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X-chromosome segregation, maternal age and aneuploidy in the *XO* mouse

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SUMMARY

Cytogenetic studies have ascertained that the segregation of the *X*-chromosome, during the first meiotic division of the oocyte in *XO* mice, occurs at random, contrary to the finding of some earlier authors. The ratio of nullo-*X* to *X*-bearing oocytes at ovulation does not change with maternal age. The usefulness of the *XO* mouse as a model for aneuploidy production in women (Lyon & Hawker, 1973) is discussed.

INTRODUCTION

Unlike *XO* women, *XO* mice are fertile, albeit subject to reproductive impairment (Lyon & Hawker, 1973).

Ever since a breeding stock of *XO* mice was established by Cattanach (1962), using the sex-linked gene tabby, as marker, controversy has existed in the literature concerning the question of segregation of the single *X*-chromosome at meiosis in the *XO* oocyte. From his own breeding data, Cattanach (1962) observed that although litter size was near normal for this particular stock, a shortfall of some 30–37% in *XO* compared to *XX* offspring occurred from *XO* mothers. He was unable to determine the reason for the reduction in *XO* progeny, but as one possibility, suggested that preferential loss of the chromosome sets lacking an *X* chromosome to the polar bodies in the meiotic divisions of the ova might have occurred. The alternative was that death of the missing classes during embryonic development had taken place. To account for the higher than expected litter size in *XO* mothers, he did not, however, discount the possibility of early loss of inviable embryos, compensated by the development of all individuals of the viable classes, some of which would have been lost in larger normal litters as a result of overcrowding in the uterus.

In a subsequent study, Morris (1968) examined reproductive performance and embryonic mortality in a large series of *XO* and *XX* females. One series of pregnant females of both genotypes was dissected after 15 days gestation and another series after $3\frac{1}{2}$ days. From his findings, he concluded that there could be both an abnormally low segregation of nullo-*X* gametes in *XO* females *and* a reduction in

viability of *XO* foetuses during the early stages of gestation. This lower viability of *XO*'s *in utero* contrasted with their seemingly normal viability after birth. Strong circumstantial evidence was also found for the death of all *OY* zygotes before implantation.

Direct cytological information on the segregation of the *X* chromosome has since been obtained by several groups of authors analysing chromosomal complements in the metaphase II oocytes ovulated by *XO* females. The results however, are conflicting (see Russell, 1976 for review). According to Evans and Ford (unpublished data), segregation of the *X* to egg or polar body is random. The data of Kaufman (1972) and Luthardt (1976), however, suggest that it is non-random, with the *X*-bearing chromosome sets being preferentially included in the egg nucleus.

A further complicating factor is the claim made by Deckers *et al.* (1981), from breeding data on *XO* mice, that the phenomenon of non-random segregation is maternal-age related. These authors found a greater recovery of *XO* progeny relative to *XX* as the age of the mother (or litter number) advanced.

The present study was initiated in a further attempt to clarify some of these issues. Breeding data on a large series of *XO* mice at a range of ages have been coupled with a cytological analysis of ovulated metaphase II oocytes. The question of whether or not segregation of the single *X*-chromosome is random has been re-investigated. Also, evidence for changing relationship between *X*-segregation and age has been sought. The question of whether the *XO* mouse constitutes a good model for the human pre-menopausal female, in terms of chromosomal nondisjunction as suggested by Lyon & Hawker (1973), is also considered.

METHODS AND MATERIALS

(i) *Animals used*

The colony of *XO* mice used was set up from mice kindly supplied by Dr Mary Lyon, MRC Radiobiology Unit, Harwell, England. The sex-linked gene, Tabby, was used as marker. Normal-coated *XO* females mated to Tabby males produced three types of phenotypically distinct offspring; normal-coated males (+/*Y*), greasy-coated females (Ta/O) and striped females (Ta/+). The Ta/O and +/*Y* offspring were subsequently used as breeders to regenerate +/O and Ta/*Y* animals, with striped females (Ta/+) again being produced. In this way all offspring could be identified from their coat colours. Brother-sister matings were avoided. The stock was checked occasionally, by blood karyotyping, to ensure that all supposed phenotypic *XO* females were in fact of the *XO* genotype.

Offspring were classified at weaning and female breeders used until they reached 36 weeks of age, after which time they were killed and used for oocyte chromosome analysis.

(ii) *Oocyte collection and chromosome preparation*

The female mice used for chromosome analysis in metaphase II oocytes constituted six groups, divided according to age and phenotype. There were two

XO groups i.e. +/O and Ta/O with Ta/+ sibs serving as controls. Analysis was carried out over two different ages within each group i.e. 8–20 weeks (young) and 30–40 weeks (old) (Lyon & Hawker, 1973 have shown that in *XO* mice, both age-related ovarian changes and reduced reproductive performance are detectable by 28 weeks).

Table 1. *Birth data from XO mice*

	Total animals			Animals at weaning			
Mating type	Pairs	Litters	At birth	*XY*	*XO*	*XX*	Dead
Ta/O♀X+/Y♂	50	177	624	188 (30·1%)	113 (18·1%)	212 (34·0%)	111 (17·8%)
+/O♀XTa/Y♂	48	248	940	339 (36·1%)	166 (17·7%)	314 (33·4%)	121 (12·9%)

Figures in parentheses represent percentages of total births.

Each female was superovulated using 2·5 i.u. pregnant mares serum (PMS) and 2·0 i.u. human chorionic gonadotrophin (HCG) given 48 h later. The oocytes were sampled 15 h after HCG injection at a time corresponding to metaphase II. Hansmann and El Nahass (1979) have previously shown that these hormone doses do not affect the incidence of non-disjunction during the first meiotic division in the mouse oocyte. Mice were killed by cervical dislocation, the oocytes being removed from the ampullae of the fallopian tubes and fixed by the method of Tarkowski (1966). The preparations were C-banded according to the method of Sumner (1972) and chromosome counts made.

RESULTS

(i) *Birth data*

Birth data were obtained from both Ta/O and +/O mothers (Table 1). Analysis showed that there was a significant difference between the two breeding groups ($\chi^2_3 = 9{\cdot}73$; $P < 0{\cdot}05$), this being due to a higher level of death among offspring from Ta/O mothers than +/O mothers between birth and weaning. Ta/O females are generally less robust than +/O females and appear to be less competent as mothers. The data at weaning, showing increased death of offspring from Ta/O mothers compared with +/O mothers, support the findings of Cattanach (1962), Morris (1968) and Deckers *et al.* (1981), although the difference between the two types of mother is lower in the present study than has been found by these other authors.

There was no significant difference in the distribution of *XY: XX: XO* offspring at weaning from the two types of *XO* mother. Neither was there any difference in the birth ratio of *XX: XO* offspring between the two maternal genotypes, the proportion being 1·88:1 for Ta/O mothers and 1·89:1 for +/O mothers. Both ratios were lower than those found by earlier investigators (Table 2) indicating that comparatively more *XO* progeny were born to *XO* mothers in our stocks.

(ii) *Chromosome counts*

A total of 379 metaphase II (MII) counts were made from 82 *XO* females and 179 counts from 28 *XX* females. The results have been grouped in Tables 3–5 according to maternal age and genotype. Cells giving counts of less than 17 were few in number, tending to be from poor quality preparations: They were discounted as unreliable.

Table 2. *Ratios of XO to XX offspring at weaning*

	+/O Mothers		Ta/O Mothers	
Author	No. of ♀♀	Ratio Ta/O:Ta/+	No. of ♀♀	Ratio +/O:Ta/+
Cattanach (1962)	661	1:2·74	276	1:3·30
Morris (1968)	966	1:2·37	926	1:2·67
Russell (1976)	118	1:2·17	192	1:3·23
Deckers *et al.* (1981)	362	1:2·45	119	1:2·84
Brook (Present Study)	480	1:1·89	325	1:1·88
Léonard & Schröder (1968)	—	—	2029	1:1·97

Table 3. *Chromosome counts from MII preparations from Ta/+ mice.*

	Chromosome number n =					
	17	18	19	20	21	Total
Young (8–20 weeks)	1	4	13	87	0	105
Old (30–40 weeks)	1	9	11	51	0	72
Total	2	13	24	138	0	177

A break-down of the chromosome counts from the *XX* (Ta/+) females is given in Table 3. This shows a proportion (22·03%) having counts below the expected $n = 20$ number. It is assumed that the vast majority of these hypomodal counts are attributable to artefactual loss of chromosomes during slide preparation.

Chromosome counts from the two genotypically different groups of *XO* mice (Ta/O and +/O) are given in Tables 4*a* and *b* respectively. These showed no significant differences ($\chi^2_{12} = 9{\cdot}94$; $P > 0{\cdot}5$) and the counts for the two genotypes were thus pooled (Table 5). From Table 5 it would appear, at first glance that segregation of the *X* chromosome, to egg or polar body, in *XO* females, is occurring entirely at random; equal numbers of $n = 19$ and $n = 20$ being recorded. From a consideration of the data obtained in *XX* females, showing a 22% level of cell breakage and chromosome loss due to preparative technique, it is by no means justifiable, however, to reach such a straightforward conclusion. If artefactual loss of a single chromosome occurred, it would result in oocytes with 20 chromosomes being spuriously classified as having only 19 thus helping to inflate the $n = 19$ total. At the same time some oocytes with 19 chromosomes would be spuriously classified as having only 18. The net result would be to deplete the number of counts in the

20-chromosome category whilst leaving the number in the 19-chromosome category approximately the same. A correction factor is thus clearly necessary in order to arrive at a true figure for the ratio of nullo-*X* to *X*-bearing ova at ovulation. This has been devised in the following way, taking into account the possibility that each chromosome count has arisen by a two-step process involving firstly, the segregation

Table 4. *Chromosome counts from MII preparations from XO mice*

	Chromosome number n =					
(*a*) Ta/O	17	18	19	20	21	Total
Young (8–20 weeks)	1	13	29	39	0	82
Old (30–40 weeks)	1	11	46	44	1	103
Total	2	24	75	83	1	185
	Chromosome number n =					
(*b*) +/O	17	18	19	20	21	Total
Young (8–20 weeks)	3	11	42	39	0	95
Old (30–40 weeks)	3	11	44	39	2	99
Total	6	22	86	78	2	194

Table 5. *Chromosome counts from MII preparations from Ta/O and +/O mice combined*

	Chromosome number n =					
	17	18	19	20	21	Total
Young (8–20 weeks)	4	24	71	78	0	177
Old (30–40 weeks)	4	22	90	83	3	202
Total	8	46	161	161	3	379

of chromosomes at anaphase I and secondly, possible breakage and loss of a chromosome (or chromosomes) by technical artefact. If it is assumed (1) that all those cells with less than 20 chromosomes in the control group (Ta/+) have arisen through breakage, and (2) that the probability of oocyte breakage is the same in both *XO* and *XX* mice, then, it is possible to estimate the proportion of all spreads from *XO* mice with 19 or less chromosomes which have arisen through breakage as follows:

If p_0 represents the proportion of unbroken cells in the control group (Table 3), p_1 the proportion losing 1 chromsome and $1-p_0-p_1$ the proportion losing more than 1 chromosome, then the following values can be assigned to each group:

$$p_0 = 0{\cdot}7797,$$

$$p_1 = 0{\cdot}1356,$$

$$1-p_0-p_1 = 0{\cdot}0847,$$

$$1-p_0 = 0{\cdot}2203.$$

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For the *XO* oocyte spreads, the number found in the 20-chromosome group ($n = 20$) is made up of the actual number ovulated with 20 chromosomes (prior to breakage) multiplied by the proportion of unbroken spreads. Similarly the number of counts in the 19-chromosome ($n = 19$) group comprises the number of non-broken 19-chromosome-bearing spreads plus the number ovulated with 20

Table 6. *Segregation ratios found by various authors, before and after correction*

	Original findings			Corrected findings		
Author	20	≦ 19	% X-bearing	20	≦ 19	% X-bearing
Evans and Ford (unpublished)	61	59	50·8	—	—	—
Kaufman (1972)	65	40	61·9	76	29	72·4
Luthardt (1976)	52	37	58·4	61	28	68·3
Brook (present study)	164	215	43·3	205	171	54·6

chromosomes which have subsequently lost 1 chromosome. The number with 18 (or less) chromosomes ($n = 18$) is made up of the number ovulated with 20 chromosomes which subsequently lose more than one chromosome plus the number ovulated with 19 losing one or more chromosome subsequently.

This can be expressed algebraically as shown below:

$$n20 = Mp_0$$

$$n19 = Mp_1 + (N - M)p_0$$

$$n18 = M(1 - p_0 - p_1) + (N - M)(1 - p_0)$$

Where N = total number of oocyte preparations scored, and M = the number in the $n = 20$ group prior to breakage.

These equations can be used to estimate M, most conveniently by the modified minimum chi-square method (Kendall and Stuart, 1961).

For the *XO* females the segregation ratio is 205:171 for oocytes with counts of 20 and 19 chromosomes respectively (Table 6). This 1·2:1 ratio does not differ significantly from the 1:1 ratio expected if random segregation is occurring ($\chi_1^2 = 3{\cdot}07 : 0{\cdot}1 > P > 0{\cdot}05$).

To simplify the calculation, the effect of non-disjunction was ignored, as there were only three disomic eggs with counts of $n = 21$. The effect of chromosome gain on the segregation ratio would be in the opposite direction to that of chromosome loss, thus slightly decreasing the 1·2:1 ratio, taking it even closer to a 1:1 ratio.

Table 6 also shows a comparison with data obtained by Kaufman (1972) and Luthardt (1976). These authors did not introduce a correction factor into their results to allow for artefactual breakage. Their data have, however, been subjected to our correction model allowing for their own levels of control breakage. When this is done the data show an even greater bias towards non-random segregation than when the uncorrected figures are considered. The discrepancy between their data and those obtained in the present study will be dealt with in the Discussion.

It is not possible to adjust the data of Evans and Ford (unpublished) to allow for breakage as no control data were given by these authors.

For the stock of mice use in the present study, the ratio of *X*-bearing to nullo-*X* eggs at ovulation (1·2:1) differs from that found at weaning, the ratio of *XX* to *XO* offspring at that time being 1·88:1. Assuming there to be an equal chance of

Table 7. *Genotype of offspring weaned from +/O mothers in terms of litter order*

Litter	No. of mothers	Total	Ta/O	Ta/+	+/Y	$\frac{\text{Ta/+ + +/Y}}{\text{Total}} \times 100$
1	48	186	39	71	76	79·03 ± 2·99
2	45	139	26	50	63	81·29 ± 3·33
3	39	123	25	43	55	79·67 ± 3·61
4	35	110	28	39	43	74·55 ± 3·60
5	28	98	14	44	40	85·71 ± 3·51
6	23	82	21	33	28	74·39 ± 4·84
7	14	42	5	20	17	88·10 ± 5·01

fertilization of *X*-bearing and nullo-*X* eggs, it would thus appear, from the altered ratios, that 36·2% of *XO* mice die between fertilization and weaning. Cattanach (1962) has shown that *XO* offspring have as good a chance of survival between birth and weaning as do *XX* offspring, and it can thus be assumed that the 36·2% death of *XO*'s occurs during gestation.

(iii) *XO segregation and maternal age*

In view of the claim made by Deckers *et al.* (1981), that a greater number of *XO* offspring are born to mothers of advanced age, the cytological data were considered, not only in relation to genotype, but also to maternal age. The data presented in Tables 3 and 4 show no significant differences however, either for Ta/O or +/O mothers, in distribution of chromosome counts in the young group compared with the old. Tables 7 and 8 moreover, show the numbers of offspring of each genotype weaned from +/O and Ta/O mothers respectively, in terms of litter order. χ^2 tests for heterogeneity, between the two sets of breeders showed no change in the relative proportions of offspring with litter order – so the two sets of data can be combined. Regression analysis on the combined data shows there to be no significant change in the proportion of progeny born to older mothers ($t = 0{\cdot}356$: $P > 0{\cdot}1$). This finding, together with the cytological evidence, gives no indication in our stock of a changing pattern of *X*-segregation with age of the mother. This constrasts with claims made by Deckers *et al.* (1981) for an increasing recovery of *XO* progeny with increasing maternal age. (see Discussion).

(iv) *Aneuploidy*

As can be seen from Table 5, three disomic eggs ($n = 21$) were found in the old age group of *XO* females compared with none in young *XO* or in control *XX* females (Table 3). These disomic eggs are assumed to have arisen by non-disjunction

in the X -bearing oocytes, and constitute 3/86 (3·5%) of the total eggs assumed to be X-bearing. Their frequency was not significantly greater, however, than in the other two groups of female (young XO and control XX). If the assumption is made that a similar level of non-disjunction occurs among nullo-X eggs, (the hyperploid ($n = 20$) products however being hidden among the normal X-bearing

Table 8. *Genotype of offspring weaned from Ta/O mothers in terms of litter order*

Litter	No. of mothers	Total	+/O	Ta/+	Ta/Y	$\frac{\text{Ta/+ + + /Y}}{\text{Total}} \times 100$
1	52	186	32	62	92	82·80 ± 2·75
2	41	110	25	43	42	77·27 ± 4·01
3	34	95	21	39	35	77·89 ± 4·25
4	21	72	17	32	23	76·39 ± 4·71
5	15	45	7	20	18	84·44 ± 5·47
6	9	33	9	13	11	72·73 ± 7·73
7	4	13	2	5	6	84·62 ± 9·90

($n = 20$) totals), a projected figure of 7 out of 202 hyperploid counts for old XO mothers would be obtained. This enlarged figure is again not significantly different from the zero level of aneuploidy of young XO and control XX females. It is also expected that for each non-disjunctional event producing a disomic egg, there would be a comparable X-bearing nullisomic ($n = 19$) egg produced. These would be hidden in the naturally occurring nullo-X bearing total. Similarly, non-identifiable double nullisomics ($n = 18$) may be produced by non-disjunction in nullo-X oocytes but these could not be distinguished from oocytes which had lost chromosomes through breakage. If the overall level of aneuploidy were thus derived by doubling again, there would then be 14/202 or a 7% frequency for the aged females and this would be statistically significant ($P < 0·05$). The assumption is made in the above calculation that for every non-disjunctional event, producing a disomic egg, a corresponding event would produce a nullisomic. This, of course, is the conventional view of aneuploidy production by non-disjunction. Recent data of Maudlin & Fraser (1978) indicate, however, that the trisomy might arise in ageing female mice without equivalent monosomy. How this could come about is not stated, but if it were to be true, these calculations would not, of course, be valid.

DISCUSSION

In view of contradictions in the literature concerning the XO mouse, the present sudy was set up in an attempt to answer three basic questions. Firstly, does the segregation of the X-chromosome, during the first meiotic division of the oocyte, occur entirely at random? Secondly, if the X-chromosome is preferentially incorporated into either egg or polar body, does this change with maternal age? Thirdly, does the XO mouse constitute a good model for the pre-menopausal human female in terms of maternally age-related aneuploidy?

The ratio of *XX* to *XO* offspring at weaning (1·88:1) in the present study is considerably lower than the ratios observed by others (Cattanach, 1962; Morris, 1968; Russell, 1976). Similarly, the ratio of ovulated *X*-bearing to nullo-*X* eggs (1·18:1) is lower than has been found in previous cytological studies. In fact, unlike the studies of Kaufman (1972) and Luthardt (1976), the corrected figures in the present study are consistent with a 1:1 segregation ratio, in agreement with Evans and Ford (pers. comm). The large difference between the results of this study, and those of Kaufman (1972) and Luthardt (1976), cannot be easily reconciled. When both previous sets of data are corrected for breakage however, (see Table 6) the proportion of *X*-bearing gametes becomes so high that, to be reconciled with the birth data from our own and other studies, it would be necessary to postulate *preferential survival* of *XO*'s during gestation. This clearly is not the case. The present study indicates a 36·2% loss of *XO* progeny during gestation, and others have shown that there is excess death for *XO* litters during early gestation, as compared with *XX*'s (Morris, 1968 and Russell, 1976). The early loss of OY embryos accounts for part of this but loss of a considerable proportion of *XO*'s prior to day 12 post-conception also seems to occur (Russell, 1976; Luthardt, 1976). As pointed out by Russell (1976), the further from randomness one postulates the segregation of the *X* chromosome to be, the lower need be the prenatal loss of *XO* embryos. To reconcile his findings, Morris (1968) concluded that there was preferential segregation of the *X*-bearing set of chromosomes into the gamete *and* death of some *XO*'s during the early stages of gestation. However, Evans and Ford (pers. comm) on re-analysing Morris's data, subsequently suggested that they could be interpretated as showing a 1:1 segregation ratio, and even an increased production of nullo-*X*, as compared to *X*-bearing gametes.

It would appear from these contradictory results that the cytological studies are unsatisfactory because of the problem of breakage and chromosome loss. Obviously it would be ideal if it were possible to identify the *X*-chromosome in the oocyte and then eggs could be simply scored as *X*-bearing or nullo-*X*. Nevertheless, it seems unlikely that the different results obtained by various authors can be explained on the basis of differing amounts of breakage encountered in each different study. One possibility is that there is a drive mechanism, which is responsible for the excess production of *X*-bearing gametes but which varies in strength from one stock to another. Genetic background may be important. Thus, in the present study, there may be little, if any, preferential loss of the chromosome set lacking an *X* to the polar body, whereas in others—such as those used by Kaufman (1972) and Luthardt (1976) the drive mechanism may be stronger. It would, however, seem unlikely that the amount of death of *XO* progeny during gestation should differ significantly in other stocks from the 36·2% found in the present study.

The second point which arises out of this study concerns the question of whether preferential segregation of the *X* to the egg changes with maternal age. Since the data in the present study give no indication of any such change, they are at variance with those of Deckers *et al.* (1981). Both the birth data and the MII counts found in the present study show no reduction in the transmission of *X*-bearing

gametes with age in *XO* mothers. Regression analysis on *X*-segregation data in successive litters of Deckers *et al.* (1981), however, showed a significant negative slope, indicating change with maternal age. Similar treatment of our data gave no such significant result, with the slope in fact being slightly positive. As a χ^2 test for heterogeneity proved negative, regression analysis of the combined data was performed producing a non-significant – even though slightly negative, slope. This would suggest that the two sets of data are homogenous but the anomalous result of Deckers *et al.* (1981) is due to their small sample size.

Finally, on the question of aneuploidy in ageing *XO* mice, and because of the similarity to the human female, in that fertility ends through depletion of oocytes, Lyon & Hawker (1973) suggested that *XO* mice may pass through a period of irregular oestrous cycles towards the end of their reproductive life, during which time hormonal imbalance may occur thus leading to aneuploidy. Consequently, *XO* mice could provide a useful model for the situation in human premenopausal females, where non-disjunction occurs with a high frequency leading to the birth of abnormal children. It is not known whether *XO* mice pass through a period of irregular cyclicity towards the end of their reproductive life although work now in progress, in this laboratory, will, hopefully, show this to be the case. Studies in other strains of mice that, before cycling ceases, a period of irregular cyclicity occurs (Thung *et al.* 1956; Thung, 1961; Brook and Gosden, unpublished data). Although an increase in disomic oocytes was observed with increasing age in the *XO* mice used in the present study, this alone was not found to be statistically significant. When *X*-bearing nullisomics and non-disjunction in nullo-*X* eggs were considered, this figure did, however, become significant: The usefulness of the *XO* mouse as an appropriate model for human aneuploidy and the maternal age effect is, however, questionable in view of these complications arising out of the estimation of the true aneuploidy frequency.

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