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Bare-patches, a new sex-linked gene in the mouse, associated with a high production of *XO* females

II. Investigations into the nature and mechanism of the *XO* production

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SUMMARY

The high *XO* production in *Bpa*+ mouse stocks appears to be due, not to *Bpa* itself, but to a closely linked factor for high *XO* production (*Fxo*) which may be a gene or a chromosomal aberration. The change of *Bpa Fxo* to *Bpa* non-*Fxo* occurs with a frequency of about 6 %; the reciprocal change from *Bpa Fxo* to *Fxo* non-*Bpa* has not been found. There is some evidence that *Fxo* might involve a structural alteration such as an inversion or a deletion. The *X*-chromosome loss is due to non-disjunction which occurs in about one-third of the oocytes, probably mainly at meiosis I. Frequencies of other chromosomal abnormalities found in the stocks are given.

1. INTRODUCTION

Bare-patches (*Bpa*), a male-lethal, sex-linked gene in the mouse, was found to be associated with a high production of *XO* offspring (Phillips, Hawker & Moseley, 1973). The genotype associated with this high *XO* production has been called *Fxo* for ease of discussion. Numerous questions remained to be answered: (*a*) on the nature of *Fxo*, such as whether the effect is due to *Bpa* itself or to some closely linked factor and whether, if a linked factor is involved, it is in fact a 'normal gene' or some chromosomal rearrangement; (*b*) at what stage in development the *X* chromosome is lost and by what mechanism.

This paper describes some attempts we have made to answer these questions.

2. MATERIALS AND METHODS

(i) *Materials*

Three stocks of *Bpa* were maintained:

(1) *Bpa*+ ♀♀ crossed to (C3H × 101)F_1 ♂♂ at each generation; the presence of *XO* animals was tested for by corneal mitotic preparations (Fredga, 1964) of the wild-type females.

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(2) Some *Bpa* females from (1) were crossed for one generation to (JU × C3H) and (C3H × JU)F_1 ♂♂; that is, males carrying different alleles of the *X*-chromosome controlling element (*Xce*) (Cattanach & Isaacson, 1967), which causes non-random *X*-chromosome activity (Cattanach & Williams, 1972).

(3) For ease of analysis for the presence or absence of *Fxo* and to collect data on the effect of *Fxo* on sex-linkage values, *Bpa* females from (1) were crossed with males carrying the sex-linked genes Tabby (*Ta*) or Blotchy (*Blo*), a different type of male being used for alternate generations. The offspring were classified three times a week until the presence or absence of *Bpa*+ and *XO* types were determined.

Females from these stocks were divided into two classes: A-line, where their parents, at least, had been shown to be *XO*-producing, and D-line, where they and/or their parents had been proved non-*XO* producing.

(ii) *Methods used in the investigation into the nature of* Fxo

(1) Most of the data came directly from the normal breeding stocks described above, in which we were able to establish *Fxo*-free lines and from which we were able to obtain information on the linkage of *Bpa* with other sex-linked genes in the presence and absence of *Fxo*. But, to assess the possibility of obtaining the reciprocal non-*Bpa Fxo* mice, two further groups of matings were set up. (*a*) Wild-type females from A-line matings in stock (1) were tested for *XX* or *XO* by counting mitotic cells from cultures of ear skin. Known *XX* females were then crossed to *TaY* and, if possible, at least 16 female offspring classified for *XO*. (*b*) Wild-type females from A- and D-line matings of stock (2) were crossed to *TaY* and those which produced any *TaO* offspring were themselves scored for *XX* or *XO* by the ear culture method referred to above. Proven *XO* mice were discarded whilst those shown to be *XX* were allowed to continue breeding to assess the percentage of *TaO* offspring produced. Finally, those females who produced no *TaO* amongst 16–20 classified female offspring were killed and their *XX* genotype confirmed by counts of corneal mitotic metaphases (Fredga, 1964). The offspring from all the matings were classified during the first week for *Bpa*+ to eliminate the possibility that any female was a normal overlap for *Bpa*.

(2) To assess whether the presence or absence of *Fxo* affected the percentage of pre- and post-implantation death, A- and D-line *Bpa*+ and wild-type sibs from stocks (1) and (2) were mated to (C3H × 101)F_1 ♂♂; the age of the females varied from $3\frac{1}{2}$ to 6 months. Pregnant females were dissected at about 14 days, corpora lutea were counted and the implants classified. The wild-type A-line females were classified as *XX* or *XO* at the time of dissection by corneal mitotic metaphases.

(iii) *Methods used in the investigations into the mechanism of* X-*chromosome loss*

(1) Oocytes from A- and D-line *Bpa*+ females from all three stocks were isolated from the ovaries by follicular puncture and cultured *in vitro* to diakinesis/metaphase I, at which stage chromosome preparations were made by the air-drying method (Henderson & Edwards, 1968).

(2) Control females (C3H × 101)F_1, A-line *Bpa*+ females and D-line *Bpa*+ females from stocks (1) and (2) were super-ovulated with 5 i.u. PMSG followed after a 48 h interval by 5 i.u. HCG. Oocytes were isolated from the ampullae and activated as described by Kaufman (1973*a*, *b*) and Kaufman & Surani (1974) at 20–21 h after the HCG injection. The parthenogenones formed (Figs. 1, 2) were

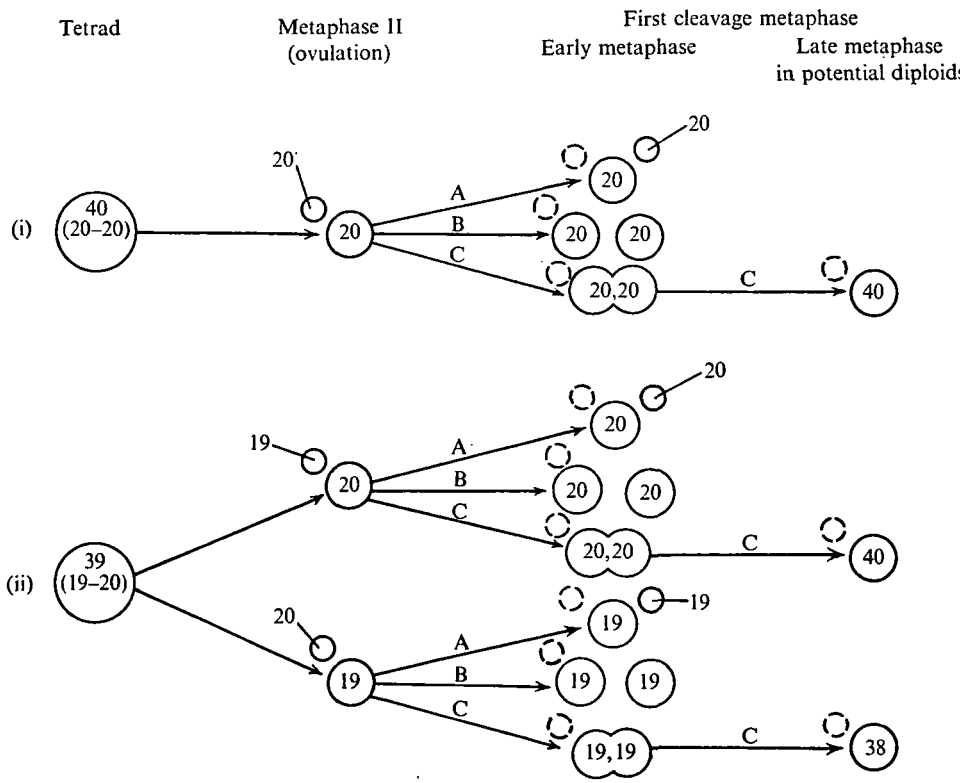

Fig. 1. Diagram showing products of ovulation and activation in (i) normal cell division, (ii) an *XO* region of the ovary. Pathway A: leads to haploid egg with a single haploid pronucleus and extrusion of a non-analysable 2nd polar body. Pathway B: retention of 2nd polar body to give an immediate cleavage, potentially haploid (2-cell) embryo. Pathway C: a potentially diploid egg with two pronuclei. In both (B) and (C) the 2nd polar body contents are analysable unless fusion of the two pronuclei (in the potentially diploid egg) has occurred.

scored at 1st cleavage for abnormal chromosome number. The experiment was coded so that the genotype of the female was not known at the time of scoring.

(3) Finally, the oocytes from six stock (2) females, themselves proven to carry *Fxo* by previous mating, were cultured in low osmolar medium directly after activation to increase the number of eggs in which second polar body suppression had occurred (Kaufman & Surani, 1974), thus allowing the two products of anaphase of meiosis II to be examined (Pathway B, Figs. 1 and 2). In this way non-disjunction at meiosis I can be distinguished from that at meiosis II.

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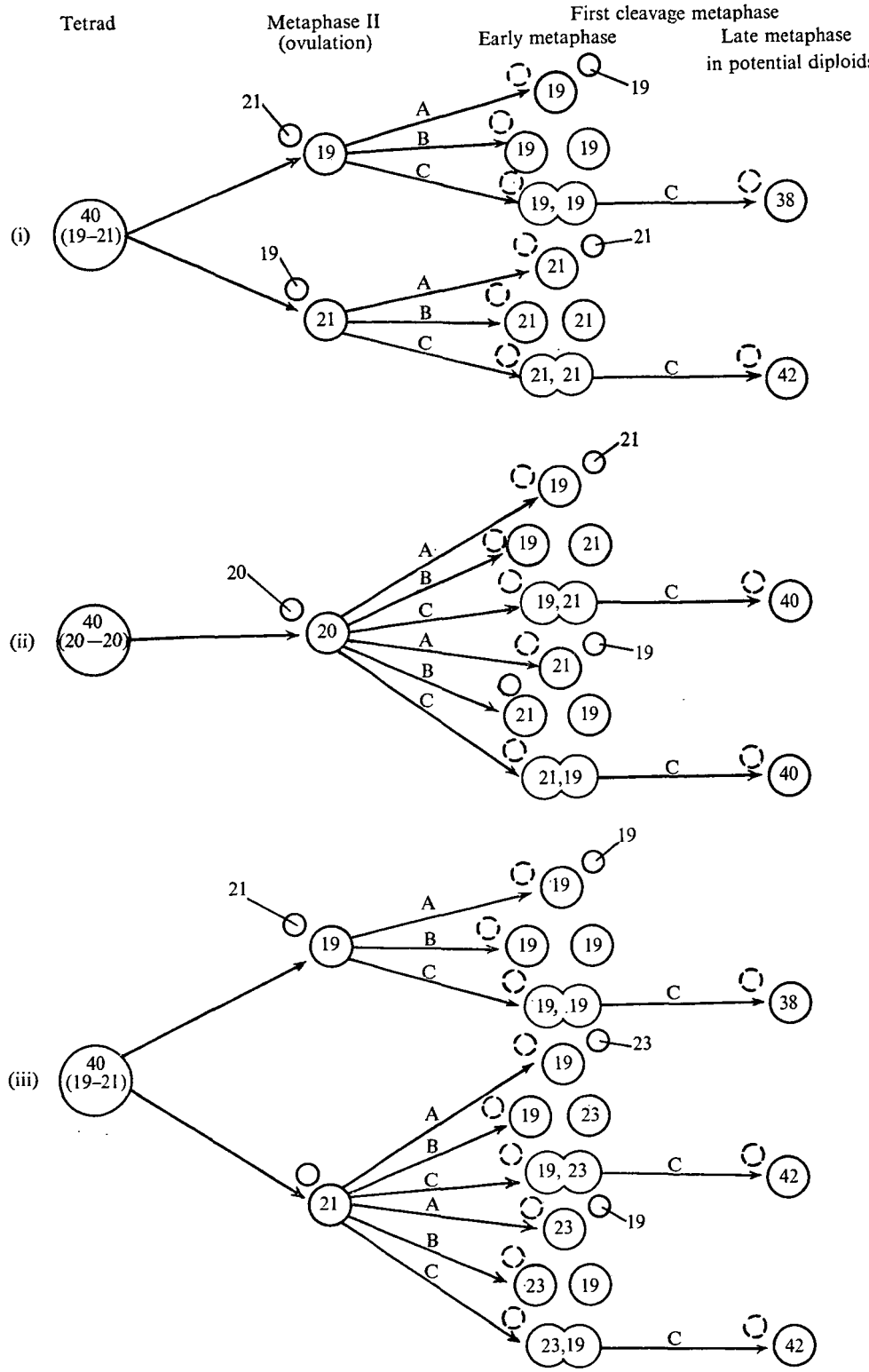
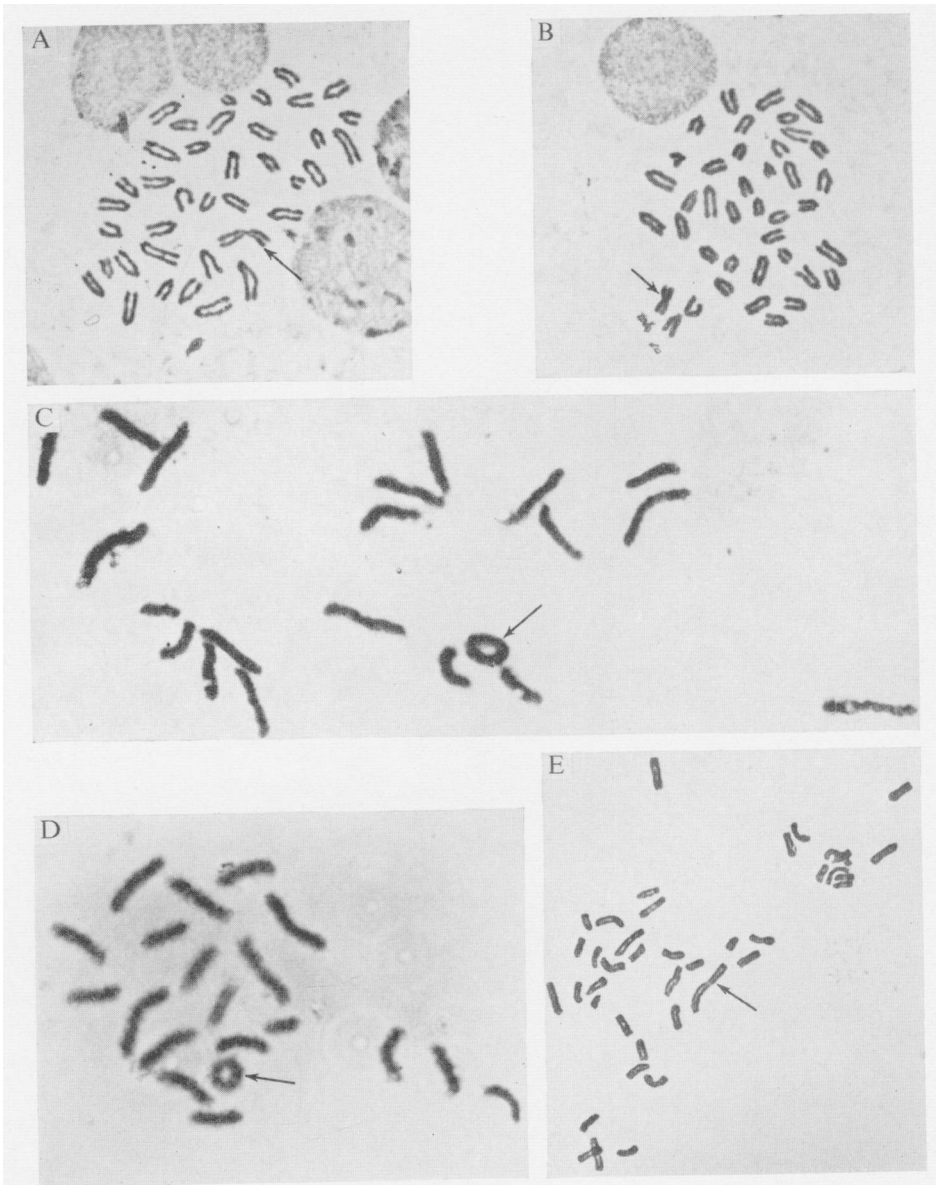


Fig. 2. Diagram showing the products of ovulation and activation from (i) cell with non-disjunction at meiosis I, (ii) cell with non-disjunction at meiosis II, (iii) cell with non-disjunction at meiosis I and II. Pathways A, B and C as in Fig. 1.



Examples of unexpected abnormal cells found during these experiments. (A) Mitotic corneal preparation of 38 chromosomes of which 1 was a metacentric (assumed *XO*). (B) Mitotic corneal preparation of 40 chromosomes of which 1 was metacentric (*XXX*?). (C) Haploid parthenogenone with 21 chromosomes including 1 ring. (D) Haploid parthenogenone with 20 chromosomes including 1 ring. (E) Diploid parthenogenone with 40 chromosomes including a possible metacentric.

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3. RESULTS

(i) *Investigations into the nature of* Fxo

(*a*) *Crossing-over between* Bpa *and* Fxo

Fig. 3 shows in diagrammatic form, the establishment of definite non-*XO* producing lines (D-lines) from known *XO*-producing matings (A-lines) in stock (2), and Table 1 gives data from all three stocks. In at least 5, and possibly 6, of the 91 matings (86 A-line matings + the 5 original D matings) considered in Table 1, the female had lost the *XO* producing factor: also in 7 of the A matings the number of offspring raised was too few to be sure of their classification. Thus the frequency of the conversion of *Bpa Fxo* to *Bpa* non-*Fxo* ranges from 5/91 to 6/84 or 5·6 to 7·1 %.

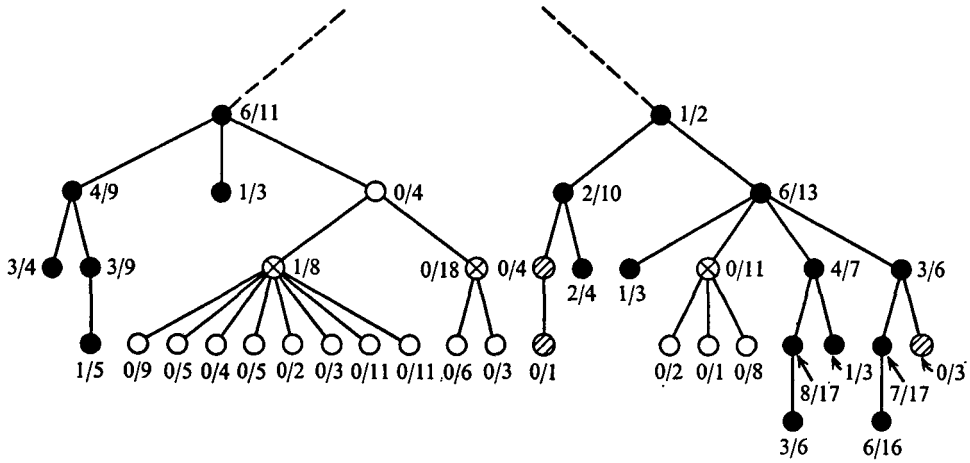

Fig. 3. Diagram showing the ancestry of stock (2). ●, A-lines; ○, D-lines; ⊘, incompletely tested; ⊗, mice from these matings used as A line in haploid 1st cleavage metaphases (group D, Table 4) later proved to be from new crossover or D-lines. Numbers = $XO/(XO+XX)$.

Overall there were five *XO* offspring out of 480 classified in the D-lines (Table 1) giving a value of 1·04 % for spontaneous *XO* production as against an average value of 36 % for the A-lines. This difference is highly significant. Whether the chromosome missing was of maternal or paternal origin was in most cases not identifiable: matings of stock (3) (Table 5), however, have shown that in A-lines it is the maternal *X* that is lost giving OX^P. Information on the frequency of production of *XO* offspring from wild-type known *XX* females from both A- and D-lines was obtained from crosses put up to search for the reciprocal *Fxo* non-*Bpa* chromosome (see below). The data are summarized in Table 2. Overall a frequency of 7/1068 or 0·6 % was found, not significantly different from that obtained for the D-line *Bpa*+ females. During routine scanning of corneal mitotic preparations, 1 out of 327 wild-type stock-2 females was found to carry a metacentric chromosome (Plate 1 A; Table 3): she was the offspring of an A-line mother.

The search for the reciprocal *Fxo* non-*Bpa* chromosome has not so far been

successful. Sixteen wild-type females from stock (1) A-lines were tested by method (*a*) (see Methods (ii) 1), but only 13 of them produced at least the required 16 classifiable female offspring. One female had one X^{MO} out of 36 daughters classified; no other *XO* mice were produced in a total of 330 female offspring (Table 2).

Table 1. *Number of* XO *animals found and the number of non-*Bpa *females tested* (N) *in all stocks from time of establishment of line D1*

Line	Totals *XO*	Totals *N*	No. matings	*XO* (%)
A1*	47	152	18	30·9
A2	119	317	37	37·5
A3	38	96	12	39·6
A4	18	41	6	43·9
A5	25	83	13	31·3
Totals	247	689	86	35·85
Line				
D1	2	147	12	1·4
D2†	2	178	20	1·1
D3†	0	24	2	0
D5†	0	22	4	0
D6†	1	109	15	0·9
	5	480	53	1·04

* Includes another possible D-line.

† D2 arose from A1 in generation 1; D5 from A2, generation 2; D3 and D6 from A5, generations 4 and 5 respectively.

Table 2. *Summary of data on the background* (*i.e. not due to* Fxo) *frequency of* XO *mice in these stocks*

Genotype of ♀ parent	Line	Stock	X^{MO}	OX^{P}	Total *XO*	Total ♀♀ classified	% *XO**
+ +	A	1	1	0	1	330	0·30
+ +	A	2	2	2	4	446	0·90
+ +	D	2	1	1	2	322	0·62
Bpa+†	D	2, 3	—	—	5	480	1·04

* Frequency not corrected for fact that only half the losses of maternal *X*-chromosomes are detectable.

† Data from Table 1; X^{MO} and OX^{P} not distinguishable.

This frequency of 1/330 or 0·3 % is within that expected for the background spontaneous level. Russell (1968) reports frequencies for X^{MO} varying from 0·1 to 1·1 % (with 0·51 % in (101 × C3H)F_1 females (Russell & Montgomery, 1966)). In one or two cases, the females were mated to (C3H × 101)F_1 males instead of *TaY* and their female offspring scanned by corneal mitotic preparations; in one of these a youngster was found with 40 chromosomes one of which was a metacentric (Plate 1B; Table 3). A further 41 A-line females, together with 21 D-line females

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for comparison, from stock (2) were tested by method (*b*). Of the 41 A-line females, 13 were proved *XO* and 28 *XX*, but six of the 28 failed to produce the required 16 young leaving 22 reasonably tested females, who produced two OX^P and two X^MO offspring out of 446 classified (0·9%) (Table 2). The four *XO* mice were all from different matings in the ratios 1/20, 1/29, 1/26 and 1/20 respectively. The wild-type D-line females produced one OX^P and one X^MO out of 322 offspring classified or 0·6% in good agreement with the value found for the wild-type A-line females (Table 2). The frequency of OX^P is much higher than the < 0·02% reported by Russell (1961) but, on the other hand, Léonard & Schröder (1968),

Table 3. *Frequency of chromosomal abnormalities other than* XO

Nature of abnormality	No. abnormal	Total tested	Abnormal (%)
X^MX^PY	1	1141	0·09
♀♀ heterozygous for a metacentric*	2	915	0·22
Parthenogenones containing a ring chromosome	2	228 haploid 13 diploid	0·8

* In addition, one diploid parthenogenone from a known *Fxo* female (Table 7, group E, and Plate 1E) may have included a metacentric chromosome.

using C3H females, found a spontaneous value of 0·13% for OX^P and 0% for X^MO in 1508 animals tested. It therefore seems certain that out of the 35 known *XX* wild-type A-line females from known *XO* producing matings, none has proved to carry *Fxo*. Using the method of Carter (1951) so that incompletely tested females (i.e. those which raised less than 16 young) are also included and taking a figure of 9/25 (36%) for the expected *XO* offspring, the number of females fully tested can be taken as $(1-(16/25)^n)$, where n is the number of offspring raised by each female. The equivalent-tested for the 16 females tested by method (*a*) and 28 females tested by method (*b*) totalled 41·65, as compared with 35 if incompletely tested females are excluded. Thus the observed incidence of *Fxo* non-*Bpa* is 0/41·65 and the upper fiducial limit at the 5% level is 9%, not significantly different from the 5·6–7·1% crossover value found for *Bpa Fxo* to *Bpa* non-*Fxo*.

(*b*) *Dissections of pregnant females*

The results of dissection of pregnant females from A- and D-lines are presented in Table 4. Both lines show a high level of prenatal death but the values for the D-line are about 7–8% lower, opening up the possibility that *Fxo* itself is causing some death; on the other hand, the D-line data are from various sources and are very heterogeneous.

(*c*) *Linkage tests*

The results from crosses of A- and D-line *Bpa*+ females on to various sex-linked stocks (stock 3) are presented in Table 5. Here there is a statistically significant

Table 4. *Dissection of pregnant females* (*a*) Bpa + ♀♀ *and* (*b*) *proven* XX *sibs*

(The females were opened at 13–15 days gestation.)

Source	Type and no. of ♀♀	Corpora lutea (CL)	Classification of implants (I)			Death (%)		
			Live (LE)	Dead	Moles	Pre-I* $1-\left[\frac{\text{I/CL }(a)}{\text{I/CL }(b)}\right]$	Post-I* $1-\left[\frac{\text{LE/I }(a)}{\text{LE/I }(b)}\right]$	Total $1-\left[\frac{\text{LE/CL }(a)}{\text{LE/CL }(b)}\right]$
Line A								
Stock (1)†	(*a*) 20	185	60	1	62	11·5	41·8	48·5
	(*b*) 19	205	129	3	22			
Stock (2)	(*a*) 8	80	30	1	19	11·5	37·1	44·4
	(*b*) 9	92	62	0	3			
Totals	(*a*) 28	265	90	2	81	11·47	40·35	47·19
	(*b*) 28	297	191	3	25			
Line D								
Stock (1)†	(*a*) 12	98	49	4	26	(−5·27)	22·5	18·4
	(*b*) 11	111	68	2	15			
Stock (2)	(*a*) 14	144	52	0	51	12·38	45·05	51·85
	(*b*) 17	196	147	3	7			
Crossovers (A ⟶ D)	(*a*) 8	82	38	1	14	14·9	19·78	31·72
	(*b*) 20	221	150	0	21			
Totals	(*a*) 34	324	139	5	91	7·54	32·87	37·94
	(*b*) 48	528	365	5	43			

* Pre-I, pre-implantation death; Post-I, post-implantation death.

† Includes data from Phillips, Hawker & Moseley (1973).

difference between the crosses of the two lines. In crosses between *Bpa* and *Ta* in the presence of *Fxo*, 0·97 % crossover has been seen amongst the male offspring (where classification is unequivocal), as opposed to 8·75 % in its absence. Again in the *Bpa*, *Blo* crosses, more crossing-over has occurred in the absence of *Fxo*. Tests are also in progress with *Gs*, *spf* and *Hq*.

Table 5. *Linkage of* Bpa *with* Ta *and* Blo *in the presence (line A) or absence (line D) of* Fxo

	Cross 1			Cross 2	
Offspring	A	D		A	D
Bpa Ta/+*Blo* and *Bpa*+/+*Blo*	51	42	*Bpa Blo*/+*Ta* and *Bpa*+/+*Blo*	48	28
Ta+/+*Blo*	101	72	*Blo*+/+*Ta*	76	36
++/+*Blo*	1	3	++/+*Ta*	0	6
BloO	51	0	*TaO*	33	0
TaY	102	73	*BloY*	47	31
+*Y*	1	7	+*Y*	3	9
Linkage value (using ♂ data only) %	0·97	8·75		6·00	22·50
Standard error	± 1·0	± 3·2		± 6·9	± 6·6
S.E. of difference between estimates (%)*	3·3			7·4	

* Standard error for the difference between two estimates, a and b, of a recombination value $= \sqrt{[(SE_a)^2 + (SE_b)^2]}$. Twice this value is then compared with the difference between the two estimates.

(ii) *Investigation into the stage at which the* X*-chromosome is lost and evidence as to what mechanism is involved*

(1) Diakinesis/metaphase I preparations were examined from A-line females. Sixty-three scorable cells were examined, all of which appeared to consist of normal configurations of 20 tetrads.

One of the females had oocytes in the dilated ampullar region, and these were also examined. One metaphase II preparation had 21 chromosomes.

(2) Results from the haploid first cleavage metaphase analyses of activated oocytes (Figs. 1 and 2, Pathway A) are presented in Table 6. A low but definite incidence of non-disjunction was observed in the (C3H × 101)F_1 stock (group A, Table 6) and a significantly higher incidence in the A-line *Bpa*+ females (group B, Table 6, $\chi^2_1 = 15{\cdot}48$, $P < 0{\cdot}001$); the latter females tended to be older (3 to nearly 6 months) than the control females ($1\frac{1}{2}$–4 months) but there was no indication of a correlation between age and the distribution of abnormalities in either group. A ring chromosome was observed in two metaphases, one with 21 chromosomes and the other with 20 chromosomes (not included as an abnormal cell in the calculations) (Plate 1C, D; Table 3).

D-line females (group C, Table 6) showed a similar frequency of abnormal cells

to the control (C3H × 101)F_1 animals. Five other females (group D, Table 6) from their ancestry included as A-line females, also appeared to behave as D-line; their classification as non-*Fxo* was later confirmed by breeding data (Fig. 3). Although there is significant heterogeneity within this crossover group (group D, Table 6, last column), there is no heterogeneity between groups when the three controls, A, C and D are added ($\chi^2_{(2)} = 0{\cdot}663$, $P > 0{\cdot}7$).

Table 6. *Classes of oocytes ovulated by* Bpa+ *and* (*C3H* × *101*)F_1 ♀♀

Group	Nos. and type of female	Chromosome complement of metaphases: 18	19	20	21	22	23	Abnormal groups (%)	Heterogeneity between ♀♀ within groups: D.F.*	χ^2	P
A	10 (C3H × 101)F_1	—	8	159	5	—	—	7·56	9	13·2	> 0·1
B	10 *Bpa*+ A-line†	1‡	5	39§	9§	—	—	27·78	9	19·5	< 0·05
C	10 *Bpa*+ D-line	—	3	94	2	—	—	5·05	8	9·9	> 0·2
D	5 *Bpa*+ crossovers from A-line to D-line (see Fig. 3 and text)	—	1	46	2	—	—	6·12	4	14·2	< 0·01
E	6 *Bpa*+ proved to carry *Fxo*	—	6	13	6	—	1	48·00	Data from all 6 lumped		

* Degrees of freedom.
† Crossover of 1 or 2 ♀♀ to *Fxo* cannot be excluded.
‡ Oocyte not included in calculations.
§ A ring chromosome present in one group of each (the cell with 20 chromosomes not included as abnormal in calculations).

Some of the D-line females had their oocytes activated and induced to develop as immediate cleavage embryos (haploids) or as diploid parthenogenones (Pathways B and C, Figs. 1 and 2). One example at late prometaphase had one pronuclear group with 21 and a second group with 19 chromosomes. This results from non-disjunction at meiosis II.

(3) The oocytes from the six females, previously tested to confirm *XO* production, were also induced to develop as diploid parthenogenones. In fact 26 cells were haploid having already formed the polar body, and are included in Table 6 (group E). One of these cells contained 23 chromosomes which could indicate non-disjunction at metaphase I and II (see Fig. 2(iii)). Of those cells in which diploidy was induced, four oocytes were at late metaphase of 1st cleavage where the 2 pronuclear contents had already fused, giving 3 cells with 40 (either 20+20 or 19+21) and 1 cell of 42 (21+21). The latter implies non-disjunction at meiotic metaphase I (Fig. 2(i)) or possibly 23+19 indicating non-disjunction at I and II, see Fig. 2(iii). Only eight oocytes were of the immediate cleavage class, where the haploid metaphase from each blastomere could be analysed; six consisted of 20, 20, one of 19, 21 and one of 21, 21. Therefore non-disjunction had occurred at meiotic metaphase II and I respectively. No 19, 19 groups were observed but one anomalous cell occurred with 19, 20 groups, and, also from a known *Fxo* female

(Table 6 E), another diploid cell with 40 chromosomes one of which was much longer than normal (Plate 1 E). The abnormally long chromosome had a slightly paler central spot and may have been a metacentric or might possibly be the product of unequal crossing-over.

The data on the frequency of production of chromosomal abnormalities, other than the *X*-chromosome loss caused by *Fxo*, are listed in Tables 2 and 3.

4. DISCUSSION

Four possible mechanisms were considered to account for the observed high frequency of *XO* offspring.

(1) That the maternal environment of *Bpa* + mothers was such that the paternal *X*-chromosome was lost during early cleavage. This has been excluded by crosses of *Bpa* + with other sex-linked genes. These have shown that it is the maternal *X* that is lost (Table 5 and tables 3 and 4 of Phillips, Hawker & Moseley, 1973).

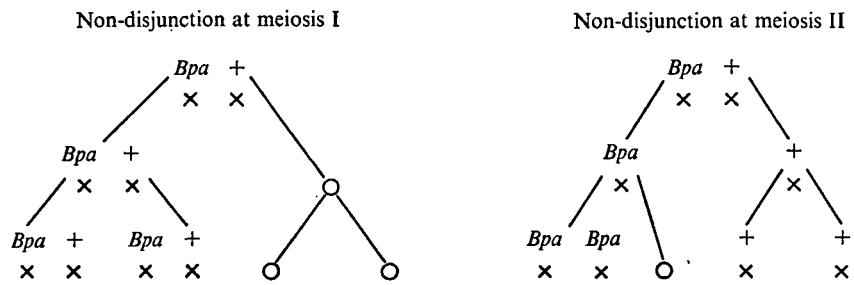

Fig. 4. Diagram showing the expected products of non-disjunction at meiosis I and II in *Bpa* + oocytes.

(2) That the *XX* and *XO* stem lines developed in the ovaries of these mice, so that the germ line was a mosaic and resulted in oocytes being ovulated with 19 or with 20 chromosomes (Fig. 1 (ii)). This was excluded by the results of the diakinesis/metaphase I preparations all of which showed the normal complement of 20 pairs of chromosomes (tetrads).

(3) That the *Bpa* chromosome was lost in (*a*) meiosis: this originally seemed the most likely explanation since *XXX* and *XXY* types were not seen (but see discussion under (4) below). Otherwise only one cell suggested chromosome loss: this cell, one of the potential diploid parthenogenones where both haploid metaphases could be analysed, consisted of one group of 19 and one of 20 chromosomes (as mentioned at end of Results section). (*b*) Cleavage stages: this, in the absence of selection for one cell type, would be expected to lead to mosaicism. No definite evidence for this was found except for one wild-type female, offspring of an A-line *Bpa* + mouse, 10% of whose corneal cells showed a count of 40 and 90% of 39. Thus a low frequency of chromosome loss both at meiosis and in the early cleavage stages cannot be excluded.

(4) That non-disjunction of the *X*-chromosome occurs during meiosis. Non-disjunction should lead to both *XXX* and *XXY* as well as *XO*, but despite the lack of evidence for these two types in the breeding data (see also Phillips *et al.* 1973), the cytological investigations have demonstrated that oocytes from A-line *Bpa+* females do undergo significantly more non-disjunction than control mice (Table 6) and thus this is the main mechanism involved in the *X*-chromosome loss. Therefore, possible reasons for the absence of *XXX* and *XXY* must be considered.

Table 7. *Segregation from A- and D-line matings in Table 1 (including only litters where all wild-type classified for* XX/XO)

(Figure in parentheses = % of total in that class.)

Source	*Bpa+*	Wild type *XX*	Wild type *XO*	*+Y*	*N*
A-line stocks (1) and (3)	89	148	76	147	460
A-line stocks (2) and (3)	69	90	57	111	327
Total	158 (20·0)	238 (30·2)	133 (16·9)	258 (32·8)	786 (100)
D-line stocks (1) and (3)	154	296	3	317	770
D-line stocks (2) and (3)	114	187	2	197	500
Total	268 (21·1)	483 (38·0)	5 (0·39)	514 (40·5)	1270 (100)

If non-disjunction were occurring in *Bpa* oocytes at meiosis II, the *XXX* and *XXY* classes would be of the constitution *BpaBpa+* and *BpaBpaY* (Fig. 4). As all *BpaY* die prenatally (Phillips *et al.* 1973) the *BpaBpaY* would obviously also do so and as 40–50 % of *Bpa+* also appear to die (Table 7) it would not be surprising if *BpaBpa+* were lethal as well. If, on the other hand, non-disjunction occurred at meiosis I, *XXX* and *XXY* would be of the constitution *Bpa++* and *Bpa+Y* and there appears to be no *a priori* reason why these should die.

Evidence from the oocytes induced to develop as diploid parthenogenones is not conclusive. Only one abnormal oocyte in which both products of anaphase of meiosis II could be examined was available from the control females; this showed non-disjunction at meiosis II (Results section (ii) 2). Therefore non-disjunction is occurring at meiosis II in the controls. From the known *Fxo* females (Results section (ii) 3) four oocytes gave information; one of 42 chromosomes and one of 21, 21 chromosomes indicated non-disjunction at meiosis I; one of 19, 21 was the result of non-disjunction at meiosis II and the haploid group of 23 (Table 6) indicated non-disjunction at both meiosis I and II. Therefore either *Fxo* is inducing non-disjunction at meiosis I and II or it acts only at meiosis I. Evidence from the breeding data tends to support the latter hypothesis.

The expectations from *Bpa+* crossed to *+Y* in the D-lines are:

$$1/4\ v\ Bpa+ : 1/4\ ++ : 1/4\ BpaY : 1/4\ +Y,$$

where v = the viability of *Bpa+*. This viability is approximately 60 % (Table 7) and the expected live-born births are therefore:

$$3/20\ Bpa+ : 1/4\ ++ : 1/4\ +Y,$$

with a total of 13/20th live and therefore 7/20th or 35·0% dead. The frequency of prenatal death (Table 4) is in good agreement with this.

In the A-lines the expectations in the various classes are complicated by the contribution from that proportion of oocytes which undergoes non-disjunction. If this proportion is taken as p then the live-born expectations from normally dividing cells become:

$$1/4\ v\ (1-p)\ Bpa+ : 1/4\ (1-p)\ + + : 1/4\ (1-p)\ + Y,$$

the products of non-disjunction at meiosis I (Fig. 4):

$$1/4\ p\ XXX : 1/4\ p\ XXY : 1/4\ p\ XO : 1/4\ p\ OY,$$

and the products of non-disjunction at meiosis II (Fig. 4):

$$1/8\ p\ XXX : 1/8\ p\ XXY : 1/8\ p\ OX : 1/8\ p\ OY : 1/4\ p\ XX : 1/4\ p\ XY,$$

assuming that non-disjunction only occurs in the *Bpa* or *Fxo* carrying oocyte.

Table 8. *The percentage live-born young and total death expected* following non-disjunction at meiosis (M) I or II compared with the observed frequencies*

	Offspring (%)				Total death (%)
	Bpa+	*++*	*+O*	*+Y*	
A-lines					
Non-disjunction M I	19·4	32·3	15·9	32·3	48·2
Non-disjunction M II	15·7	38·9	6·4	38·9	35·8
Observed†	20·0	30·2	16·9	32·8	47·19
D-lines					
Non-disjunction M I	22·6	37·7	2·0	37·7	37·0
Non-disjunction M II	22·0	38·5	0·96	38·5	35·1
Observed†	21·10	38·03	0·39	40·47	37·9

* Expected where the viability of *Bpa+* = 60% and the proportion of *Bpa*-carrying oocytes undergoing non-disjunction = 0·33 (groups B and E, Table 4) for A-lines and 0·05 (groups C and D, Table 4) for D-lines.

† Data from stock (i), Tables 4 and 7.

The value of p observed in activated oocytes from A-line females (Table 6 B, E) averaged 33·3% and this figure was used to solve the expectations above, from which were then calculated the percentage expected live-born in each class assuming that the XXX, XXY and OY genotypes are lethal. The results are given in Table 8, together with the observed percentages obtained from Tables 4 and 7. From this table it is obvious that the expectations from meiosis I non-disjunction fit the data more closely than those from meiosis II. The more obvious explanation for the lack of the XXX and XXY types (death of *BpaBpaX* and *BpaBpaY*) does not seem to be valid. Whether X^MX^MX and X^MX^MY are lethal or are eliminated in most cases by cell selection after fertilization is not known: nor is it known whether this lack is due to some property of *Fxo* or is general for the mouse; X^MX^MY definitely seems to be viable in man (Edwards, 1971). There is some

evidence that *Fxo* might be a structural alteration rather than a straightforward gene (see below) and this may contribute to the elimination of these multiple types.

The investigation into the nature of *Fxo* has shown that it can be separated from *Bpa* but the non-*Bpa Fxo* crossover type has not been identified. This may be chance or may indicate some interaction so that *Fxo* only expresses itself in the presence of *Bpa*.

The finding of significantly less crossing-over between *Bpa* and *Ta* and between *Bpa* and *Blo* in the presence of *Fxo* (Table 5), together with a possible increase in prenatal death (Table 4), suggests that *Fxo* might be a structural alteration. A number of such cases of crossover-suppression have been reported for the mouse, for instance with sex-linked translocations and tobacco mouse translocations (Cattanach, 1966; Cattanach & Moseley, 1973; Lyon & Newport, 1973), with inversions (Roderick & Hawes, 1970) and with a presumed deletion (Wallace, 1972). No evidence of a translocation has been seen cytologically in 63 meiotic metaphases from A-line females but this is not conclusive as some translocations may have as low as 8% abnormal configurations (Ford *et al.* 1969). It is not possible to distinguish between an inversion and a deletion as a cause of crossover-suppression on the present evidence; all that can be said is that no obvious abnormalities were visible in meiotic preparations. On the other hand the abnormally long chromosome in a diploid A-line cell (see Results (ii) 3 and Plate 1E) and the two-ring chromosomes found in A-line parthenogenones (Tables 3, 6; Plate 1C, D) may be connected with *Fxo*. No ring chromosomes were found in 172 $(C3H \times 101)F_1$ controls nor in approximately 1000 haploid first cleavage stages from $(C57Bl \times A_2G)F_1$ ♀♀ (M .H. Kaufman, unpublished). The presence of some structural alteration as an explanation of the phenomenon seems more likely than that two point-mutations occurred so close together in one animal (see Phillips *et al.* 1973). Obviously much more information is needed before the whole story is elucidated.

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