A 41, XYY Mouse

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Summary. Cytogenetic analyses of bone marrow and meiotic cells in an apparently normal male mouse clearly revealed the presence of an extra Y chromosome leading to 2n = 41, XYY. During meiosis the sex chromosomes formed all possible types of combinations (XYY, XY/Y, YY/X and X/Y/Y). Compared to 40, XY normal mice, the sperm count was significantly less, but the incidence of sperm head abnormality was at the normal level.

A number of XYY cases have been described in man since the first report in 1961[2], while in the mouse there have been only a few reports[3-7]. To add further information on the XYY condition in mice, the present communication reports on bone marrow and meiotic chromosome analyses and quantitative and qualitative aspects of sperm in a 41, XYY mouse.

This 41, XYY mouse was one of about 1500 Swiss mice *(Mus musculus)* cytologically examined by us since 1966. It was discovered in the course of our investigations of the effects of drugs on mitotic, meiotic and post-meiotic cells, but it belonged to a control line. The fertility and behaviour of the exceptional individual were unfortunately not noted. Its body weight (29.5 g) however, did not differ markedly from that of other individuals of the same age group (28.0 g). The right and left testes weighed 104 mg and 84 mg respectively.

For bone marrow and meiotic preparations generally-used air-drying techniques were followed. Slides of the meiotic preparations were also used for the study of morphological changes of the testicular sperms. Quantitative estimation of epididymal sperm was made haemocytometrically following the method adopted recently by us[8].

In this strain the Y chromosome is as short as, or sometimes shorter than, the chromosomes of the last autosome pair (chromosome 19). The other chromosomes (Nos 1-18) are slightly larger. Thus in the karyotype of normal males the Y is one of the 3 smallest chromosomes. All the 100 metaphases scored at random from bone marrow preparations were found to contain 41 chromosomes, and karyotype analysis (fig. 1) revealed 4 smallest chromosomes instead of the usual 3.

Each of the 100 diakinesis-metaphase-I cells examined at random also contained 1 extra chromosome (E). However, the extra chromosome strongly resembled the Y in shape, size and staining behaviour (figs 2-4). In no case was the extra chromosome found to be associated with any autosomal bivalent. Rather, in most of the cells it showed a close association with X and/or Y. The incidences of different types of association are given in the table.

In this particular mouse the total count of the epididymal sperm population (mean of left and right) was found to be 6.33×10^6 while the corresponding value for normal males of the same age group was 10.07×10^6. A qualitative analysis of sperm heads, however, did not reveal any remarkable change in the incidence of abnormality in the XYY male compared with that of normal males.

The cytogenetic analysis of mitotic and meiotic cells clearly indicates the existence of an extra chromosome in the mouse described here. Various types of association recorded between E and sex-chromosome(s) as well as lack of association between E and autosome bivalents in spermatocytes rule out the possibility that E is an autosome or an autosomal fragment. Rather, these observations, plus the close resemblances noted between Y and E in morphology

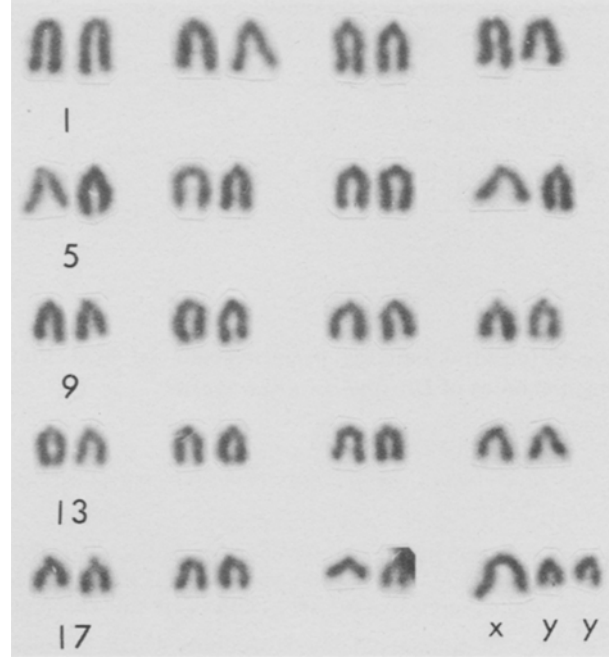
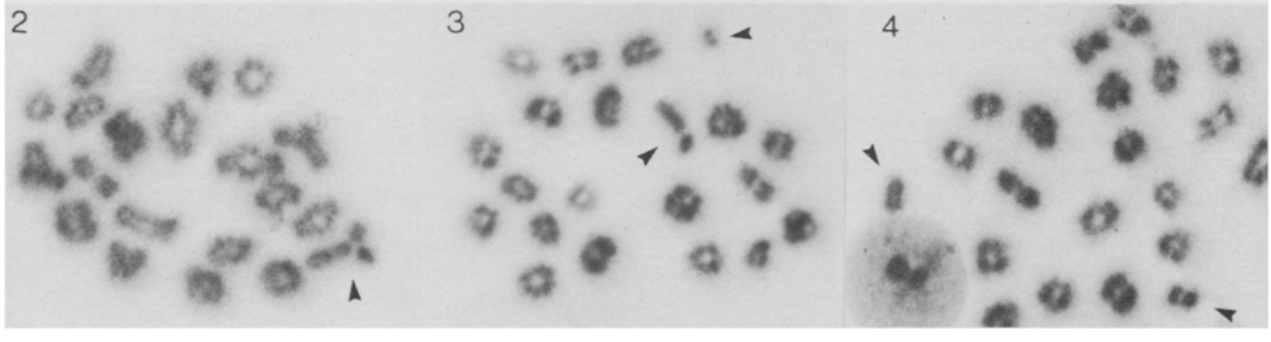


Figure 1. Karyotype of a bone marrow metaphase plate showing 4 smallest chromosomes.



Figures 2-4. *2* Diakinesis stage showing XYE trivalent. *3* Metaphase-I stage showing (XY/E) or (XE/Y) association. *4* Metaphase-I plate showing (X/YE) association.

and behaviour, strongly suggest that the extra chromosome is another Y. So the exceptional mouse carried an XYY sex-chromosome constitution.

To date, there is no evidence for fertility in XYY mice. About the present individual we have no record. However, our observations are in good agreement with those of Rathenberg and Muller[5] who also reported apparently normal-sized testes with considerable amounts of spermatozoa in their XYY mouse, in which fertility was also not tested. The mice of Cacheiro and Generoso[6] had very small numbers of sperms. The first case reported by Cattanach and Pollard[3] was sterile, with small testes and a few sperms only. Thus a great deal of variation has been noted with regard to testis size and sperm production in XYY mice. Interestingly, no XYY mouse has so far been reported to exhibit aspermia (an XO/XYY mosaic mouse of course showed aspermia[4]). So the possible occurrence of fertile XYY individuals cannot be ruled out. In radiation experiments, reduction of the sperm population to a level below 10% of normal is supposed to lead to sterility[9]. But in our case the epididymal sperm population was only reduced to 63% of the normal level. It is therefore not obvious that our individual was sterile.

The sex-chromosomes in the primary spermatocytes in our case exhibited all possible types of association (table). 2:1 ratio of types 2–3 association in the present individual suggests randomness of the pairing tendency of X and Y, which is in accordance with the findings of Rathenberg and Muller[5] but contradicts an earlier report[4]. Further evidence of equivalent pairing is provided by sex trivalent formation. The absence of certain association types in some earlier findings[3,4] may be due to the fact that the observations were based on limited data.

Incidences of different types of association involving X, Y and extra (E) chromosomes. 100 cells scored.

Association types	No. observed	Figure
1. XYE trivalent	15	2
2. XY bivalent and E univalent, or XE bivalent and Y univalent	55	3
3. X univalent and YE bivalent	27	4
4. X, Y, E univalents	3	-

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The differential banding pattern produced by Actinomycin-D/Acridine-Orange counterstaining in metaphase chromosomes of *Drosophila melanogaster*

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Summary. A longitudinal differentiation is found in fixed metaphase chromosomes of *Drosophila melanogaster* after treatment with Actinomycin-D and subsequent Acridine-Orange staining. We postulate that our findings are strictly related to the presence of AT-rich DNA in specific chromosomal areas of this species.

A number of counterstaining procedures which produce longitudinal differentiation in mammalian metaphase chromosomes are known[1-6]. These methods involve the coupled use of compounds which bind and, in some cases, emit secondary fluorescence when interacting with specific DNA base sequences. 2 main points are invoked for explaining metaphase banding patterns after counterstaining: 1. The binding competition between the compounds used. 2. The transfer of electronic energy from one compound to the other[6].

With the exception of the data reported by Lin et al.[7], however, counterstaining has thus far been carried out to increase the fluorescence contrast of the producible, standard banding pattern induced in metaphase chromosomes by fluorochromes such as Quinacrine, 33258 Hoechst, DAPI, DIPI, Chromomycin A_3, Olivomycin and so on, which are known for showing enhanced secondary fluorescence when interacting with adenine-thymine (AT) or guanine-cytosine (GC)-rich polynucleotide sequences[1,2,4,6]. Acridine-Orange (AO), on the contrary, is a planar molecule which shows the same fluorescence response as a consequence of binding any DNA base composition[8]. As the fluorometric properties of AO are identical when this fluorochrome interacts with both AT or GC-rich DNA, a uniform green secondary fluorescence is shown when this dye stains native, double-stranded DNA in fixed chromosomes[8].

Actinomycin-D (AMD), on the other hand, is a compound which is known to be a guanine binder agent[9]. Its interaction at GC sites in untreated fixed chromosomes would thus possibly affect both binding and fluorescence emission of AO according to the greater or smaller amount of such GC sites present in specific chromosome areas[7].

In this paper we attempt to contribute to the reports of fluorescent bandings as tools for investigating eukaryote chromosme structure, by describing and discussing in terms of DNA base composition the findings obtained in metaphase chromosomes of *Drosophila melanogaster* after AMD/AO counterstaining procedures.

Material and methods. Standard cytological preparations are obtained from third instar larvae of *Drosophila melano-*