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From the Department of Animal Genetics, Nutrition and Hygiene,
Royal Veterinary College, Stockholm, Sweden.

SOMATIC CHROMOSOMES OF THE CAT

By
Ingemar Gustavsson

The progress in human cytogenetics due to introduction of hypotonic pretreatments and tissue culture techniques has given an increased knowledge of the etiology of several disease conditions in man. Knowledge about the chromosomes of domestic animals has not had corresponding progress. Although there are many disease conditions worthy of study not only to benefit veterinary medicine but also to give valuable information about homologic conditions in man. To obtain a background for such studies, a systematic examination of the normal chromosomes in different domestic animals has been carried out. Short descriptions of the somatic chromosomes of the dog (*Gustavsson* 1964 a) and the rabbit (*Gustavsson* 1964 b) have been reported earlier from our laboratory.

In this investigation the somatic chromosomes of the domestic cat (Felis domestica) have been studied. For a long time the cat has been a popular subject for chromosome studies, but investigators have often obtained different conclusions about the normal chromosome morphology. Many research workers have tried to explain the spontaneous occurrence of male tortoiseshell cats as being due to a change in the sex chromosome set. According to one theory such cats have XXY in their cells, thus the condition is assumed to be homologous to the Klinefelter's syndrome in man. One observation (*Thuline & Norby* 1961) seems to confirm this theory, but the problem is not as yet definitely solved.

MATERIAL AND METHODS

Four normal male and six normal female mongrel cats were used (Table 1). In earlier investigations on the chromosomes of domestic animals a short tissue culture method of leucocytes from peripheral blood has been used. This method is superior if you desire a simple technique to examine chromosome number and morphology of an individual. However, with that method you must use several ml of blood. Under certain circumstances, e. g. in a long term investigation with repeated blood samples, it is sometimes disadvantageous to take this much blood from the animal repeatedly. In this investigation a modified method has been used (*Arakaki & Sparkes* 1963). This has hitherto been used especially in the study of human chromosomes, but probably will be very valuable in the future when studying chromosomes of different animals. With this method you can use whole blood without removing the erythrocytes prior to culturing. After taking the blood from *vena femoralis*, 0.05—0.25 ml blood was carefully suspended in 5 ml tissue culture medium Parker 199 containing 15 % calf serum. In the description of the method, fetal calf serum is recommended, but in our use calf serum has also produced good results. Before the cultures were incubated at 38°C 0.1 ml phytohaemagglutinin was added to every 5 ml tissue culture medium. On the third day colchicine was added to make

Table 1.

Animal number	Sex	Tissue	Chromosome number								Cells counted
			34	35	36	37	38	39	40	41	
1	♀	bone-marrow			1	3	33				37
2	♀	bone-marrow			1	1	29				31
3	♀	bone-marrow				3	31	1			35
4	♀	bone-marrow				1	27				28
5	♂	bone-marrow					21				21
6	♂	bone-marrow			1	3	33				37
7	♂	blood				2	10			1	13
8	♀	blood		1		3	16				20
9	♀	spleen, lung, kidney, heart		1	1	6	70	1			79
10	♂	spleen, lung, kidney, heart	1			8	66	1			76
Total for all animals investigated			1	2	4	30	336	3		1	377

a concentration of 10^{-7} M. Three hours later the tissue culture medium was diluted to three times its own volume of distilled water to obtain a hypotonic solution. Twenty minutes later the cells were centrifuged down and fixed in 9 parts of 60 % HAc plus 1 part 0.1 N HCL for twenty minutes. Then a manual squash in acetic orcein (2 % orcein in 60 % acetic acid) was performed in the usual way, and the preparations were made semi-permanent with Krönig cement.

Long term tissue cultures were performed according to the method described by *Basrur et al.* (1963). Small biopsies of spleen, lung, kidney, and heart were taken after the kittens had been killed by a blow on the head. The biopsies were then cut to as small fragments as possible and rinsed in tissue culture medium before implanting between two coverslips in Leighton tubes. Tissue culture medium Parker 199 containing 30 % calf serum was added and the tissue cultures were incubated at 38°C. Cellpatches were observed on the third day and chromosome preparations were made one week later. Colchicine and hypotonic pretreatments were performed as above and the coverslips with the outgrowths of cells were fixed in glacial acetic acid, ethanol (1 + 3). They were then stained in acetic orcein and mounted in Euparal.

Besides the in vitro studies, bone-marrow cells were studied in vivo according to the technique of *Tjio & Whang* (1962). In some cases (animals 2, 3 and 5) 1 ml colchicine in a concentration of 0.05 % was injected intraperitoneally 2 hours before killing the animals. The femurs were removed from the dead animals and the bone marrow put into a solution of physiologic NaCl containing colchicine in a concentration of 0.05 % for three hours. The cells were then exposed to a solution of 1 % aqueous sodium citrate for twenty minutes, stained in acetic orcein, and made semi-permanent as above.

Cells that were apparently not disrupted with well spread and clear chromosomes were analysed, the chromosomes were drawn and photographed. With a pair of scissors the chromosomes were cut out of the photography, put into pairs and placed into groups according to similarity in appearance.

RESULTS

A total of 377 metaphases were analysed concerning chromosome number and morphology. The number was within the

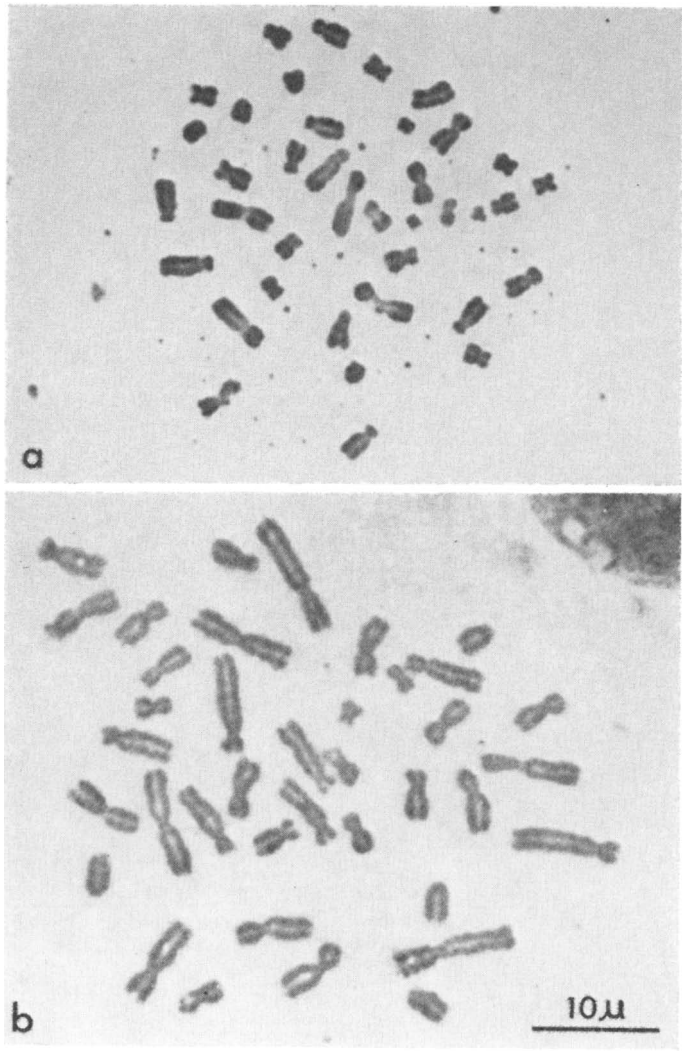


Fig. 1 a. Mitotic chromosomes derived from a short time culture of leucocytes (male).

b. Mitotic chromosomes from a primary culture of spleen (female).

range of 34 and 41 with a mean value of 38 (about 89 %). The distribution in different animals is shown in Table 1. In addition to the chromosome numbers given here, some polyploid cells were observed. On studying the chromosomes in bone-marrow cells, two categories of cells according to chromosome appearance

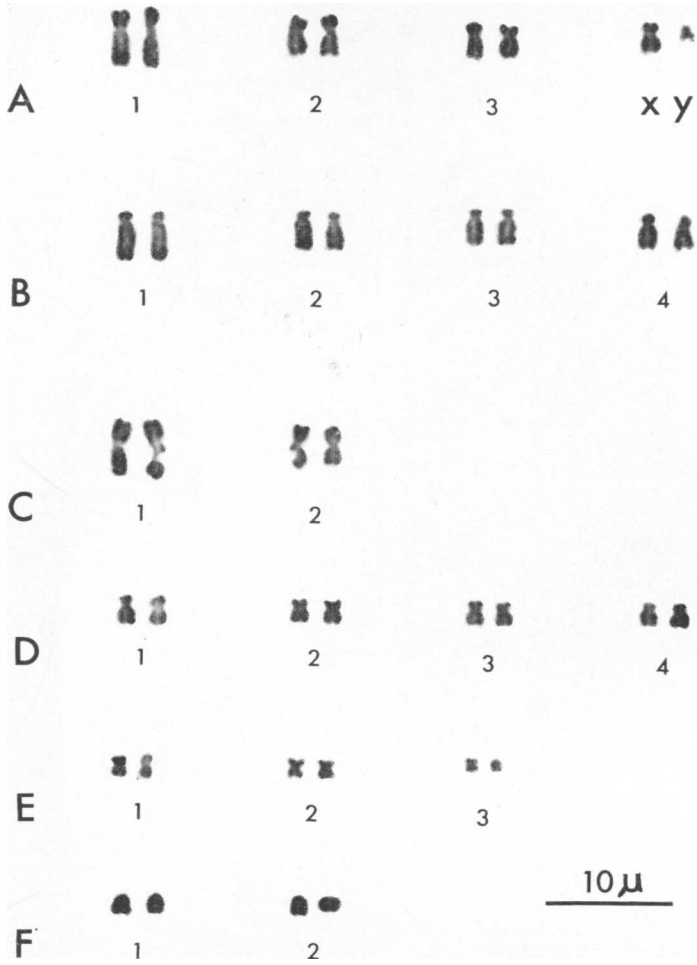
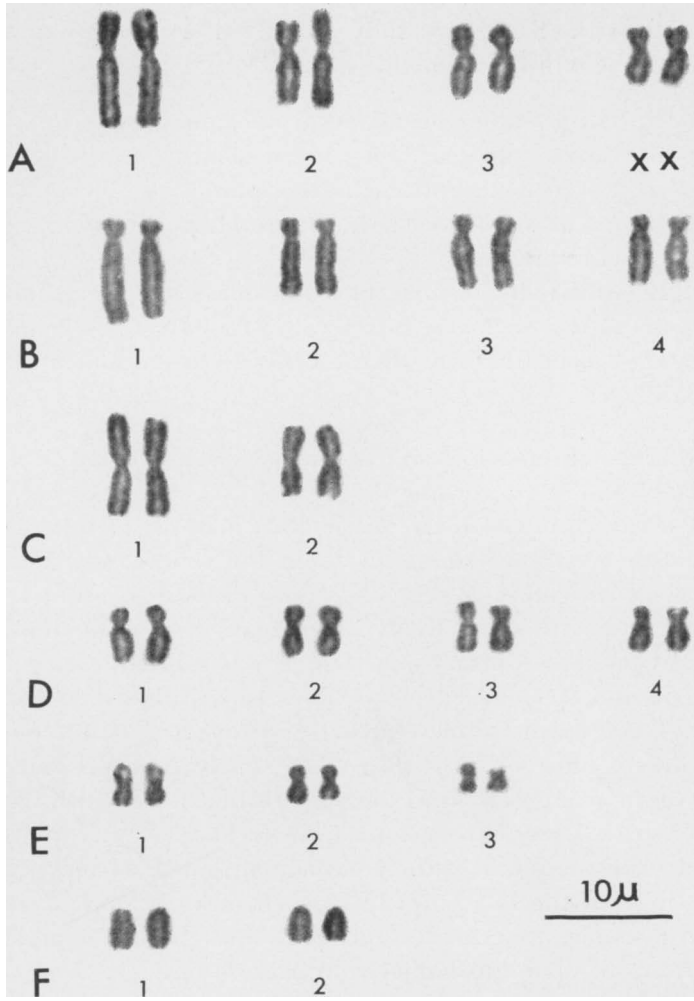


Fig. 2. Karyotype of a cat male (chromosomes taken from Fig. 1 a). "Accidental" breaks in chromosomes pairs C1 and C2.

could be distinguished. In one the chromosomes were slender and stained very distinctly. In the other type the chromosomes were more contracted and diffuse. The squashed cells of the bone-marrow usually had their cytoplasm around the chromosomes relatively undisturbed and therefore chromosome scattering was rare in this tissue. Conversely it was very difficult to get enough spreading of the chromosomes without overlapping and thus difficulties often arose at the identification of the individual pairs. In cell preparations studied in vitro most chromo-



F i g. 3. Karyotype of a cat female (chromosomes taken from Fig. 1b).

some pairs could usually be distinguished (Fig. 1). To get a practical system of analysing the chromosomes, they were put into groups according to size and centromere position (Fig. 2 and 3). Nearly consistent satellites could only be observed in one chromosome pair, but rarely secondary constrictions were observed in some other chromosomes. The following description of the chromosome complement is based on the most obvious and consistent features found in the analysis of the mitotic

chromosomes. According to the system for the family Felidae proposed by the San Juan study group*), the chromosomes have been divided into the following groups:

Group A	Large submetacentric chromosomes
Group B	Large subtelocentric chromosomes
Group C	Large metacentric chromosomes
Group D	Small submetacentric and subtelocentric chromosomes
Group E	Small metacentric chromosomes, including satellited pair
Group F	Telocentric and/or acrocentric chromosomes
Sex pair	XY

The cat chromosomes in Fig. 1 have been ordered according to this system in Fig. 2 and 3.

In many cases, however, difficulties arise to identify certain pairs. The X chromosome is of about the same size as pair A 3 and its identification is very uncertain. Pairs B 2, 3 and 4 differ slightly in length but correct identification of individual pairs is hardly possible. Chromosome pair D 1 is somewhat larger than D 2, D 3 and D 4 and can sometimes be distinguished from the latter. These chromosomes consist of two pairs with submedian centromeres and one pair with more subterminally located centromere. In group F we find the only chromosomes with terminal centromeres. These chromosomes can in an easy way be distinguished from the other in the complement, but it is very difficult to distinguish the two pairs from each other. Pair E 3 and the Y chromosome, the smallest chromosomes of the complement, are often not distinguishable from each other.

DISCUSSION

The distribution of chromosome numbers found in this work is well in agreement with the variations found by *Court-Brown et al.* (1960) in man. Scoring the chromosome number in a large number of cells, they found the distribution to be negatively

*) At preparing this manuscript some research workers on the 14 November 1964 in San Juan, Puerto Rico, during the Conference on Mammalian Cytology and Somatic Cell Genetics agreed to a uniform karyotype arrangement for the family Felidae.

skewed. 12.1 % of the cells gave counts of less than 46 in contrast to 2.9 with counts greater than 46. The same workers ascribed deviation in chromosome numbers to be artefactual due to cell rupture. The small number of polyploid cells found in vitro is due to endomitosis and is a common character of primary cell cultures treated with colchicine.

The two clearly different types of chromosome appearance found in the bone-marrow cells have earlier been observed by *La Cour* (1944), who ascribes them to two different celltypes. The polyploid cells observed in vivo in this work are probably megakaryocytes.

Diverging from results in older investigations (*Winiwarter & Sainmont* 1909, *Longley* 1911, *Stricht* 1911, *Winiwarter* 1914, 1919, 1922, 1934), the chromosome number must now be considered to be definitely determined. The first work establishing the exact number was done by *Gutherz* (1918, 1920), who in oogonia material found the chromosome number to be 38. This number was also found by *Minouchi* (1928 a, b) and *Minouchi & Ohta* (1932, 1934). The latter workers could also identify a heteromorphous sex chromosome pair. They found the chromosome complement to consist of "... 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 ..." The chromosome number and the heteromorphous sex chromosomes were later confirmed by *Matthey* (1934, 1935, 1936), *Pletnev* (1941), *Vara & Pesonen* (1947) and *Muldal* (1948). In his analysis about the cat chromosomes, *Koller* (1941) could not find any distinct heteromorphous sex chromosome pair. Later *Husted & Walker* (1956) also failed to do this. On the other side *Tateishi* (1941), *Makino & Tateishi* (1952) and *Ishihara* (1955, 1956 a, b) could identify a very distinct heteromorphous sex chromosome pair. In a comparative study on the chromosomes of the lion, the Chinese leopard cat and the house cat, *Makino & Tateishi* (1952) found "a diploid complement consisting of 18 homologous pairs of autosomal elements and an X—Y complex. The homologous members ranging from the first pair to the 9th pair are V-shaped ... Each of the members of the 10th to the 18th pairs carries a remarkable globular segment at

the proximal end of the main body, and therefore assumes a J-shape". Using tissue culturing *Nakanishi* (1960) observed in primary cultures of a kitten cell strain "... six pairs of V-, ten pairs of J-, and two pairs of rod-shaped autosomes. The X chromosome is J-shaped and the Y-element is of the rod type." If we look at the data about chromosome morphology given above, we will find that it is difficult to do a comparison due to differences in materials, methods and nomenclatures used. From other works, e. g. in the rat (*Tjio & Levan* 1956 a, b), we know that the distinction between chromosomes with subterminal and terminal centromeres often depends on the method used. The same is the case about the morphology of the Y chromosome, that earlier in cat and also in many other species has been described as rod-shaped, but in reality has got a median — submedian centromere. Using cells in vivo *Ohno et al.* (1962), in their study on the early meiosis of male germ cells in fetal testis, shortly described the cat chromosome complement. They have characterized the X chromosome as one of the shortest with median centromere and not much larger than the Y chromosome. Also *Gimenez & Lopez-Saez* (1962) have lately in bone-marrow cells studied in vivo found the X chromosome to be a small chromosome with median centromere. In studying cat chromosomes in cultured cells from lung and spleen, *Awa et al.* (1959) described all autosomes as having subterminal or submedian centromeres. The X was found to have a subterminal centromere and to "correspond to the 12th or 13th pair in magnitude", thus to be a relatively small chromosome. The Y was considered to have a subterminal centromere. Also *Matano* (1963) and *Chu et al.* (1964) have suggested that the X is a small chromosome. *Nafstad* (1963) in a study in bone-marrow and skin in vitro could not distinguish the X chromosome from two other large pairs with subterminal centromeres. Secondary constrictions were observed in several chromosomes, corresponding to pairs A 1, B 2, C 1, E 1 and E 2 in this work. *Hsu et al.* (1963) has given a comparative chromosome study of some members of the Felidae, and the San Juan study group has adopted on principle the general scheme of the Felidae given by him.

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SUMMARY

The number of chromosomes of the cat (Felis domestica) was determined at 38, and both the X and the Y chromosomes have submedially situated centromeres. X is of medium size while Y is one of the smallest chromosomes of the complement. For practical purposes, the chromosomes have been divided into groups according to their appearance.

ZUSAMMENFASSUNG

Die somatischen Chromosomen der Katze.

Die somatischen Chromosomen der Katze (Felis domestica) wurde zu 38 festgestellt. Sowohl das X-Chromosom als das Y-Chromosom haben ein submediales gelegten Centromer. Das X-Chromosom ist von Mittelgrösse und das Y-Chromosom ein von den kleinsten Chromosomen in dem Komplement. Aus praktischen Grund sind die Chromosompaaren von ihren Aussehen in Gruppen eingeteilt.

SAMMANFATTNING

Kattens somatiska kromosomer.

Antalet kromosomer hos katt bestämdes till 38, där både X- och Y-kromosomen har en submedialt belägen centromer. X är av medelstorlek, medan Y är en av de minsta kromosomerna i komplementet. Av praktiska skäl har kromosomparen efter sitt utseende indelats i grupper.

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