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New evidence of unbalanced sex-chromosome constitutions in the mouse

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INTRODUCTION

Russell, Russell & Gower (1959) and Welshons & Russell (1959) have shown that mice of the X/O sex-chromosome constitution can arise through failure to receive any sex chromosome (either an X or a Y) from the father. The X/O animals are fertile females. Assuming that the failure is due to the occurrence of non-disjunction during male gametogenesis, they estimate that the resulting O-sperms (lacking both X and Y chromosomes) must constitute about 0·6% of all sperms.

Welshons & Russell point out, however, that their extensive genetic tests failed to yield evidence for two predictions which can be made on the basis of these conclusions: (1) non-disjunction should also occur in the female, leading to the production of X/O females through fertilization of an egg lacking an X chromosome by an X-bearing sperm, and (2) X/X/Y animals should occur through fertilization of the egg by an XY sperm (the other possible product of male non-disjunction).

The above-mentioned work led me to re-examine data obtained in the course of investigating anomalous transmission of sex-linked factors in mice. As a result, not only was confirmation obtained of the phenomenon described by Russell, Russell & Gower and by Welshons & Russell, but also evidence came to light of the existence of the two missing categories listed above. In each case one example only was found, but, as will be seen, the rarity of the phenomena involved would make the collection of additional instances extremely arduous.

MATERIAL

Two stocks of mice were used. In the ancestry of stock A, the C57BL and C3H inbred strains, a multiple recessive stock, and a stock obtained from Prof. H. Grüneberg containing the sex-linked marker *Bent-tail* (*Bn*) (Garber, 1952) were represented in varying proportions, with C57BL predominating. Stock B was extracted from crosses between Stock A and a stock obtained from Dr D. S. Falconer containing the sex-linked marker *Tabby* (*Ta*) (Falconer, 1953).

RESULTS

1. *X/O females resulting from non-disjunction in the male parent*

One method of searching for females of the above type is to examine the daughters of a male carrying a totally sex-linked gene. Table 1 shows the offspring

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of hemizygous *Bent-tail* males. Any daughter not carrying *Bn* is presumptively X/O. Unfortunately, with this marker the diagnosis cannot be made on phenotypic appearance alone since *Bn* is not fully penetrant in heterozygous females. Samples

Table 1. *Stock A matings to detect non-transmitters among the daughters of* Bent-tail *males. A female was classified as a non-transmitter if she gave no phenotypically* Bent-tail *mice out of more than twenty fully classified offspring. This criterion corresponded to a P value of less than 0·001*

Type of cross	No. of daughters	No. of wild-type daughters (non-manifesters plus non-transmitters)	No. tested	No. not transmitting *Bn*
Wild-type ♀♀ × *Bent-tail* ♂♂	373	58	36	1
Bent-tail ♀♀ × *Bent-tail* ♂♂	191	53	13	1

of phenotypically wild-type daughters were therefore test-mated in order to identify non-transmitters of the *Bn* gene.

It will be seen that 2 non-transmitters were identified out of 49 tested. The first gave 21 offspring, all wild-type, 9 males, 12 females, and the second gave 41 offspring, all wild-type, 15 males, 26 females.

An X^+/O female mated to a normal X^+/Y male is expected to produce four classes of young. Of these classes, Russell, Russell & Gower (1959) brought forward evidence to show that the Y/O type is lethal (half of all males die before birth), and the X/O type of female is inferior in viability to the X/X type (in their material only 60% as viable). The resulting sex-ratio among the young should be about 1·6 : 1. The sex-ratio among the offspring of the two non-transmitters (38 females, 24 males) agrees excellently with this expectation, but does not differ significantly from a 1 : 1 ratio.

Two further series of crosses, using stock B, were such as to reveal X/O females of similar origin, but failed to find any. In one, 746 daughters of *Tabby Bent-tail* males were raised, but all were phenotypically *Tabby* and hence must have received an X chromosome from their father. In the other series, *Tabby* females were mated to non-*Tabby* males. Any *Tabby* daughters failing to receive an X chromosome from their father should show hemizygous manifestation of the *Tabby* condition as demonstrated by Welshons & Russell (1959). Of 216 *Tabby* daughters, none did so.

The proportion of females which are X/O was calculated by Welshons & Russell (1959) to be approximately 1·2% in their material. For the present data, the upper line of Table 1 yields an estimate (allowing for the fact that only 36/58 normal daughters were test-mated) of 0·4% ($=\frac{1}{373}\times\frac{58}{36}$). The data in the lower line of Table 1 unfortunately cannot be used to improve the precision of this estimate, because the three frequency classes which it includes (*Bent-tail* females, non-*Bent-tail* transmitters, and non-*Bent-tail* non-transmitters) yield only two degrees of

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freedom for purposes of estimation, and this is insufficient for estimating three unknown quantities, in this case (1) the viability of *Bn* homozygotes, (2) the penetrance in *Bn* heterozygotes, and (3) the frequency of O sperm (i.e. of sperm lacking both X and Y chromosome). The failure to find any X/O females of similar origin in the stock B crosses, out of a total of 962 relevant females, suggests that the estimate of 0·4% derived above may be too high.

2. *X/O female resulting from non-disjunction in the female parent*

Of the 746 daughters of *Tabby Bent-tail* fathers, all except one showed the typical female heterozygous manifestation of *Tabby*. The one exception, however, showed the more extreme phenotype characteristic of the hemizygous *Tabby* male or the homozygous female (there were no hairs, for instance, on the tail). The mother was an F_1 hybrid between two C3H substrains, and was of normal agouti phenotype. The exceptional animal had also inherited *Bn* from the father. Its tail was strongly but not acutely kinked, to a degree consistent with either heterozygous or hemizygous manifestation (although male hemizygous manifestation of *Bn* is much more marked than that of female heterozygotes, the two classes overlap). It appeared to have normal female external genitalia, with patent vagina, but died at the age of 10 weeks without having produced any young. This is not surprising in view of the low viability and fertility of *Tabby Bent-tail* males which it resembled in outward appearance.

The presumption is that the animal was an X/O female. Welshons & Russell (1959) report a total of six females with hemizygous *Tabby* manifestation which proved on analysis to be of X/O constitution. All six obtained their single X chromosome from the mother. The present female, on the other hand, must have obtained its X chromosome from the father, hence non-disjunction must have occurred in the *maternal* parent, leading to the production of an egg-cell with no X chromosome. No such patroclinous X/O female was observed by Welshons & Russell among 275 female offspring of appropriate crosses.

It could be argued that the anomalous female was an exceptional heterozygote showing full homozygous expression. Falconer (1953), in discussing variability of expression in *Tabby* heterozygotes, mentions that out of several hundred presumed heterozygous females, one showed the full phenotype characteristic of homozygotes. Since it failed to breed, its genotype could not be determined. Since then, two other similar cases have been observed (Falconer, personal communication), one involving *Brindled* (*Br*) and the other *jimpy* (*jp*). For these genes, the difference between heterozygous and hemizygous expression is so marked that the anomalous females cannot reasonably be regarded as being heterozygotes with extreme manifestation. In the light of this evidence, and also that of Welshons & Russell, it now seems more likely that Falconer's anomalous *Tabby* female was also an X/O hemizygote rather than a heterozygote with extreme manifestation. If so, it represents an additional case of non-disjunction in the maternal parent, since it is clear from Falconer's Table 1 (Falconer, 1953) that the

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animal must have obtained its X chromosome from the father. In the appropriate cross, 1 out of a total of 48 *Tabby* females showed hemizygous expression.

On the other hand, the *Brindled* and *jimpy* X/O females must have obtained their sex-linked markers from the mother, since both genes are lethal in males; and must therefore have resulted from non-disjunction in the male parent, resembling in this respect the *Tabby* X/O females of Welshons & Russell.

3. *X/X/Y male resulting from non-disjunction in the male parent*

Non-disjunction in the male should result in the production of XY sperms as well as sperms lacking both X and Y. It is detectable by the transmission of a totally sex-linked gene from father to son.

Table 2 summarizes the relevant crosses which were made. The single anomalous mouse which appears in the table appeared on external examination to be a

Table 2. *Crosses in which the male parent carried a totally sex-linked gene or genes absent in the female parent. One case of father-to-son transmission was detected. The data given in the first line of the table derive from the same twenty-one matings as those in the first line of Table 1*

			Male progeny	
Stock	Type of cross	No. of matings	Receiving sex-linked gene(s) from father	Total
A	Wild-type ♀♀ × *Bent-tail* ♂♂	21	0	399
B	*Tabby* ♀♀ × *Bent-tail* ♂♂	28	0	516
B	Wild-type ♀♀ × *Tabby Bent-tail* ♂♂	42	1	747
	Total	91	1	1662

male, but the manifestation of both *Ta* and *Bn* was of the heterozygous female type. For *Bn* the distinction between hemizygous and heterozygous expression is not absolute, since, as explained above, the hemizygous and heterozygous conditions constitute a continuous though bimodal distribution; but with *Ta* the hemizygous expression is extremely constant and distinctive, and no possibility of misclassification arises in the male. The presence of hairs on the tail, for instance, has never been reported in a hemizygous male. The animal was stunted in growth and died at the age of 25 days. Its sex was checked by post-mortem dissection. No macroscopic signs of intersexuality were observed. The mother was a C3H × C57BL F_1 hybrid.

On the hypothesis of non-disjunction we must presume this male to have been X/X/Y in chromosomal constitution, the result of fertilization of a normal egg-cell containing one X chromosome by a sperm bearing both an X and a Y chromosome. Not only is this conclusion indicated by the mode of inheritance of the sex-linked markers, but also by the highly anomalous expression of the *Tabby* gene. The estimate of 1/1662 for the frequency of X/X/Y males obtained from Table 2 is an

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underestimate, since, from what is known of *Bn* manifestation in female heterozygotes, not all the $X/X^{Bn}/Y$ males produced by the first two types of cross would be expected to show the *Bent-tail* phenotype.

DISCUSSION

The anomalous observations described above were of three distinct types. The chief merits of the hypothesis of unbalanced sex-chromosome constitution, arising presumably as a result of non-disjunction, are (1) that it enables them all to be accounted for under a single theory, and (2) that it is rendered *a priori* likely as a result of the work of Welshons & Russell (1959). Their combined genetical and cytological analysis has established that females of X/O chromosome constitution which have received their single X chromosome from the mother may occur with a frequency as high as 1%: if the very considerable number of mice bearing sex-linked markers which was raised in the course of the work described in the present paper had *not* yielded some similar animals, the discrepancy would itself have required explanation. The other two types of unbalanced sex-chromosome constitution implicated by the present work, namely X/X/Y animals, and X/O females which have received their single X chromosome from the father, might both *a priori* be expected to arise as a result of the same type of failure of orderly meiotic distribution of the sex chromosomes as is responsible for the production of X/O females receiving their single X chromosome from the mother. The single animal of each type which came to light can therefore be considered as further confirmatory evidence for the phenomenon uncovered by Russell, Russell & Gower (1959) and Welshons & Russell (1959).

Similar unbalanced sex-chromosome constitutions have recently been reported in the human species. Ford, Jones, Polani, de Almeida & Briggs (1959) report a case of Turner's syndrome (a condition of women, in which the gonads are absent or rudimentary, other congenital malformations are present, and characteristically the female sex chromatin is absent from the skin cells) where on the basis of chromosome counts it was concluded that the sex-chromosome constitution was X/O. In this case there was no way of determining which parent had transmitted the single X chromosome; but in four other cases of chromatin-negative Turner's syndrome, studies of the inheritance of sex-linked recessive colour-blindness suggested that the non-disjunctional event leading to the production of a gamete lacking any sex chromosome occurred in the male parent (for references see Ford, Jones, Polani, de Almeida & Briggs, 1959). Evidence has also been presented (Jacobs & Strong, 1959; Ford, Jones, Miller, Mittwoch, Penrose, Ridler & Shapiro, 1959) that two cases of Klinefelter's syndrome (male-type intersexes, with under-developed testes) showing female sex chromatin in the skin cells were X/X/Y in chromosome constitution. The three cases of colour-blind Klinefelter individuals which have been detected so far (Nowakowski, Lenz & Parada, 1959) apparently arose through fertilization of an XX ovum by a normal Y sperm (Stern, 1959), rather than through fertilization of a normal ovum by an XY

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sperm, as in the presumptive case of an X/X/Y mouse described in the present paper.

A single case has been reported of a human female with forty-seven chromosomes, apparently X/X/X in chromosomal constitution (Jacobs, Baikie, Court Brown, MacGregor, Maclean and Harnden, 1959). In *Drosophila* such 'super females' are often inviable; but in Man only the genital tract seems to be affected. A proportion of the buccal mucosa cells contained two sex-chromatin bodies, rather than the normal female complement of one. The term 'super female' is accurate only in respect of chromatin, since the genitalia were under-developed, and the woman almost certainly sterile.

It is interesting to note that in humans the 'female' sex chromatin appears to be associated with the possession of two or more X chromosomes, since it is present in X/X females and X/X/Y males, present in larger quantity in the X/X/X 'super female', but absent in X/O females and X/Y males. The gross morphological characteristics of maleness and femaleness seem, however, to depend on the presence or absence of the Y chromosome, both in the human species and in the mouse.

Welshons & Russell (1959) discuss whether in the mouse the X/X/Y constitution would be expected to lead to intersexual or to fully male development. The single presumed X/X/Y animal reported in the present paper appeared to be fully male, but it did not survive long enough to enable its fertility to be tested.

The phenotypes now believed to correspond, in the mouse and Man respectively, to the unbalanced sex-chromosome constitutions discussed above are summarized in Table 3; while Table 4 lists the various non-disjunctional mechanisms by which these constitutions may arise, assuming non-disjunction in one parent only.

Table 3. *Phenotypes corresponding to anomalous sex-chromosome constitutions*

	Species	Phenotype	Reference
X/O	Mouse	Fertile female	Russell, Russell & Gower (1959); Welshons & Russell (1959); Present paper
	Man	Sterile female; Chromatin negative ('male'); (Turner's syndrome)	Ford, Jones, Polani, de Almeida & Briggs (1959)
X/X/X	Mouse	Not known	—
	Man	Female, genital tract underdeveloped; Chromatin positive, some cells with two chromatin bodies ('super female')	Jacobs, Baikie *et al.* (1959)
Y/O	Mouse	Probably inviable	Russell, Russell & Gower (1959)
	Man	Not known	—
X/X/Y	Mouse	Male, fertility not known	Present paper
	Man	Sterile male; Chromatin positive ('female'); (Klinefelter's syndrome)	Jacobs & Strong (1959)

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If X/X/Y mice are indeed viable males or near-males, why do they apparently occur so rarely compared with X/O females? Welshons & Russell (1959) presume that their eight matroclinous X/O females arose through failure of the paternal XY bivalent to disjoin at meiosis, resulting in sperm which carried neither an X nor a Y chromosome. They do not offer any explanation of their failure to detect the complementary non-disjunctive type, that is the X/X/Y animals which should result from fertilization by sperm carrying both an X and a Y chromosome,

Table 4. *Modes of origin of anomalous sex chromosome constitutions*

(Chromosomal constitutions of gametes produced as a result of non-disjunction are printed in bold type)

Type	Sperm	Egg	Genotype	Species	Reference
Non-disjunction in female parent	X	**O**	X/O	Mouse	Present paper
					Falconer (1953)
				Man	Not reported
		XX	X/X/X	Mouse	Not reported
				Man	Jacobs, Baikie *et al.* (1959)
	Y	**O**	Y/O	Mouse	Russell, Russell & Gower (1959)
				Man	Not reported
		XX	X/X/Y	Mouse	Not reported
				Man	Stern (1959)
Non-disjunction in male parent*	**O**	X	X/O	Mouse	Russell, Russell & Gower (1959)
					Welshons & Russell (1959)
					Present paper
				Man	Ford, Jones, Polani, de Almeida & Briggs (1959)
	XY	X	X/X/Y	Mouse	Present paper
				Man	Not reported

* Non-disjunction during the second meiotic division could in theory lead to the production of XX and YY sperm also, and hence to X/X/X and X/Y/Y animals.

though these might in theory be expected to occur as frequently as the X/O types. However, in *Drosophila*, where non-disjunction of the X chromosomes has been intensively studied, the frequency of XX eggs is about 1 in 2500, while the frequency of no-X eggs is 1 in 600, or more than four times as high. To quote Sturtevant & Beadle (1939): 'This difference is evidently due to the fact that failure of one of the X chromosomes to be included in a daughter nucleus at the first division is a more frequent deviation from normal than is the inclusion of both X's in a single daughter nucleus.' A corresponding asymmetry in the assortment of the paternal X and Y chromosomes in mammals would account for the higher incidence of the X/O than the X/X/Y chromosome constitution.

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SUMMARY

Some anomalous results are described of genetical tests using the sex-linked markers *Tabby* and *Bent-tail*. The results are shown to be consistent with the demonstration by Welshons & Russell (1959) that the X/O chromosome constitution is female, and suggest that the condition can arise from non-disjunction in the female as well as from non-disjunction in the male parent. A single animal of presumed X/X/Y constitution appeared anatomically to be fully male, but died before breeding tests could be performed.

Note added in proof

In writing this paper, it was assumed that the non-disjunctional event leading to the production of unbalanced sex-chromosome constitutions occurred in the parent, during the course of gametogenesis. Genetically identical results would be observed if non-disjunction of the sister chromatids of one of the sex chromosomes took place in the first cleavage division of the zygote, provided that the other aneuploid blastomere either died or failed to contribute to the further development of the relevant parts of the embryo. Ohno, Kaplan & Kinosita (1959) have subsequently reported that cytological examination of 2192 meiosis figures from fifteen male mice belonging to six inbred strains 'failed to reveal a single instance of mishap which might lead to the formation of XY- and O-sperm'. The discrepancy between this result and Welshons & Russell's estimate of 0·6% O-sperm is suggestive evidence that most or all of the non-disjunction occurs at the first cleavage division rather than during gametogenesis. But it is not conclusive, since the frequency of non-disjunction might vary widely between different stocks of mice, between different individuals, or even in the same individual at different ages.

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