The Chromosomes of the Horse (*Equus caballus*)
*(Chromosome studies in domestic mammals, 1)*

By

**Sajiro Makino**

Zoological Institute, Faculty of Science,
Hokkaido Imperial University, Sapporo

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The karyological investigations of the domestic mammals are a matter of both scientific and practical importance because of their practical value. At the present time, however, our knowledge regarding them remains in an unsatisfactory status, despite a considerable amount of works devoted to them by previous investigators, such as Wodsedalek (’13, ’14, ’16, ’20, ’22), Masui (’19a, b), Hance (’17), Painter (’24), Sokolov (’30), Krallinger (’31), Shiwago (’31), Butarin (’35), Berry (’38, ’41). It is desirable that this field be thoroughly explored in order that the geneticists and breeders of the future may have a fundamental information upon which to build. Being in response to this request, the present investigation has been instituted with the suggestion and guidance of Prof. Oguma, and in the series of papers to be continuously published hereafter will be dealt with the chromosomes of various domestic mammals.

The chromosomes of the horse with which the present paper is concerned have been studied by several investigators, but, as in the case of many other mammals, there is marked disagreement on the fundamental question of chromosome numbers. The early work by Kirillow (’12) indicated that the haploid number was not less than 10 nor more than 16. A little later Wodsedalek (’14) published a study on spermatogenesis in which he reported the diploid number as 37 and the haploid as 19. Masui (’19) found the diploid number to be between 33 and 38, and 19 in the haploid. Somewhat later Painter (’24) published an extensive paper on spermatogenesis in which he placed the diploid number at proximately 60 and the haploid at 30 chromosomes. Recently Ranquini (’34) also gave the diploid number of chromosomes as 60. It is highly probable that the wide discrepancy in the reported chromosome numbers as above mentioned does not primarily depend upon the difference of the animals they used but is only due to the incomplete fixation of chromosomes in-

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duced by the inadequate technique. Particularly the extreme low chromosome counts in the earlier works are probably accounted for by the unfavourable technique used. The purpose of the present investigation is to establish, on the one hand, the exact accounts of the chromosomes in the horse, not only in the number but also in other morphological details, and on the other hand, to learn any kind of difference in the chromosomes, if present, between the highly improved breed and the primitive native breed of horses.

Before going further it is the author's pleasant duty to express here his sincere gratitude to Prof. K. Oguma for his kind guidance and valuable suggestions. The expense of carrying out this work was partly borne by a grant from the "Nippon Keiba-kai" and by the Scientific Research Expenditure of the Department of Education.

**Material and Method**

The horses which furnished the testicular material for the present study are the Percheron breed and the Ryūkyū Pony. The former, Percheron, is famous as one of the heavy draught-horses of France. The testes from two individuals ranging in age from two- to three-year-old, fixed in May of 1932, comprise the material. They were obtained by means of castration in the Veterinary Hospital of this University.

The Ryūkyū Pony which is said to be indigenous to the Ryūkyū (Loo Choo) Islands, is an extremely small, native breed, often not more than 1 metre high, carrying a number of primitive characters. Despite the smallness of its size, this pony is capable of doing a good deal of hard work. The ponies readily cross with the other imported breed producing fertile offsprings. At present they have been more or less crossed with alien blood, and the true old breed has been kept in the Miyako-Zima, one of the Ryūkyū Islands. During his sojourn at the Ryūkyū Islands, taking place in the spring of 1941, the present author had a chance to get the testicular material of the Ryūkyū Pony at Naha, through the courtesy of Mr. H. Yashiro, to whom the author's hearty thanks are due. The pony in which the castration was performed, was three years old in age. It was imported from the Miyako-Zima in September of 1940.

In both cases the testes were cut into small pieces as soon as possible immediately after castration and put into the fixative. For preservation of chromosomes Champy's solution and Flemming's mixture without acetic acid were used and excellent result was obtained in both of them. Sections were cut 10–12 micra thick and stained according to the Heidenhain's iron-haematoxylin method.

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1 Not of completely pure blood.
Observations

1. Accounts regarding the Percheron breed

**Spermatogonia:** A considerable amount of time has been devoted to a study of the morphology of the spermatogonial chromosomes, in order that the small size of the cells in the horse coupled with the prevalence of small chromosomes has made counting very difficult. For the study of the number, therefore, the metaphase plates of the primary spermatogonia were selected on account of their large size of the cells. Figs. 1–4 are the representatives. In these plates the chromosomes are found sharply defined from one another and show no sign of obscure individuality. There are definitely found 66 chromosomes (as 2n) in every spermatogonial plate without any slight doubt. No such variations in number (60, 58, 57) as given by Painter ('24) could be found. Though no conclusive statement can be made since these metaphase plates are not sufficiently good enough to make precisely the morphological analysis of the individual chromosomes, however, it is possible to state that the diploid complex contains at least 12 atelomitic chromosomes in 6 homologous pairs along with the remaining telomitic elements of varying lengths. Of the atelomitic group some are median and others submedian in their fibre attachments. Those of telomitic range in shape from straight or slightly curved long rod to short pointing rod. The evidence showing that the number of the spermatogonial chromosomes is even, naturally suggests the possibility that the sex chromosomes contained should be of the ordinary XY-type. The identification of the X and Y elements in the diploid complement, however, is practically a matter of difficulty, since they assume no remarkable features distinguishable from the other elements, while the condition that is encountered in the metaphase of the first division, as stated in the next

Figs. 1–4. Chromosomes of the Percheron breed. Spermatogonial complexes in polar view, 66 chromosomes in each. ca. ×4000.
section, is very suggestive of the fact that the X-element must be represented by one of the long rod-shaped chromosomes and the Y-element by one of the short ones. There is entirely absent such a peculiar element as described by Wodsedalek ('14) as the sex chromosome of this animal.

**Primary spermatocytes:** Fortunately excellently preserved metaphase plates were found abundantly everywhere in the sections. Determination of the chromosome number is made with extreme clearness in this stage on account of its reduced number, and therefore this stage is very available for the confirmation of the diploid number. A sufficient number of excellent equatorial plates as shown in Figs. 5 to 8, in which all chromosomes show no obscure clumped condition that makes the exact study of individual elements impossible, were studied for determination of the chromosome number. Thirty-three distinct bivalents having various shapes are found in every plate observed (Figs. 5–8). They are arranged in the most regular manner, the larger tetrads being situated at the outer circle of the spindle surrounding the smaller ones in the central space. The tetrads lying in the marginal zone are of ring-, V- or cross-shape. The largest of them is provided with the form of the compound ring. This is undoubtedly the descendant of the largest pair of the V-shaped chromosomes formerly observed in the diploid group. Those scattering in the central space considerably vary in their shape and size, some being small ring- or V-shaped and the others assuming elongated dumbbell shape. A remarkable fact is the constant presence of a heteromorphic tetrad which is composed of two unequal components, a striking long rod element and a smaller one, connected end to end with the former. On account of its remarkable structure, this tetrad appears in shape distinction to the other ordinary tetrads. In the metaphase arrangement it lies almost always in the most peripheral circle (Figs. 5–8, xy). Viewing all characteristic conditions it is selfevident that this particular tetrad is nothing other than the XY-complex, the longer one being the X-element and the shorter the Y.

It is in the side view of the metaphase plate that the configuration and structure of the XY-complex become most evident (Figs. 9–11). The X-element is apparently characterized by acquiring a diffused texture with somewhat vague outline, and morphologically it seems to be provided with the telomitic fibre attachment, being represented by one of the longer elements, probably the longest of all. The Y-element, which seems to range in size a little longer than the smallest autosome, is always stained as deep as the autosomes exhibiting a solid and defined contour. The X and the Y are connected to each other end to end by means of a fine but distinct fibrous element. Examination of many metaphase plates is very suggestive of indicating that the mode of conjugation between the X and the Y of this animal is probably analogous to that reported in some
species of *Rattus* (cf. Minouchi '28, Oguma '35, Makino '42a, b). Namely, in conjugation the X is approximately horizontal to the equatorial plate being vertical to the long axis of the spindle, whereas the Y stands perpendicular to the equatorial plate, in parallel to the spindle axis. And therefore, it is quite reasonable to accept that the spindle fibres come to attach in the inner proximal portion of the X where the latter associates with the Y, while in the Y-chromosome, the fibre attaches to the free end opposite the point where it comes in contact with the X.

Remarkable and noticeable is the segmentary configuration characterized by the X-chromosome. The X represents, at least in preparations adequately preserved and a little destained, a striking structure provided with two transverse constrictions which subdivide the entire body of the X into three consecutive segments. Clear pictures illustrating this feature are seen in Figs. 6 to 11. The proximal segment, at the free end of which the Y comes in connection, is quite distinct, due to its compact texture, from the other two segments, and is nearly identical in its relative magnitude and configuration to the Y-element. It is in this proximal segment that the attachment locus of the spindle fibres is involved. The median and the distal segments assume a less stained and diffused appearance with vague outline, sometimes elongated to a considerable extent, probably due to drawing out of the inner chromonema.
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spirals. The X-element occasionally appears to be irregularly bent at the two constricted portions due to their loose texture. As thus noted, the condition displayed by the X evidently shows a striking similarity to that described in the cases of rats, mice and some others (Oguma '35, '37a, b, Makino '41, '42). The tripartite structure seems to be a remarkable characteristic and of wide occurrence in the X-chromosome of mammals.

At anaphase, all the tetrads divide synchronously. The X always disjoins from the Y in this division. There are no chromosomes which divide a little in advance of the others, contrary to the evidence described by Painter ('24) in which a conspicuous precocious division of XY-bivalent seems to be of due course in the horse. Thus the division of the primary spermatocytes gives rise to the secondary spermatocytes containing X-chromosome in the one class and in the other including the Y-chromosome.

Secondary spermatocyte: Unexceptional segregation of the X- and Y-chromosomes in the first division gives rise to two different garnitures of chromosomes in the secondary spermatocyte as regards the X and Y, the number of chromosomes being similarly 33 in both kinds. One kind of garniture is the X-class cell containing the X-element, the other the Y-class, including the Y-element. The former garniture is composed of 6 atelomitic double V-shaped, 26 telomitic double rod-shaped and a single V-shaped dyad which is considered, from its character, to be X-element (Figs. 12–13). The X lies always in the peripheral zone of the spindle and shows a remarkable distinguishing feature from other autosome dyads, assuming a vague outline being less stained than others. The X-element is evidently composed of two telomitic rod-shaped monads, jointed at one end pointing the center, thus assuming the form of V's. The other kind of garniture consists of 6 atelomitic double V-shaped and 27 telomitic, double rod-shaped dyads in which the Y-element may be included (Figs. 14–15). The identification of the Y-element in the complex is impossible since it indicates no marked character distinguishable from the other autosomes. The secondary spermatocyte of the horse is very small in size and this makes more precise examination difficult.

It may be entirely needless to discuss here regarding whether the double reduction or second pairing of chromosomes in the second division as described by Wodsedalek ('14) in the horse is a natural process or not, since our recent knowledge of cytology pointed out clearly that this old misconception is derived from the haphazard supposition which is conveniently constructed to explain misunderstanding induced by artificial agglutination of chromosomes.
2. Accounts regarding the Ryūkyū Pony

There is a distinct breed of pony, about 1 metre in height, in the Ryūkyū Islands. Though nothing has been known on the origin of this animal, it has generally been accepted that this pony is indigenous to the Ryūkyū Islands and carries a number of primitive characters. It seems very likely that, since the Ryūkyū group consists of a number of desert islands in the ocean, ancient characters have been kept unaltered so long in this pony, without being affected by alien blood.

The number of chromosomes determined in the study of the Ryūkyū Pony is in complete agreement with that previously established for the Percheron breed, 66 chromosomes being attained for the diploid complement in the spermatogonium (Fig. 16) and 33 for the haploid in the primary spermatocyte (Figs. 17–18). So far as the observations go, the morphological characteristics of the chromosomes seem likewise apparently identical between both breeds. In the present material is fortunately obtained a favourable metaphase plate of the spermatogonium as given in Fig. 16, which is excellently preserved sufficient enough to admit the morphological analysis of the individual chromosomes to some extent. The diploid complement contains, as clearly recognizable by referring to the figure, six or more pairs of atelomitic V-shaped chromosomes with varying size, together with the remaining rod-shaped ones ranging in length from long rod to short small ones. By the mating up of the supposed homologous chromosomes based on comparison of their shape and size, a rod-shaped chromosome which is a member of longer chromosomes, is remained destitute of its homologous mate. It is the

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Figs. 16–20. Chromosomes of the Ryūkyū Pony. ca. ×4000. 16, spermatogonial complex, 66 chromosomes. 17–18, primary spermatocyte metaphases, 33 elements in each. 19, side view of the primary spermatocyte spindle, illustrating the XY-complex. 20, telophase figure of the first division, showing the segregation of the X and Y.
element marked with an asterisk in Fig. 16 and this is, in all probability, the X-chromosome. The X-element, which, in the Percheron breed, was difficult to be pointed out among the diploid group, was thus apparently identified in the present case, while the Y-chromosome is entirely impossible to be pointed out in the diploid complement from the autosomes due to prevalence of closely similar elements.

With regard to the spermatocyte chromosomes, there is also present no new evidence especially noticeable and added for this breed, not only in their morphological features but also in their behaviour. The reference to the annexed figures will bear witness to this fact and illustrate better than any verbal description (compare Figs. 17–18 with Figs. 5–8). The X-element likewise bears a segmental configuration showing two constrictions (see x in Figs. 17–19). The X conjugates with the Y in quite a similar manner as observed in the Percheron (Fig. 19), and they segregate in the first division running to the opposite poles (Fig. 20). Though not actually observed, it may be beyond question that there are produced two kinds of secondary spermatocytes, one containing the X-element and the other the Y. Hence, these observations lead to the conclusion that there are detected no apparent and noteworthy differences between the chromosomes of the Ryūkyū Pony and the Percheron breed, so far as the general morphological characteristics of chromosomes are concerned.

Some Considerations on the Chromosomes of the Horse

The chromosome number: So far as the literature shows (cf. Oguma and Makino '37), the cytological study of the horse has been made by five investigators until the present time. Kirillow ('12) is the first author who reported the chromosome number in the horse, announcing that the haploid number was not less than 10 nor more than 16. Somewhat later, Wodsedalek ('14), in his paper dealing with the spermatogenesis of the horse, reported that 37 chromosomes occur in the spermatogonia and 19 in the primary spermatocyte. Then Masui ('12) published a paper on spermatogenesis, expressing nearly similar opinion with Wodsedalek ('14), in which he counted 33–38 chromosomes in the spermatogonia and 19 in the primary spermatocyte. Later Painter ('24), in his study on the chromosomes of the horse, confirmed that the number of chromosomes is not so small as given by earlier investigators. He said as follows: “Spermatogonial counts range from 57 to 60, the best plate showing the latter number. Favourable haploid counts with one exception, have given consistently 30 chromosomes” Recently Ranquini ('34) attained to the same conclusion giving 60 for the diploid chromosome number of the horse. Thus, the number of chromosomes of the horse
differs according to the authors. How are brought forth such discrepant statements as to the number of chromosomes in one and the same animal, as mentioned above? There are probably two explanations for these disagreements: 1) the numerical difference of chromosomes according to the race of horses, 2) the difference in counting introduced from the improperly fixed material. The first mentioned question was settled in the present study carried out on the Percheron breed and the Ryūkyū Pony¹, by confirming that the number of chromosomes is not varying according to the breed of the horse. The second problem seems to be the most probable source which causes the numerical difference in mammalian chromosomes, and this is the matter of dispute. Previous investigators of the chromosomes of the horse, except Painter (’24), failed to preserve them in their natural state. The metaphase chromosomes in their figures are clumped together beyond hope of recognition of their individuality. To make definite statements as regards the chromosome number, applying such imperfect material with quite obscure individuality of chromosomes, is a task of adventure. In the best metaphase plate he could find, Painter (’24) who succeeded to some extent in preserving the spermatogonial chromosomes, was unable to count more than 60 chromosomes in diploid. However, there are present some chromosomes of indefinite individuality in his figure. It is impossible therefore, to determine the exact number of chromosomes in such a preparation. For such disputable respects in chromosome counts, he apologized as follows: “On the basis of spermatogonial counts alone, the number 60 plus or minus 2 is as close as one would be justified in maintaining”. In addition to this, he stated secondarily, after examination of the primary spermatocyte, that “all things considered, the first spermatocyte counts were the most favourable, and as 30 chromosomes have been found here, we may give the diploid number as 60” But his figures of primary spermatocytes in which he made counts are also quite doubtful; the chromosomes are so closely overlying one another that one can not determine the exact number. This evidently induces a source of error in counting. Inasmuch as his preparations are imperfect as stated above, counting of Painter (’24) cannot be considered as valid.

Hence it is evident that the chromosome number hitherto reported by five investigators is not exact one and that these disagreements as to the number do not depend on the racial difference of the material employed in the study, but result from improper poor fixation. In the present study, the author has carefully examined numerous metaphase plates in both sides of diploid and haploid groups and has found that there are 66 chromosomes in diploid and 33 in haploid without exception.

¹ In addition to these forms, the chromosomes of the Anglo-Norman impure breed were examined, establishing likewise 33 chromosomes in the primary spermatocyte.
The explanation for the lower counts given by earlier investigators such as Kirillow ('12), Wodsedalek ('14) and Masui ('12) is undoubtedly faulty preservation. Judging from his figures it is very likely that Painter's counts ('24) were handicapped by a fusion of some chromosomes. The numbers 66 in diploid and 33 in haploid are, it seems to the author, the natural number characteristic to the horse dealt with.

**The form of chromosomes:** It is a matter of difficulty to determine precisely the form of chromosomes of a spermatogonial metaphase. In well preserved condition, however, this will become clear to some extent by the examination of chromosomes not only in the spermatogonia but also in the primary and secondary spermatocytes. In the present study it is ascertained with all probability that the diploid complement shows at least six pairs of atelomitic V-shaped elements, and this fact was confirmed again in the secondary spermatocyte by pointing 6 atelomitic chromosomes. In Painter's best figure ('24) one can point out nearly 12 V-shaped chromosomes, though he did not touch decidedly this point.

As the interesting feature of spermatogonial chromosomes in the horse, Painter ('24) described the form of the third largest pair of chromosomes. According to his description, each element of this pair is made up of two components connected by a more or less attenuated chromatic bridge and the occurrence of this pair is constant. Supporting his view on fragmentation hypothesis of mammalian chromosomes, he stated on these chromosomes as follows: “the very high number in the horse together with the large proportion of small elements at once suggests the possibility that this condition has been brought about by a fragmentation of larger chromosomes, and that the third pair of chromosomes shows a step in this fragmentation.” Though careful examination is made, however, the author has failed to find such curious shaped elements in the spermatogonial complex. The presence of such elements is only accounted for by unfavourable fixation.

In this place, it is interesting to compare the chromosomes of the horse with those of some other hoofed animals. Krallinger ('31, '36), in his extensive study on this group of animals, reported 60 chromosomes as the diploid number respectively in the cattle, sheep and goat, 38 in the pig and 30 in Pecari. According to his statement, 60 chromosomes of the cattle are composed of 59 telomitic and 1 atelomitic ones, and those of the sheep consist of 56 telomitic and 4 atelomitic, while in the goat they are all telomitic. So far as the number and form are concerned, therefore, the chromosomes of the horse are of the most complicated among the members of the ungulates. More detailed comparison may be made later when the author's own studies being carried out on these hoofed animals will have been accomplished.

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1 Sokolov ('30) and Shiwago ('31) also gave the same number 60 for the goat.
The sex chromosome: The previous investigations are not in agreement concerning the evidence for sex chromosome in the horse. Wodsedalek ('14) and Masui ('19) reported the existence of an odd X-chromosome in the horse. It is doubtless that these earlier investigators entirely failed, as already mentioned, in the identification of the real sex chromosomes. The X-chromosome adopted by them in the first division is evidently no other than a displaced tetrad. On the other hand, Painter ('24) and Ranquini ('34) agree in finding the sex chromosome of the XY-type. Referring to his drawings it seems probable to the author that the XY-elements designated by Painter ('24) in his paper do not exhibit any natural feature, not only in their configuration but also in behaviour. The magnitude of the X-element, and of the Y as well, vary considerably according to the individual cells. In some cases, he failed in the identification of the true sex chromosomes. Thus the conditions, as above mentioned, are sufficient to show that the accounts on the sex chromosome of the horse given by the previous investigators were in a very insufficient status. As mentioned in the foregoing descriptions, the present investigation was able to furnish, with indisputable clearness, the correct evidence for the sex chromosome in the horse, which can be regarded as conclusive, establishing various morphological characters of the X- and Y-elements to a great extent.

The tripartite segmentary structure of the X-chromosome which has been described and discussed for the first time by Oguma ('35) in his study of some rodents, and whose occurrence has been broadened over several other forms of mammals by his extensive studies (Oguma '35, '37a, b), has been proved to occur also in the horse herein dealt with. As already described in detail, the X of the horse is subdivided into three successive segments in a linear series by the presence of two transverse constrictions. The tripartite structure is possibly of wide-spread occurrence in the X-chromosomes of mammals, and has undoubtedly an important bearing upon considering the origin and evolution of the sex chromosome in animals, as the suggestion made by Oguma ('37a, '42) shows.

Summary

The chromosomes of the horse were investigated in the following two distinct breeds, the Percheron and the Ryūkyū Pony. So far as the number and other general morphological characters of chromosomes are concerned, the two breeds under consideration show no remarkable and significant difference.

The number of chromosomes in the horse is not so small as described

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1 Nothing can be said further regarding the work of Ranquini ('34), since his original paper was not accessible to the present author.
by previous authors. The spermatogonium contains 66 chromosomes in
which are contained at least 12 atelomitic V-shaped chromosomes along
with the remaining rod-shaped elements ranging in length from long
rod to short one.

The primary spermatocyte metaphase shows 33 tetrads. A hetero-
morphic tetrad of XY-complex is always found lying at the periphery
of the equatorial plate. Both elements of the XY-bivalent seem to be
of telomitic nature. The X-element assumes a characteristic tripartite
structure, being divided by two distinct constrictions into three consecu-
tive segments.

There are distinguishable two different garnitures of chromosomes
in the secondary spermatocyte as regards the X and Y chromosomes,
both being 33 in number. The one is X-class cell consisting of 32 autosome
dyads (6 atelomitic and 26 telomitic) and an X-element of single V-shape.
The other is Y-class and contains 6 atelomitic and 27 telomitic dyads in
which the Y-element is included. The X-element bears a characteristic
structure distinguishable from the other autosomes in the metaphase,
whereas the Y is difficult to be discriminated among the others.

Literature Cited

Berry, R. O. 1938. Comparative studies on the chromosome numbers in sheep, goat, and
sheep x goat hybrids. Jour. Hered. 29.

Butarin, N. S. 1935. The chromosome complex of Arkhar (O. polii karelini Serv.),

Hance, R. T. 1917. The diploid chromosome complexes of the pig (Sus scrofa) and their


Krallinger, A. F. 1931. Cytologische Studien an einigen Haussägetieren. Arch. Tierern-

Makino, S. 1941. Studies on the murine chromosomes. I. Cytological investigations of
mice, included in the genus Mus. Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI,
(Zool.), 7.
— 1942a. Studies on the murine chromosomes. II. Morphological comparison of the
chromosomes between the wild form and the domesticated variety of Rattus
— 1942b. A comparative morphology of the sex chromosomes in five species of Rattus.

Masui, K. 1939a. The spermatogenesis of domestic mammals. I. The spermatogenesis
— 1919b. The spermatogenesis of domestic mammals. II. The spermatogenesis of cattle

Zool. 1.

— 1937a. The segmentary structure of the human X-chromosome compared with that of
— 1937b. Absence of the Y-chromosome in the vole, Microtus montebelli Edw. with supple-
mentary remarks on the sex-chromosomes of Evotomys and A. edemus. Cytologia,
Fujii Jubilee Vol.


