On the Chromosome Number and the Sex-chromosomes in the Germ-cells of Male and Female Cats

By

Osamu Minouchi and Takeo Ohta

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Introduction

Winiwarter published an excellent work on the spermatogenesis of the cat in 1920. He stated that there are 35 chromosomes in the spermatogonia and 18 in the first spermatocytes. According to him the sex-chromosome goes undivided to one pole in advance of the autosomes in the first maturation division, and one of the daughter sets of chromosomes receives 18 chromosomes, the other 17; thus two kinds of cells are produced in the second division. But Minouchi (1928) found 38 chromosomes in the spermatogonia and 19 in the first meiosis, the sex-chromosome being composed of two unequal elements as is the case with other mammals. Before Minouchi, Gutherz (1920) described the number of chromosomes in the female cat as follows: "...übrigens konnte ich mehrfach mit Sicherheit mindestens 38 Chromosomen-zählen." That was the first paper in which the diploid number of chromosomes in the cat was reported as 38. But there are no figures in his paper and we do not know the degree of his certainty. Therefore we tried to clear up this problem.

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Material and method

The male cats came to our possession by the courtesy of Mr. G. Ida of the Institute of Dendrology, Kyoto Imperial University. These cats attacked *Actinida* sp., a frutex, and were caught. They are very fond of the smell of this frutex, bite the bark and dance around it mewling,

though why they behave so has not yet been explained. The animals were killed by decapitation and the testes taken out from the body immediately. At the same moment they were cut into small pieces and dropped into Champy's mixture or Flemming's strong solution without any trace of acetic acid. Sections were cut 8–10 μ thick.

Two newly born cats were used for the study of the female chromosomes. The animals were killed by decapitation three days after their birth. The ovaries were fixed in Flemming's strong solution (without acetic acid) diluted with the same volume or with two parts of water. The latter gave better results than the former in this case.

All the figures were drawn under the microscope (oc. Zeiss K 18; obj. Zeiss Ap. 1.5, N.A. 1.3; tube length 160 mm.), the drawing paper being about 30 mm. below the stage, and were reduced in reproduction to 4/5, except the figure 2 which was enlarged to the scale of 1 $\frac{1}{2}$.

Observation

The connective tissue is well developed in the testis of the cat, and the seminal tubules cannot be pulled out separately. A great many dividing germ-cells are present in the seminal tubules of the testis. The spermatogonia and Sertoli's cells are in contact with the basal membrane. Inside of the spermatogonial zone many first and second spermatocytes are found. The spermatogonia become gradually smaller in every division.

Figs. 3–5 represent polar views of the metaphase of spermatogonia showing 38 chromosomes in each plate. The morphological feature of the chromosomes is especially well preserved in Figs. 2, 3, 4. We lined up the chromosomes in pairs from the real figure under the microscope

Fig. 1. Alignment of chromosomes in pairs.

(not from the drawing) and show the result in the accompanying Text-Figure 1. The paired chromosomes 2, 4, 5, 8, 11 and 16 look as if they were not homologous, apparently differing from each other in size, owing to the fact that the figure is a projected shadow of magnified

chromosomes and some of the chromosomes appear shortened in this figure: the magnified image of the real X-chromosome is probably longer than it appears in the drawing. Even from this example it may be clear that it is very dangerous to arrange chromosomes in pairs from pictures of polar view of the equatorial plate without considering the original preparation. It sometimes leads to an erroneous conclusion.

Three pairs of large chromosomes with median or submedian fiber attachment, four pairs of large ones with subterminal attachment, four pairs of intermediate subterminal or terminal ones, nine pairs of small ones, of which three are submedian and the other six are terminal or subterminal, and a heteromorphous pair with terminal attachment are present in the equatorial plates of spermatogonia. The last pair of heteromorphous chromosomes seems to be the XY group. We have carefully examined many dividing spermatogonia, but we could not find any case where the number of chromosomes is 35 as reported by Winiwarter (1920).

Fig. 2. Figure 3 enlarged ($\times 1\frac{1}{2}$ the original drawing).

Oogonia

Winiwarter et Sainmont (1909) published a fundamental work on the oogenesis and organogenesis of the cat. They mentioned that oogonial divisions are still found in individuals 16-21 days old. But we could not find any dividing oogonia in individuals 14 days old, all of the germ-cells having already been developed into oocytes enclosed by follicle cells. Judging from their figures, they may perhaps have mistaken the division figures of another kind of cells for oogonia. Guthertz's figure 1 in 1920 is also considered to be an oocyte, because it is enclosed by follicle cells. Such cells enter the growth period without division. In the cat the ovaries grow rapidly after birth, and many dividing figures of germ-cells are seen in the cortical layer of such ovaries, as is the case with the dog (Minouchi 1928). We therefore killed the animals three days after birth. The oocytes enclosed by follicle cells are not yet found in these ovaries. The oogonia are

present in the matrix arranged in clusters, not isolated from each other. They are larger than the surrounding matrix cells and contain a large round nucleus, so that we can easily identify them in the ovary. The oogonia clustered together in one group show almost the same division-phase.

Out of many dividing oogonia six metaphase plates are given in Figs. 6-11. They were preserved in Flemming's strong solution (containing no trace of acetic acid) diluted with two parts of water. This fixative seems to be a little too strong for such quite young material, the chromosome plate appearing more contracted and the cytoplasm showing more evident shrinkage than in spermatogonia. The chromosomes

Figs. 3-5. Polar views of the spermatogonial metaphase showing 38 chromosomes. Figs. 3-4. Treated with Flemming-Minouchi. Fig. 5. Treated with Champy-Minouchi. Figs. 6-11. Polar views of the oogonial metaphase showing 38 chromosomes. Treated with diluted Flemming-Minouchi.

could perhaps be separated better and their form be conserved like in the living state, if they were fixed with a more diluted mixture. It is a pity that we are not able to compare these chromosomes with those of spermatogonia, because they are more deformed than the latter. But the preparations are sufficiently clear to enable us to determine the number of chromosomes. The number of chromosomes in oogonia is 38 as shown in these figures, without exception.

The chromosomes in meiosis

The general appearance of the first spermatocyte of the cat is like that of the racoon dog (Minouchi 1929). At the beginning of the growth period the nucleus shows a foggy appearance, then it becomes fibrous. In this stage the chromosomes consist of loosely coiled chromonemata and a small amount of ground substance which seems to have been dispersed throughout the nuclear sap in the resting stage. It begins to converge upon the chromonemata at the outset of the growth period and this process continues until the middle of the metaphase. During this change the chromosomes show a granular or zig-zag appearance on their surface. When this process is finished the surface becomes smooth.

Fig. 12 shows the stage prior to the metaphase and Fig. 13 is a side-view of the early metaphase representing the granular or zig-zag appearance on the surface of chromosomes. Fig. 14 shows a side view of the late metaphase in which the surface of the chromosomes has

Fig. 12. A stage prior to the metaphase in the first maturation division. XY = sex-chromosome.

Figs. 13-14. Side views of the metaphase.

become smooth. In every one of these figures there is a chromosome made up of two heteromorphous elements, a dot-like and an elongated rod being connected lineally. This is the sex-chromosome of the XY type, which has the same structure in the case of man, of the dog, of the albino rat etc. It shows also heteropycnosis during the growth period. Figs. 15-21 show polar views of the metaphase, in which 38

chromosomes are present. These cells are not very well preserved and the sex-chromosome is sometimes displaced from the margin to the inside of the equatorial plate, whereas it should be found at the periphery of the equatorial plate in the natural condition. There are two large tetrads of triple rings in the equatorial plate as shown in Figs. 18, 20 and 21. They are derived from two pairs of large V's in spermatogonia, although it is difficult to identify the pairs in the spermatogonia from which they are derived.

Figs. 15-21. Polar views of the metaphase, presenting 19 chromosomes.

Figs. 22-26. Polar views of the metaphase in the second division, presenting 19 chromosomes.

Figs. 22-26 represent polar views of the equatorial plate in the second division. 19 chromosomes can be counted in every one of these plates without exception. They should have the same features as those found in spermatogonia in the natural condition, but the figures are too poor to make it possible to verify the relationship.

General considerations

Winiwarter and his followers uphold the idea that man and mammals carry sex-chromosomes of the XO type: Winiwarter (1912), Oguma

et Kihara (1923), and Winiwarter et Oguma (1926) reported the presence of sex-chromosomes of the XO type in man; Winiwarter (1920) in the cat; Mols (1928) in the guinea-pig. Masui (1919) published a paper on the spermatogenesis of the ox, the horse and the rabbit. He reached the same conclusion with regard to the type of sex-chromosome. But we have never been able to verify such a conclusion in the course of our studies on mammalian chromosomes. It is certain that the guinea-pig has sex-chromosomes of the XY type, notwithstanding the fact that its diploid and haploid numbers are not yet known with certainty. Painter succeeded in verifying the presence of the XY elements in the rabbit (1924) and in the horse (1926). We have also reached the same conclusion in the rabbit, but no report was published because the result was quite identical with Painter's (1926). Recently Minouchi and Krallinger have ascertained the presence of the XY elements in the spermatocytes of the ox, the chromosome number of which had been already reported by Krallinger, in 1931.

Besides, there are a great many papers reporting the presence of an XY group in wild and domestic mammals and also in man. Of these, the following are considered to give a correct analysis of the number of chromosomes and the presence of XY elements in mammalian germ-cells: Hoy and George, (1928) in the opossum; Drummond (1933) in five species of marsupials; Painter (1924; 1926; 1929) in the horse, the monkey, the rabbit and the mouse; Masui (1923) in the mouse; Minouchi (1928 a, b, c, d, 1929) in the cat, the albino rat, the dog, the mouse and the racoon dog; Pincus (1927) in the Norway rat and the black rat; Shiwago and Andres (1932), and Minouchi and Ohta (1933) in man. From these works it is to be concluded that all mammals have sex-chromosomes of the XY type in the male and of the XX type in the female.

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