Absence of the Y-Chromosome in the Vole, Microtus montebelli Edw.
with Supplementary Remarks on the Sex-Chromosomes
of Evotomys and Apodemus 1)

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(With 3 Text figures and one Plate)

Shortly after publication of the present writer's work (1935) on the sex-chromosomes of a vole, Evotomys bedfordiae, in which the absence of the Y-chromosome was observed, Matthey and Renaud (1935) produced a short account of the sex-chromosomes of Evotomys glareolus, announcing, in contrast to the present author's results, the existence of the Y-chromosome as a mate of the X, and emphasizing, at the same time, the idea of the constant occurrence of the XY-type of sex-chromosomes through all kinds of Mammalia. Such an idea has already been elaborated by many cytologists since Painter (1925) claimed it for the first time.

Adverse to such an opinion, the writer holds that the sex-chromosomes of Mammalia may sometimes be XY-type, or sometimes XO-type, as in the course of more widely extended observations examples were found in which the Y-chromosome is really absent. Especially among the rodents, Apodemus speciosus ainu (1934) and Evotomys bedfordiae (1935) exhibit XO-type of the sex-chromosomes as the writer believes in striking contrast to species belonging to the genera Rattus and Mus, in which also the XY-complex of the sex-chromosomes was found by the present writer.

It is not surprising to the writer to know that his statements obtained from Apodemus and Evotomys may sometimes be considered by some authors as if resulting from an erroneous interpretation, because, they certainly disturb to a great extent the idea of the constant occurrence of the Y-chromosome throughout a definite systematic group of animals. But the writer still feels, after repeated observation of his slides, that no revision or alteration of his ideas is necessitated. Furthermore, the writer's chromosome survey of Japanese wild Muridae has been extended to discover one more example in which the Y-chromosome is certainly absent. That is a

1) Contribution No. 111 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University.
vole possessing the scientific name, *Microtus montebelli* Edw.,<sup>1</sup> belonging to the subfamily Microtinae together with *Evotomys* and *Arvicola*. The vole lives in farms in the main island of Japan and is well known not only from its injurious effect on crops but also as the propagator of certain local epidemics. It is the chief purpose of the present paper to communicate what could be observed in the chromosomes of this species of Muridae, but some additional comments will be made upon previous results obtained from the studies on *Evotomys* and *Apodemus*, as they have connection with the present findings.

**Morphological analysis of spermatogonial chromosomes**

It will be sufficient to show here four metaphase plates of spermatogonia of the best fixation, selected from four different individuals, in order to determine the chromosome number 2n, characteristic to the vole now dealt with (Text fig. 1, A–D; Pl. 29, Figs. 1–3). Without the slightest ambiguity, thirty-one chromosomes (an odd number) can be counted in every single garniture shown in the figures. The same number is further proved in many other spermatogonia which are not here reproduced, and it will be noticed that this is one of the smallest number so far recorded from Mammalia (*vide infra*). In addition to such a small number, the chromosomes, as well as the cell in which they are included, are remarkably large in size. This, therefore, makes the counting very easy and allows further morphological analysis, by which the homologous mates are discriminated. The chromosomes assume V-shape in majority, in striking contrast to all other species of Muridae hitherto studied, except one case, *Arvicola scherman*,<sup>2</sup> in which similarly shaped chromosomes have recently been informed by Matthey and Renaud (1935). In the figures shown in Text fig. 1, the present writer has tried to label with a's to o's the thirty autosomes constituting fifteen pairs, after close comparison of their size and shape, and one X for the single sex-chromosome.

1) Material was collected and prepared at Niigata through the cordial assistance of Dr. K. Ikeda, to whom sincerest thanks are now expressed. Fourteen testes obtained from seven adult males were fixed by (a) Flemming without acetic acid and (b) Champy, but the best figures of chromosomes were confined in material treated with the former fluid. Sections were stained by iron haematoxylin after Heidenhain with counter staining of light green.

2) In their original paper Matthey and Renaud (1935) adopted the name *Arvicola scherman terrestris* L. But the species employed by them as the material of their study seems to have correctly the name *Arvicola scherman exitus* Miller, indigenous to Switzerland (cf. Miller's Catalogue of the Mammals of western Europe, pp. 746–749, 1912, and Hinton's Monograph of Voles, pp. 410–414, 1926).
Chromosome pair a. They are sharply characterized by their exceedingly huge size as compared with the remainder, presenting a V-shape with two arms of considerably different length, the fibre attachment being submedian. The longer arm usually extends twice as long as the shorter (In Text fig. 1, A, one of the a's, found in upper left hand quarter, appears to be mediomitic by the apparent shortening of the longer arm, of which the terminal part is bent in the direction of vision). In equatorial arrangement they are found in major cases at opposite positions in the peripheral zone though approaching in some instance as Text fig. 1, C.

Chromosome pairs b to k. These twenty chromosome constituting ten homologous pairs are also represented by V's but considerably smaller than the a's. They show gradatory diminution of length from b to k in alphabetical order, with the apicis of the V's directed towards the center of the equator. Every two chromosomes regarded as homologous usually lie near each other with occasional exceptions. Among these ten pairs some possess a median fibre attachment or mediomitic, having two arms of equal length, while some are of submedian attachment, having consequently two arms of different length. It is not absolutely certain at present how many pairs out of the ten belong to the former kind, but two pairs b and c, at least, seem invariably to have arms of equal length. In some of the latter kind a terminal constriction in the longer of one V can frequently be observed. A terminal portion, thus constricted off from the main bulk of the arm, becomes occasionally separated from the latter having an achromatic connection. Chromosome pairs d and g (Text fig. 1, C) for instance show very clearly such occurrence, and as the satellite structure in chromosomes of some rodents as squirrels as described by Cross (1931) is recalled.

Chromosome pair l. These chromosomes may sometimes be longer than the k, but they have a subterminal fibre attachment, instead of submedian as those of the preceding category, and thereby assume a J-shape with terminal bendings.

Chromosome pair m. They seem like telomitic rod-chromosomes with a constriction near one extremity in each. At the point of constriction they bend to give rise to the J-shape as chromosomes l, in approximately the same frequency with the case where they do not show bending (Compare m's in Text fig. 1, A–D). It is not certain at present whether they should be classed as telomitic chromosomes or atelomitic ones in which the spindle fibres should be found at the point of constriction.
Chromosome pair \( n \). They show also an ambiguous figure in respect to fibre attachment as do chromosomes \( m \). With most probability, however, they are telomitic rod-chromosomes having satellite structure at one extremity, although this structure becomes obscure when the chromosomes acquire heavy condensation (Text fig. 1, A).

Text fig. 1. *Microtus montebelli*. Chromosomes in metaphase of spermatogonial division (polar view). \( \times ca \) 5500.

Chromosome pair \( o \). The smallest chromosomes thus designated by the letter “\( o \)” are obviously rod-chromosomes with a terminal attachment of spindle fibre. They attain a length not more than one third that of chromosomes \( n \), and by this minor size they show so striking feature in a spermatogonial garniture as the \( m \)-chromosomes, which are not noticeable in any species of Muridae. Corresponding to the \( m \)-chromosomes in other kinds of animals these
"o" chromosomes of minute size always arrange themselves at the central region of the equatorial plate.

To summarize the above descriptions the whole thirty chromosomes in fifteen pairs are classified into three different kinds in their apparent configuration as follows:

V-chromosomes (V's), a, b, c, d, e, f, g, h, i, j, k.

J-chromosomes (J's), l, m.

Rod-chromosomes (R's), n, o.

In the V's the spindle fibre attaches beyond doubt at the bent point or on the apex of the V. while in the R's it is inserted at the terminal part as indicated telomitic. The J's, on the contrary, may or may not have the fibre attachment at the point of bending. In the l chromosomes for instance the fibre attachment seems with all probability to be subterminal, but in the m's the matter is different, for, in a large number of cases the entire body of the chromosome is stretched into a straight rod, showing no bending, and the terminal globular parts are invariably directed to the center of the equatorial plate as ordinary R's do, but in minor cases they clearly show bending and thereby present the J-shape. If the essential structure of the m's be telomitic, then the J-shape thus produced can not be accounted for otherwise than as a temporal and superficial figure, and it should be distinguished from the true J's of atelomic nature.

Sex-chromosome. After labelling a's to o's for the fifteen pairs of autosomes as mentioned above, there is eventually left a single chromosome destitute of its synaptic mate. This corresponds to the sex-chromosome as labelled "X" in the figures. The X-chromosome thus identified is found in major cases (though not absolutely) on the peripheral zone of equatorial arrangement of chromosomes. In respect to size it belongs to the chromosome of medium size with approximately similar magnitude to the J-chromosomes, and assumes, without exception, a V-shape, of which one arm is remarkably longer than the other arm. It has a characteristic of remaining somewhat vague in contour or not heavily condensed as compared with the autosomes. Moreover, special attention should be called to the fact that in the longer arm of this V-shaped X-chromosome are frequently visible two partial swellings of chromatin (Text fig. 1, C), which are almost universally observable in X-chromosomes of Muridae as well as in that of man, and will be referred to again later.

There is no other sex-chromosome corresponding to the Y-chromosome. The figures shown in Text fig. 1 are so clear as to show undoubtedly the absence of the Y, and in reality the diploid group of chromosomes does not involve a sex-chromosome in addition to the X, showing an odd number 31.
Tetrads and the X-chromosome in the primary spermatocytes

When observed from the pole of division the primary spermatocyte always shows sixteen chromosomes, of which fifteen are bivalent autosomes or tetrads and the remaining one is the univalent X-chromosome (Text fig. 2, A and B). The former present very characteristic and different forms from those of ordinary Rattus or Mus. A majority appear like thick crosses, or, to put it otherwise, one bivalent is composed of two thick V's connected with each other of their apices. A few, however, still take the form of horizontal rings which usually occur in descendants of simple rod-chromosomes found in other species of Muridae. The smallest chromosomes, the o's, are of course noticeable as the smallest bivalent with the appearance of a solid dumbbell.

In striking contrast to those bivalents described above, the X-chromosome remains in univalent condition destitute of its synaptic mate, and in good preparations it is sharply distinguished from the autosome bivalents not only by its V-shape but also by its less affinity to haematoxylin. In well differentiated preparations it takes a gray colour while the autosomes are stained deep black (Text fig. 2, A). Such a peculiarity in respect to stains has been known in many different kinds of animals covering both vertebrata and invertebrata. Before trying further descriptions of its morphology the autosomes will be described from the side of equatorial arrangement. In profile the essential structure of the bivalents will first clearly be understood, especially in rather strongly destained preparations (Text-fig. 2, C; Pl. 29, Fig. 4). In short, most of them belong to vertical ring-tetrads, similar to those of urodelan Amphibia, but there are produced many variations caused chiefly by the occurrence of chiasmata.

Now to continue about the morphology of the X-chromosome in metaphase. As already known from the polar view of the metaphase plate, the X-chromosome takes a V form, and the two component arms differ from each other in thickness as well as in length. The thicker arm is always longer than the thinner one, the former assuming a clavate shape while the latter usually keeps the same diameter throughout its entire length (Text fig. 2, A, D and F; Pl. 29, Figs. 5 and 7). The former corresponds to the distal segment and the latter to the proximal segment in terminology of the segmentary structure of X-chromosomes as is discussed in another paper (in press). The present writer has described in that paper, how in the X-chromosome of man and rodents the distal segment is frequently subdivided
into two distinct parts, $D_1$ and $D_2$, while the proximal segment always remains in single $P$, which may sometimes grow thinner having a small spherical swelling at the extremity, or sometimes become con-

![Text fig. 2. Microtus montebelli. Chromosomes in metaphase and anaphase of primary spermatocytes in division. $\times$ca 5600. A and B, metaphase, polar view. C, D, E, F, metaphase, lateral view. G and H, anaphase, lateral view, notice the lagging X-chromosome of pale colour.](image)

verted into a comparatively large globule at the expense of the elongate part. It is in the latter case that the $P$-segment is very frequently regarded as if it were the $Y$-chromosome. A feature, suit-
ing the above descriptions, can also be discovered in the case of the present vole. The X-chromosome shown in Text fig. 1, E (= Pl. 29, Fig. 6) for instance, strongly resembles the XY-complex, but in
reality the two contiguous segments (upper part) represent the D₁ and the D₂ parts, while the single segment (lower part) having chromatic connection with the former is nothing more than the P-segment, not to be considered as the Y. It is of great importance to know that such a tripartite structure, D₁-D₂-P, can be followed back to the spermatogonial stage (Text fig. 1, C). In case where the chromosomes are too much destained and therefore become thinner in general, one will not overlook the presence of the proximal granule at the junction of D₁ and P (Text fig. 2, C and H; Pl. 29, Fig. 4).

In parallel to the tripartite structure displayed by the X-chromosome in question, it is very important to observe in detail its behavior during meiosis. Up to the commencement of the separation of dyads, the X-chromosome occupies its position on the same plane with tetrads, hesitating to move to the pole with the separated dyads (Text fig. 2, G). When the latter approach, however, to the poles of division to which they belong, the X-chromosome starts to follow after one of the daughter groups of chromosomes (Text fig. 2, H). No disintegration occurs between the D- and P-segment of the X-chromosome, as both two component segments, with a proximal granule between them, are clearly observable in the same configuration and constitution as in the previous stage (Text fig. 2, G). By this phenomenon it will be made absolutely clear that the P-segment is by no means to be considered the Y-chromosome, but merely a definite part of the X-chromosome, discriminated as the P-segment by the present writer.

Discussion

On the chromosome number. To the author of this paper it came as a surprise when Krallinger (1936) informed the chromosome number of Pecari tajacu, in which he found only 30 chromosomes in diploid, a small number incomparable with any species of Eutheria hitherto recorded. But soon Koller (1936) discovered the still lower chromosome number, 28, in the grey squirrel, Sciurus carolinensis leuconotus. Thus our knowledge on the chromosome number has gradually been accumulated and the previous idea concerning the fundamental number of Eutheria chromosomes had necessarily been altered and revised. At present 28 found in the gray squirrel holds, at any rate, as the lowest chromosome number in Eutheria, followed by 30 in Pecari and then the number 31 now found in the vole, Microtus montebelli.

Knowledge on chromosomes of any species belonging to Microtus has been very meager, as only a short account is available from one
of American species, *Microtus townsendii*, by Cross (1931) who reports the chromosome number (2n) of this species as 50, of which all appear as rods and spherules. In the present species, *Microtus montebelli*, on the contrary, the chromosome number is remarkably lower being only 31, and a majority of the chromosomes are represented (at least eleven pairs from a to k) by V's which may probably be multiples. As these eleven pairs may be explained as multiples, however, the chromosome numbers of these two different species can closely approach each other, by analysing each V into two rods. Such an analysis of V-chromosomes seems to be more reasonable when an attempt is made to compare the present species with *Evotomys bedfordiae*, in which the writer has already recorded (1935) 55 chromosomes of rod-shape. For, if the twelve pairs of V and J chromosomes, a's to l's, be considered as multiples, then there is obtained 48 R's (rods) by breaking every individual V into two R's. This number 48 will suffice to give rise to the number 55, similar to that of *Evotomys bedfordiae*, by adding 6 remaining autosomes, (m's, n's and o's) and one X-chromosome. But there remains a great doubt whether the chromosomes m's are essentially rod-chromosomes or whether the l's are in reality multiples.

In respect to chromosome morphology, *Microtus montebelli* is much more closely allied to *Arvicola scherman* studied recently by Matthey and Renaud (1935) than to the American *Microtus* studied by Cross (1931) and *Evotomys*. Because, they found 36 chromosomes in *Arvicola scherman*, a majority of which being V-shape. Their information, to the regret of the present writer, is confined to a very short note, not extending so far as to allow any morphological analysis and therefore it is impossible to know at present how many V's are actually present in a garniture of chromosomes.

On the problem of the sex-chromosomes. As stated in the introduction to this memoir, the present writer holds the view, adverse to most investigators of Mammalian chromosomes, that the formula of the sex-chromosomes varies in Mammalia either XY or XO in different species, and it seems very questionable whether the chromosome which is interpreted as XY-complex by some authors is always in reality of a multiple nature composed of two distinct chromosomes X and Y, or whether it merely possesses an appearance of a multiple chromosome as a result of segmentary construction.

It is beyond doubt clear that in figures of spermatogonial chromosomes reproduced in the present paper no one can count 32 chromosomes instead of 31. What is meant by this fact of an odd number of chromosomes? Moreover, the X-chromosome, thus identified
by the writer, is constantly found in auxocytes with a constant configuration. In striking contrast to this, Matthey and Renaud (1935) found 36 chromosomes (diploid number) in Arvicola scherman, without any difficulties according to their statement, and one XY-complex, composed of two distinct chromosomes connected bout à bout, in the reduced garniture of chromosomes in the auxocyte. The writer does not know whether such a discrepancy is due to the taxonomical differences existing between an oriental and an occidental species of voles.

On the contrary, however, they doubt the writer’s previous account of Evotomys bedfordiae, in which were described and figured 55 distinct chromosomes in the spermatogonium, as they presume the chromosome number of Evotomys glareolus to be probably 56 (they could not actually count it). They say that in auxocytes il est aisé d’identifier un couple XY. At present, the writer is quite ignorant of the morphology of that complex, as their memoir has no accompanying figures. If there exists homology between their species and the writer’s, however, they might have considered the minute globular body attached to one end of X as a distinct Y-chromosome. That minute body with a globular appearance denotes, so far as observed in Evotomys bedfordiae, nothing other than the proximal granule to which the spindle fibre comes to attach itself. Because, if it be the Y-chromosome as they may interprete, eventually one extremely minute spherule in the spermatogonial garniture should be discovered, but unfortunately the writer can not find at all such a chromosome corresponding to that minute spherule (see previous paper, 1935, Figs. 25 and 26).

On closing of present discussion it seems necessary to the writer to touch on the problem of the sex-chromosomes of Apodemus, since he recorded (1934) for the first time that in Apodemus speciosus aino the sex-chromosome separates postreductationally in a quite different manner from cases hitherto known in Mammalia. A similar behaviour has also been ascertained to occur in sex-chromosomes in several other species of Apodemus, by Tateishi (1934), Raynaud (1936) and Matthey (1936a, 1936b). But all these authors hold the view, different from the present writer’s, that the short arm of the V-shaped sex-chromosome represents the Y-element of an XY-complex, instead of being a part of a single X-chromosome, as the writer holds. The writer’s opinion is based upon two valid facts: (1) both arms, long and short, of a V-shaped chromosome develop within one and the same chromosome vesicle, and (2) the diploid number of chromosomes found in the spermatogonium is 47, among which is invariably observed one V-shaped chromosome with a correspondent
configuration to some extent with the sex-chromosome in auxocytes. In the writer's previous paper (1934) the spermatogonial garniture of chromosomes was shown by only one example. It is with reference to this case that Matthey (1936a, 1936b) expressed unwillingness to accept the writer's interpretation, including doubt that there may exist cells in which 48 chromosomes compose a diploid garniture, as

**Text fig. 3.** *Apodemus speciosus ainu*. Chromosomes in metaphase of spermatogonial division (polar view). ×ca 3000. X-chromosomes are indicated by solid black; it is not distinguishable in F, for all chromosomes are extremely thickened.
Raynaud (1936) actually sketches from *Apodemus sylvaticus* (only one figure is shown by the latter author and no V-shaped chromosome is visible). The single figure shown in the writer's previous paper does not represent the sole example from which the conclusion was drawn, but is only one representative out of a great many cells of best fixation under close observation. On the present occasion, therefore, the writer wishes to publish here, with a supplementary purpose, some of his sketches of spermatogonia (Text fig. 3; Pl. 29, Figs. 8–10) from the same material, in which every single garniture is invariably composed of 47 distinct chromosomes and in most of them is noticed a chromosome having a more or less V-shape (solid black in figures) and without any apparent mate.

Thus the writer's comparative study of rodents results in a conclusion that the Y-chromosome is really absent in one field mouse, *Apodemus speciosus ainu*, and two voles, *Evotomys bedfordiae* and *Microtus montebelli*. This is striking contrast to the house mouse, *Mus molossinus*, and the rats, *Rattus norvegicus* and *Rattus rattus*, in all which species the Y-chromosome is proved to exist. It is advisable to any one who investigates the Mammalian sex-chromosomes, before he decides to explain a small chromatic segment as the Y-chromosome, that he should pay special attention to the segmentary structure of the X-chromosome as discussed in detail in another paper by the present author (in press) in connection with the human sex-chromosome. The X-chromosome seems in general to show a tripartite structure not only in man and in rats, but also in species of *Lepus* as have recently been studied by Tateishi (1936). Occasion will be found in the near future to discuss again the problem of the sex-chromosome, because a study of the chromosomes is going on in another species of *Apodemus*, indigenous to Japan.

**Literature cited**


**Explanation of Plate 29**

All photomicrographs were taken under magnification ×2000.

*Microtus montebelli*

Figs. 1–3. Spermatogonial chromosomes (polar view).
1. corresponds with Text fig. 1, A
2. " " " " B
3. " " " " C

Figs. 4–7. Chromosomes of primary spermatocytes (profile), x denotes the X-chromosome.
4. corresponds with Text fig. 2, C
5. " " " " D
6. " " " " E
7. " " " " F

*Apodemus speciosus ainu*

Figs. 8–10. Spermatogonial chromosomes (polar view).
8. corresponds with Text fig. 3, K
9. " " " " A
10. " " " " F
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