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Inheritance of sperm head abnormality types in mice – the role of the *Y* chromosome

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SUMMARY

Four inbred strains of mice were used, differing in the total percentages of spermatozoa with abnormal heads (KE, 22·1 %; C57, 26·4 %; KP, 7·7 %; CBA, 5·5 %) and in the frequency distribution of abnormality types, as divided into four arbitrary classes. The most variable class 2 (narrow heads with canals inside the nuclear material) accounted for 47 % of all abnormalities in KE strain, was common in CBA (29 %) and almost missing in KP and C57 strains. F_1 hybrids from the diallel crosses of these strains exhibited highly significant heterosis effects and significant reciprocal differences in the total percentage of abnormalities. The relative frequency of class 2 ranked in F_1 hybrids in a similar order as calculated from the mid-parental values. After seven generations of backcrosses performed to introduce the *Y* chromosome from CBA to the genetical background of the KE strain, the total percentage of abnormalities was significantly reduced, although the relative proportion of class 2 was similar to that in KE strain. Also the *Y* chromosome from C57 strain, introduced into the genetical background of KE strain, caused a significant reduction of total abnormalities, but again the relative frequency of class 2 was not affected. It is concluded that the *Y* chromosome plays an important role in determining the total percentage of sperm head abnormalities, but does not seem to be involved in influencing specific abnormality types.

1. INTRODUCTION

Inbred strains of mice show different proportions of abnormal spermatozoa (e.g. Beatty & Sharma, 1960; Mori, 1961; Krzanowska, 1962; Brożek, 1970; Bruce, Furrer & Wyrobek, 1974). In experiments involving some inbred strains these differences were found to be polygenically determined by a small number of genes, and the role of the *Y* chromosome was detected (Krzanowska, 1966, 1969, 1972).

These investigations were based on the total percentage of all sperm head abnormalities. One can expect, however, that such an analysis might be much more accurate if different abnormality types were taken into account. For this purpose four inbred strains were characterized from the point of view of the relative proportions of different sperm head abnormalities, divided into four arbitrary classes. It appeared (Krzanowska, 1976) that not only the total percentage of abnormalities,

but also the frequency distribution of abnormality classes were characteristic for a given strain.

The present experiments were performed to find out whether the *Y* chromosome, which was shown to be partly responsible for the total percentage of sperm head abnormalities, might be also involved in influencing specific abnormality types.

2. MATERIALS AND METHODS

The first series of experiments involved F_1 hybrid males obtained from the diallel crosses between four inbred strains of mice: C57BL/Kw, CBA/Kw (referred to later on as C57 and CBA, respectively), KE and KP. When denoting the parentage of hybrids the strain of the mother is always written first.

In the second series, aimed at transferring the *Y* chromosome from the CBA strain to the genetical background of the KE strain, repeated backcrosses were performed according to the scheme:

$$♀\,KE \times ♂CBA = F_1,$$
$$♀\,KE \times ♂F_1 = B_1,$$
$$♀\,KE \times ♂B_1 = B_2, \quad \text{etc.}$$

After seven generations the following reciprocal crosses were made:

$$♀\,KE \times ♂\,B_7 = B_8,$$
$$♀\,B_7 \times ♂\,KE = B_7 \times KE,$$

and the resulting males were tested.

In the third series the analogous experiment was made to introduce the *Y* chromosome from C57 to the genetical background of KE. The reciprocal crosses were performed in the sixth generation.

As it is known that in very young males the proportion of abnormal spermatozoa is highly elevated (Hancock, 1972; Krzanowska, 1972), only adult 10- to 15-week-old males were used for testing, and killed by cervical dislocation. After pressing the cauda epididymis, allowing sperm to pass to the vas deferens, the content of the latter was expressed into a small drop of 0·95 % NaCl. Four minutes later a smear was prepared, air-dried and fixed for 1 h in acetic alcohol (3 parts of absolute ethylene alcohol and 1 part of glacial acetic acid). Initially the preparations were stained with Feulgen reagent, using the method adapted for testicular preparations (Ford, 1962). This method makes possible a detailed analysis of disturbances in chromatin distribution, but is rather laborious. Therefore, later on for routine analysis smears were stained with a 5 % aqueous solution of eosin, which was found to be sufficiently reliable to distinguish abnormality types. Stained smears were dehydrated in alcohol, cleared with xylene, mounted in Canada balsam and covered with coverslips.

Preparations were examined under oil-immersion objective (100 ×). Two hundred spermatozoa from each male were counted and the percentages of normal and abnormal heads were calculated. The classification of abnormal forms was based on a previous one (Krzanowska, 1976), four main classes being distinguished and divided into subclasses (Fig. 1):

Class 1. Almost normal head but with slightly bent acrosome (1*b*) or with a changed curvature in the distal part of the head (1*a*); both forms were counted together.

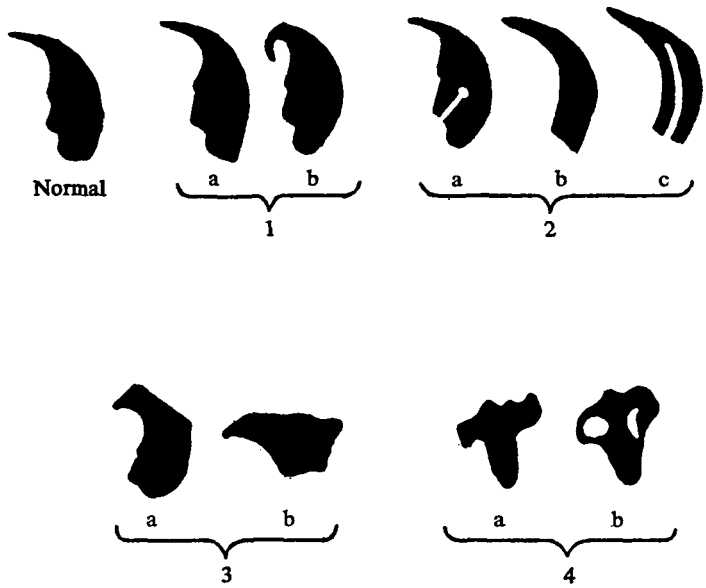

Fig. 1. Normal spermatozoan head and typical examples of abnormal heads divided into arbitrary classes (1–4). Drawn from the photographs.

Class 2. The most typical form has a narrow, condensed head and contains a longitudinal canal of very low stainability (2*c*); sometimes the canal is not visible (2*b*). This class includes also heads of completely normal shape, except that they possess canals of low stainability, situated transversely, obliquely or longitudinally to the long axis of the head (2*a*) and often ending with a round vacuole. On Feulgen-stained smears the stainability of class 2 spermatozoa is highly variable.

Class 3. Misshapen heads distended mainly in the apical part (3*a*) or in the distal part as well (3*b*); they are flat and stain normally.

Class 4. Severely misshapen heads showing abnormalities in distribution of chromatin. On Feulgen-stained smears the distal part is usually very dark in colour, while the apical part stains normally (4*a*). Some of these heads possess vacuoles or short canals of less stainable material (4*b*).

For statistical treatment the percentages of abnormal spermatozoa were transformed to angles (Snedecor, 1955).

3. RESULTS

Table 1 shows the percentages of abnormal spermatozoa in all experimental animals. Data referring to the pure strains (Krzanowska, 1976) are also included for comparison. They show that the males of both the C57 and KE strains are characterized by very high percentages of abnormal spermatozoa ('high' strains) although

Table 1. *Proportion of different types of sperm abnormalities in inbred strains of mice and their hybrids*

Genotype of males	No. of males	Percentages of abnormal spermatozoa of the following classes: 1	2 a	2 b	2 c	3 a	3 b	4 a	4 b	All abnormal spermatozoa together %	In angular transformation $\bar{x} \pm$ S.D.	Relative proportion of class 2 a+b+c %	Mid-parental value
Inbred KE†	20	2·0	1·7	1·0	7·6	5·7	0·6	1·4	2·1	22·1	27·83 ± 4·27	46·6	
Inbred CBA†	20	0·4	0·7	0·0	0·9	1·2	0·8	0·5	1·1	5·5	13·50 ± 2·00	28·8	
Inbred KP†	20	1·3	0·0	0·0	0·2	3·8	0·9	0·5	0·9	7·7	15·13 ± 6·06	3·6	
Inbred C57†	20	2·5	0·0	0·0	0·0	8·5	6·7	5·4	3·2	26·4	30·52 ± 6·87	0·0	
F_1 KE × CBA	20	0·2	0·4	0·1	1·8	0·8	0·3	0·3	0·3	4·2	11·73 ± 2·46	55·1	37·7
F_1 CBA × KE	20	0·3	0·9	0·1	3·0	0·6	0·2	0·1	0·5	5·8	13·65 ± 2·97*	70·3	
F_1 KE × KP	20	0·9	0·1	0·0	0·7	2·2	0·6	0·4	0·4	5·5	13·44 ± 1·93	15·9	25·1
F_1 KP × KE	20	1·4	0·3	0·1	1·8	2·2	0·4	0·3	0·5	7·1	15·21 ± 2·78*	32·1	
F_1 KE × C57	20	0·9	0·3	0·1	0·4	0·8	0·5	0·4	0·6	4·0	11·26 ± 2·85	20·5	23·3
F_1 C57 × KE	20	1·0	0·0	0·1	0·2	0·7	0·7	0·4	0·2	3·4	10·48 ± 1·56	11·1	
F_1 KP × CBA	10	0·1	0·1	0·0	0·2	0·7	0·2	0·0	0·1	1·6	6·95 ± 2·25	25·0	16·2
F_1 CBA × KP	10	0·3	0·0	0·0	0·1	0·7	0·2	0·1	0·2	1·7	7·29 ± 1·84	11·8	
F_1 C57 × CBA	20	0·2	0·0	0·0	0·1	0·8	0·3	0·4	0·2	2·0	7·86 ± 1·95	6·3	14·4
F_1 CBA × C57	20	0·2	0·0	0·0	0·2	1·0	0·6	0·4	0·3	2·9	9·49 ± 2·76*	10·3	
F_1 C75 × KP	10	1·3	0·1	0·1	0·0	1·7	0·7	0·2	0·2	4·3	11·52 ± 3·78	4·6	1·8
F_1 KP × C57	10	2·0	0·1	0·2	0·0	2·1	1·2	0·9	0·5	7·0	15·27 ± 2·18	5·0	
Backcrosses from CBA to KE													
B_8	16	0·7	1·0	0·3	3·1	2·1	0·8	0·9	0·2	9·2	17·55 ± 2·03	48·0	
B_7 × KE	14	1·9	1·0	0·8	7·9	5·0	1·3	1·1	1·4	20·4	26·73 ± 3·22**	48·0	
Backcrosses from C57 to KE													
B_6	10	2·1	0·7	1·1	4·6	4·0	0·8	0·4	1·0	14·9	22·69 ± 1·92	43·5	
B_5 × KE	13	4·9	1·4	0·8	7·2	5·2	0·8	0·9	1·1	22·4	28·21 ± 1·65**	42·1	

** Difference between the reciprocal hybrids significant at 0·01 level; * at 0·05 level. † From Krzanowska (1976).

the frequency of abnormality classes is different. In KE males the most frequent is class 2 which accounts for 47% of all abnormalities. This class is absent in C57 spermatozoa. On the other hand, KP and CBA males possess low levels of abnormal spermatozoa ('low' strains) but they again differ in abnormality types. Class 2 is common in CBA males (about one third of abnormalities belong to this category), while it is almost missing in KP males.

Table 2. *Differences between F_1 hybrids and their parental strains in the total proportion of abnormal spermatozoa (in angular transformation)*

F_1 hybrid	'Higher' parental strain	Mid-parental value	'Lower' parental strain
F_1 KE × CBA	−16·10**	−8·93**	−1·77*
F_1 CBA × KE	−14·18**	−7·01**	+0·15
F_1 KE × KP	−14·39**	−8·08**	−1·69
F_1 KP × KE	−12·62**	−6·27**	+0·08
F_1 KE × C57	−19·26**	−17·91**	−16·57**
F_1 C57 × KE	−20·04**	−18·69**	−17·35**
F_1 C57 × CBA	−22·66**	−14·15**	−5·64**
F_1 CBA × C57	−21·03**	−12·52**	−4·01**
F_1 C57 × KP	−19·00**	−11·30**	−3·61**
F_1 KP × C57	−15·25**	−7·55**	+0·14
F_1 KP × CBA	−8·18**	−7·36**	−6·55**
F_1 CBA × KP	−7·84**	−7·02**	−6·21**

** Difference significant at 0·01 level; * at 0·05 level

(i) *F_1 hybrids*

F_1 hybrid males showed marked effects of heterosis, as far as total percentages of abnormal spermatozoa were concerned. Their level was always significantly lower than in the mid-parental value, and in most cases also lower than in the lower parental strain (Table 2). Moreover, when crossing low and high strains the reciprocal hybrids differed significantly, the level of abnormalities being always higher in the hybrids whose male parent was derived from the high strain (Table 1). Only when both parents belonged either to high strains (C57 and KE) or to low strains (KP and CBA) was there no difference between the reciprocal hybrids.

To analyse the distribution of abnormality types their relative proportions were calculated (the absolute percentage of spermatozoa belonging to the given class divided by the total percentage of all abnormal spermatozoa) and are presented graphically in Fig. 2. Because of the low level of abnormalities in F_1 hybrids the relative proportions could not be accurately determined. However, it is clear that the frequency distribution of abnormality classes in F_1 hybrids was strongly influenced by both parental strains. This is particularly evident when analysing class 2. The right column of Table 1 shows that the relative frequency of this class ranked in F_1 hybrids in a similar order as calculated from the mid-parental values. The test of independence revealed that the differences in the relative proportion of class 2 between the reciprocal hybrids were significant only in crosses between KE and CBA

strains ($P < 0{\cdot}05$) and between KE and KP strains ($P < 0{\cdot}01$). In both cases a higher proportion of class 2 was found in hybrids sired by KE males.

(ii) *Backcrosses from CBA to KE strain*

After seven generations of backcrosses aimed at introducing the *Y* chromosome from the CBA strain into the genetical background of the KE strain, the level of abnormal spermatozoa was tested in males of generation B_8 and in $B_7 \times$ KE males,

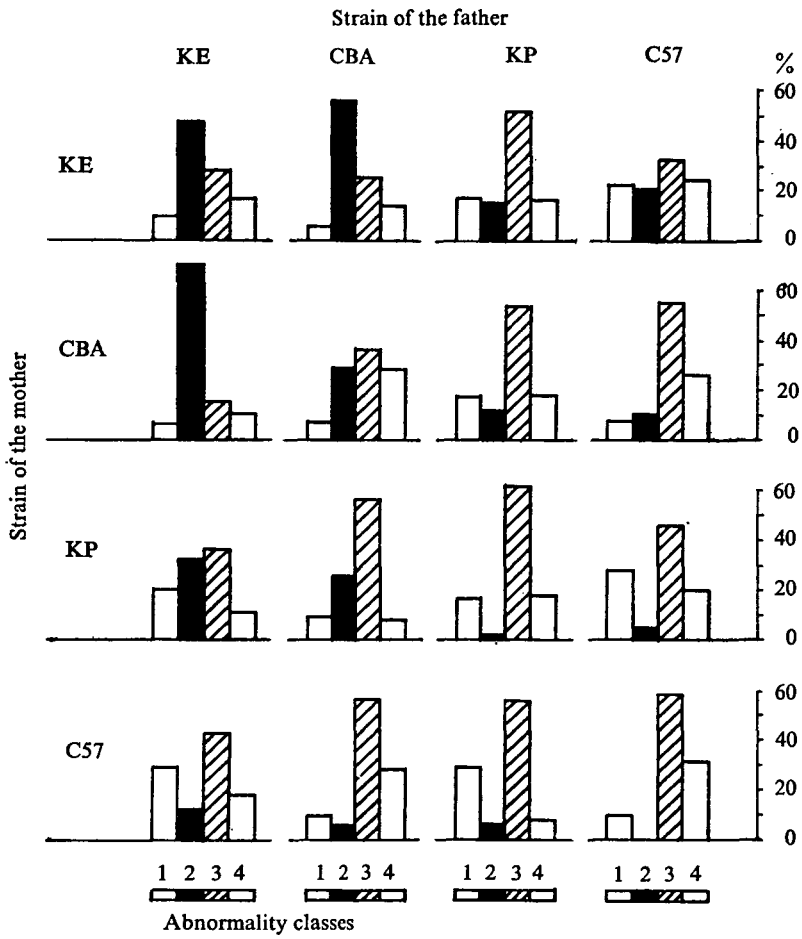

Fig. 2. Distribution of relative percentages (%) of abnormality types (1–4) in males from the dialled crosses between four inbred strains.

resulting from crosses between B_7 females with KE males. The animals from these reciprocal crosses differed significantly (Table 1) in the total percentage of abnormal spermatozoa, which was lower in B_8 males. On the other hand the percentage of abnormal spermatozoa in $B_7 \times$ KE males was not significantly different from that in KE strain males.

The difference between B_8 and $B_7 \times$ KE males shows the net effect of the different

Y chromosomes on the same genetical background. Thus the *Y* chromosome derived from the CBA strain caused a significant reduction of the total percentage of abnormal spermatozoa, although the level typical for the CBA strain has not been reached. The difference between B_8 males and CBA males was still highly significant ($P < 0{\cdot}01$).

The *Y* chromosome has not exerted any effect on the frequency distribution of abnormality classes which did not differ between B_8 and $B_7 \times KE$ males and was almost the same in the KE strain (Fig. 3). However, it should be noted that even in the CBA strain this distribution was not very different.

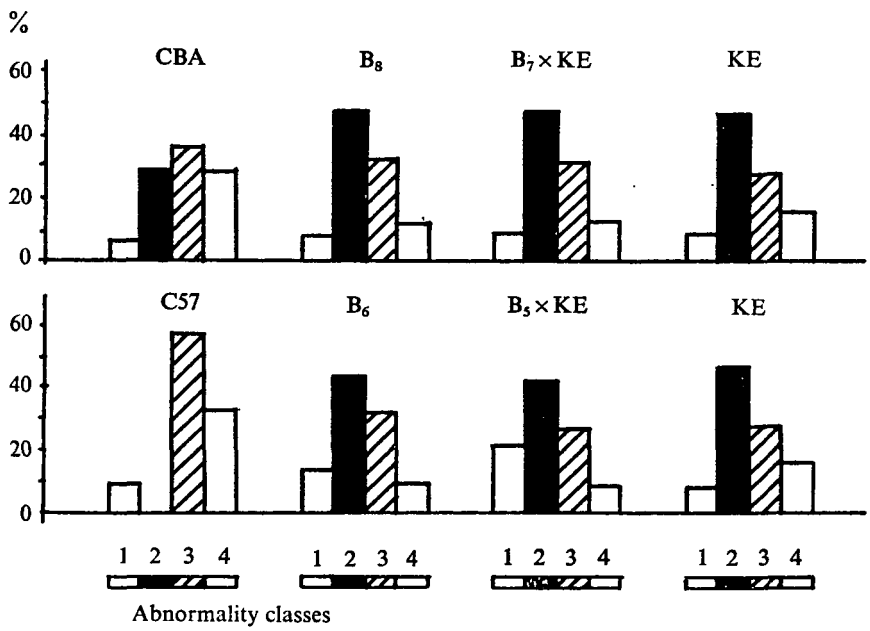

Fig. 3. Distribution of relative percentages (%) of abnormality types (1–4) in original strains and in backcrosses; top row, from CBA to KE; bottom row, from C57 to KE.

(iii) *Backcrosses from C57 to KE strain*

This experiment, analogous to the preceding one, was performed to introduce the *Y* chromosome from C57 into the genetical background of KE. This time both strains belonged to high strains but differed diametrically as far as frequency distribution of abnormality classes is concerned.

The level of abnormal spermatozoa was tested in males of B_6 and in $B_5 \times KE$ males, resulting from crosses between B_5 females and KE males. The animals from these reciprocal crosses differed significantly (Table 1) in the total percentage of abnormal spermatozoa, which was lower in B_6 males. Again, there was no significant difference between $B_5 \times KE$ males and KE strain males.

The difference between $B_5 \times KE$ and B_6 males shows that the *Y* chromosome, although derived from the high C57 strain, nevertheless caused a significant

reduction of the total percentage of abnormal spermatozoa. However, it should be noted that this reduction was significantly ($P < 0{\cdot}01$) less pronounced than in the case of introducing the Y chromosome from the CBA strain.

Similarly, as in the preceding experiment, the Y chromosome has not exerted any effect on the frequency distribution of abnormality classes, which was very similar in B_6 and $B_5 \times$ KE males (Fig. 3).

4. DISCUSSION

The present results have confirmed the earlier findings (Krzanowska, 1969, 1972) about the important role of the Y chromosome in determining the total percentage of abnormal spermatozoa. This conclusion has been again directly supported by the fact that the Y chromosome derived from the low percentage CBA strain caused a significant reduction of sperm abnormalities when tested on the genetical background of the KE strain. Similarly as in previous experiments the level characteristic for the CBA strain has not been reached, indicating that autosomal and/or X-linked genes are also important.

Somewhat unexpectedly, the Y chromosome derived from the high percentage C57 strain also caused a reduction of the level of abnormalities in the KE strain. This effect, although less pronounced than in the case of the CBA-derived Y chromosome, was nevertheless significant. This might be explained by some sort of interaction occurring between the Y chromosome of C57 and the KE genetical background.

The reciprocal differences found in F_1 hybrids from the diallel crosses between the four inbred strains might also have been caused by Y-linked loci, although the involvement of X-linked loci or cytoplasmic effects cannot be excluded.

The strains used in the present investigations differ markedly in the relative frequencies of abnormality types and seem to be suitable for studies on the inheritance of these characters. Genetic factors may cause specific sperm defects (for review see: Beatty, 1970; Hancock, 1972) by interfering with the normal process of spermatogenesis at specific points. It seems also that some of these genetic effects could be simulated by environmental factors. Wyrobek & Bruce (1975) in their paper on chemical induction of sperm abnormalities in mice, noted that there were significant differences in the types of abnormalities caused by the different agents and at different times. Judging from the pictures in their paper, at least some of these malformations were similar to those occurring in our strains.

The present analysis of F_1 hybrids leads to the conclusion that the frequency distribution of abnormality classes remains under a strong genetical control. Although the total percentages of abnormalities were greatly reduced owing to heterotic effects, and therefore the relative proportions of abnormality types could not be very accurate, nevertheless they showed obvious influences of the respective parental strains (Fig. 2).

Among the defects observed in the present investigations the group of abnormalities designated as class 2 is particularly interesting. Its frequency shows the highest variability between strains, being predominant in KE and lacking in C57 sperm. As found on electronmicrographs by Kaczmarski (1972) many spermatozoa of the

KE strain show incomplete condensation of chromatin or the presence of large vacuoles and canals containing remnants of cytoplasm, in various regions of the head. Owing to the fact that the canals and vacuoles are visible also under the optical microscope on stained smears, the frequencies of these defects could be easily estimated. On methyl green–pyronin stained smears, spermatozoa of class 2 differ from the other types in that they appear violet or reddish, instead of green (Krzanowska, 1976). This points to the conclusion that the biochemical composition of chromatin is not normal in these spermatozoa. Moreover, when examining serial sections from the testes it was found that this violet stain is characteristic for normal spermatids of stage 13–15 (B. Godowicz, personal communication). This seems to suggest that spermatozoa of class 2 do not pass through the final steps of chromatin maturation occurring normally during spermiogenesis. It is worth mentioning that the formation of certain misshapen sperm heads in the bull involves some defects in binding nuclear protein to DNA during spermateliosis (Gledhill, Darżynkiewicz & Ringertz, 1971); and as pointed out by Fawcett, Anderson & Phillips (1971) the shape of the sperm head is largely determined by a genetically controlled pattern of aggregation of DNA and proteins.

On these grounds special attention was focused on the genetical factors influencing class 2 abnormalities, with the aim of inquiring into the possible role played by the *Y* chromosome. In F_1 hybrids the relative percentages of this class ranked in a similar order as calculated from the mid-parental values (Table 1). The fact that in the crosses between KE and CBA, as well as between KE and KP strains, the relative frequencies of class 2 were significantly higher in F_1 hybrids sired by KE males, might suggest that the *Y* chromosome was involved. However, this does not seem to be convincing. Firstly, no reciprocal difference was found in F_1 hybrids between the two most contrasting strains (KE and C57), and secondly, in the backcrosses neither the *Y* chromosome from CBA nor from C57 has exerted any effect on the frequency of class 2 abnormalities, when tested on the genetical background of the KE strain.

It appears therefore that the *Y* chromosome is involved in determining the total level of these abnormalities which are characteristic for the given genetical background, but does not by itself influence specific abnormality types.

In this connexion it is worth recalling that in *Drosophila* – where the role of the *Y* chromosome was most thoroughly investigated – the following conclusion was reached: 'the *Y* chromosomal factors control the co-ordination of the various synthetic and morphogenetic processes during critical phases of spermatid development rather than contributing primary structural information'. And 'the phenotype determined by the factors on the *Y* chromosome is sensitive to changes in the genetic background' (Meyer, 1972).

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