Re-examination of the chromosome homology between two subspecies of Japanese raccoon dogs *(Nyctereutes procyonoides albus* and *N.p. viverrinus)*

MASAYASU Y. WADA 1.2, TAMAKI SUZUKI 3, and KIMIYUKI TSUCHIYA4

I Amagi Wild Boar Park, Amagiyugashima, Shizuoka-Pref., 410-32, Japan; 2 Imamichi Institute for Animal Reproduction, 1103 Fukaya, Dejima, Niihari, Ibaragi-Pref., 300-01, Japan; 3 Miyoshi Animal Hospital, 45Hoei, Miyochi, Miyoshi, Nishikamo-gun, Aichi-Pref., 470-02, Japan; 4 Experimental AnimalCenter, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki-Pref., 889-16, Japan.

SUMMARY- We investigated the chromosomal relationships between subspecies of Japanese raccoon dogs from Hokkaido *(Nyctereutes procyonoides albus;* NP A) and all of the other major Japanese Islands (N. *p. viverrinus;* NPV). Chromosomes of wild-caught NPA were analyzed by conventional stain, G-banding and C-banding techniques. The basic karyotype of this subspecies is composed of met acentric (M) and acrocentric (A) chromosomes plus a variable number of B chromosomes *(2n* = 38 (= 26M + 10A + XY or XX) + Bs) and is the same as that of NPV. This study of NP A suggests that the Island type *(2n* = 38) only occurs in Japanese islands including Hokkaido, whereas all animals from mainland Asia and eastern Europe that have been examined have the Continental type *(2n* = 54).

INTRODUCTION

The genus *Nyctereutes* (the raccoon dog) is monospecific and comprises five or six subspecies distributed throughout Asia and Japan (W ARD and WURSTER-HILL 1990). The Japanese raccoon dog comprises two subspecies *Nyctereutes procyonoides viverrinus* (NPV) of Honshu, Shikoku and Kyushu, and N.*p. albus* (NPA) of Hokkaido (KURODA 1938). The diploid number of *2n=42* for NPA was first described by TSUCHIYA (1979) using conventionally stained chromosomes. In a study of banded (G and C band) chromosomes, the karyotype of NPA has been described as *2n* = 42, similar that to of NPV and exhibiting chromosome homology with NPV (YOSHIDA *et al.* 1982) . However , recent work has indicated that the NPV karyotype is *2n* = 38 (=26M+10A+XY or XX)+Bs (YOSIDA *et al.* 1983; WURSTER-HILL *et al.* 1986; W ADA 1987). The recognition of the basic chromosome number of NPV as *2n* = 38 + Bs comes from interpretation that the karyotype of NP A also has *2n* = 38 + Bs (W ADA *et al.* 1991). However, this interpretation of the karyotype of NP A without comparative evidence of the chromosomal homology between

these two subspecies is not necessarily correct. Moreover, Robertsonian polymorphisms were found in the NPV (YOSIDA and W ADA 1984; OBARA and NAKANO 1989; WADA and IMAI 1991). To investigate karyotypic homology and the possible existence of a karyotype intermediate in form between those of NP A and NPV, we re-analyzed the chromosomes of wild-caught NP A.

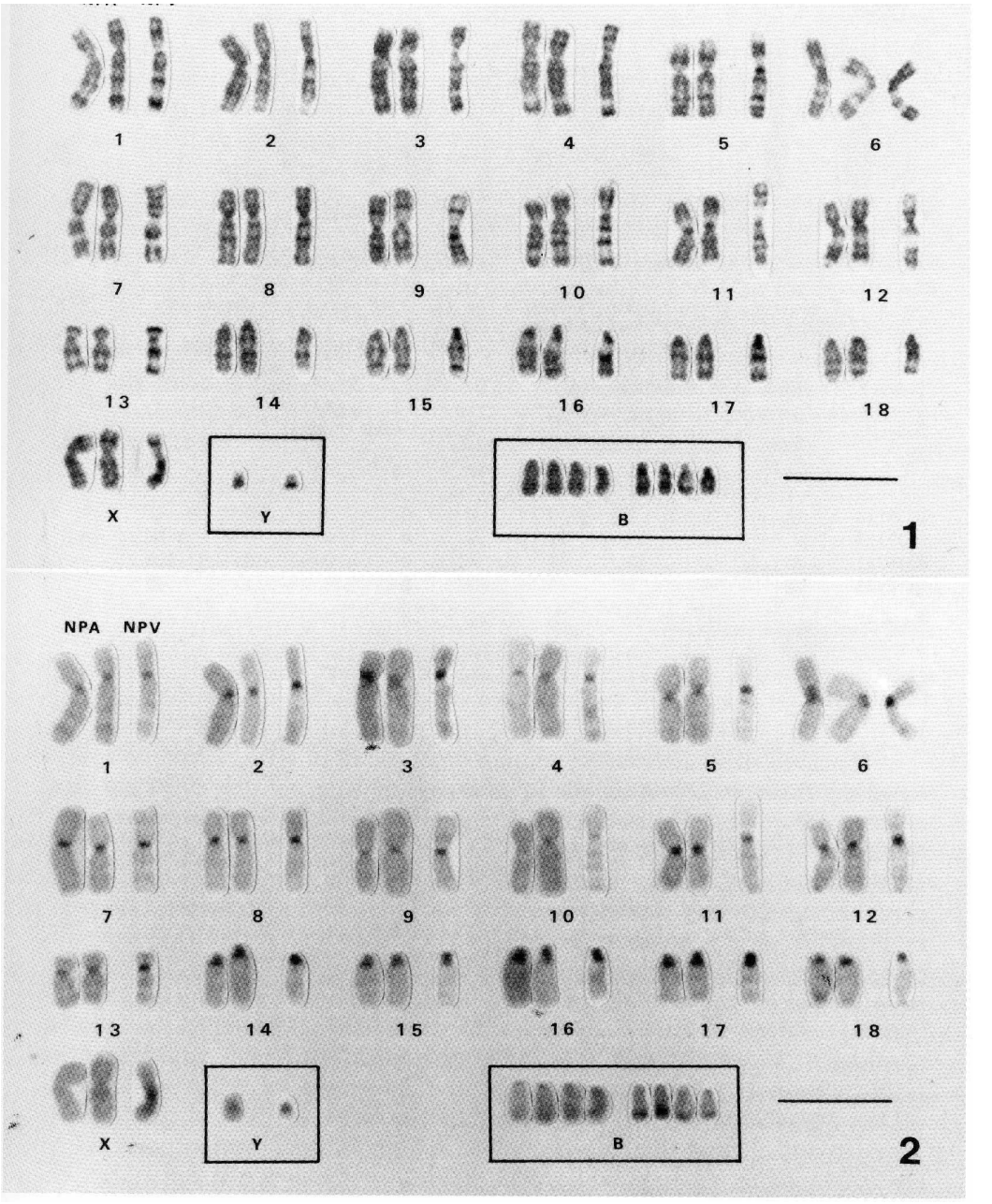
MATERIALS AND METHODS

Four specimens of *Nyctereutes procyonoides albus* (NP A) were studied. Three mature individuals (two males Nos. RD9114, RD9343 and female No. RD9115) were wild-caught in Kushiro city, Hokkaido and kept at Kushiro Municipal Zoo. One mature male (No. RD9231) was wild-caught in Sapporo city, Hokkaido and kept at Sapporo Municipal Maruyama Zoo. A haploid G and C banded karyotype including X and Y chromosomes of a male N. *procyonoides viverrinus* (NPV), caught in Yokohama city, Honshu, was used to compare with the G- and C-banding patterns of NP A. An another male NPV, caught in Kumamoto city, Kyushu, was used in a morphological comparison with the conventionally-stained Y chromosome of NP A.
Fibroblast cultures were established from skin biopsies, and chromosome preparations were made as described previously (W ADA *et a!*. 1989) . At least three metaphases per individual were selected for karyotypic analysis using conventional staining, G-banding and C-banding by methods published previously (W ADA *et a!*. 1989). In this paper we used the chromosome numbering system of NPV proposed by WADA (1987). A total of 214 well spread metaphases of NPA were counted to establish the modal diploid number for the species.

OBSERVATIONS

The distribution of chromosome numbers found in NP A is shown in T able 1. Chromosome numbers in 214 cells varied from 40 to 46. Thirteen pairs of meta- or submetacentrics, 5 pairs of acrocentrics, the met acentric X and the minute acrocentric Y chromosome were indistinguishable from those of NPV described previously by G-banding and C-banding patterns (Figs. 1 and 2). Small unpaired acrocentrics, presented in Fig. 1 varied in number from cell to cell and animal to animal, and were indistinctly G-banded and lacked centromeric C-bands (Fig. 2). Chromosomal satellites were observed at the ends of three pairs and the Y chromosome of NPA. The long arms (q), short arm (p) of No. 11q, 12q, 18q, and Yp, are also identical with those seen in NPV.

Fig. 1. - Comparison of the G-banded karyotype of female *Nyctereutes procyonoides albus* (NPA) (left) and the G-banded haploid set of N. *p. viverrinus* (NPV) (right). Center inset shows y chromosome. Right inset shows unpaired acrocentric chromosomes of NPA and B chromosomes of NPV. Bar equals 10 m.
Fig. 2. - Comparison of the C-banded karyotype of female *Nyctereutes procyonoides albus* (NPA) (left) and the C-banded haploid set of N. *p. viverrinus* (NPV) (right). Center inset shows y chromosome. Right inset shows unpaired acrocentric chromosomes of NP A and B chromosomes of NPV. Bar equals 10 m.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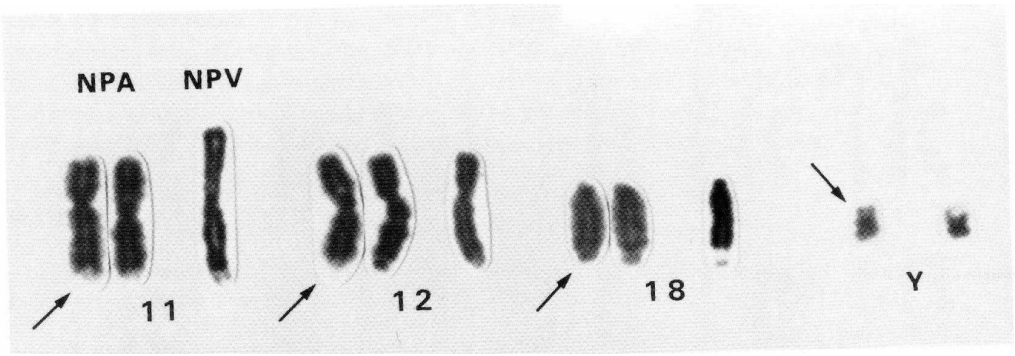

Fig. 3. - Comparison of the satellite positions of No.11, 12,18 pairs and y chromosome from NPA (left) and No.11, 12, 18 and y chromosomes of NPV (right).

TABLE 1 - Distribution of chromosome counts in 4 individuals of *Nyctereutes procyonoides albus*.

Animal No.	Sex	Chromosome count							
		40	41	42	43	44	45	46	Total
RD9114	M	1	8	5	1	0	0	0	15
RD9115	F	4	19	21	6	0	0	0	50
RD9231	M	2	57	37	4	0	0	0	100
RD9343	M	4	8	15	10	8	3	1	49
Total		11	92	78	21	8	3	1	214

DISCUSSION

Unpaired small acrocentric chromosomes found in NP A should be considered the B chromosomes for the following reasons. In not only NPV but also in other subspecies, the presence of B chromosomes is a distinctive feature in *Nyctereutes* (YOSIDA *et al.* 1985; MAKINEN *et al.* 1986; WURSTER-HILL *et al.* 1986; W ARD *et al.* 1987; W ADA *et al.* 1991) . The characteristics of these B chromosomes are very similar to those of small unpaired acrocentrics observed in NP A in several regards: 1) ambiguous G-banding pattern, 2) lack of centromeric C-bands, 3) varying numbers from cell to cell, 4) varying numbers from animal to animal. On the other hand, morphological, and G- and C-banding analysis in this study suggest that remaining paired chromosomes (autosomes) and the X and Y of NP A were indistinguishable from those of NPV. Therefore, we concluded that the karyotype of NPA is *2n* = 38 (26M + 10A + X(M) + Y(A)) + Bs(A) and is the same as that of NPV. It is considered likely that the diploid number of 42 published previously for NP A (TSUCHIYA 1979; YOSHIDA *et al.* 1982) is erroneous due to the difficulties of identification of the B chromosomes from other acrocentric autosomes.

WADA *et al.* (1991) proposed that the karyotype of *Nyctereutes* was consists of two types: 1) Continental type: $2n = 54$ (= 10M + 42A + XY or XX) + Bs, distributed in various regions of China, eastern Europe and Korea, and 2) Island type $2n = 38$ (= 26M + 10A + XY or XX) + Bs in Honshu, Kyushu and Shikoku of Japan. This study of NP A suggests that the Island type only occurs in the Japanese islands including Hokkaido. The cytological mechanism which distinguishes the two distinct karyological types within this monospecific genus is still unclear (W ARD *et al.* 1987; WADA *et al.* 1990; WADA and IMAI *et al.* 1991). On the other hand, Robertsonian polymorphisms have been found in the NPV (YOSIDA and WADA 1984; OBARA and NAKANO 1989; WADA and IMAI 1991) . These polymorphisms are considered very important to an explanation for this interesting phenomenon which occurs in the *Nyctereutes* karyotypes. Further population surveys of the NPV and NP A have been carried out by us, and these results will be published elsewhere

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