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CHROMOSOMAL ANALYSIS OF THE JAPANESE RACCOON DOG BASED ON THE G- AND C-BANDING TECHNIQUES*

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The chromosomes of two subspecies of the Japanese raccoon dog (*Nyctereutes procyonoides viverrinus*; 4 individuals & *N. p. albus*; 2 individuals) were analysed using the G- and C-banding techniques.

The Japanese raccoon dog had 42 chromosomes in dipliod number and the fundamental arm number (NF, 'nombre fundamental') was 70. The autosomes comprised 13 pairs of meta or submetacentrics and 7 pairs of acrocentrics. The X chromosome was metacentric and intermediate in size between Nos. 12 and 13 while the Y was the smallest acrocentric with a satellite.

The G- and C-banding patterns of *N. p. albus* were similar to those of *N. p. viverrinus*. The homologous pair of chromosomes could be identified by the G-banding pattern. Most of the chromosomes were C-band positive and contained centromeric heterochromatin.

In one male individual (*N. p. viverrinus*), modal chromosome numbers showed 41, XY because of the elimination of one acrocentric autosome. However, this male did not show any abnormal features except in diploid number.

INTRODUCTION

Recent advances in methods of differential chromosome staining have made it possible to identify each genome correctly and easily, and to determine the inner structural changes of chromosomes. Differential staining methods are being used in comparative mammalian cytogenetics, leading to the accumulation of more information on karyotoxnomy.

The raccoon dog (*Nyctereutes procyonoides*) is a monospecific genus in Canidae. There are two subspecies of raccoon dogs in Japan: *N. p. viverrinus* ranging from Honshu and Shikoku to Kyushu; and *N. p. albus* in Hokkaido.

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The chromosomes of the raccoon dog have been studied by several investigators who have shown that the chromosomes of *N. p. viverrinus* are 2n=42 (MINOUCHI, '29; WURSTER, '69; TODD & PRESSMAN, '69; HUS & BENIRSCHKE, '71; TSUCHIYA & YOSHIDA, '71), and for *N. p. albus*, 2n=42 (TSUCHIYA, '79). However, MÄKINEN ('74) reported that the chromosomes of the Finnish raccoon dog (*N. p. procyonoides*) were 2n=56. There are chromosomal variations in diploid number between the Japanese and Finnish raccoon dog.

Cytogenetic studies of the raccoon dog using differential staining methods has been carried out in the Finnish raccoon dog (MÄKINEN & FREDGA, '80), but not in the Japanese raccoon dog.

The present report describes the G- and C-banding patterns of chromosomes in the Japanese raccoon dog.

MATERIALS AND METHODS

Six raccoon dogs (farm bred near Sapporo, Japan) consisting of *N. p. viverrinus* (2 males & 2 females) and *N. p. albus* (1 male & 1 female) were subjected to chromosomal analysis.

Blood samples were drawn from the cephalical vein of each animal into a heparinized sterile syringe. The leukocytes were cultured in Eagle's minimum essential medium (Nissui) supplemented with 10% fetal calf serum (Gibco). Phytohemagglutinin-M (Difco) was added as a mitogen. The cells were cultured for 3 days at 37°C.

Cell harvest and chromosome preparation were then performed in the usual manner and stained through the following three procedures: (1) conventional Giemsa, (2) trypsin G-banding (SEABRIGHT, '71) and (3) BSG C-banding (SUMNER, '72). One male of *N. p. viverrinus* which showed the chromosomal variation in diploid number was killed in November, and macroscopical and histological examinations were performed.

RESULTS

The results of this chromosomal analysis are summarized in table 1. Among the 6 raccoon dogs studied, five individuals of both subspecies had 42 chromosomes in diploid number. Figures 1 and 4 show the photographs of *N. p. viverrinus* and *N. p. albus*, and figures 2, 3, 5 and 6 show a conventional karyotype of *N. p. viverrinus* and *N. p. albus*. The autosomes of 5 individuals of both subspecies were comprised of 13 pairs of meta or submetacentrics and 7 pairs of acrocentrics, and sex chromosomes XX in the female and XY in the male. The X chromosome was metacentric and intermediate in size between Nos. 12 and 13. The Y chromosome was the smallest acrocentric with a satellite, but the region of the satellite was less stainable than the main body. Relative length, arm ratio and chromosome type of the pair chromosomes in both subspecies

TABLE 1 *Karyotypes of the 6 raccoon dogs used in this study*

INDIVIDUAL NO.	SUBSPECIES	SEX	AGE (mo.)	KARYOTYPE
1	*N. p. viverrinus*	male	0.5	42, XY
2	"	"	"	41, XY
3	"	female	?	42, XX
4	"	"	?	42, XX
5	*N. p. albus*	male	?	42, XY
6	"	female	?	42, XX

TABLE 2 *Relative length of chromosomes in N. p. viverrinus and N. p. albus* (average of five cells)

PAIR NO.	*N. P. VIVERRINUS*			*N. P. ALBUS*		
	Relative* length	Arm ratio	Chromosome** type	Relative* length	Arm ratio	Chromosome** type
1	69.7	1.9	SM	74.7	1.9	SM
2	66.5	2.1	SM	66.8	2.2	SM
3	64.0	1.5	M	64.5	1.5	M
4	61.9	2.2	SM	63.0	2.6	SM
5	59.9	1.4	M	59.9	1.3	M
6	58.2	1.3	M	57.6	1.3	M
7	56.9	1.5	M	55.5	1.5	M
8	55.0	1.5	M	53.7	1.3	M
9	52.3	1.2	M	52.0	1.3	M
10	50.2	2.1	SM	50.8	2.0	SM
11	48.2	1.3	M	48.4	1.5	M
12	45.8	1.7	SM	47.2	1.7	SM
13	36.0	2.0	SM	38.7	2.0	SM
14	39.4		A	40.4		A
15	37.6		A	37.7		A
16	35.2		A	35.8		A
17	33.1		A	32.8		A
18	29.8		A	29.4		A
19	26.5		A	26.0		A
20	24.8		A	24.8		A
X	43.2	1.6	M	40.7	1.6	M

* Chromosome length expressed as permillage of total autosome length per cell
** SM: Submetacentric M: Metacentric A: Acrocentric

are summarized in table 2.

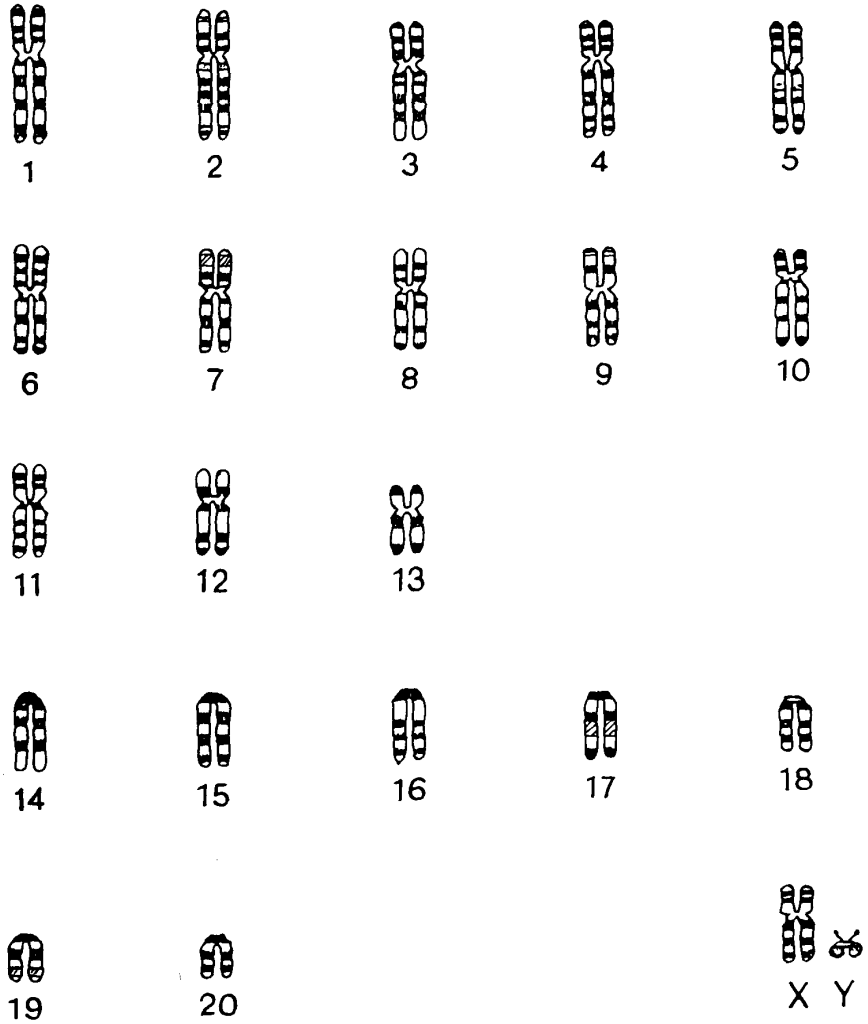
Using the G-banding patterns, chromosomes can be identified and arranged homologous pairs; Figures 7 and 8 show the G-banded karyotypes of *N. p. viverrinus* and *N. p. albus* respectively. It was possible to identify every chromosome in the two subspecies. The number of G-bands in both subspecies is shown in table 3, and the schematic drawing of *N. p. viverrinus* is illustrated in diagram. The number and location of the G-bands of *N. p. albus* were similar to those of *N. p. viverrinus*. The X chromosome was correctly identified as the second smallest metacentric among the biarmed chromosomes.

The C-banding patterns revealed the amount and the distribution of constitutive heterochromatin in the chromosomes. Figures 9 and 10 show the C-banded karyotypes

TABLE 3 *Number of G-bands in each chromosome pair of N. p. viverrinus and N. p. albus*

PAIR NO.	*N. P. VIVERRINUS*		*N. P. ALBUS*	
	Short arm	Long arm	Short arm	Long arm
1	3	4	2	4
2	3	6	3	6
3	3	4	3	3
4	3	4	2	4
5	3	5	3	4
6	3	4	3	4
7	3	3	2	3
8	2	4	2	4
9	2	3	2	3
10	2	4	2	4
11	2	4	2	4
12	1	3	1	3
13	1	2	1	2
14		3		3
15		4		4
16		4		4
17		3		3
18		3		3
19		3		3
20		2		2
X	1	3	1	3
Y		1		1

DIAGRAM *A schematic drawing of the G-banding pattern* in a male N. p. viverrinus*



* Strongly stained bands are shown in black, and faintly stained ones by oblique lines

of *N. p. viverrinus* and *N. p. albus*. In both subspecies, most of the autosomes were C-band positive and deeply stained in the centromeric area; however, some variations of C-band were observed. In *N. p. viverrinus*, one weak interstitial C-band was observed in the long arm of submetacentric pair No. 4, and the entire arm of acrocentric pair No. 19 was stained. In *N. p. albus*, a terminal C-band was observed both in the short and long arm of submetacentric pair No. 12, and acrocentric pair No. 19 was entirely stained on all of its arms as in the case of *N. p. viverrinus*. The X chromosome showed a centromeric heterochromatin, and the proximal half of the long arm was deeply stained. The Y chromosome was C-band negative and did not show any cen-

tromeric heterochromatin. There were few differences of C-banding patterns between the two subspecies.

In the case of one male raccoon dog (individual No. 2, *N. p. viverrinus*), the modal chromosome number showed 41, XY. Table 4 shows the distribution of the chromosome number of this individual, and figures 11, 12 and 13 show its photograph and conventional karyotype. Because of the elimination of one acrocentric autosome, the number of chromosomes decreased the normal complement. Figures 14 and 15 show the G- and C-banded karyotypes of this individual with 41, XY. It was correctly surmised that the elimination occurred only in acrocentric pair No. 17. However, this individual did not show any abnormal features except in diploid number. Figures 16 and 17 show the testis cross sections of this individual. There were a few primary spermatocytes but no spermatid and sperms in the seminiferous tubules.

TABLE 4. *Distribution of chromosome numbers in individual No. 2*

CELLS OBSERVED	NO. OF CHROMOSOMES				
	39	40	41	42	43
91	7	14	57	12	1

DISCUSSION

Using the testis section method, MINOUCHI ('29) first reported the figure 2n=42 with the XY sex chromosome type in *Nyctereutes viverrinus*. This result was later confirmed by several investigators using the tissue culture method.

In the present study, we reported on the chromosomes of two subspecies of the raccoon dog in Japan using the G- and C-banding techniques. As a result, it became clear that the Japanese raccoon dog has 42 chromosomes in diploid number, and that the G- and C-banding patterns of *N. p. albus* are similar to those of *N. p. viverrinus*. The NF was 70 in both subspecies.

The amount of constitutive heterochromatin revealed by the C-band in the Japanese raccoon dog is large compared to that in other Carnivora as reported by PATHAK & WURSTER-HILL ('77).

The NF of *N. p. viverrinus* was found to be 68 in previous reports (WURSTER, '69; TSUCHIYA & YOSHIDA, '71), however, it was 70 in this study. The difference between the NF observed in the present study and that of previous reports is due to the morphology of the X chromosome. The X chromosome in this study was metacentric, which is the same result as that reported by TODD & PRESSMAN ('69). However, several reports revealed that the X chromosome was the acrocentric. HSU & BENIRSHKE ('71) identified the acrocentric X chromosome by autoradiography. The acrocentric

X chromosome of the raccoon dog is unlike the metacentric one of all the Canoidea (WURSTER, '69). The Finnish raccoon dog has 56 chromosomes in diploid number and the NF is 68 (MÄKINEN, '74), and its autosomes are comprised of 5 pairs of meta or submetacentrics and 22 pairs of acrocentrics. The X chromosome is metacentric and the Y is the smallest metacentric with a satellite (MÄKINEN & FREDGA, '80). The number of acrocentrics is more than that in the Japanese raccoon dog. When compared to the X chromosome of the raccoon dog in Japan and Finland, the G- and C-banding patterns have a similarity in number and distribution.

The X chromosome polymorphisms reported in mice (BIANCHI & CONTRERAS, '67) and rats (KAMALI, '75) were due to deletion, those in Leggata (MATTHEY, '72) to translocation of the autosome, and those in bats (HAIDUK et al., '81) to pericentric inversion.

The reason for the difference reported in the X chromosome of the Japanese raccoon dog is not clear, but it appears that the pericentric inversion occurred in the X chromosome. This suggests that there are two groups of raccoon dogs in Japan which have either acrocentric or metacentric X chromosomes.

The present result also showed the existence of intraindividual chromosomal polymorphism in one male raccoon dog (*N. p. viverrinus*). In this case, little spermatogenesis was observed; the reason for this is thought to be ageing and seasonal variations. TODD et al. ('69) found an individual having a modal chromosome number of $2n=40$, and FUKUOKA ('80) also observed a mosaicism of $2n=42$, 43 and 44 in the male *N. p. viverrinus*. In addition, two mosaic males with $2n=56$ and 57 were observed in the Finnish raccoon dog (MÄKINEN & FREDGA, '80).

The increase or decrease of the chromosome numbers is mainly due to the nondisjunction of chromosomes in meiosis or mitosis, but variations of the chromosome number by nondisjunction are demonstrated in tumors or culture cells and occur rarely in the autosomes in animals (YOSHIDA, '80).

Autosomal polymorphism of the intraspecies karyotype is found in several mammals such as the deer mouse (SPARKES & ARAKAKI, '66), the Leggata (MATTHEY, '72), the black rat (YOSHIDA, '80), the red fox (GUSTAVSSON & SUNDT, '65; SASAKI et al., '68), the arctic fox (GUSTAVSSON & SUNDT, '65; MÄKINEN & GUSTAVSSON, '80; SWITONSKI, '80), the water buffalo (WURSTER & BENIRCHKE, '68), and the Sika deer (GUSTAVSSON & SUNDT, '69). Most reports explain that polymorphism occurs as the result of pericentric inversion, centric fusion or the presence of supernumerical chromosomes. MAKINO ('79) suggested that intraspecies polymorphism was the cytogenetic process or mechanism by which some species differentiate and evolve.

The geographical distribution of the raccoon dog, according to CORBET ('78), is the woodland zone of East Asia from the River Amur to Yunnan and North Vietnam, west to Shansi and East Szechuan and all the main islands of Japan. Introduced first into European Russia from East Asia, it is now widespread in the European regions.

Until now, there have been no cytogenetic studies conducted on the raccoon dog of mainland East Asia in the zone of interference between Japan and Finland. Thus the reason for intrasubspecific chromosomal polymorphism in the Japanese and the Finnish raccoon dog is not clear. However, it would seem that the intraindividual polymorphism in chromosome number and the morphological differences in the X chromosome exist with high frequency in these subspecies.

We are planning a further study on intrasubspecific and intraindividual chromosomal polymorphism in the raccoon dog.

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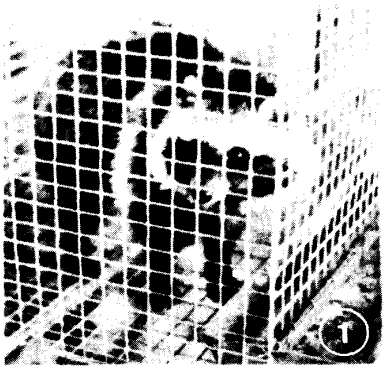
Explanation of plate

Plate I

Fig. 1	Photograph of *N. p. viverrinus*
Figs. 2 & 3	Conventional karyotype of a female *N. p. viverrinus*
Fig. 4	Photograph of *N. p. albus*
Figs. 5 & 6	Conventional karyotype of a male *N. p. albus*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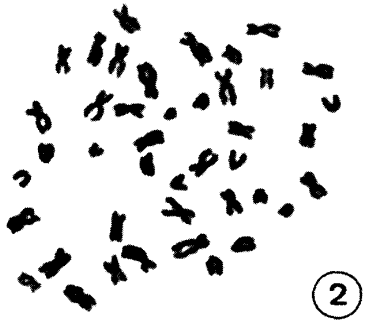
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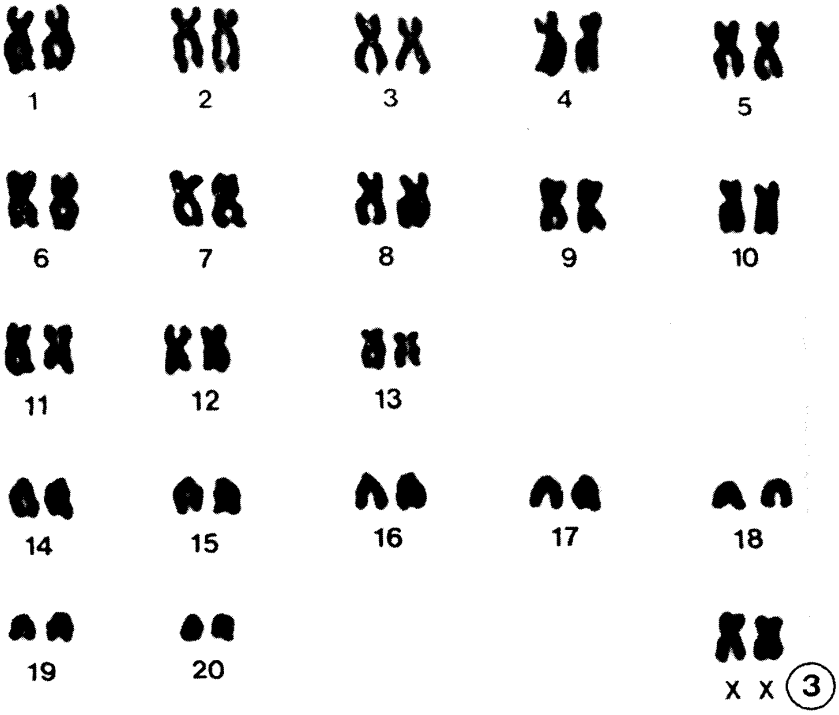
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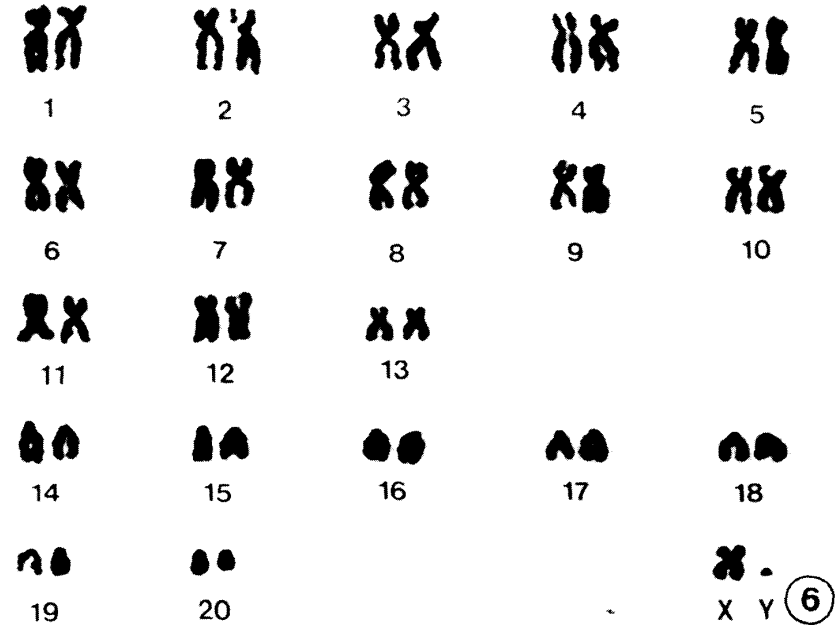
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PLATE II

Fig. 7 G-banded karyotype of a male *N. p. viverrinus*

Fig. 8 G-banded karyotype of a male *N. p. albus*

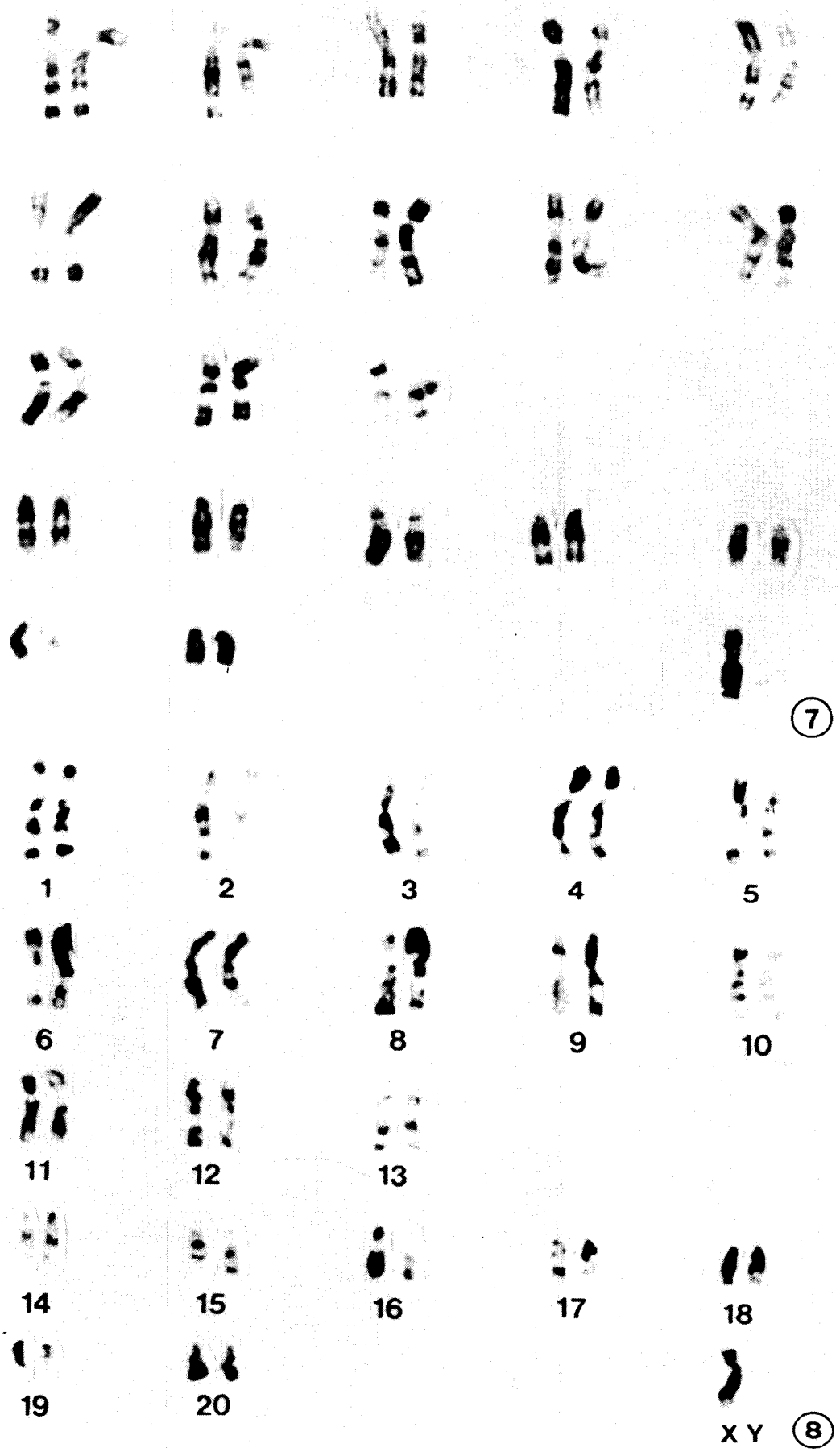
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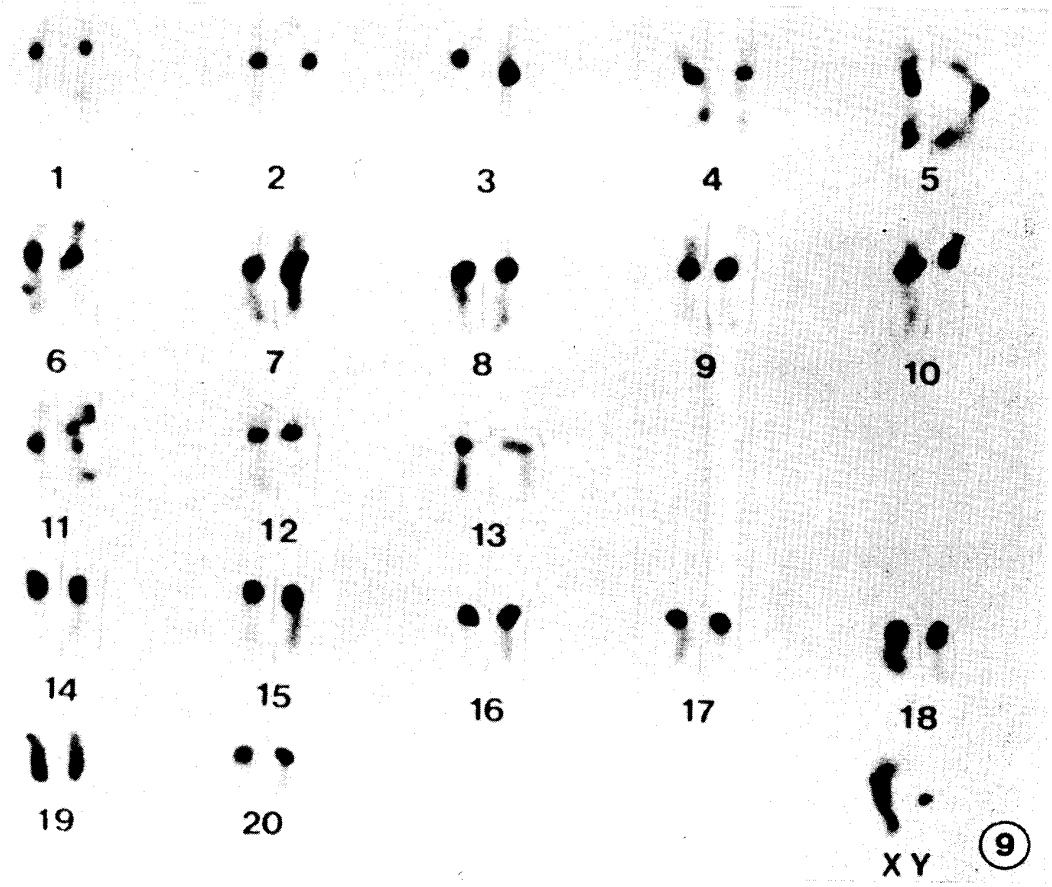
PLATE III

Fig. 9 C-banded karyotype of a male *N. p. viverrinus*

Fig. 10 C-banded karyotype of a male *N. p. albus*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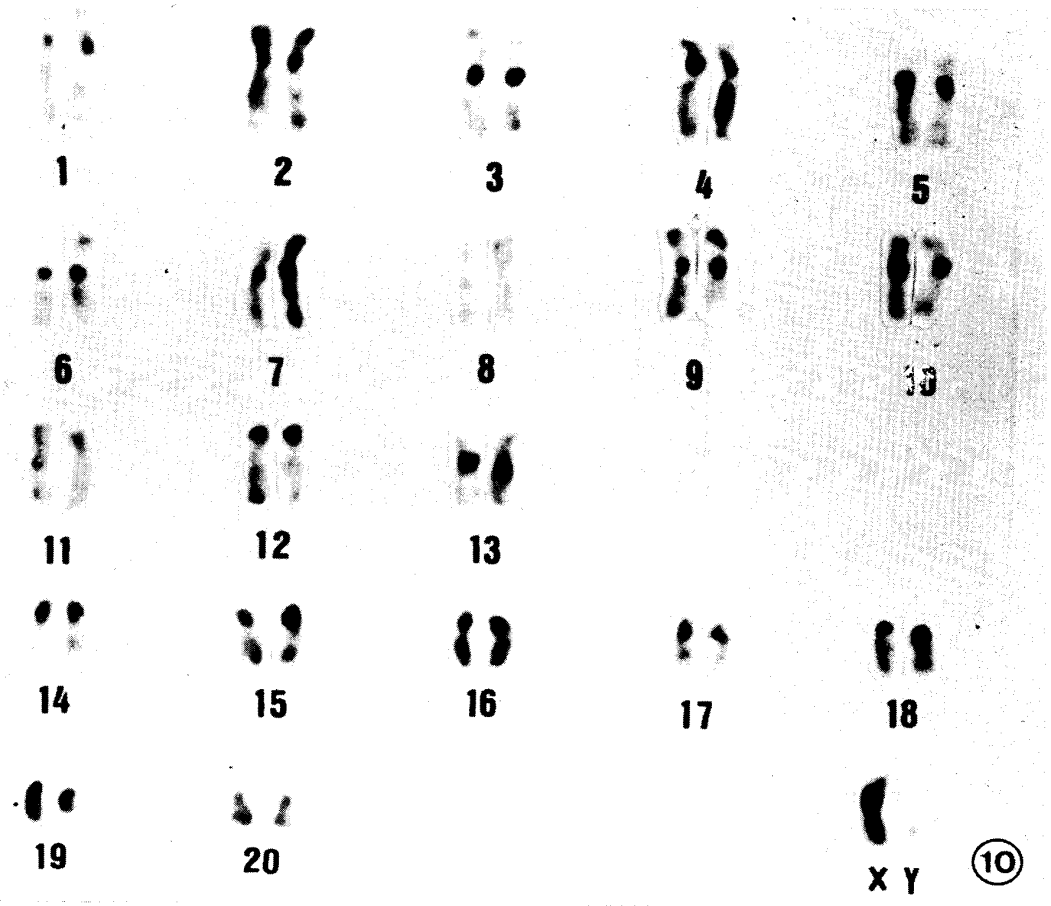
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PLATE IV

Fig. 11 Photograph of a male *N. p. viverrinus* (individual No. 2)

Figs. 12 & 13 Conventional karyotype with 2n=41

Fig. 14 G-banded karyotype of a male *N. p. viverrinus* with 2n=41 (individual No. 2)

Fig. 15 C-banded karyotype of a male *N. p. viverrinus* with 2n=41

Fig. 16 Cross section of the testis of a male *N. p. viverrinus* with 2n=41 (individual No. 2) Little spermatogenesis is observed. No spermatid and sperm are observed in the seminiferous tubules. (H & E) ×110

Fig. 17 Cross section of the testis of a male *N. p. viverrinus* with 2n=41 (individual No. 2) Primary spermatocytes (arrow) are ovserved but they are few in number. (H & E) ×220

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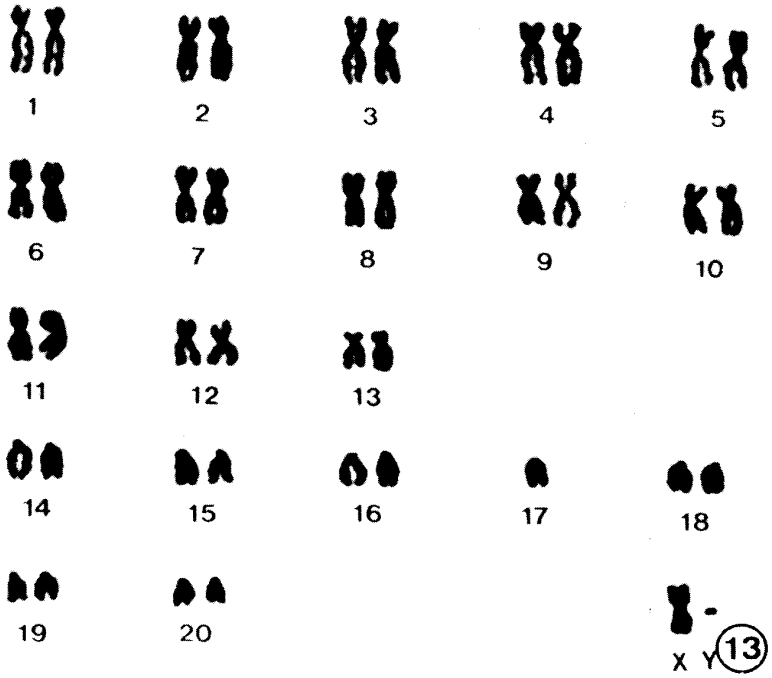

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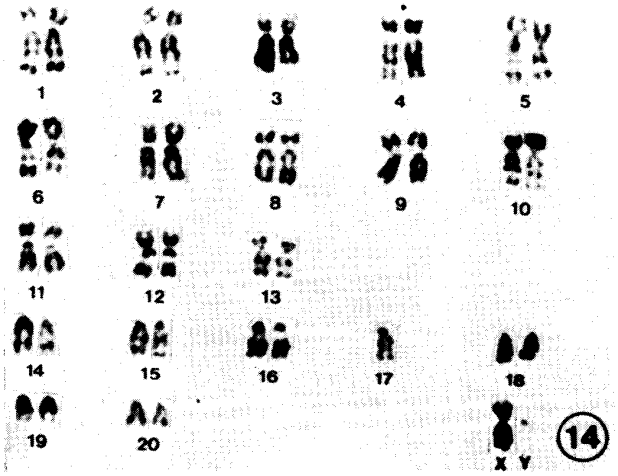

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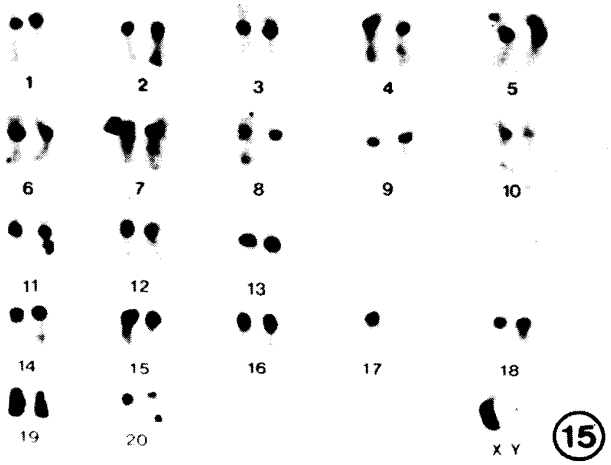

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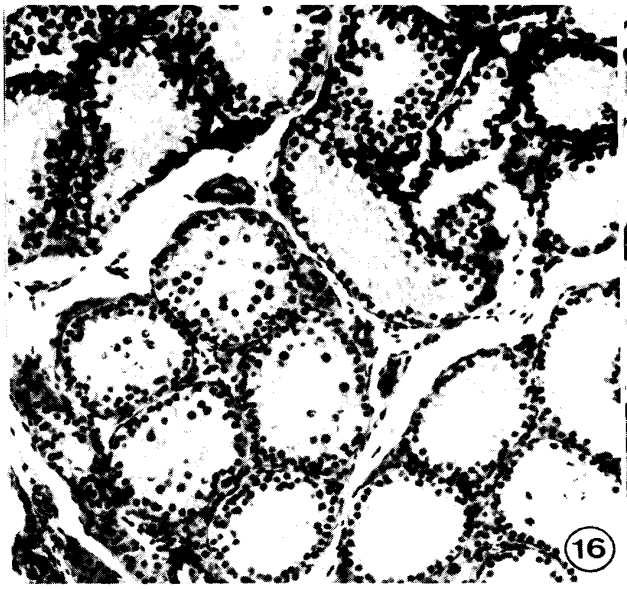

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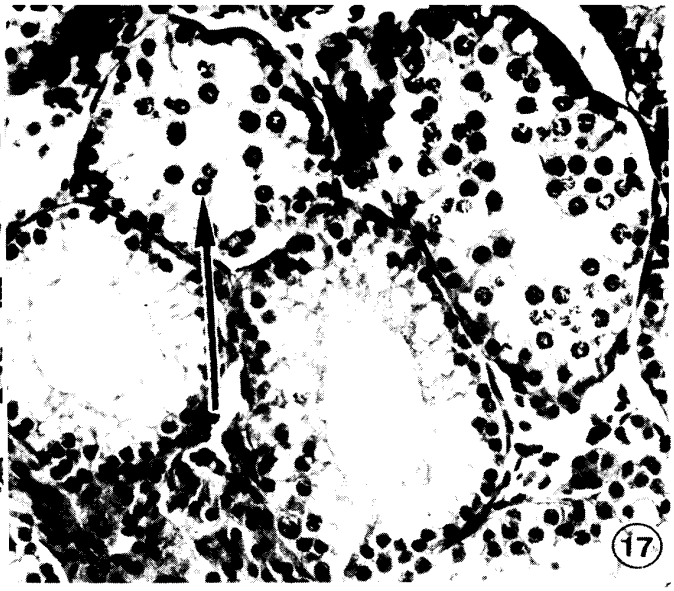

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