may account for the maternal age-effect on non-disjunction in man.

##### 5. SUMMARY

The frequencies of non-disjunction at first and second meiotic division, in both male and female of *Drosophila melanogaster*, are estimated. Numbers of exceptional individuals scored in several broods of increasing parental age are also presented. These results fail to show any evidence of an age-effect. Work claiming to demonstrate the existence of an age effect in *Drosophila* is reinvestigated. The importance of these results and their relevance to human studies are discussed.

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