THE GENETICAL AND MECHANICAL PROPERTIES OF THE SEX CHROMOSOMES

VII. *APODEMUS SYLVATICUS* AND *A. HEBRIDENSIS*

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(With Nine Text-figures)

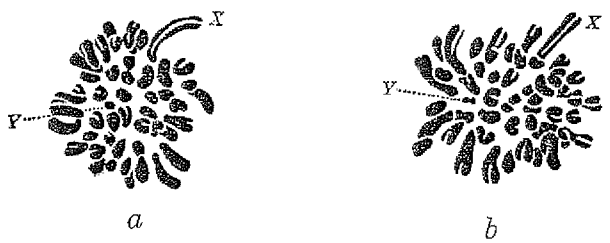
Introduction

A study was made of the chromosome complements in the male sex of two species of *Apodemus*, *A. sylvaticus*, the common field-mouse, which inhabits the mainland of Britain and is also commonly found in Central Europe (Brohmer, 1929; Heinrich, 1929), and *A. hebridensis*, which is restricted in its distribution to the Outer Hebrides, off the north-west coast of Scotland (Koller, 1939).[1] Besides the specific morphological differences, such as coat colour, size, etc., a difference was detected in the sex-determining mechanism. The structural peculiarities of the sex chromosomes in *A. sylvaticus* and in various other species excluding *A. hebridensis* have been described by several cytologists (cf. Oguma, 1934; Tateishi, 1934, 1935; Matthey, 1936*a*, *b*, 1938; and Raynaud, 1936), and attempts have been made to interpret the behaviour of the sex-determining mechanism during meiosis. These interpretations are based upon assumptions which are contradictory not only to the interpretation put forward by the present author to explain the genetical and mechanical properties of sex chromosomes in various mammals (mouse, rat, golden hamster, mole, ferret, squirrel, marsupials, man), but also to those principles which are shown, by the most extensive cytogenetical investigations, to govern chromosome behaviour in general. Because of the repeated appearance of these erroneous conceptions (see Minouchi, 1928; Oguma, 1934, 1937; Matthey, 1936*a*, *b*, 1938), a correction was felt to be imperative; hence the present paper, besides describing the structural differentiation in the sex chromosomes of *A. sylvaticus* and *A. hebridensis*, will deal in general with the controversial subject of the structure and behaviour of the morphologically unequal sex chromosomes.

[1] The author is greatly indebted to Mr T. Warwick, of the Royal (Dick) Veterinary College, Edinburgh, for kindly supplying him with the material.

STRUCTURE AND BEHAVIOUR OF THE SEX CHROMOSOMES DURING MEIOSIS AND MITOSIS

The diploid chromosome number in the male sex is 48 in both species (Fig. 1*a*, *b*). While the same number was counted in *A. sylvaticus*, *A. flavicollis*, *A. agrarius* (Matthey, 1936*b*; Raynaud, 1936), *A. semotus*, *A. agrarius ningpoensis*, and *A. speciosus speciosus* (Tateishi, 1934, 1935), Oguma (1934, 1937) claims that only 47 chromosomes are present in the spermatogonia of *A. speciosus ainu* and *A. geisha*. At mitotic metaphase the chromosomes show great variation in size; large, medium-sized and small chromosomes can be distinguished in both species under discussion. The length of the chromosomes varies between 5·5 and 1 μ. The position of the centromere is apparently nearly terminal because all the chromosomes are rod-shaped. An analysis of bivalent configurations during meiosis also shows that the loci of the centromeres are not terminal but



a *b*

Fig. 1. Mitotic metaphase in *A. sylvaticus* (*a*) and in *A. hebridensis* (*b*). (×3200.)

subterminal. Though the short arm may not be identifiable at mitotic metaphase, it is easily recognized in the larger chromosomes during meiosis. Contrary to the author's observations, Matthey (1938) is of the opinion that the majority of the chromosomes in *A. sylvaticus* as well as in *A. agrarius* are "telomitic" or telocentric, having a terminal centromere. Recent observations concerning the function and structure of the centromere (Darlington, 1939*a*, 1940) have shown that telocentric chromosomes are the result of misdivision of the centromere and, as a consequence of this, exhibit abnormalities which lead to their elimination in the course of evolution. Erroneous interpretations of mammalian chromosome structure are due to their great variability, attributable only to fixation. The primary constrictions, which are assumed to represent the loci of the centromeres, are particularly affected by fixation. Descriptions of telocentric chromosomes are often given by those investigators who have satisfied themselves with a limited number

of observations and have failed to compare chromosome structure with chromosome behaviour during mitosis and meiosis (Matthey, 1938).

An analysis of chromosomes in the diploid complex of *A. sylvaticus* and *A. hebridensis* has shown that the smallest and one of the largest chromosomes have no corresponding partners. This unequal chromosome pair is assumed to represent the sex chromosomes, *X* and *Y*. It was furthermore discovered that the two species differ in respect of the *Y*-chromosome, it being at least twice as large in *A. sylvaticus* as in *A. hebridensis* (Fig. 1*a*, *b*). This appears to be the only detectable difference in the chromosome morphology of the two species.

In both species, during the leptotene and the zygotene stages of meiosis, a nucleolus-like structure can be distinguished as a permanent constituent of the nucleus. Its shape and size vary and it contains two regions, one stained deeply and the other lightly.

By following the behaviour of this structure in successive stages of meiosis, it was ascertained that it is the "sex-chromosome nucleolus", and that it represents the two associated and precociously condensed sex chromosomes. The structural differentiation of the sex-chromosome nucleolus is clearly discernible during the growth period of the meiotic prophase, when the autosomal bivalents temporarily lose the ability of staining, but the sex chromosome nucleolus stains and shows its double structure (Fig. 2). No difference was observed in the form and behaviour of the sex chromosome nucleolus in *A. sylvaticus* and *A. hebridensis* during the prophase of meiosis.

At the first meiotic metaphase in both species the *XY* complex has a well-differentiated structure; it is composed of a deeply stained terminal region to which a thin, diffuse, lightly stained thread of varying thickness is attached (Figs. 3, 4). The size of these two regions of the *XY* varies in different primary spermatocytes, indicating a time-lag in the behaviour of the different parts of the sex bivalent. As mentioned previously, the *X*- and *Y*-chromosomes are represented by an unequal chromosome pair in the diploid chromosome complement, and hence during meiosis they necessarily form an unequal bivalent which is made up of a pairing and a differential segment. Such bivalents are easily recognizable on account of their asymmetrical shape, and have been reported by various investigators in widely different organisms (Robertson, 1916; Wenrich, 1916; Carothers, 1926, 1931). But it is known now that unequal chromosomes may form an apparently "symmetrical" bivalent; the shape of the metaphase bivalent is determined by the position of the pairing segment in relation to the centromere and differential segment

Fig. 2. Various configurations of the precociously condensed sex-chromosome during the growth period of meiotic prophase. (×3200).

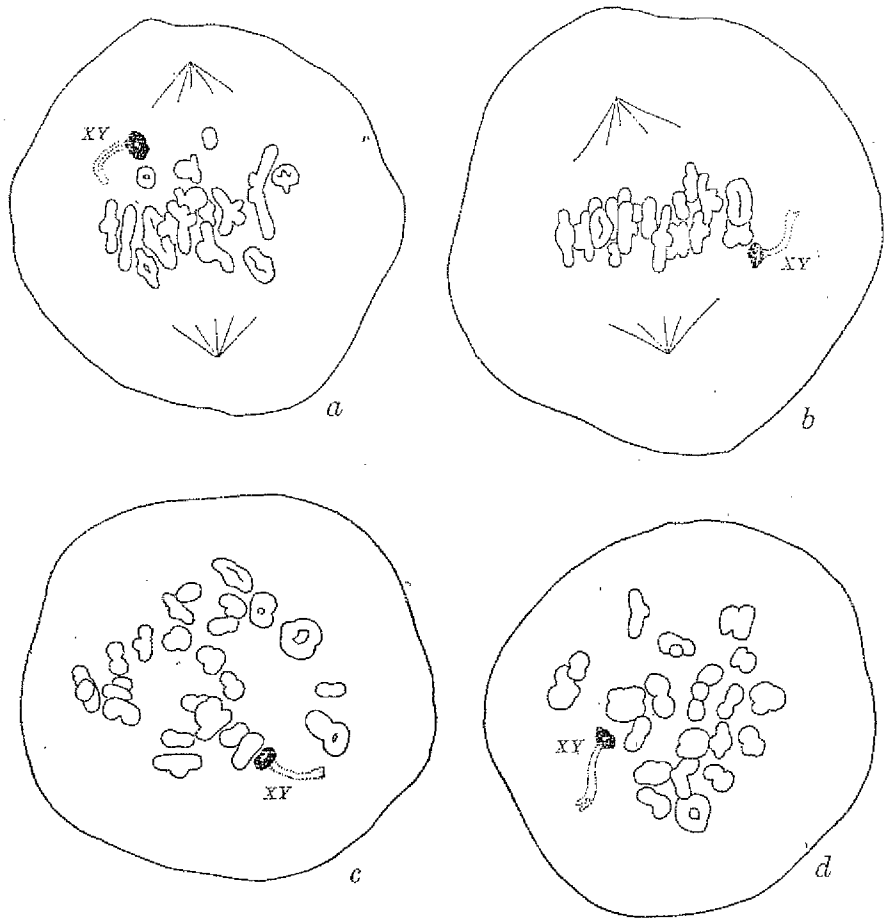


Fig. 3. Side view (*a*, *b*) and polar view (*c*, *d*) of first meiotic metaphase in *A. sylvaticus*, showing the deeply stained and diffuse regions of the sex bivalent. (×3200.)

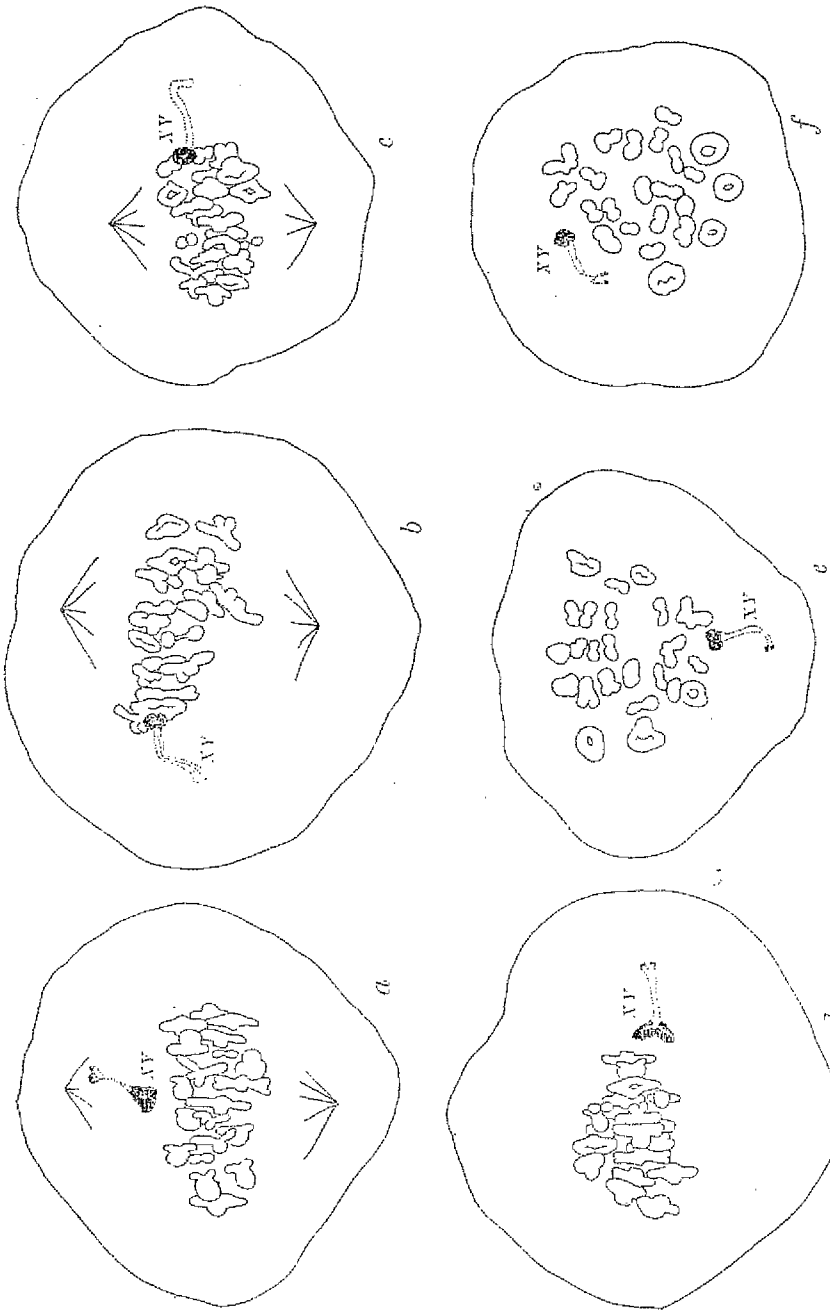


Fig. 4. Side view (*a*, *b*, *c*, *d*) and polar view (*e*, *f*) of first meiotic metaphase in *A. hebridensis*. The sex bivalent is very similar in form to that in *A. sylvaticus*. (×3200.)

(Koller & Darlington, 1934). The XY bivalent of *A. sylvaticus* and *A. hebridensis* closely resembles the symmetrical sex bivalent, first observed in the rat (Koller & Darlington, 1934) and later identified in several other organisms (Koller, 1937).

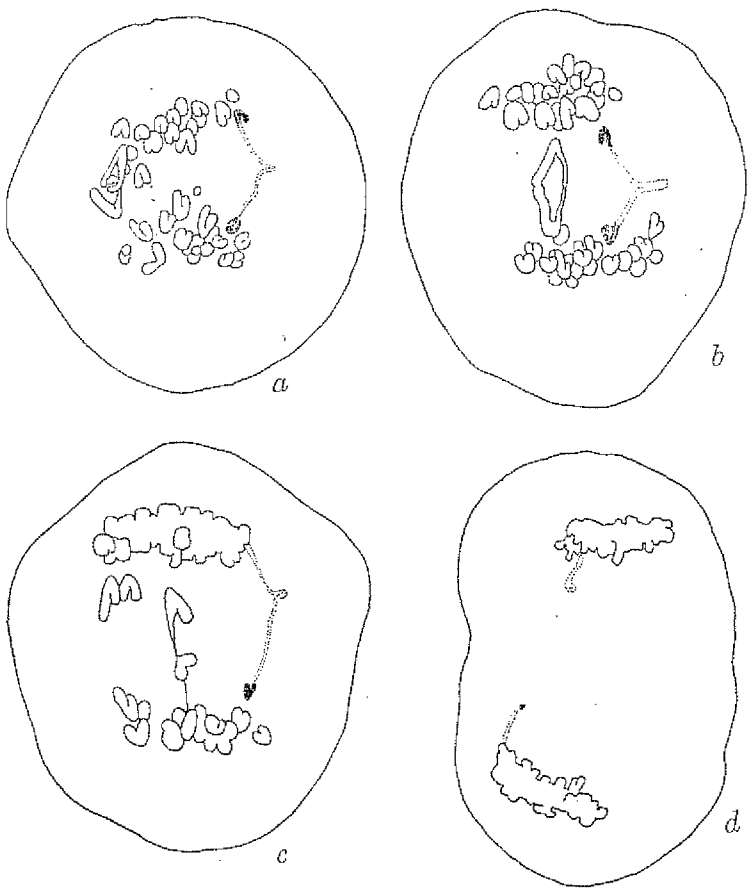


Fig. 5. First (*a*, *b*) and second (*c*, *d*) meiotic anaphase in *A. hebridensis*. The segregation of the *X*- and *Y*-chromosomes is invariably post-reductional. (×3200.)

Though the configuration of the XY bivalent at meiotic metaphase is very similar in both species, the method of segregation of the *X*- and *Y*-chromosomes during first meiotic anaphase is different. In *A. hebridensis* the sex bivalent always divides equationally at the first anaphase (Fig. 5), and reduction of the X and Y takes place at the second meiotic anaphase; in *A. sylvaticus* about 8% of the primary spermatocytes show

reductional segregation of the X and Y during the first anaphase (Figs. 6, 7), i.e. while pre-reduction of X and Y is facultative in *A. sylvaticus*, it is obligatory in *A. hebridensis*. The behaviour of the

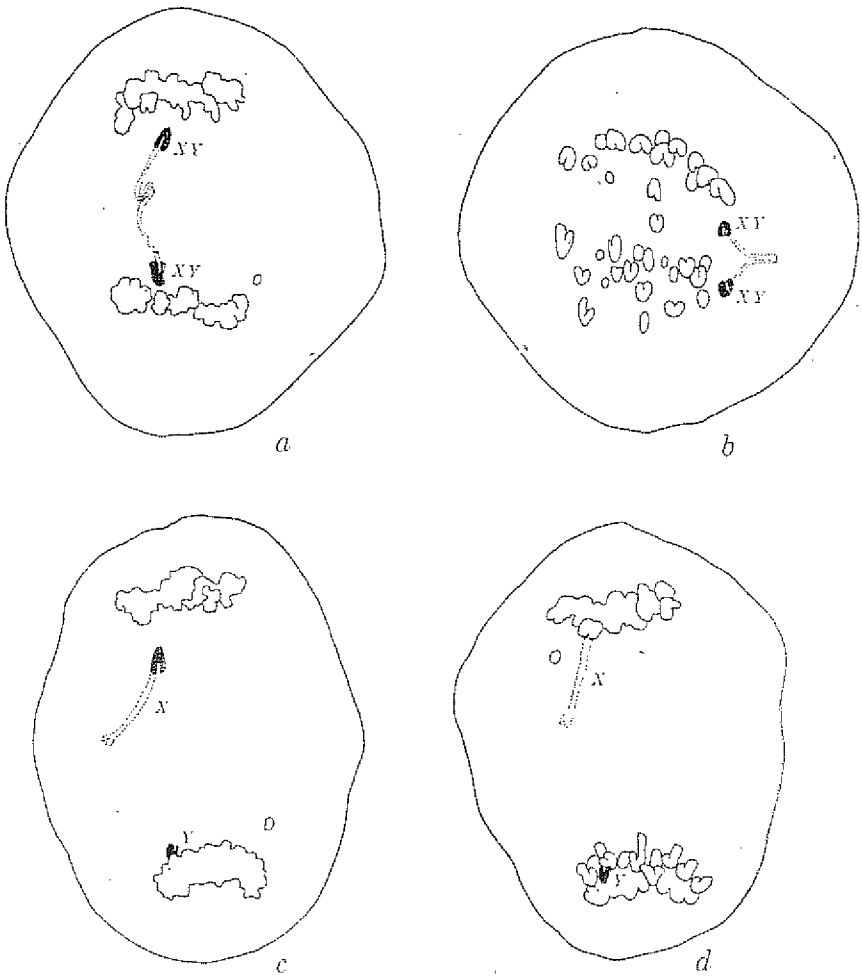


Fig. 6. First meiotic anaphase in *A. sylvaticus* showing post-reductional (*a*, *b*) and pre-reductional (*c*, *d*) segregation of X- and Y-chromosomes. (× 3200.)

sex bivalents in *A. agrarius* and *A. flavicollis* and *A. agrarius ningpoensis* is similar to that observed in *A. sylvaticus*. It is interesting to note that no visible differences were detected in the configuration of the pre- and the post-reductional sex bivalents of *A. sylvaticus*. This observation

clearly indicates that an unequal chromosome pair may form bivalents which are similar in appearance but different in internal structure.

The behaviour of sex bivalents at meiotic metaphase and anaphase enables us to determine the structure of these sex chromosomes. It is

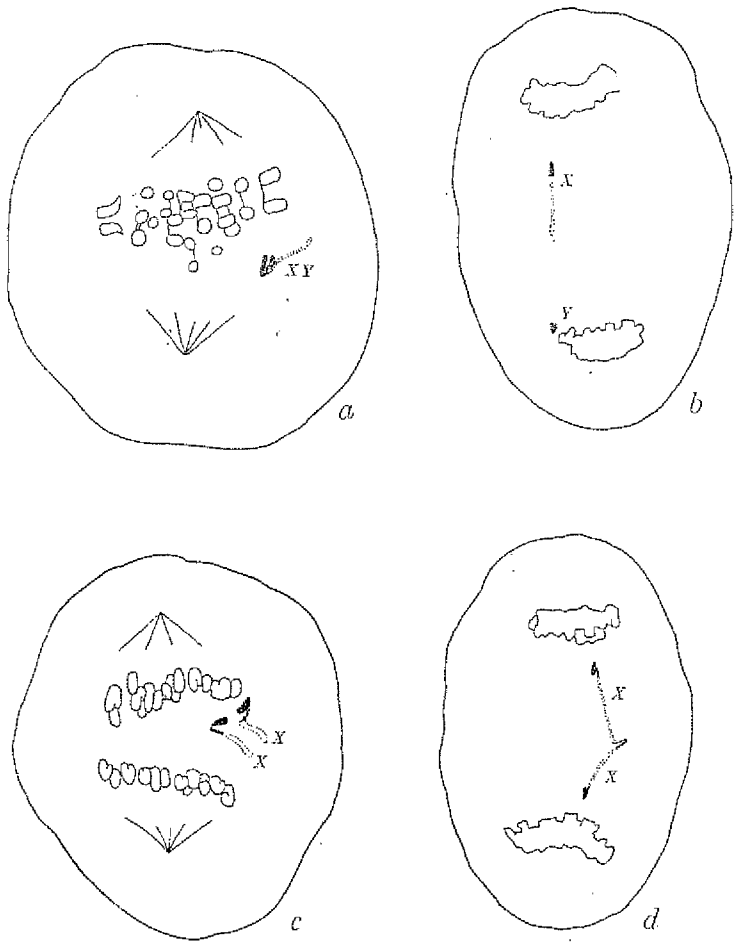


Fig. 7. Second meiotic anaphase and telophase in *A. sylvaticus*, showing post-reduction (*a*, *b*) and pre-reduction (*c*, *d*) of the *X*- and *Y*-chromosomes. (×3200.)

accepted that metaphase association of chromosomes and their segregation at anaphase are normally conditioned by chiasmata formed during meiotic prophase (cf. Darlington, 1937). It has already been demonstrated that the sex chromosomes (with a few exceptions) are subject to the same mechanism of chromosome pairing and post-pachytene

association as other chromosomes (Koller, 1937). A structurally unequal chromosome pair such as the *X* and *Y* of *A. sylvaticus* and *A. hebridensis* is divided into a pairing and a non-pairing or differential segment. Chiasmata are formed only in the pairing segment, the differential segment having no partner. The position of the pairing segment in relation to the centromere and to the differential segment is the factor which determines the meiotic behaviour, particularly the pre- or post-reductional segregation of the unequal chromosomes. The existence of both kinds of segregation in *A. sylvaticus* indicates that the centromere is interstitial and that chiasmata may be formed on either side. The pairing segment is divided by the centromere into a distal (or outer) and a proximal (or inner) portion; the latter is proximal, the former distal, to the differential segment. Crossing-over or chiasma formation in the outer pairing segment leads to pre-reductional segregation, while crossing-over in the inner pairing segment is responsible for the post-reduction of the *X* and *Y*. Segregation of one type only indicates the presence of one pairing segment. Thus, the complete lack of pre-reduction of *X* and *Y* in *A. hebridensis* suggests that only the proximal pairing segment is present in the *X* and *Y*, or that, if a distal pairing segment is present, it is so short that no chiasma is ever formed in it. When both kinds of segregation of *X* and *Y* are encountered during the first meiotic anaphase, the relative lengths of the distal and proximal pairing segments may be estimated by the frequencies of pre- or post-reduction in the primary spermatocytes. In *A. sylvaticus* 8% of these show pre-reduction and 92% post-reduction; consequently we may infer that the distal pairing segment is very short compared with the proximal one. In *A. sylvaticus* the apparently similar configuration of the pre- and post-reductional sex bivalents during metaphase may be explained by assuming that the repulsion which normally operates between pairs of homologous regions lapses in the proximal pairing segment of the *X* and *Y*. It is suggested that the distal region of the *Y*, though no chiasma is formed in this segment, remains joined to the corresponding region of the *X*-chromosome. This similarity of the pre- and post-reductional *XY* bivalent during metaphase is responsible for the erroneous interpretations which have been put forward by several investigators (see Matthey, 1938). A detailed analysis of the behaviour of the *XY* bivalent during the various stages of meiosis, however, enables us to reconstruct the internal differentiation of the sex chromosomes in *A. sylvaticus* and *A. hebridensis*, and this is illustrated in Figs. 8 and 9.

Discussion

It can be seen from the description given above that the sex chromosomes in *Apodemus* are subject to the laws which govern chromosome behaviour in general. The variations seen in the behaviour of the *X* and *Y* are only secondary results of the internal and external differentiation

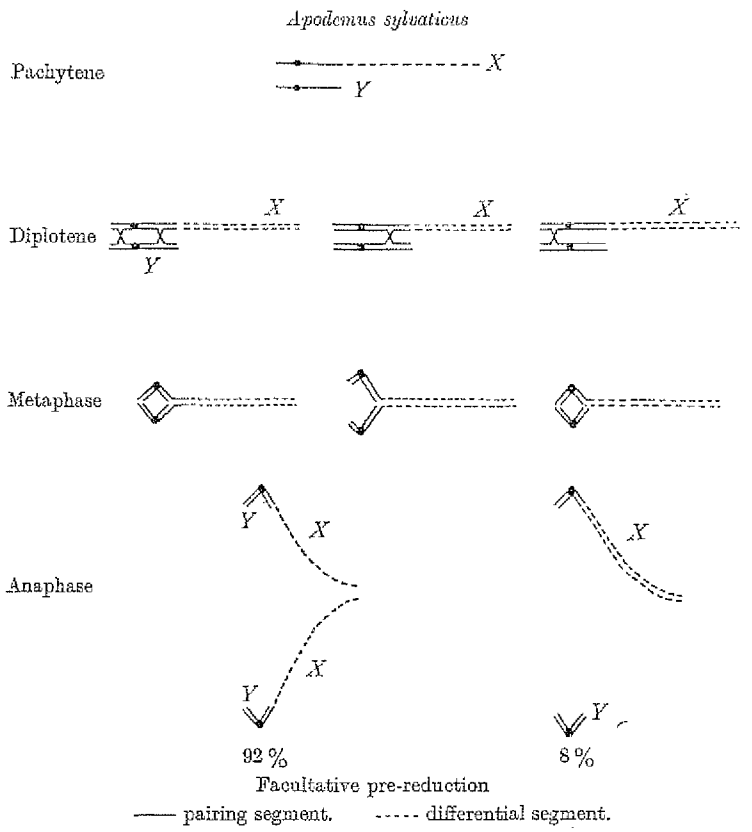


Fig. 8. Diagram illustrating the structure of the sex chromosomes in *Apodemus sylvaticus*. The pairing segment is divided by the centromere into a distal (outer) and a proximal (inner) region.

which occurred in these chromosomes. The changes in time relationship in the different regions of the sex chromosomes, expressed by precocity and heteropycnosis, result from a qualitative differentiation, while the size difference between the sex chromosomes is due to structural differentiation. It is shown that their segregation at meiosis depends on metaphase association which is conditioned by chiasma formation or

genetical crossing-over in the regions which are represented in the *X*- and *Y*-chromosomes. During the meiotic prophase and metaphase the position and number of chiasmata in the sex bivalent of *Apodemus* are obscured because of its peculiar properties resulting from qualitative differentiation. The segregation of *X*- and *Y*-chromosomes, however, can easily be followed at the first meiotic anaphase in various *Apodemus*

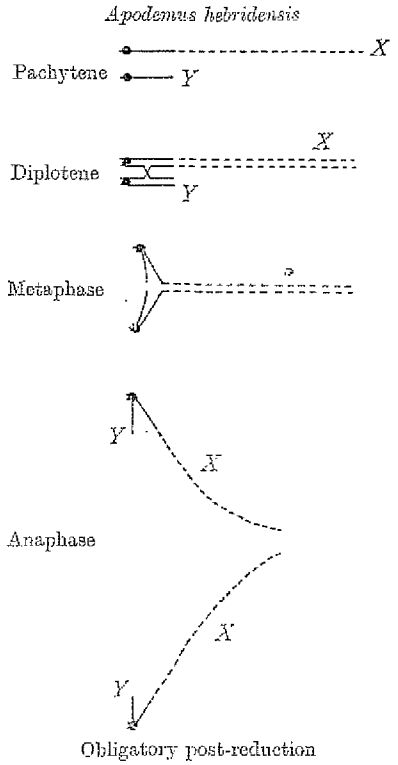


Fig. 9. Diagram illustrating the structure of the sex chromosomes in *Apodemus hebridensis*. The distal pairing segment is either absent or too small for chiasma to have formed.

species and has already been reported by several investigators (Matthey, 1936*a*, *b*; Oguma, 1934; Raynaud, 1936; Tateishi, 1935). Matthey alone made an attempt to explain how post- and pre-reductional segregation of the *X* and *Y* can occur in *A. sylvaticus* and *A. agrarius* (Matthey, 1936*a*, *b*, 1938), assuming that post-reduction is brought about by crossing-over between the paired *X*- and *Y*-chromosomes, while pre-reduction is due to lack of crossing-over. Other erroneous conceptions of Matthey are the association of non-homologous regions in the chromo-

somes, crossing-over of sister chromatids, and a temporary inactivity of the centromere during the first meiotic anaphase. The analysis of chromosome behaviour given above, however, has shown that there is no justification for attributing such peculiar properties to the sex chromosomes in order to explain the metaphase configuration and anaphase segregation of the *X* and *Y* in *Apodemus*.

It has already been mentioned that the diploid chromosome number in the male of various *Apodemus* species is 48, except in the two Japanese species, *A. speciosus ainu* and *A. geisha*, in which Oguma counted 47 chromosomes. According to him, in *A. speciosus ainu* the *Y*-chromosome is absent and the single *X* always divides equationally during the first meiotic anaphase. The configuration of the sex complex in this species during the first meiotic metaphase is similar to that found in *A. sylvaticus* and *A. hebridensis*, and it is more than probable that this represents not a single *X* but the *XY* bivalent. Tateishi (1935), in another Japanese species, *A. agrarius ningpoensis*, observed a chromosome configuration exactly similar to that found in *A. speciosus ainu*, which he identified as the *XY* bivalent. The only evidence which favours Oguma's assumption that the heterogametic sex in *A. speciosus ainu* and *A. geisha* is of the *X-O* type is that he actually counted 47 chromosomes in the spermatogonial division. It is, however, very probable that a *Y*-chromosome is present but is too small to be discerned during mitotic metaphase in the spermatogonia. This point has already been stressed by Matthey (1938), who also favours the view that *A. speciosus ainu* and *A. agrarius ningpoensis* are probably the same species.

The various *Apodemus* species may be arranged in the following series, according to the size of the *Y*-chromosome: *A. speciosus ainu*, with the smallest *Y*, *A. agrarius ningpoensis*, *A. geisha*, *A. speciosus speciosus*, *A. hebridensis*, *A. agrarius*, *A. flavicollis* and *A. sylvaticus*. The question may be asked how such differences in the size of the *Y*-chromosome of closely related species came about. It may be assumed that the differentiation of the sex chromosomes is a gradual evolutionary process, in which the following steps may occur: (1) the origin, by mutation, of a gene pair, or sex differentials; the segregation of these genes determines the two sexes; (2) suppression of crossing-over in the region where the sex differentials are localized; the suppression is genotypically controlled; (3) the development of the differential segment through structural changes in the region which contains the sex differentials.

The evolution of the sex chromosomes depends primarily on the

genetic history of the differential segments (Darlington, 1939*b*). In the homogametic sex, the differential segment of the *X* may cross-over while in the heterogametic sex the *Y* is excluded from crossing-over and consequently the size of the differential segment may alter not only as between related species but frequently within the same species. Thus the great variation observed in the size of the *Y*-chromosome in the various *Apodemus* species may have been brought about by suppression of crossing-over in the differential segment. To explain the difference in the method of segregation of the sex chromosomes of *A. hebridensis* and *A. sylvaticus* during the first meiotic anaphase, one must assume that another divergence has taken place, namely a change in the position of the centromere, which is located interstitially in the pairing segment. The change in the position of the centromere is brought about by a pericentric inversion (Muller, 1940) which includes the centromere; the distal breakage point is near the end of the chromosome, while the proximal is next to the centromere. This structural change will lead to the reduction of the distal or outer pairing segment to such an extent that it may be represented by only a very few genes, and crossing-over will be absent; hence a post-reduction of the *X*- and *Y*-chromosomes will be obligatory. We may visualize the differences in the structure and behaviour of the *X*- and *Y*-chromosomes in *A. sylvaticus* and *A. hebridensis* as being due to secondary structural changes, one of which involves the differential and the other, the pairing segment. It can be seen that an analysis of the structure and behaviour of *X* and *Y* in various species of *Apodemus* enables us to gain an insight into those processes through which the sex-determining mechanism has evolved.

SUMMARY

1. The diploid chromosome number in the spermatogonia of *Apodemus sylvaticus* and *A. hebridensis* is 48.

2. The sex chromosomes are unequal in size; the *X* is the largest chromosome in the complement, the *Y* is the smallest. The *Y* chromosome is smaller in *A. hebridensis* than in *A. sylvaticus*.

3. The sex bivalent shows a uniform symmetrical configuration during meiotic metaphase in both species.

4. In *A. hebridensis* post-reduction is obligatory, the *X* and *Y* segregate at the second meiotic anaphase. In *A. sylvaticus* 8% of the primary spermatocytes show pre-reduction, and 92% post-reduction, of the *X* and *Y*.

5. The behaviour of the *X*- and *Y*-chromosomes of *A. sylvaticus* during meiosis suggests that the pairing segment is composed of a distal and a proximal region, i.e. that the centromere is located interstitially and chiasmata can be formed on either side of it.

6. In *A. hebridensis* the behaviour of the *XY* bivalent suggests that the distal pairing segment is either entirely absent or is too small for chiasma to have formed, and hence segregation of the *X* and *Y* is always post-reductional.

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