Studies on the Murine Chromosomes. II. Morphological Comparison of the Chromosomes between the Wild Form and the Domesticated Variety of *Rattus norvegicus* Berkenhout[1]*

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(With 30 Text-figures)

The Norway rat, *Rattus norvegicus* Berkenhout, is one mammal easily obtainable at the present time both in the wild state and as a domesticated form, the latter being represented by the albino rat so common in our laboratories. The wild brown rat is, as well known, cosmopolitan having world wide distribution and always living in close connection with man. The albino variety is known at present only as a domesticated strain. This strain is, as a rule, far removed from its wild ancestor and moderately inbred.

Rattus norvegicus has offered the classical and favourite object for cytology. A considerable number of investigations have been made on this material, the number of papers so far published being over thirty (*cf.* the list published by Oguma and Makino '37). The majority of works have been carried out on the chromosomes of the domesticated albino variety, only a few having been concerned with those of the wild form. Setting aside the classical studies with unsatisfactory results, there should be mentioned some papers published by Allen ('18, '40), Pincus ('27), Painter ('28), Minouchi ('28), Swezy ('28), Bryden ('32, '33), Koller & Darlington ('34) and Matthey ('36) which are concerned with the domesticated albino material and those by Swezy ('28), Oguma ('35) and Tateishi ('35)

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undertaken on the wild form. The interests of these authors, however, have been concerned merely with the chromosome number and the sex chromosome, and have been extented in no cases to the question as to how morphological difference or likeness occurs in the chromosomes between the wild form and its domesticated variety. No attempt has ever been made, therefore, to work out a caryological analysis between these two forms from the viewpoint of comparison. While working with the chromosomes of some species of *Rattus* obtainable in Japan, the present author first became intensely interested in this question. Accordingly a close comparative investigation of the chromosomes was undertaken in this study with the sufficient material coming from different strains and localities. Furthermore, the cytological study of this character carried on by means of an exact method may play an important role in considering the evolutional course of the domesticated animals, particularly that of useful domestic mammals.

This work has been done under the direction of Prof. K. Oguma to whom the author wishes to express his sincere gratitude for kind criticism and valuable suggestion. The funds expended in collecting the rats used in this study were furnished in most part by the Scientific Research Expenditure of the Department of Education.

Material and Method

Specimens of the wild form of *Rattus norvegicus* used in the present study were collected in different and widely separated districts, such as Sapporo (Hokkaido), Taihoku (Taiwan) and Naha (Okinawa). They were obtained by trapping in a warehouse and sometimes in the field.

The domesticated albino rats coming from two different colonies furnished the material for this study. One of them consists of albino rats of the Wistar strain which were introduced wthin recent years and bred pure since then in the Zootechnical Laboratory of the Tokyo Imp. University. These rats were obtained through the generosity of Prof. K. Masui to whom the author wishes to acknowledge his indebtedness at this opportunity. In addition to these rats, white rats commonly reared by breeders for supply to the laboratory were purchased and employed for study. The origin of the latter strain found in Japan is not wholly clear. These have been referred to in the following descriptions as the common albino strain.

The taxonomical determination of the rats used was made in each specimen by observation of the character of the skull with the kind cooperation of the specialist, Dr. M. Tokuda of the Kyoto Imp. University to whom the author's cordial thanks are due.

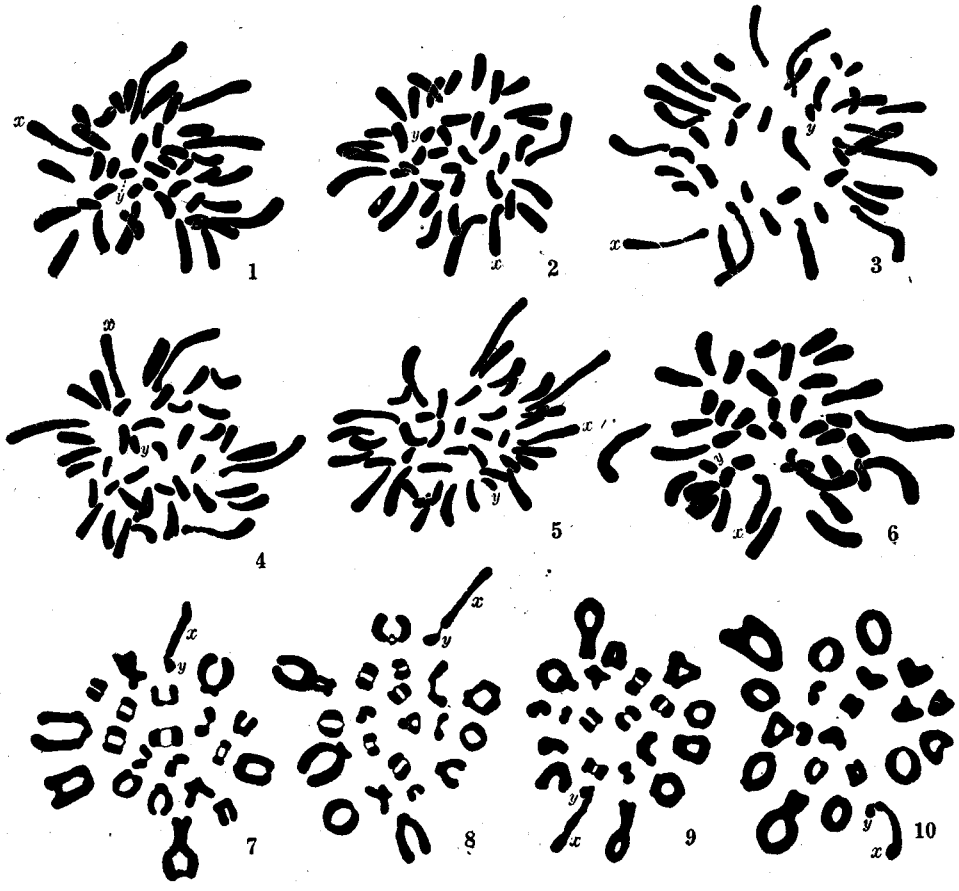
In every case the testes preserved exclusively with Flemming's strong solution containing no trace of acetic acid provided the material for the present investigation. The testicular material obtained from three to five specimens was ready in each experiment for investigation. The sections were made by the usual procedure of paraffin method and stained with the iron-haematoxylin method after Heidenhain.

The morphological analysis of the chromosomes and their comparison were undertaken exclusively with the spermatogonial chromosomes of the diploid constitution, since they are most suitable to see the general characteristic picture of the chromosomes. The technical proceduce by which the material was treated was entirely identical in all cases as mentioned above, since different methods produce unequal results. In every case five to fifteen adequate equatorial plates were selected in the excellently preserved material and used for the following observations. In making the observations special attention has been paid to the careful estimation of the real length of chromosomes and the comparison of their shape to make an exact identification of the homologous mates. A close microscopical examination was first made as accurately as possible, on the individual chromosomes. Then they were carefully drawn with the aid of a camera lucida and each of the supposed homologous mates thus pointed out was numbered in alphabetic order. Then the chromosomes were copied again and finally placed in the serial alignment according to descendant order of size. As proved in the author's previous study regarding the chromosomes of *Mus* (Makino, '41) this method will involve only the slightest degree of error, so far as the measurement of chromosomes is made on those preserved in excellent condition.

The general morphology of the chromosomes of *Rattus norvegicus*

The chromosome number of the rats dealt with agrees essentially with that reported by the previous authors, being 42 in diploid and

21 in haploid for both the wild and domesticated albino forms (*cf.* Figs. 1–10). Fluctuation from the normal number was encountered



Figs. 1–10. Chromosomes of *Rattus norvegicus* Berkenhout. ×3400.

1–6. Spermatogonial chromosomes (polar view)
- 1–2, from the albino rats of the Wistar strain.
- 3, from the white rat of the common albino strain.
- 4, from the wild rat (Taihoku).
- 5, from the wild rat (Sapporo).
- 6, from the wild rat (Naha).

7–10. Chromosomes of the primary spermatocyte (polar view)
- 7, from the Wistar albino.
- 8, from the common albino.
- 9, from the wild rat (Taihoku).
- 10, from the wild rat (Sapporo).

in no case. The chromosomes are all of the simple rod-type, and take the usual radial arrangement with their inner ends pointing toward the centre of the equatorial plate. According to the general conception, therefore, these chromosomes may be of the type characterized as telomitic. But the fact must be noticed here that there are some pairs of chromosomes which are characterized by a satellite-like globular body attached at their inner terminals. Detailed accounts on this structure will be given in another paper.

Generally speaking, the individual chromosomes bear no characteristic appearance beyond that of length. The mating up of the homologous chromosomes was done by means of the procedure already described, and they were placed in pairs and arranged serially according to the descendant order of length. Thus it is established that each series consists of 20 homologous pairs of autosomes ranging from *a* to *t* and an unequal pair made up of the X (large) and Y (small) chromosomes placed at the extreme right (*cf.* Figs. 11–30). Of the autosome pairs two are comprised of conspicuously large chromosomes (*a*'s and *b*'s in Figs. 11–30), and from the third largest pair (*c*'s) downwards the elements form a closely graded series. There is also an uniform evidence that the X element thus identified is represented by the chromosome ranging between the *b*'s and *c*'s of the autosome series in relative magnitude; in other words, the X is the third largest, being slightly larger than the *c*'s, when all the elements come under comparison. The Y chromosome approximately simulates in size the chromosomes of the smallest pair (the *t*-pair), sometimes presenting a more slender appearance than the latter (cf. *xy* in Figs. 1–6 and Figs. 11–30).

In the primary spermatocyte division there occur 21 bivalent chromosomes at metaphase, 20 of which are autosomal tetrads, the remaining one being a heteromorphic tetrad made up by the end-to-end connection of a large X with a small Y (Figs. 7–10). In this division the X and Y segregate without exception migrating towards the opposite poles, as do the autosomal tetrads, and this results in the production of two sorts of secondary spermatocytes, one including the X and the other the Y.

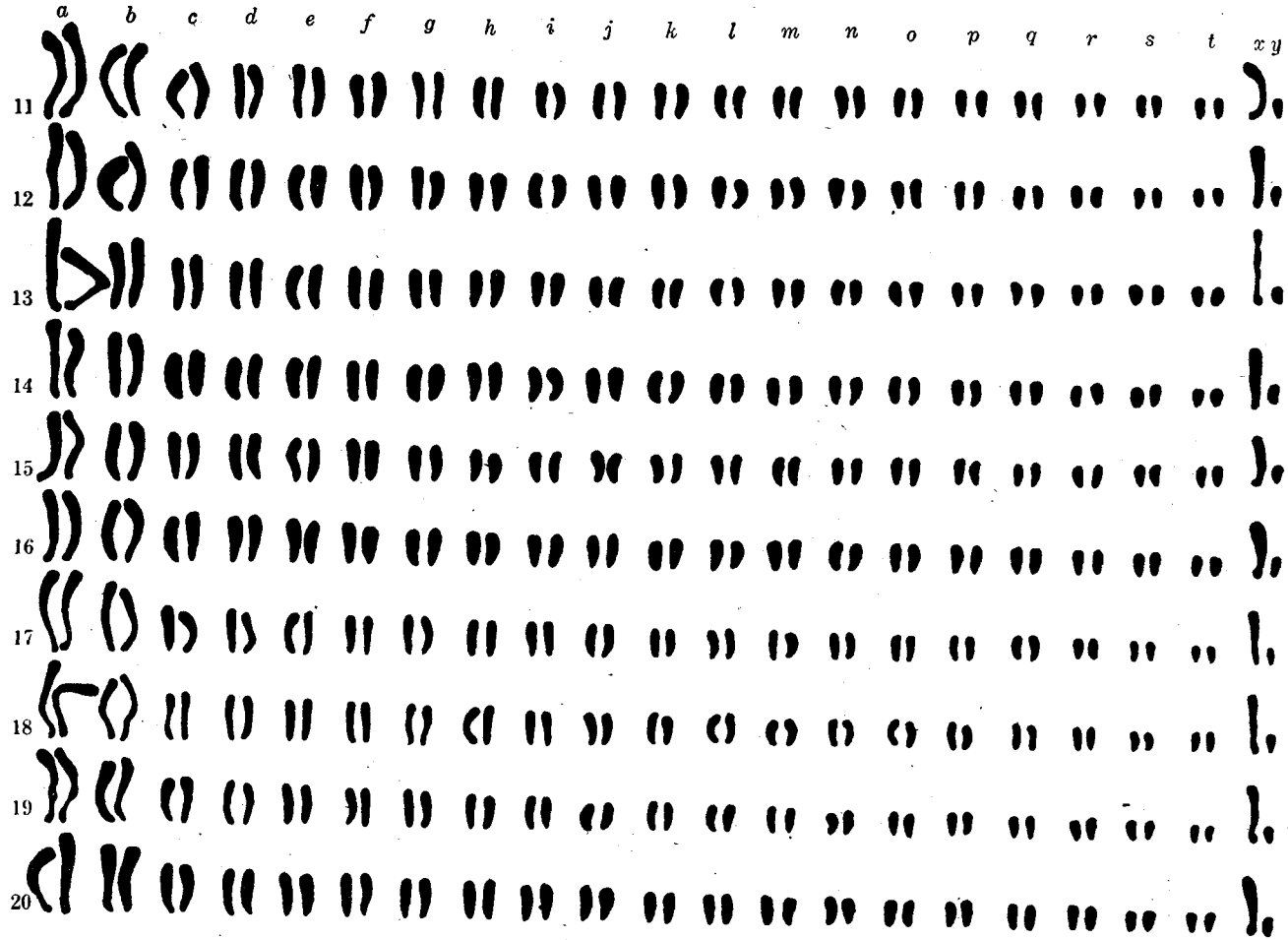
Comparison of the chromosomes between the wild form and the domesticated variety

The comparison of the chromosomes was made for the sake of convenience in the spermatogonial complex arranged in the serial

alignment, all of which were derived from the material treated with identical technique. In the case of the wild form, 20 series of the alignmental arrangement of chromosomes were ready for study, of which 10 series are derived from the specimens secured in Taihoku (Taiwan), five from Naha (Okinawa) and also five from Sapporo (Hokkaido). In the albino material, 22 series of the alignmental arrangement were provided for study, 15 of which come from rats of the Wistar strain and the remaining seven from those of the common albino strain. Examples are given in Figs. 11 to 30. Thus in total, 42 series of the alignmental arrangement of chromosomes served for this study where the comparison of chromosomes was to be made between the wild and domesticated forms.

The chromosomes shown in Figs. 11 to 20 are all from the domesticated albino rats, of which those from Fig. 11 to Fig. 16 are examples of the chromosomes offered by the albino rats of the Wistar strain and those from Fig. 17 to Fig. 20 by white rats of the common albino strain. The serial alignments shown in Figs. 21 to 30 are representative of the chromosomal complement of the wild rats coming from different districts such as Taihoku (Figs. 21–26), Naha (Fig. 27) and Sapporo (Figs. 28–30). A general comparison roughly made by reference to these figures exhibits that there is an extensive homology between the chromosomes of the wild form and of the domesticated variety. The uniformity is firstly conspicuous in the fact that there occur two distinct, large pairs of autosomes labelled *a* and *b*, the one (*a*'s) being considerably larger than the other (*b*'s). From the third largest pair (*c*'s) downwards the elements show a serial gradation in diminution of length, so that the curve, if plotted, will approach a straight line. Another uniform fact is that through all the cases the X is represented by an element which ranks in magnitude between the *b's* and *c*'s while the Y simulates in size the members of *t*-pair. .

Wishing to get some data which would make the comparison more appreciable, some mensural observations were made on these chromosomes. The ratio of length between each member of the largest (*a*) and smallest (*t*) pair of the autosomes, which are extremely distinctive and therefore very easy to pick out, comes under consideration in the first place. As proved in the author's previous paper (Makino '41), the size of the chromosomes varies in a quite proportional relation between the large and small cells, so that the



Figs. 11–20. Chromosomes of the domesticated albino variety of *Rattus norvegicus* Berkenhout. ×3400. Paired alignments of homologous mates from spermatogonial chromosomes in descending order. 11–16, from the Wistar strain. 17–20, from the common albino strain.

ratio of length between certain chromosomes may be applicable as the basis of comparison in different cells. Table I shows the mean value of the ratio of length of the *a*'s to that of the *t*'s by way of comparison in observed cases.

TABLE I

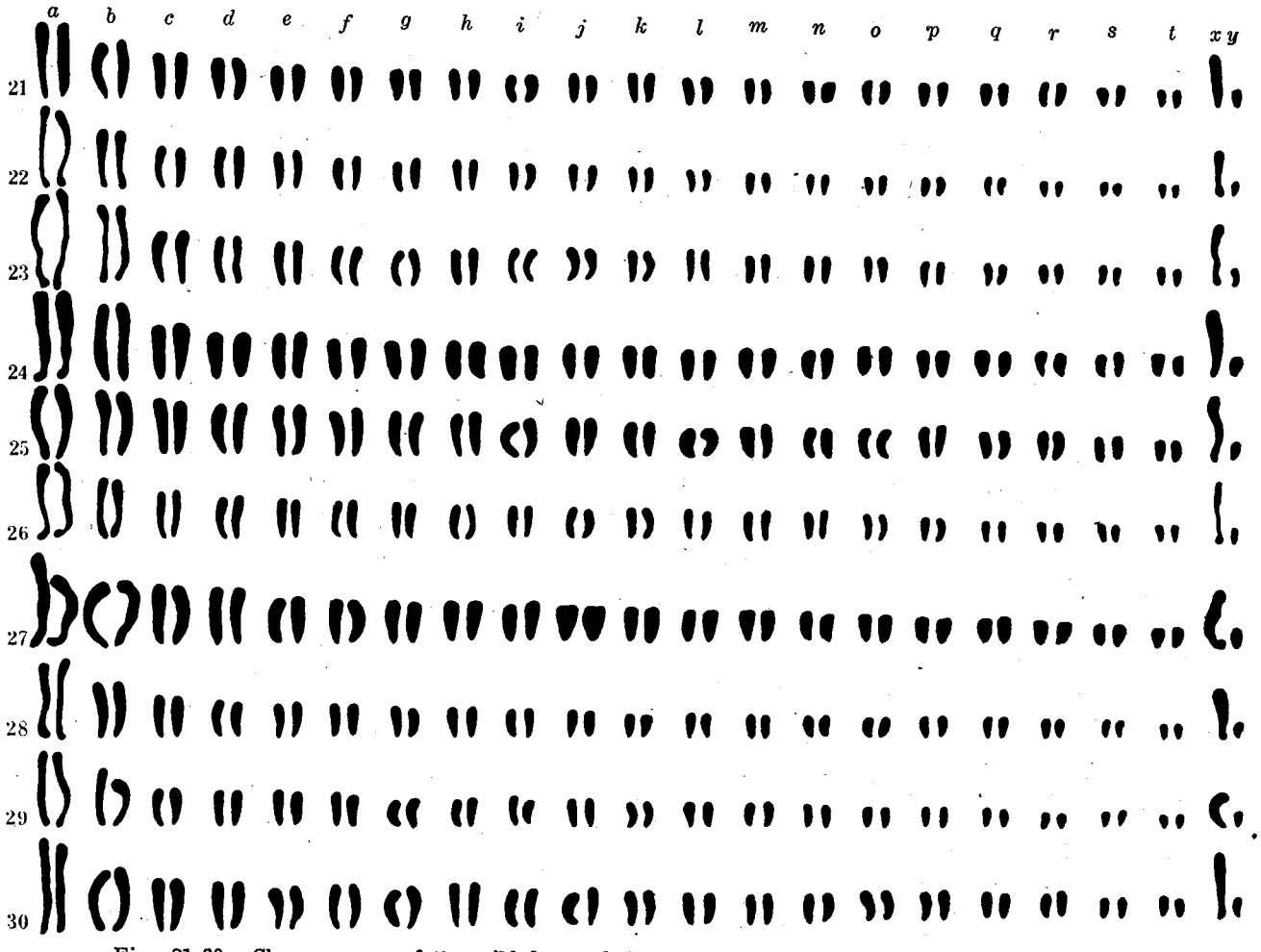
Mean value of ratio in length between the *a*'s and *t*'s, and also between the *b*'s and *c*'s, observed in the domesticated and wild forms of *R. norvegicus*

Material	Mean value of ratio in length ($a:t$)	Mean value of ratio in length ($b:c$)	Number of cells observed
Domesticated albino rats:			
Wistar strain	3.68±0.20	1.34±0.05	15
Common albino strain	3.70±0.28	1.44±0.20	7
Wild rats:			
Taihoku specimens	3.69±0.16	1.31±0.05	10
Sapporo specimens	3.84±0.10	1.31±0.01	5
Naha specimens	3.60±0.15	1.30±0.01	5

Referring to the above table, it will be found that the ratio herein obtained shows no appreciable difference, but is nearly identical among the rats compared.

In the next place, the ratio in length between the second largest (*b*'s) and third largest (*c*'s) autosomes was compared and the data are also arranged in Table I. Examining the results it is seen again that there occurs no significant difference between the domesticated and wild forms compared.

As thus above noted, the data obtained in this study are sufficient to show that there is present no slight feature to prove any morphological difference in the chromosomes between the wild form and the domesticated albino variety of *R. norvegicus*: both the general aspect of the chromosome complex in the wild form and the morphology of all elements, as well as the number of chromosomes and the ratios in length between certain chromosomes are quite identical with all the characters of the domesticated variety. On the strength of this investigation, therefore, it can be said, that, so far as the general morphological characteristics of chromosomes are concerned, there is found no visible shifting in the chromosomes between the



Figs. 21–30. Chromosomes of the wild form of *Rattus norvegicus* Berkenhout. ×3400. Paired alignments of homologous mates from spermatogonial chromosomes in descending order. 21–26, from Taihoku specimens. 27, from Naha specimen. 28–30, from Sapporo specimens.

domesticated variety of *R. norvegicus* and its original wild form. At the same time it is also clear that the chromosomal condition of the wild rat is entirely identical among the individuals coming from different localities, so far as the scope of the present observation is concerned.

In addition it may be necessary to note here that the meiotic course proceeds quite regularly in rats, both wild and domesticated, no aberrant behaviour of chromosomes taking place during meiosis. Of 164 metaphase figures of the primary spermatocytes observed in the Wistar rats, no one case has been observed in which an aberrant number of chromosomes occurred due to univalent formation, or the irregular behaviour of the sex chromosomes as occasionally reported by some authors takes place. In this regard detailed accounts will be given in another paper together with necessary discussion.

Critical remarks

Though a number of papers have appeared on the cytology of *Rattus norvegicus*, none of these investigators has directed his studies towards the morphological analysis of the chromosomes from the standpoint of comparison between the domesticated and wild forms. It is quite recently that the morphological details of chromosomes have been fully cleared up in this form and the exact count of its chromosome number (as 42 in $2n$ and 21 in n) has been reported. Allen ('18, '40), Pincus ('27), Painter ('28), Minouchi ('28), Swezy ('28), Bryden ('32, '33), Koller & Darlington ('34) and Matthey ('36) have investigated with the albino rats, while the studies of Swezy ('28), Oguma ('35) and Tateishi ('35) have been carried out on material derived from the wild rats. All these investigators agree in respect of the number of chromosomes, being 42 in diploid and 21 in haploid, in both the wild and domesticated forms, except some controversies in respect of the behaviour and morphology of the sex chromosomes. The present study is the first close morphological analysis of the chromosomes and direct comparison of them made between the wild and domesticated forms.

Swezy ('28) is the only author to report an unusual chromosomal condition in *R. norvegicus*, a condition which heretofore had not been known. In the cytological study on a hybrid colony of rats established by interracial crosses of the albino rats with the wild rats, Swezy ('28) reported two kinds of individuals, one having

a diploid number of 42 and haploid numbers of 21 and 31, and the other showing 62 diploid and 31 and 21 haploid numbers. Matings attempted between members of the colony, produced litters some of which had 62 and some 42 chromosomes. These findings of Swezy are quite unusual and no reference to any other such like case has been found in the literature either in animals or in plants. Any adequate explanation for this phenomenon was not made by Swezy ('28). Referring to her original paper it becomes apparent at once that the evidences shown by Swezy ('28) are not indisputably clear-cut and absolutely convincing. The point is fully discussed in the author's previous paper (Makino '41) ; one cannot expect any reliable result from her observations. A reinvestigation is thus quite desirable at present upon the chromosomes of rats of the same strain with the application of the modern improved technique.

There are many animals obtainable both wild and domesticated. The cytological condition of these animals is mostly unknown. Kawaguchi ('28) made a comparative study of the chromosomes between the domesticated silkworm, *Bombyx mori* and the wild silkworm, *Bombyx mandarina*, the latter having been considered as the ancestral form from which the former was derived. According to him, the domesticated slikworm shows the haploid number of 28, while in the wild silkworm it is 27, one less than that of the former. The interpretation for the chromosomal relationship between these two forms has been given by him as thus; "Eines der 27 Chromosomen von *B. mandarina* in zwei Teile zerbrochen ist und so bei *B. mori* die Gesamtzahl auf 28 Chromosomen erhöht hat". Recently Tateishi ('36) published his comparison of the chromosomes of the formosan hare, *Lepus formosus* and the rabbit, *Lepus cuniculus*. The number of chromosomes of the hare[1] was 48 for diploid, while that of the rabbit was 44, four less than the former. With regard to the chromosomal relationship existing between the hare and rabbit Tateishi ('36) stated that the numerical and morphological differences found between them are too great to be accounted for on the basis of a breaking in two of a chromosome or the fusion of two pairs.

1) At this opportunity the author wishes to note here that from his own observations on the hare found in Hokkaido (*Lepus gichiganus ainu*) there occur likewise 24 chromosomes (as *n*) at metaphase of the primary spermatocyte.

Contrary to the above cases, the present comparative investigation undertaken on the wild and domesticated forms of *R. norvegicus* reveals that the chromosomal condition is quite identical in all characters and that there are no numerical nor morphological differences of chromosomes between these two forms[1]. This finding agrees with the fact of the taxonomical similarity existing between these two forms showing that the two are systematically one and the same species designated as *Rattus norvegicus* Berkenhout. The domesticated albinos readily cross with the wild brown rats producing fertile offsprings. The brown coat of the wild form is dominant for the white coat in crosses between them. It is interesting and important to see that the chromosomes of the domesticated variety of *R. norvegicus* undergo no visible shifting from those of the wild form under the influence of domestication and inbreeding of many years.

Summary

With the purpose of finding whether any cytological difference is present or not between the domesticated and wild forms of *Rattus norvegicus*, the present study was carried on directed to the morphological analysis of the chromosomes and the close comparison of the corresponding elements in these forms. For material of the domesticated rats, albinos coming from the different strains, one being the Wistar strain and the other the common albino strain, were employed, while for the wild rats those secured in Sapporo (Hokkaido), Taihoku (Taiwan) and Naha (Okinawa) were used for study.

The investigation reveals that so far as the general morphological characteristics of the chromosomes are concerned, the chromosomes of the wild brown rat are quite identical in all characters with those of the domesticated albino form, and that there is no visible difference in the chromosomes between the individuals coming from the different localities, not only in the number but also in the other external features. The evidence may be clearly understood by reference to the accompanying figures and table.

1) Recently Tateishi ('41) who studied the chromosomes of *Felis bengalensis*, a wild cat, reported that the chromosomes are nearly identical in their number and other morphological characters with those of the domestic cat, *Felis domestica*.

Literature cited

ALLEN, E. 1918. Studies on cell division in the albino rat (*Mus norvegicus albinus*). III. Spermatogenesis, the origin of the first spermatocytes and the organization of chromosomes, including accessory. Jour. Morph., vol. 31: 133–185.

———— 1940. Variations in the accessory chromosomes in metaphase and anaphase of the first spermatocytes of the albino rat. Jour. Morph., vol. 66: 453–462.

BRYDEN, Wm. 1932. Cytogenetic studies on the rat. Somatic chromosome complex, meiosis and chiasma frequency. Jour. Genet., vol. 26: 395–416.

———— 1933. A comparison of the chromosomes of the rat and the mouse. Jour. Genet., vol. 27: 421–434.

KAWAGUCHI, E. 1928. Zytologische Untersuchungen am Seidenspinner und seinen Verwandten. I. Gametogenese von *Bombyx mori* L. und *Bombyx mandarina* M. und ihrer Bastarde. Zeits. Zellf. mikr. Anat., Bd. 7: 519–552.

KOLLER, P. Ch. and C. D. DARLINGTON. 1934. The genetical and mechanical properties of the sex-chromosomes. I. *Rattus norvegicus*, ♂. Jour. Genet., vol. 29: 159–173.

MAKINO, S. 1941. Studies on the murine chromosomes. I. Cytologial investigations of mice, included in the genus *Mus*. Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zoology, vol. 7: 305–380.

MATTHEY, R. 1936. Le problème des hétérochromosomes chez less Mammifères. Arch. Biol., T. 47: 319–383.

MINOUCHI, O. 1928. Spermatogenesis of the albino rat (*Mus norvegicus albus*). Jap. Jour. Zool., vol. 1: 235–254.

OGUMA, K. 1935. The chromosomes of four wild species of Muridae. Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zoology, vol. 4: 35–57.

OGUMA, K. and S. MAKINO. 1937. A new list of the chromosome numbers in Vertebrata (March 1937). Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zoology, vol. 5: 297–356.

PAINTER, T. S. 1928. A comparison of chromosomes of the rat and mouse with special reference to the question of chromosome homology in mammals. Genetics, vol. 13: 180–189.

PINCUS, G. 1927. A comparative study of the chromosomes of the Norway rat (*Rattus norvegicus* Erxl.) and the black rat (*Rattus rattus* L.). Jour. Morph., vol. 44: 515–532.

SWEZY, O. 1928. On the existence of two chromosome numbers in a mixed rat strain. Jour. Exp. Zool., vol. 51: 135–161.

TATEISHI, S. 1935. On the sex chromosomes of 11 species of the Muridae, etc. (Abstract in Japanese). Proc. Jap. Assoc. Adv. Sci., vol. 10: 1007–1012.

———— 1936. The chromosomes of the Formosan hare, *Lepus formosus* and of the rabbit, *Lepus cuniculus*. (Japanese with English Resumé). Zool. Mag. (Japan)., vol. 48: 617–624.

———— 1941. The domestic cat and *Felis bengalensis*, a wild cat. (In Japanese). Kagaku-no-Taiwan, vol. 9: 1–7.