Title	Notes on the Chromosomes of Four Species of Small Mammals (Chromosome Studies in Domestic Mammals, V) (With 35 Textfigures)
Author(s)	MAKINO, Sajiro
Citation	北海道大學理學部紀要, 9(4), 345-357
Issue Date	1948-07
Doc URL	https://hdl.handle.net/2115/27067
Type	departmental bulletin paper
File Information	9(4)_P345-357.pdf

Notes on the Chromosomes of Four Species of Small Mammals[1] (Chromosome Studies in Domestic Mammals, V)

By

Sajiro Makino

Zoological Institute, Faculty of Science, Hokkaido University

(With 35 Textfigures)

The present paper deals with the chromosome investigation in male germ cells of the following four kinds of small domestic mammals, namely the silver fox (*Vulpes vulpes*), the Japanese mink (*Mustela itatsi itatsi*), the guinea pig (*Cavia cobaya*) and the nutria (*Myocastor coypus*). The former two forms belong to the Carnivora and the latter two are members of the Rodentia. The silver fox and the guinea pig have been subjects of repeated investigations in cytology by a number of previous workers heretofore, but no accurate accounts have yet been furnished for the chromosomes of these animals, the whole field requiring therefore reinvestigation. The Japanese mink and the nutria provided a new material for the field of cytology, and, so far as the writer is aware, there is no reference to these animals in the literature prior to the present study. It is the writer's intention to demonstrate the exact features of the chromosomes of these mammals in this study, with special regard to the number of chromosomes, as well as to the type and behaviour of the sex chromosomes. The morphological analysis of the karyotypes was also a subject which received attention.

In every case the animals were stunned by a blow on the head, and then the testes were removed, cut into small pieces and put into fixatives. Flemming's strong solution containing no acetic acid and Champy's mixture were the fixing solutions exclusively employed with excellent result. For staining the sections were subjected to the Heidenhain's iron-haematoxylin method with counterstaining of light green.

---

1. The chromosomes of the silver fox (*Vulpes vulpes*)[1]

Referring to the literature, there are accessible two papers contributed to the chromosome study of the fox, the results of which point to two different numbers of chromosomes. Wodsedalek ('31) informed that the number of chromosomes of the red fox is 42 in $2n$ and 21 in n, and the male has X-Y chromosomes. Then the study of Andres ('38) has appeared reporting the haploid number as 17, one of which is a heteromorphic element. These reported evidences do not agree with the results obtained in the present study, not only in respect of the chromosome number but also of the detail of the sex chromosomes as mentioned below.

The diploid number of chromosomes observed in the spermatogonial division was determined as 38 and the haploid number was ascertained to be 19 in the primary spermatocyte division. Figs. 1 and 2 are the metaphase groups of the spermatoginal division each of which contains 38 chromosomes, and Figs. 3, 4 and 5 show those of the first division demonstrating 19 bivalents in each. To give a further comfirmation for the chromosome number here obtained, a morphological analysis of the chromosome complex was attempted, since the result of this study strikingly differs from those reported in the previous studies. By examination of the morphological character of the individual chromosomes it becomes evident that the diploid complement consists of 33 atelomitic chromosomes of varying size and shape, and the remaining five minute grain-like elements, in a striking contrast in shape to the former. Among the group of atelomitic chromosomes, 32 elements were observed to constitute 16 homologous pairs by comparison of the shape and size, and the remaining one characterized by its elongated form and subterminal attachment is

---

1) Preliminary notes were published in the Zoological Magazine (Tokyo), Vol. 56, 1944.

destitute of its homologous mate. This element represents in all probability the X chromosome. It is also highly probable that one of the five minute chromosomes is no other than the Y.

The chromosome number here determined for the silver fox is just the same as that attained in the cat by Minouchi & Ohta ('34). From the study of the karyotypes by way of comparison of drawings, however, it is apparent that the chromosome complement of the silver fox is more complicated than that of the cat, since in the chromosome complex of the former the number of the V-shaped elements is much prevalent. And it is further noticeable that the silver fox is also characterized by the existence of five minute chromosomes, quite remarkable in their size and shape.
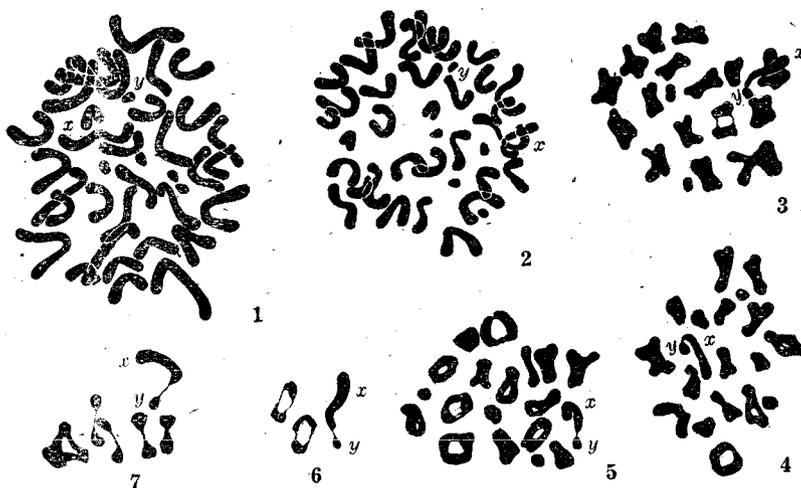
Figs. 1-7. Chromosomes of the silver fox (*Vulpes vulpes*). × 3200. 1-5, Spermatogonial metaphases, 38 chromosomes in each. 3-5, Primary spermatocyte metaphases, 19 bivalents in each. 6-7, Side views of primary spermatocyte spindles, showing the XY complex.

As is the case with other mammals, the X and the Y conjugate forming a heteromorphic bivalent in the first metaphase (Figs. 3–5). The configuration of the XY bivalent is clearly observable in the lateral aspect of the metaphase spindle of the first division (Figs. 6–7). It may be needless to add that the X and Y segregate as usual in the first division, and this results in the production of two sorts of secondary spermatocytes, one of which possesses the X and the other the Y.

The chromosome number of the red fox reported as 42 by Wodsedalek ('31) is astonishingly different from that by the present investigation. The number, 42, is the same as that attained in the racoon dog by Minouchi ('29). From the fact that the silver fox is regarded as a variant of the red fox, the similarity of the chromosome constitution between these two forms is to be highly expected. In view of this fact, the chromosome number reported by Wodsedalek ('31) seems very questionable to us, but it is now impossible to make any discussion about it, since his study appeared in the form of a brief abstract. Reference to the chromosome figures of Andres' paper, on the other hand, makes it very probable that the low chromosome count given by Andres ('38) may be attributed to inadequate preservation of material, the counting being handicapped by an irregular fusion of some chromosomes. For the same reason the chromosome number given by him for *Vulpes lagopus* (*n*, 26) cannot either be regarded as valid.

Referring to the literature, the fox appears to possess the smallest chromosome number among the Canidae so far studied, since the previous investigations have recorded the chromosome numbers of the dog and racoon dog as 78 and 42 respectively (Minouchi '28, '29).
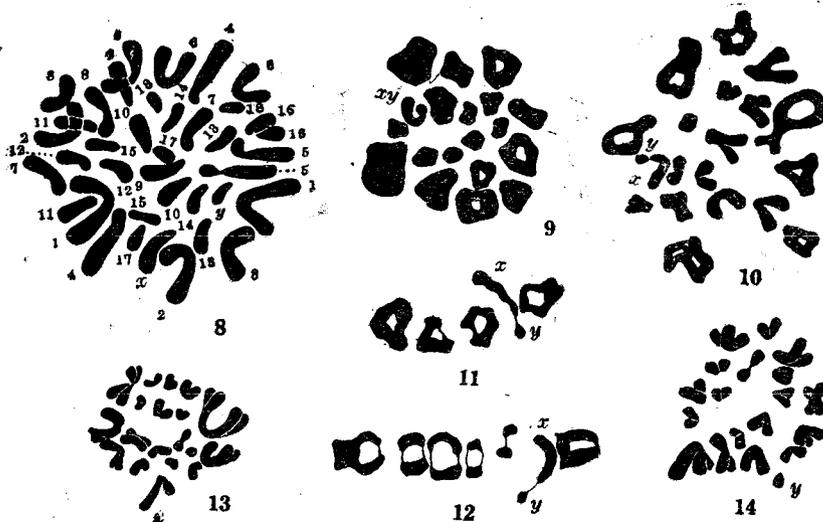
2. The chromosomes of the Japanese mink
(*Mustela itatsi itatsi*)

The Japanese mink, *Mustela itatsi itatsi*, on which the present study was based, is a member of the Mustelidae (Carnivora) and highly valued as one of the important fur animals, common in the wild rather than under domestication. In the field of cytology there has appared heretofore no paper dealing with the present form. Recently Koller ('36) investigated the chromosomes of a closely allied species, *Putrius furo*, announcing 34 chromosomes in the male diploid complex, which markedly differs from the result of the present species as mentioned below.

The chromosome counts were made in this study in several spermatogonial metaphases and the diploid number was determined as 38 in every case. It will be sufficient to reproduce here an example of the excellent metaphase plates in order to show the diploid complex of this form (Fig. 8). As clearly visible in this figure, the morphology of the individual chromosomes is highly distinct, so that the

identification of the homologous mates can be made with easiness. After a close comparison of the individual chromosomes, the homologous pairs with identical shape and size were mated, and then they were numbered in the approximate order of size, as indicated in Fig. 8. Thus the thirty eight elements constituting the diploid complex are arranged here into 18 homologous pairs and one unequal pair which consists of a rod-shape element of medium size and an extremely small one. From the above fact it seemed warranted to speak of this latter unequal pair as the XY complex, the larger being the X and the smaller the Y. In magnitude the Y element seems to be slightly larger than the smallest autosomes (the 18th pair). Of the autosome pairs, the members forming from the first to the eighth pair are very evident in having remarkable atelomitic structures of submedian and subterminal attachment. The members of the remaining ten pairs (from the ninth to the eighteenth pair) are regarded as telomitic in their structure, since they assume a simple rod-shape with a taper end.

The metaphase of the primary spermatocyte was found to exhibit

Figs. 8-14. Chromosomes of the Japanese mink (*Mustela itatsi itatsi*). ×3200. **8,** Spermatogonial metaphase showing 38 chromosomes. **9–10,** Primary spermatocyte metaphases, 19 bivalents in each. **11–12,** Side views of the primary spermatocyte spindles, showing the XY complex. **13,** Specondary spermatocyte metaphase, including the X. **14,** The same containing the Y.

19 chromosomes with indisputable clearness, in which eighteen are the ordinary bivalents and the remaining one is the XY bivalent (Figs. 9–10). The entire configuration of the latter is well understood by reference to the side view of the first metaphase plate (Figs. 11–12), where it exhibits a sharp distinction in shape to the others by its heteromorphic structure composed of the long X connected in its one end with the small Y. As a result of the segregation of the X and Y in the first division, there are produced two kinds of secondary spermatocytes as regards the distribution of the X and Y elements. Every metaphase of the secondary spermatocytes always indicates 19 chromosomes, but they are different in the fact that one sort of them contains the X (Fig. 13) and the other the Y (Fig. 14).

Looking upon the reported evidences, it becomes evident that the chromosome number herein determined for the Japanese mink is the same as those of the cat (Felidae) (Minouchi & Ohta '34) and of the silver fox (Canidae) formerly studied. General comparison of the karyotypes among these three forms points out that the Japanese mink seems to be close to the cat in the constitution of the chromosomes rather than to the silver fox. In *Putrius furo* which is an allied species to the Japanese mink, Koller ('34) reported 34 chromosomes in the male diploid complex. It seems very probable to the author that *Putrius* would be expected to be identical in its chromosome constitution to the Japanese mink, in view of the close taxonomic relationship occurring between them. A revised study is desirable to ascertain the chromosome feature of this animal by application of the improved technique.

3.   The chromosomes of the guinea pig, *Cavia cobaya*

Cavia cobaya here dealt with is a member of the Caviidae (Rodentia) and is so common by the name of the guinea pig in our laboratory, living always in close connection with us. The guinea pig has furnished the classical object for cytology. Starting with the work of von Bardeleben appeared as early as 1892, over ten papers have been published until now, devoting to the chromosome study of this form. Setting aside the classical studies with unsatisfactory results such as those by von Bardeleben ('92), Flemming ('98), Moore & Walker ('00), Lams ('13) and Harman & Root ('26), there should be mentioned the works published by Stevens ('11), Painter

('26), League ('28) and Mols ('28). That the chromosome number of the guinea pig is not so small as announced by the earlier authors, was firstly pointed out by the intense study of Stevens ('11). She reported the spermatogonial diploid number as approximately 56 and the haploid number as 28, establishing the sex chromosomes as of the XY type. Later, Painter ('26) and League ('28), based on the same material, have published extensive papers on the spermatogenesis of this animal, in which they placed the diploid number at approximately 62±2 and the haploid number at 31, demonstrating the XY sex chromosomes. Then Mols ('28) made a comprehensive study on the chromosomes in germ cells as well as in somatic cells, and arrived at a conclusion that does not coincide with the results of the above American authors. According to Mols ('28), the number of chromosomes of the guinea pig is 65 in the spermatogonial cell, 33 in the primary spermatocyte, and 32 and 33 in the secondary spermatocyte. Contrary to the results of the former studies, Mols ('28) is in view that the guinea pig possesses the sex chromosome of the XO type. Reviewing these reported evidences, it is surprising to know that the questions relative to the chromosome number as well as to the sex chromosomes have not yet been conclusively cleared up in such a familiar animal as the guinea pig.

The present material was favourably provided with a considerable number of dividing cells, which were available for the precise study of chromosomes. The results of counting the chromosomes of the clear metaphase plates in this study revealed that the spermatogonial number was decidedly 64 without exception (Figs. 15–16). This diploid number has received a further confirmation by the observations extended upon the primary and secondary spermatocytes, since the haploid number showing 32 was established in both of them beyond question (Figs. 17–19). The approximate results furnished by Painter ('26) and League ('28) thus arrived at the final conclusion in this study. The present author does not hesitate in claiming that the chromosome number here determined is the definite one characteristic to the guinea pig.

The diploid complex of this animal is very remarkable in containing few chromosomes of striking shape and size. Two chromosomes, labelled *a* in the drawings (Figs. 15–16) are very prominent because of their elongated rod-shape, being in size the largest of all. The other two marked with *b* are also conspicuous in assuming a distinct V-shape, submedian in attachment. These two pairs (*a*'s

and b's) are regarded as homologous ones respectively, since they are nearly identical in size two by two.   The remaining chromosomes are all of telomitic nature having each a simple rod-shape with a taper end, two of which may be the X and Y chromosomes.   The

Figs. 15-25. Chromosomes of the guinea pig (*Cavia cobaya*). × 3200. **15-16,** Spermatogonial metaphases, 64 chromosomes in each.   **17-19,** Primary spermatocyte metaphases, 32 bivalents in each.   **20-22,** Side views of the primary spermatocyte spindles, illustrating the XY complex.   **23,** Anaphase configuration of the first division, showing the segregation of the X and Y.   **24,** Secondary spermatocyte metaphase, including the X.   **25,** The same having the Y.

chromosomes seem to form a graded series ranging in shape from long rods to short ones. The X chromosome looks slightly smaller than the members of the largest pair ($\dot{a}$'s), because of its solitary and outstanding occurrence amongst the rod-shaped group. However, the Y element is impossible to be pointed out among the group on account of the abundance of the chromosomes with similar size.
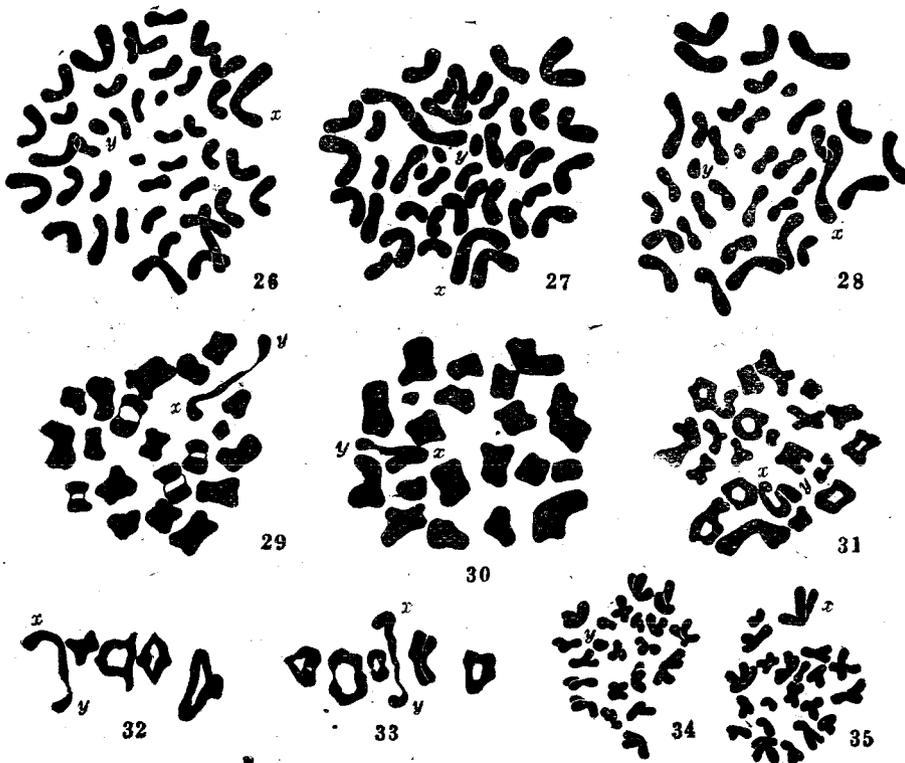
The metaphase plate of the primary spermatocyte shows well-defined 32 bivalents with indisputable clearness (Figs. 17–19). One element is quite prominent among the others by its remarkably large size. This is in all probability the bivalent made up of the a-pair. In every plate there is found at the periphery of the equatorial plate a chromosome composed of two unequal elements, a gently curved rod and a small grain-like one connected end-to-end. This is no other than the XY complex. The feature of the XY complex seen in profile view of the spindle is very interesting. The X element invariably bends inwardly at the two joints so as to give an elliptical configuration. At the inner extremity of the X, the Y comes in contact. What is very remarkable, at this stage the Y element lies always horizontally in the equatorial plate, holding its long axis parallel to the latter. Clear illustrations of this point are shown in the drawings given in Figs. 20–22. The behaviour displayed by the X shows a close approach to that revealed in *Rattus confucianus culturatus* by the author (Makino '43), while the feature observed in the Y seems to be comparable to that described in cattle, zebu and water-buffalo (Makino '44). Thus the morphological peculiarity expressed by the X and Y seems to be a characteristic of the guinea pig, being unique in appearance, and there has been found no comparable case in animals so far investigated. In the light of these findings, therefore, it is quite evident that the odd X chromosome claimed by Mols ('28) is very doubtful. It seems very probable to the author that the largest autosomal bivalent was erroneously mistaken by Mols ('28) as the X. The XY complex indicated in League's paper does not exhibit either any natural feature, being irregularly deformed due probably to poor fixation. With such material it was entirely impossible to study the morphology of chromosomes so precisely as done in the present observation.

The segregation of the X and Y takes place in the first division as seen in Fig. 23, and this results in the production of two kinds of secondary spermatocytes, (i) those carrying the X element together with 31 autosomal dyads (Fig. 24) and (ii) those containing the Y

with an equal number of the autosomal dyads (Fig. 25). Among the autosomal dyads, the long *a*-element and the atelomitic *b*-element are very prominent as is the case with the spermatogonial complement.

4. The chromosomes of the nutria, *Myocastor coypus*

The present material, *Myocastor coypus*, which is rather familiar to us under the name of nutria (or beaver-rat), is an aquatic and herbivorous rodent, belonging to the family Capromyidae, a group close to the Caviidae embracing the guinea pig. The original home

Figs. 26-35. Chromosomes of the nutria (*Myocastor coypus*). ×3200. 26-28, Spermatogonial metaphases, 42 elements in each. 29-31, Primary spermatocyte metaphases, 21 bivalents in each. 32-33, Side views of the primary spermatocyte spindles, showing the XY complex. 34, Secondary spermatocyte metaphase containing the Y. 35, The same including the X.

of this animal is Argentine, South America, mainly distributed abundantly along the river La Plata. Recently the nutria has received much attention on account of the high value of its fur, and has been largely cultivated under domestication by breeders. Of late years they were imported from America to our country and have continuously been bred with successful results as one of the most important fur animals. The material on which the present study was carried out was secured through the courtesy of a breeder, Mr. J. Kawasima, to whom the author's thanks are due. Looking over the literature, the author has been unable to get access to any investigator engaged with the chromosomes of this interesting rodent.

The author was surprised at finding the chromosome complex of this species highly different from that of the guinea pig, V-shaped elements of various sizes being very prevalent, whilst he had expected that the karyotypes of these two animals would show a close approach to each other, owing to the taxonomical relation existing between them. Careful examination of several clear metaphase plates makes it certain that the diploid number of chromosomes is undoubtedly 42 (Figs. 26–28). The majority of the members constituting the diploid complement seem to be of atelomitic nature, each bearing a marked constriction, except three of extremely small size, which are very prominent in their dot-like configuration in sharp contrast to the others. The atelomitic elements, being 39 in number, do not exhibit so apparent a size-difference, the reduction in magnitude being relatively slight. The larger ones among them assume conspicuous V-shape showing median and submedian attachment, while smaller ones gently bend at the point of constriction. Noticeable is that one V-shaped chromosome with submedian structure is very outstanding amongst the atelomitic group by its prominent large size, being probably the largest of all. Because this element is destitute of its homologous mate, there is enough reason for its interpretation as the X chromosome. Turning to the three dot-like chromosomes, it was found that the smaller two form a homologous pair owing to their similarity in size, whereas the remaining one, which is a little larger than the former, remains unpaired. It is this element that was identified as the Y.

The diploid number of chromosomes above obtained was fairly confirmed by the examination of the haploid chromosomes in the spermatocytes. The haploid number was found with certainty to be 21 in both of the primary and secondary metaphases. Figs. 29 to

31 are metaphase plates of the primary spermatocytes, and Figs. 34 and 35 indicate those of the secondary spermatocytes. One of the twenty-one bivalents in the primary spermatocyte is the XY complex having a distinguishable structure, composed of a large X element and a small Y element coming in end-to-end conjugation. The whole configuration of the XY complex is fully detected in the lateral view of the spindle as shown in Figs. 32 and 33. The X is distinctly characterized by a submedian structure and to its longer arm the Y comes in contact. As occurred in the foregoing cases, there are two kinds of secondary spermatocytes with regard to the distribution of the X and Y. One of them possesses the X (Fig. 35) and the other contains the Y (Fig. 34).

The family Capromyidae embracing the nutria is known to be taxonomically related to the family Caviidae to which the guinea pig belongs. From this fact the author expected a resemblance of the karyotype between the two animals. The karyotype of the nutria, however, is essentially different as mentioned above from that of the guinea pig in every point of number and morphology of the chromosomes. Indeed, no intimate relationship can be detected in the karyotypes of these animals. On the other hand, the number of chromosomes established for the nutria is the same as those obtained in the following murid rodents, *Rattus norvegicus, R. rattus* and *R. losea* (cf. Makino '43), while the chromosome constitutions of these forms are entirely dissimilar to that of the nutria, since in the former animals all of the members constituting the diploid complement are of the simple roy-type.

Summary

The chromosomes of four forms of small domestic mammals, two carnivores and two rodents, were morphologically investigated in male germ cells during the course of spermatogenesis, with special regard to the construction of the karyotype and to the feature of the sex chromosomes. The species under study and their chromosome numbers obtained are listed below:

Species	$2n$	n	s x-chrom.
Canidae (Carnivora)			
Vulpes vulpes	38s (33 V's+5 r's)	19 (I, II)	XY
Mustelidae (Carnivora)			
Mustela itatsi itatsi	38s (16 V's+22 r's)	19 (I, II)	XY

Caviidae (Rodentia)
Cavia cobaya   64s (2 V's+62 r's)   32 (I, II)   XY

Capromyidae (Rodentia)
Myocastor coypus   42s (39 V's+3 r's)   21 (I, II)   XY

    s: spermatogonium.   (I, II): primary and secondary spermatocytes.
    V: V-shaped chromosome.   r: rod-shaped chromosome.

Throughout the species under study there are invariably present the sex chromosomes of a simple XY type. The sex chromosomes assume in each the characteristic feature, specific to the species. They conjugate in meiosis forming a heteromorphic bivalent and segregate to opposite poles in the first division.

Literature

Andres, A. H. On the chromosome complex in several Canidae. Cytologia 9.

Bardeleben, K. von 1892. Ueber Spermatogenese bei Säugetieren, besonders beim Menschen. Verh. Anat. Gesell. Wien, 6.

Flemming, W. 1898. Ueber die Chromosomenzahl beim Menschen. Anat. Anz. 14.

Harman, M. T. and F. P. Root 1926. Number and behaviour of the chromosomes in *Cavia cobaya*. Biol. Bull. 51.

Koller, P. C. 1936. Chromosome behaviour in the male ferret and mole during anoestrus. Proc. Roy. Soc. London, 121.

Lams, H. 1913. Etude de l'oeuf de cobaya aus premiers stades de l'embryogenèse. Arch. Biol. 28.

League, B. B. 1928. The chromosomes of the guinea-pig. Jour. Morph. 46.

Makino, S. 1943. Studies on the murine chromosomes, III. A comparative study of chromosomes in five species of *Rattus*. J. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, 9.

———— 1944 a. A contribution to the study of the chromosomes of domestic mammals. (In Japanese). Zool. Mag. (Tokyo), 56.

———— 1944 b. Karyotypes of domestic cattle, zebu and domestic waterbuffalo. (Chromosome studies in domestic mammals, IV). Cytologia 13.

Minouchi. O. 1929. On the spermatogenesis of the racoon dog (*Nyctereutes viverrinus*), with special reference to the sex-chromosomes. Cytologia 1.

Minouchi, O. and T. Ohta 1934. On the chromosome number and the sex-chromosomes in the germ cells of male and female cats. Cytologia 5.

Mols, G. 1928. Etude comparative des mitoses chez le cobaya. (Mitoses séminales hétérochromosome—mitoses de l'amnions). Arch. Biol. 38.

Moore, J. E. S. and J. Walker 1906. The mitotic process in the Mammalia. Liverpool Univ. Rep.

Oguma, K. and S. Makino 1937. A new list of the chromosome numbers in Vertebrata (March 1937). J. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, 5.

Painter, Th. 1926. The chromosomes of Rodents. Sci. 64..

Stevens, N. M. 1911. Heterochromosomes in the guinea pig. Biol. Bull. 21.

Wodsedalek, J. E. 1931. Sermatogenesis of the red fox, *Vulpes fulvus*. (*Abstract*). Anat. Rec. 51.