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THE GENETICAL AND MECHANICAL PROPERTIES OF THE SEX-CHROMOSOMES

IV. THE GOLDEN HAMSTER

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(With Plate VIII and Twelve Text-figures)

INTRODUCTION

CRICETUS AURATUS, the golden hamster, is a small rodent whose distribution is restricted to the neighbourhood of Aleppo, Asia Minor. Dr S. Adler, of the Microbiological Institute, Jerusalem, introduced the species into Britain in 1931 and Prof. E. Hindle, of the University of Glasgow, bred it and later distributed animals to various places in the country. It is considered by Bruce & Hindle (1934) to be a geographical race of the Caucasian hamster, *C. raddei*. *C. auratus* was found to be excellent material for cytological investigation and a study was undertaken of the number and morphology of its chromosomes. Cytological investigation has shown that the golden hamster possesses a peculiar sex-determining mechanism in that a differential segment is present in one of the sex-chromosomes during meiosis and consequently there are formed two kinds of gametes with different sex potencies.

The present paper describes the structural differentiation and the behaviour of the sex-chromosomes during mitosis and meiosis.

MATERIAL AND TECHNIQUE

Several adult males were received through the kindness of Dr A. S. Parkes, F.R.S., National Institute of Medical Research, London, to whom the author is greatly indebted. The animals were descendants of those bred by Prof. E. Hindle. Small pieces of testes were fixed in the following solutions: medium and strong Flemming, San Felice, Navashin, Minouchi's modification of Flemming, Carnoy, and Benda solution. Excellent results were obtained by Navashin's and medium Flemming fixation. Sections were made at 15–24μ thickness and stained with gentian violet and iron haematoxylin. The former were the more satisfactory. Drawings were made with the aid of Zeiss' drawing apparatus,

using 0·90 oil-immersion objective n.a. 1·4 and ×20, or 30 comp. eyepiece. The scale of magnification is given with the figures.

Chromosome behaviour during mitosis

The resting nuclei of the spermatogonial cells, which form two layers at the periphery of the seminiferous tubules, contain two or more small

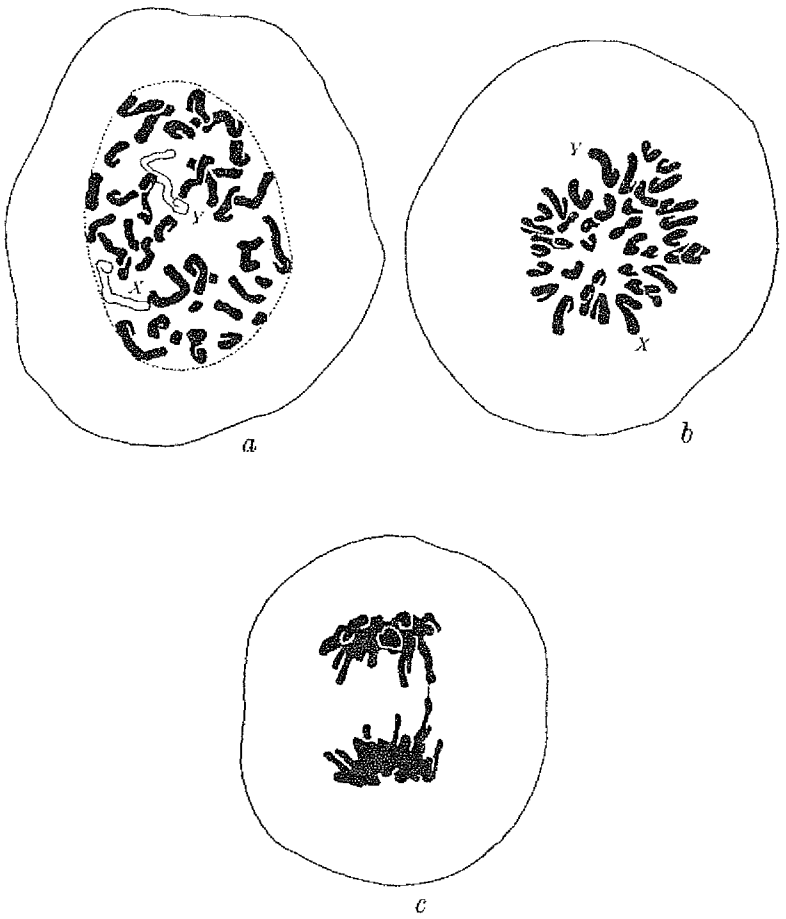


Fig. 1. *a*, mitotic prophase; *b*, metaphase. The sex-chromosomes are indicated; *c*, anaphase, showing the delayed separation of the long chromosomes. ×2·800.

aggregates, which stain well with iron-haematoxylin but little with gentian violet. They are the remnants of a large nucleolus which breaks up into smaller elements and completely disintegrates during the early stages of mitotic prophase.

The chromosomes during mitotic prophase are evenly distributed within the oblong nucleus (Text-fig. 1*a*). The chromatids and their association by relational coiling could not be seen. The prophase chromosomes show 1–4 relic coils.

The diploid chromosome number was determined in four spermatogonial cells and found to be 38. This number was definitely verified at meiosis by counting 19 bivalents as the haploid chromosome number. The chromosome complement is made up of chromosomes of different lengths. The size difference between members of the complement during mitotic metaphase is gradual; the longest chromosome is about 4·5–4·8μ and the smallest 2·0μ or less. The chromosomes vary in shape, which indicates that the centromere may have a median, submedian or subterminal position (Text-fig. 1*b*).

The two largest chromosomes are assumed to be the sex-chromosomes, because an analysis of chromosome behaviour during meiosis revealed that the largest bivalent, morphologically as well as structurally, differs greatly from the others and exhibits peculiarities characterizing sex-chromosomes in various species. If the largest chromosomes of the mitotic complex are actually the sex-chromosomes, it must be pointed out that they do not exhibit a clearly marked size difference during mitotic metaphase; consequently no *X* and *Y* can be identified morphologically in the chromosome complement of the male. Furthermore, they cannot be distinguished from the other chromosomes during the mitotic nuclear cycle, because they show the same degree of contraction as the others. Very similar behaviour of the sex-chromosomes was found in the grey squirrel (Koller, 1936*a*).

A more detailed analysis of chromosome morphology was found to be impossible owing to the great number of chromosomes, the 38 somatic chromosomes being crowded in a small equatorial plate. While the longer chromosomes commonly lie at the periphery of the spindle, the small members invariably occupy the centre of the metaphase plate. Very often the complete separation of the longer daughter chromosomes towards opposite poles is delayed at anaphase (Text-fig. 1*c*). The sex-chromosomes are probably amongst these lagging chromosomes.

CHROMOSOME BEHAVIOUR DURING MEIOSIS

The primary spermatocytes can be distinguished from the spermatogonial cells by their smaller nucleus, in which there is a fine network and a varying number of chromatin granules or aggregates. The appearance of the fine network indicates the beginning of meiotic prophase. Among

the fine and thin threads which probably represent the single chromosomes, one or sometimes two large closely associated aggregates of varying shapes can be seen, which differ from the small chromatin granules (Text-fig. 2*a*, Pl. VIII A). By following the behaviour of these

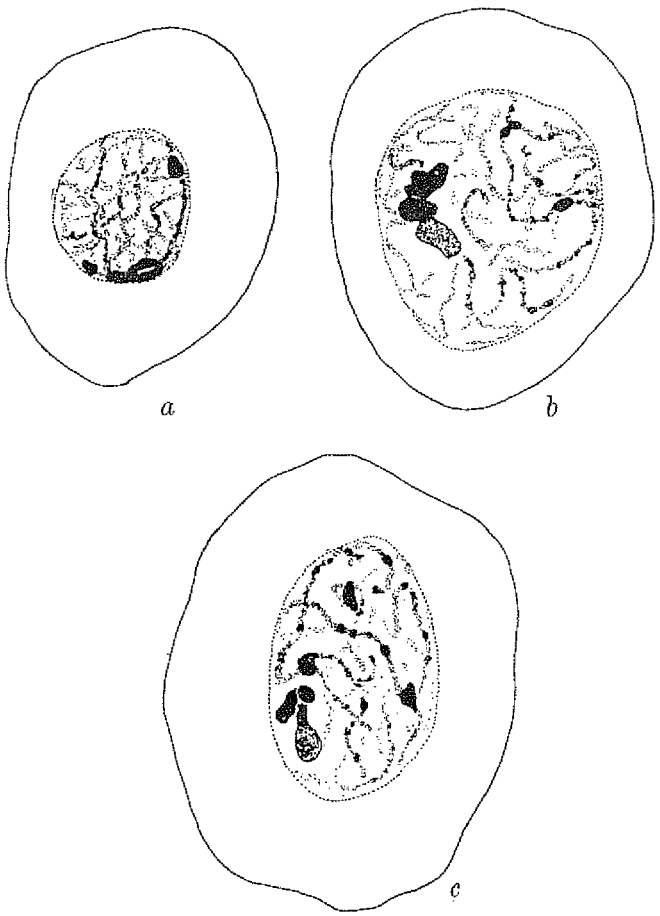


Fig. 2. *a*, , *c*, early meiotic prophase, showing the precocious sex complex, which is associated with a nucleolus. ×2·500.

aggregates during the subsequent stages of meiosis, they were identified as the two associated sex-chromosomes. In the leptotene and zygotene nucleus only the deeply stained portion of the sex complex could be seen, but during pachytene, it was found that the sex complex is associated with a "nucleolus" (Text-fig. 2*b*, *c*), which stains lightly,

apparently being similar in its staining ability to the true nucleolus, commonly developed from the organizer localized at the secondary constriction of mitotic chromosomes (Heitz, 1931).

The structure of the XY complex (Text-fig. 3) is very variable during pachytene. The variation is due partly to the variable position, shape and area of the diffuse region, and partly to the varying degree of condensation of the different parts. The sex complex usually lies near to the nuclear membrane and never has association with other chromosome threads.

The exact method of association of the two sex-chromosomes during the earlier stages of meiotic prophase could not be determined owing to the strong precocious condensation and to the variable configuration of the whole complex. The structure of the sex-bivalent can first be seen more or less distinctly at diakinesis, when two regions can be distinguished: (*a*) a precociously condensed portion, and (*b*) a diffuse or non-

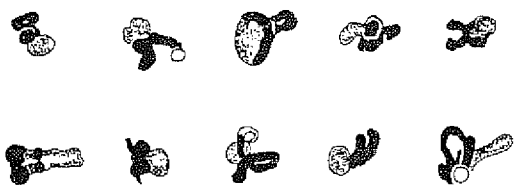


Fig. 3. The various configurations of the XY bivalent during diakinesis. ×2·500.

condensed portion with very little staining capacity. Similar observations were made in Marsupials (Koller, 1936*b*) and in *Apodemus* (Oguma, 1934). The diffuse region is associated either terminally or interstitially with the precociously condensed part; if its position is intercalary, the condensed region is bipartite. The terminally located diffuse portion is globular, but becomes gradually elongated and its volume decreases. The decrease in volume and the thickening of the peripheral part strongly suggests that a process of regional condensation takes place. At the end of diakinesis the globular diffuse part completely disappears and a long, thin, lightly stained thread is formed in its place, which is associated either with one or with both ends of the condensed region.

At the meiotic metaphase the sex-bivalent can easily be identified by its characteristic shape. Two types of sex-bivalent were found: (*a*) asymmetrical, and (*b*) symmetrical XY. The asymmetrical sex-bivalent closely resembles the shape of a shepherd's crook (Text-fig. 4, Pl. VIII B, D, F) and is made up of two easily distinguishable regions. One is thick and deeply stained, forms the whole of the Y- and part of the X-chromosome and

Fig. 4. Metaphase of meiosis, showing the structure of the asymmetrical XY bivalent, $\times 2500$.

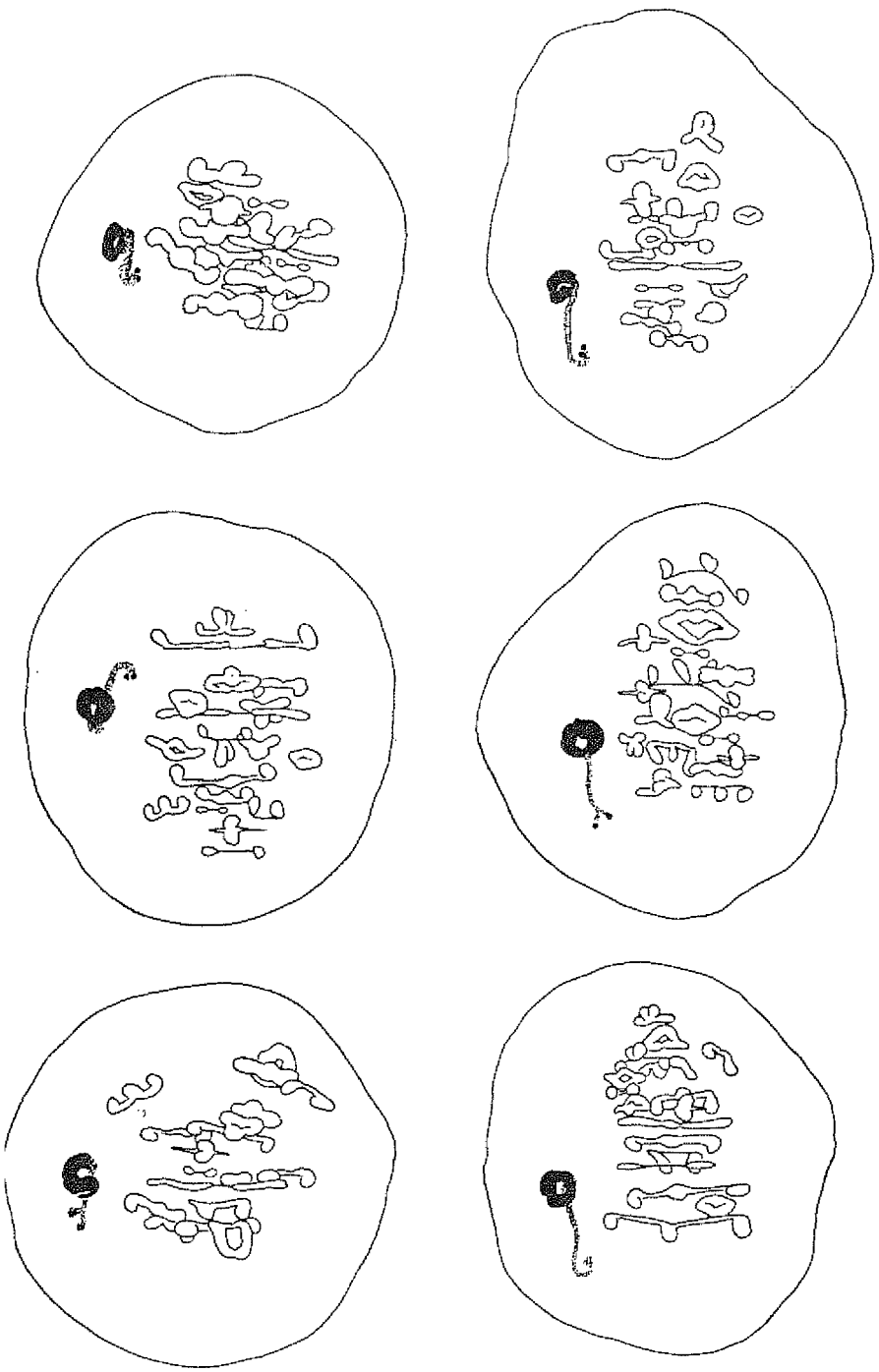


Fig. 5. The symmetrical sex-bivalent; the pairing segments form a ring. × 2·500.

is identified as the homologous pairing segment. The other segment is represented as a long, thin, double thread. This part is the differential segment of the *X*-chromosome and is altogether absent from the *Y*. The differential segment of the asymmetrical *XY* bivalent segregates reductionally at the first meiotic division, hence this type belongs to the pre-reductional type, which is described as the most common *XY* bivalent in the various animal species.

The symmetrical *XY* bivalent consists of a ring made up of the deeply stained pairing segments of the *X*- and *Y*-chromosomes, with the differential segment attached laterally (Text-fig. 5, Pl. VIII C, G). If the structure of the metaphase sex-bivalents is interpreted on Janssen's partial chiasmatype hypothesis, then the existence of the two types indicates that the centromere is localized in the pairing segment and chiasmata between *X* and *Y* can be formed on either side of the centro-

TABLE I

The frequency of the various types of sex-bivalent at meiosis

Specimen	No. of spermatocytes	*XY* bivalent	
		Asymmetrical	Symmetrical
A1	75	58	17
A4	34	29	5
B3	103	87	16
B4	41	32	9
Total	253	206	47
Percentage	—	81·6	18·4

mere. The frequency of the different *XY* types may be taken as a measure of the relative size of the two arms. An analysis of sex bivalents in 253 primary spermatocytes has shown that the asymmetrical *XY* is more frequent that the symmetrical, which indicates that the section of the pairing segment situated between the centromere and the differential segment is smaller than the other section.

At mitotic metaphase the two largest chromosomes of the complement, assumed to be the *X* and *Y*, have a subterminal centromere. The short arm is about one-fifth of the length of the longer arm. Similar relationship was found in the asymmetrical *XY* bivalent, the short arm of the *Y* being 4–5 times smaller than the long arm. By determining the position of the centromere in the pairing segment, the relative frequency of the asymmetrical and symmetrical *XY* bivalents could be predicted with accuracy. It was expected that the number of the symmetrical *XY* is 4–5 times less than the number of the asymmetrical type. The observed data (Table I) are in close agreement with the expected number of the two

types. The frequency of symmetrical XY is higher than in the rat (Koller & Darlington, 1934) but less than in the ferret (Koller, 1936*a*). It was found that the chiasma frequency in the arms of the sex-bivalent is proportional to the length.

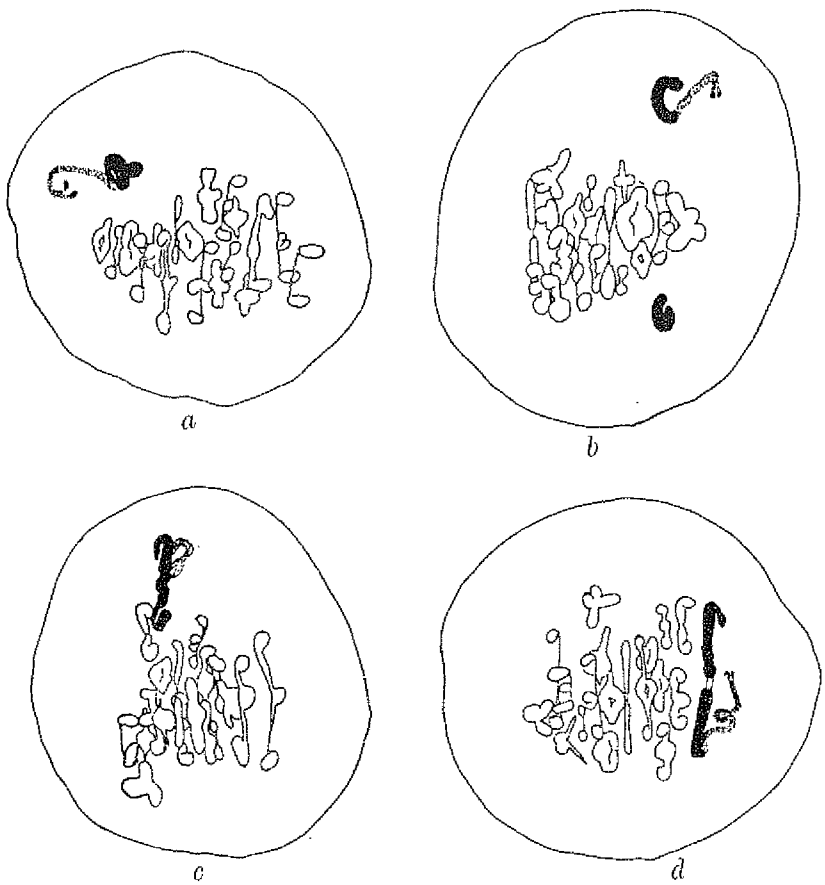


Fig. 6. *a*, symmetrical XY, showing the terminal knobs of the differential segment; *b*, unpaired sex-chromosomes at meiotic metaphase; *c*, asymmetrical XY, lying off the equator; *d*, asymmetrical XY, showing relic coil in the differential segment. ×2·500.

The asymmetrical XY bivalent lies at the periphery of the spindle either on the equatorial plate or near to one pole (Text-fig. 6*c*). The pairing segments of the X and Y, if the sex-bivalent lies on the equator, are orientated towards opposite poles. The short arm of the Y-chromosome shows very clearly, while in the X its length is difficult to estimate, because it continues into the differential segment. The differential

segment of the asymmetrical XY is either pulled out and more or less straight, or is bent into a shepherd's crook. The distal end shows two small knobs which sometimes stain more deeply than other parts (Text-fig. 6*a*), indicating that the differential segment is composed of two chromatids. The double structure was often seen in intercalary regions, where the two chromatids formed relational coiling.

During meiotic metaphase the pairing segments of the X- and Y-chromosomes in the asymmetrical configuration are associated terminally; only 8 out of 206 asymmetrical sex-bivalents had interstitial chiasma. In the symmetrical sex-bivalent, no interstitial chiasma was seen in the long arm of the pairing segment, while in the short arm it was localized at the region where the differential segment is joined to the short arm of the pairing segment.

During meiosis the sex-chromosomes are commonly associated by chiasmata and form asymmetrical or symmetrical XY complexes. Analysing 276 primary spermatocytes, in respect of the sex-bivalents, 23 (8·3%) had unpaired sex-chromosomes at meiotic metaphase. In 16 cases the X and Y lay on the opposite sides of the equatorial plate and the corresponding position of the unpaired chromosomes strongly suggests that they were associated during the prophase but failed to form chiasmata (Text-fig. 6*b*, Pl. VIII H, I). Owing to the absence of chiasmata, the precociously separated X- and Y-chromosomes very frequently fall apart towards the opposite poles, ensuring reductional segregation. The frequent failure of chiasma formation is probably due to the peculiar behaviour of the sex-chromosomes, namely, the strong precocity in condensation and the frequent intercalary position of the diffuse portion during meiotic prophase.

The metaphase chiasma frequency in the XY bivalent was calculated and compared with that of the autosomal bivalents (Table II).

TABLE II

The chiasma frequency in the sex and autosomal bivalents

Bivalent	No. of bivalent	No. of chiasmata				Total no. of chiasmata	X frequency per bivalent
		0	1	2	3		
XY	276	23	206	47	—	300	1·08
Autosomes	180	—	121	58	1	240	1·33

It can be seen that chiasma frequency in the sex-bivalent is below that of the autosomal bivalents. The difference is significant and is brought out more clearly when it is remembered that the pairing segment is longer than the largest bivalent, but its chiasma frequency is less.

During meiotic metaphase the differential segment shows two kinds of coiling: (*a*) relic (Text-fig. 6*d*), and (*b*) relational coiling (Text-fig. 4). The direction of coiling was determined in a few instances and is given in Table III.

TABLE III

The direction of coiling in the differential segment

Direction	Relic coils	Relational coils
L	21	6
R	7	3
L/R	3	4
Total	31	13

At the end of the differential segment the chromatids are separated and sometimes a small loop is formed, because the ends are still associated while the chromatids at the adjacent surterminal region are repelled. Usually, however, the differential segment appears as a uniform, thin and not well-stained thread. Its shape varies, drawn out diagonally to the pairing segment or its end appears to be attracted towards the end of the short arm of the *Y*-chromosome, though no actual association of the two was found.

At first meiotic anaphase the differential segment of the *X* can be seen distinctly (Text-figs. 7*a*, *b*, *c*, *d*, 8*a* and Pl. VIII E). Its length is decreased and the proximal region becomes thicker. The two chromatids lie far apart at the distal region. In several instances it was possible to determine the type of the previous metaphase association of *X* and *Y*, by analysing primary spermatocytes showing anaphases.

TABLE IV

Frequency of the various sex-bivalents

	Metaphase			First anaphase		
	Asymmetrical	Symmetrical	Total	Asymmetrical	Symmetrical	Total
	206	47	253	87	24	111
Percentage	81·6	18·4	—	77	21	—

A comparison of the data observed at metaphase and anaphase shows a consistent behaviour of the *XY* bivalent with respect to the number and position of chiasmata.

There is no interval between the first anaphase and second metaphase, the second metaphase plate being formed soon after the members of the bivalents have arrived at the opposite poles. The differential segment is still recognizable if the *X*-chromosome has undergone pre-reduction

(Text-fig. 8*b*, *c*). The differential segment is very slender and the two chromatids can clearly be seen lying apart. The thin threads, which

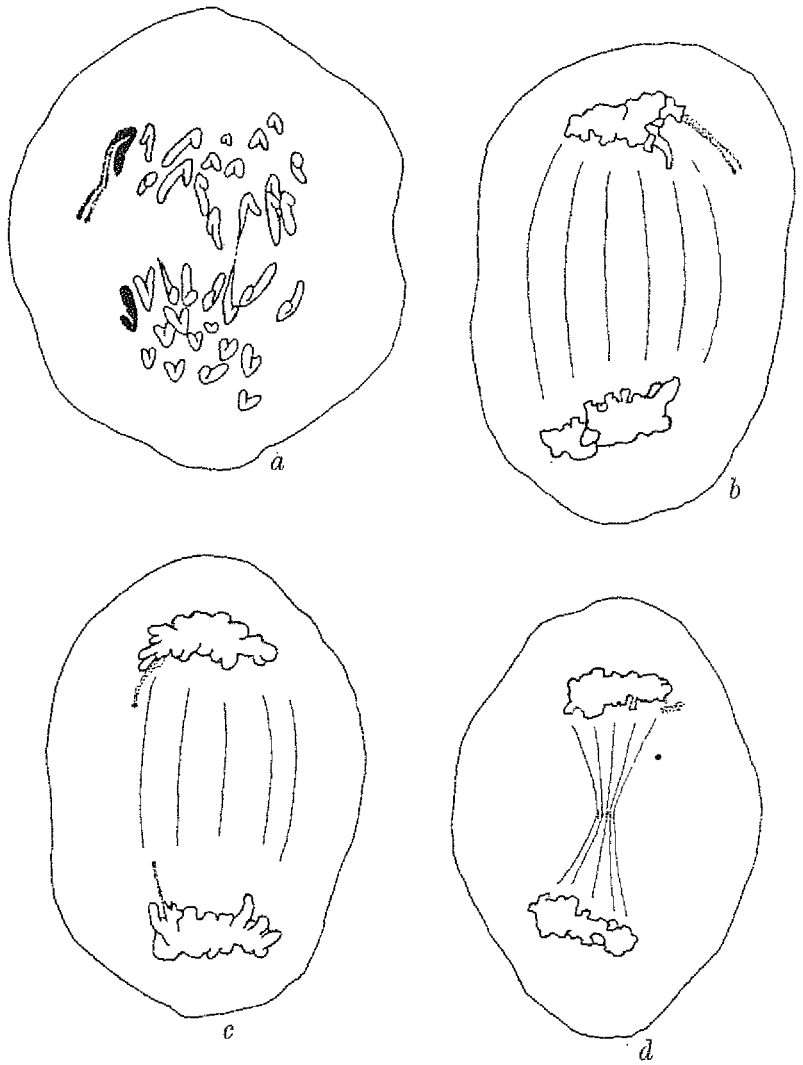


Fig. 7. *a*, early first meiotic anaphase; *b*, *c*, *d*, late anaphase, showing the differential segment of the asymmetrical XY bivalent. ×2·500.

represent the chromatids, have a tendency to fold back on the adjacent region of the pairing segment.

In those spermatocytes in which the sex-bivalent divides post-reductionally, the differential segment of the dividing XY could not

always be identified. Few second-metaphase plates were encountered where a large chromosome shows a small terminal knob, which may be interpreted as the coiled, single differential segment of the *X*.

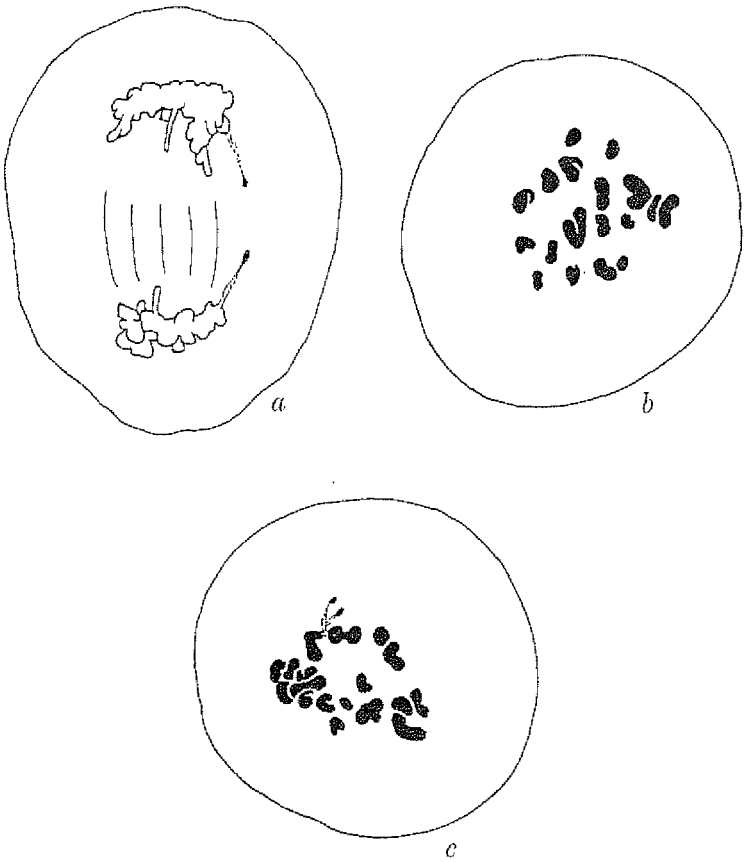


Fig. 8. *a*, late first meiotic anaphase, showing the post-reductional segregation of the symmetrical sex-bivalent; *b*, *c*, second meiotic metaphase. ×2·500.

In the globular spermatid nucleus, several deeply stained inclusions are present. Their shape, size and number are very variable. It was found that their distribution is random; hence spermatids with different sex potencies, carrying the *X* or *Y* chromosome respectively, could not be distinguished by morphological structure.

Discussion

During mitosis no significant difference was observed in the length of the two largest chromosomes of the complement, which are assumed to be the X and Y. These chromosomes have a subterminal centromere and consequently are composed of a long and a short arm. Measurements in five spermatogonial metaphases have shown that the short arm of the X, which is made up of a pairing and a differential segment according to the structure of the XY bivalent at meiosis is as long or nearly so as the short arm of the Y, in which the differential segment is entirely absent. In order to explain the discrepancy between the behaviour of the differential segment during mitosis and meiosis, one may assume that during mitosis it is (*a*) either completely absent, or (*b*) contracted to a maximal degree.

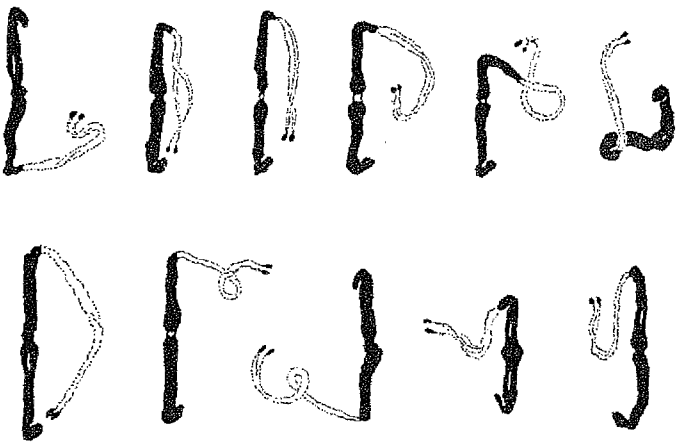
In the hamster during meiosis the differential segment develops from a nucleolus-like diffuse portion of the XY complex and remains associated with the pairing segment throughout the cycle of meiosis. It was found

Fig. 9. The various configurations of the symmetrical XY bivalent. ×2·500.

that the appearance of the diffuse part invariably coincides with the formation of the sex complex, which suggests that the differential segment constitutes a permanent portion of the X-chromosome and it is not an independent nuclear aggregate, associated with the largest bivalent only during meiosis.

It was found in various genera of Marsupials (Koller, 1936*b*) that the sex-chromosomes are much longer at meiosis than at mitosis and it was suggested that the difference in size is due to the presence of the non-condensed segment, which has a specific function during meiosis and represents the uncoiled differential segment. It is not represented as a whole during mitotic division. The differential segment of the sex complex in the hamster is fully developed at meiotic metaphase (Text-figs. 9 and 10). It is a thin, double thread 4–6 μ long and exhibits little staining capacity; the paired segment of X and Y is about 4·5–5 μ long, has a greater diameter than the differential segment and is deeply stained. These facts indicate that there is a difference in the internal

organization between the pairing and the differential segments. The pairing segment, as the external structure and behaviour suggest, has coils of the same type as the autosomal bivalents, while the differential segment, on the contrary, forms minor coils only, i.e. its condition is similar to that found in chromosomes during the early mitotic prophase.



Fi 10. Various types of the asymmetrical sex-bivalent. ×2·800.

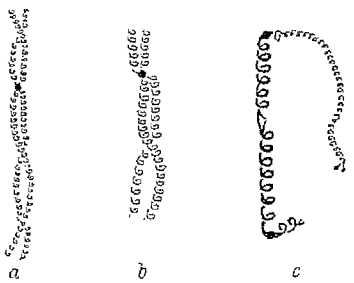


Fig. 11. Diagram showing the method of coiling: *a*, at mitotic prophase; *b*, at mitotic metaphase; *c*, in the *XY* bivalents of the hamster.

In plants the minor coiling of somatic chromosomes is initiated at prophase before the old minor or relic coils have been eliminated. When the relic coils are eliminated, the prophase chromosomes are tightly coiled and their length is considerably reduced. Before metaphase the diameter of the chromosome is increased and the length further decreased, presumably by a decrease in the number of gyres and an increase in the diameter of the remaining ones, without a great change in the pitch of the spiral (Text-fig. 11*a*, *b*) (Husted, 1937).

Since in the hamster the length and diameter of chromosomes are about the same at meiosis and at mitotic metaphase, major coils, such as are characteristic of many meiotic chromosomes in plants, are probably absent. The meiotic as well as the mitotic chromosomes are both spiralled in the form of minor coils.

The development of the minor spiral in the pairing segment of the *X*-chromosome takes place precociously, but its development in the differential segment is delayed, so that at metaphase this portion of the chromosome shows the prophase type of minor coiling, i.e. the gyres are smaller in diameter and more numerous than in the pairing segment (Text-fig. 11*c*). Presumably the presence of the nucleolar-like diffuse substance in association with the differential segment may be a factor in the delayed development of this portion of the sex-chromosomes.

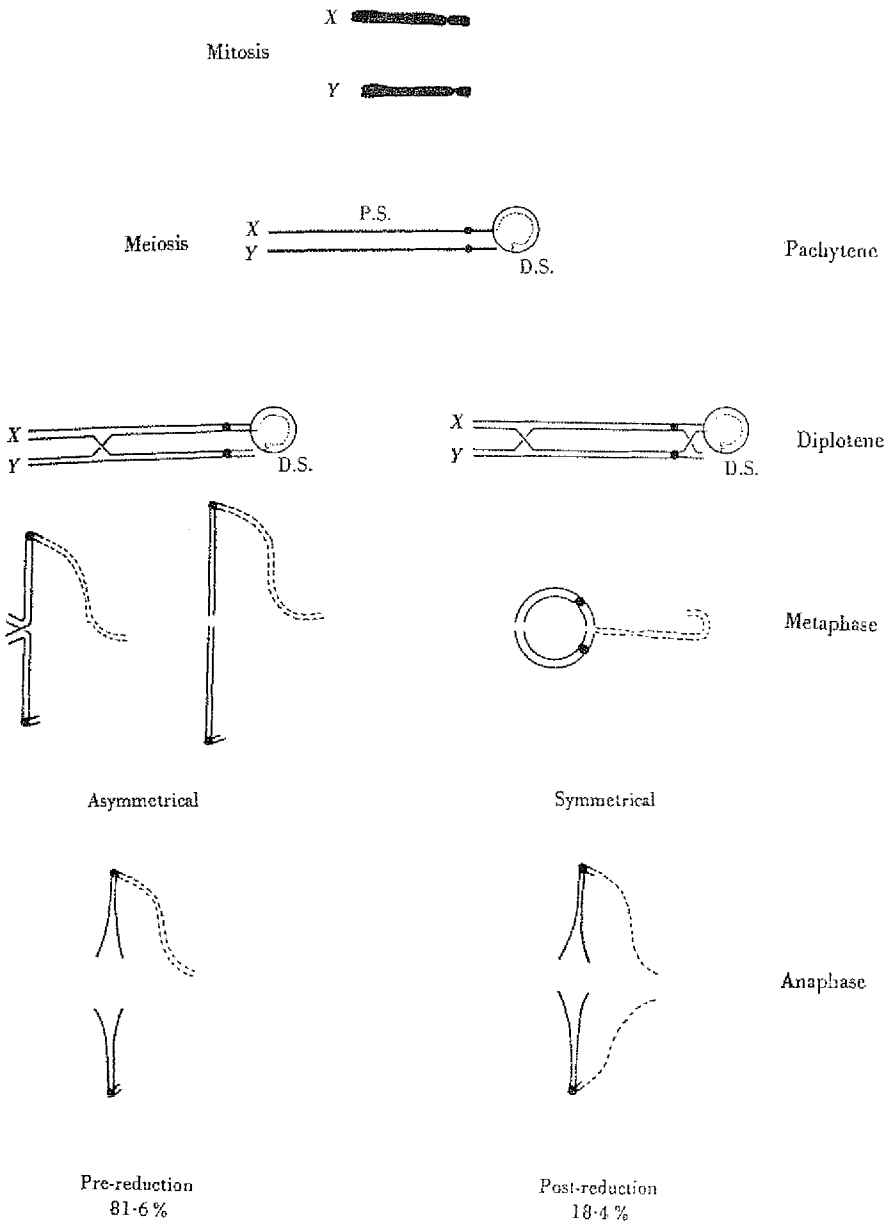
The analysis of the structural properties of the two segments has shown that there is a great time lag in the behaviour of the different parts of the sex complex. The pairing segment of both chromosomes is very precocious; the association of the pairing segments of *X* and *Y*, chiasma formation, spiralization, condensation to a maximum degree, are followed in rapid succession and are completed at pachytene.

The differential segment first appears as a nucleolus-like part of the *XY* complex after the completion of those processes in the pairing segment. It is very probable that the frequent failure of chiasma formation is due to the strong precocity, characteristic of the pairing segment.

The locus of the centromere was determined by analysing the distribution of chiasmata and consequently the type of metaphase bivalent. While the presence of the symmetrical sex-bivalent indicates that the centromere lies in the pairing segment, its locus within this segment can be more exactly determined by counting the number of the asymmetrical and symmetrical *XY* bivalents at metaphase. It was found that 81·6% of the primary spermatocytes have an asymmetrical and 18·4% have a symmetrical *XY* bivalent, which indicates that the length of the two segments, separated by the centromere, is about 1 : 4. The structure and behaviour of the sex-chromosomes during meiosis is illustrated in Text-fig. 12.

During meiotic metaphase the *XY* complex very frequently lies off the equatorial plate, and sometimes is left outside the spindle unorientated. This position suggests a profound change in the time relationship between the centromere of the sex-chromosomes and the centrosome.

The behaviour of the differential or non-condensed segment may be



—— Pairing segment

----- Differential segment

Fig. 12. Diagram illustrating the structure and behaviour of the sex-chromosomes in hamster during meiosis.

compared to that of the genetically inert region of the X- and Y-chromosomes in the salivary gland nuclei of *Drosophila*. According to Muller & Gershenson (1935), the inert region of the Y has the property of breaking at very few points; the genes are united in blocks which do not change their linear arrangement as readily as in the active regions of the chromosomes. It is assumed that the precociously condensed segment represents the genetically active region in the sex-chromosome of Marsupials. On the other hand the non-condensed, diffuse portion is probably inert or contains only those genes which have a very specialized function in sex differentiation. If this is accepted, then the genetically active region in the XY-chromosomes of the hamster is very large; it is calculated to be more than 50 morgans long and consequently it must contain a great number of genes. Those genes which are near the differential segment will exhibit partial sex-linkage, while those localized at the distal end of the long arm of the pairing segment will show very little or none at all.

Summary

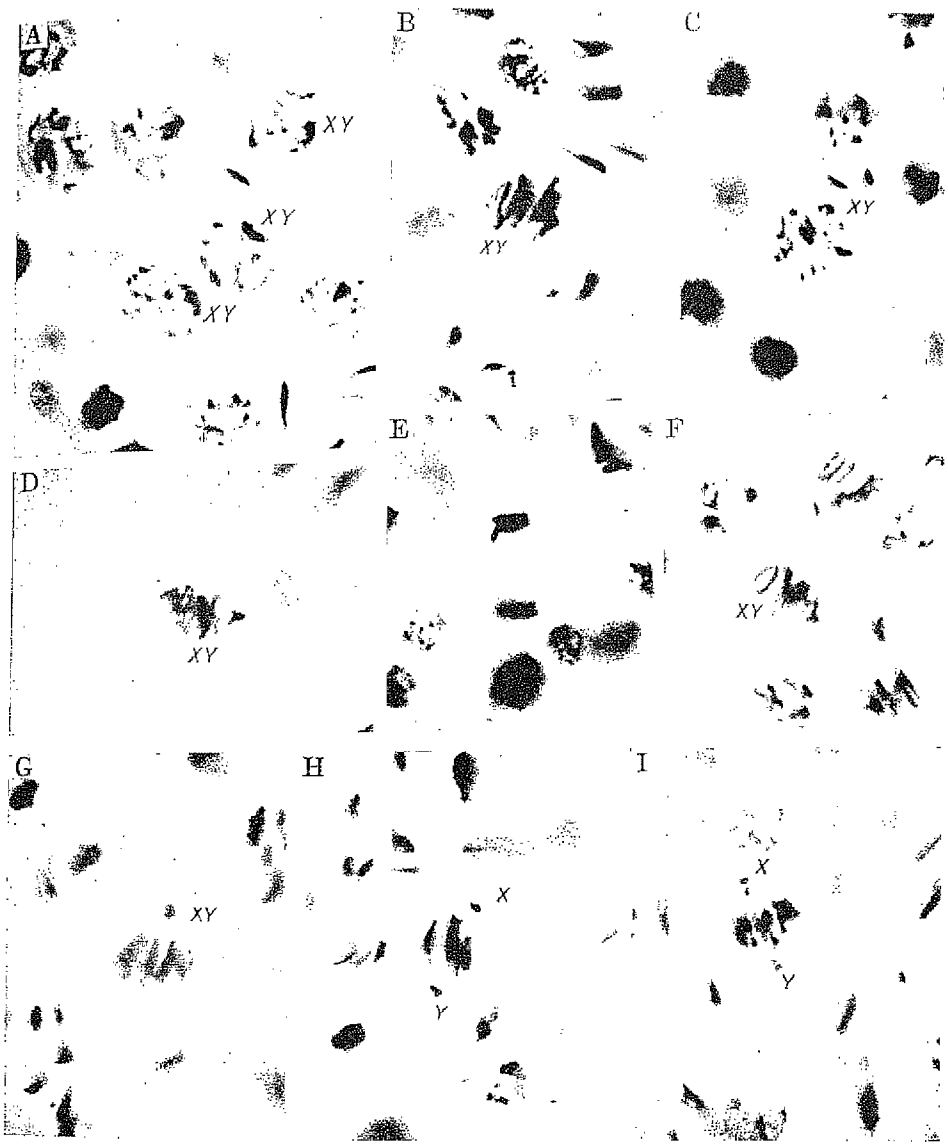
1. The diploid number of chromosomes in the golden hamster (*Cricetus auratus*) is 38. The chromosomes at mitotic metaphase show differences in size and shape.

2. The sex-chromosomes are the largest of the complement. The X- and Y-chromosomes of the male are morphologically very alike and show no significant size difference. They are approximately 4·5–5 μ long, and have a subterminal centromere. The ratio of the length of the two arms is about 1 : 4.

3. During meiotic prophase the sex complex is composed of two regions; one a precociously condensed, the other non-condensed or diffuse. The former is identified as the associated homologous or pairing segments of the X and Y; the latter gives rise to the differential segment of the X.

4. Two types of sex-bivalent are formed with unequal frequency at meiotic metaphase; asymmetrical (81·6 %), indicating pre-reduction, and symmetrical (18·4 %) conditioning the post-reductional segregation of the differential segment.

5. The centromere is localized in the pairing segment; consequently chiasmata, representing genetical crossing-over between the X- and Y-chromosomes, can be formed on both sides of the centromere. At metaphase the chiasma-frequency in the sex-bivalent is less than in the autosomal bivalents.


A
XY
XY
XY
B
XY
C
XY
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Y

6. The sex-chromosomes are unpaired or fail to form chiasmata after pairing in about 8% of the primary spermatocytes. The failure is brought about by the strong precocity of the pairing segment.

7. Analysis of the morphological structure and behaviour of the differential segment suggests that at meiotic metaphase it has minor relic and interchromatid relational coiling. Coils, which are characteristic of the pairing segment and of the autosomal bivalents, are absent.

8. It is predicted that in hamster there are great numbers of genes, which will exhibit very little sex-linkage, though they are borne in the sex-chromosomes. These genes are localized in the pairing segment of the *X* and *Y*.

REFERENCES

BRUCE, H. M. & HINDLE, E. (1934). "The golden hamster *Cricetus (Mesocricetus) auratus* Waterhouse. Notes on its breeding and growth." *Proc. zool. Soc. Lond.* Part 2, pp. 261–366.

HEITZ, E. (1931). "Nukleolen und Chromosomen in der Gattung *Vicia*." *Planta*, **15**, 495–505.

HUSTED, L. (1937). "Relational coiling in *Tradescantia*, *Allium* and *Vicia faba*." *Cytologia* (in the Press).

KOLLER, P. C. (1936*a*). "Chromosome behaviour in the male ferret and mole during anoestrus." *Proc. roy. Soc.* B, **121**, 192–206.

—— (1936*b*). "The genetical and mechanical properties of the sex-chromosomes. II. Marsupials." *J. Genet.* **32**, 451–72.

KOLLER, P. C. & DARLINGTON, C. D. (1934). "The genetical and mechanical properties of the sex-chromosomes. I. *Rattus norvegicus*." *J. Genet.* **29**, 159–73.

MULLER, H. C. & GERSHENSON, S. (1935). "Inert regions of chromosomes as the temporary products of individual genes." *Proc. nat. Acad. Sci., Wash.*, **21**, 69–75.

OGUMA, K. (1934). "A new type of the mammalian sex-chromosome, found in a field mouse, *Apodemus speciosus*." *Cytologia*, 5, 460–71.

EXPLANATION OF PLATE VIII

A. Meiotic prophase, showing the precociously contracted *X Y* complex. × 1·500.

B, D, F. Meiotic metaphase. The *X Y* bivalent is asymmetrical. × 1·500.

C, G. Meiotic metaphase with symmetrical *X Y* bivalent. × 1·500.

E. Meiotic anaphase showing the differential segment of the asymmetrical *X Y*. × 1·500.

H, I. Unpaired *X*- and *Y*-chromosomes at meiotic metaphase. × 1·500.