34. Cytogenetical Studies on the Japanese Raccoon Dog. VII

Karyotype Analysis of 22 Specimens collected in Ishikawa, Yamaguchi, Osaka and Wakayama Prefectures, Japan^{†)}

By Toshihide H. YOSIDA,^{*)} Masashi HARADA,^{**)}
and Masayasu Y. WADA^{***)}

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As already described by us (Yosida *et al.* 1983, 1984; Yosida and Wada 1984a-d) the Japanese raccoon dogs collected in Shizuoka, Kanagawa and Aichi prefectures had 38 chromosomes in the basic number, but the chromosome numbers of these specimens increased always from the basic one by the presence of the B-chromosomes and also by the Robertsonian fission of some metacentric chromosomes. The karyotypes of 22 Japanese raccoon dogs collected in Ishikawa, Yamaguchi, Osaka and Wakayama prefectures were also characterized by having several complicated chromosome numbers by the occurrence of the B-chromosomes and also by the Robertsonian fission. In the present paper the karyotype analysis in these 22 specimens will be described preliminarily, with special reference to the distribution of B-chromosomes and the Robertsonian fission.

Material and methods. Twenty-two Japanese raccoon dogs used in the present study were collected from the following places: 8 specimens (nos. 1827, 1829, 1830 and 1840 to 1844) were collected in Komatsu, Ishikawa-ken; 6 specimens (nos. 1984 to 1989) in Oshima, Yamaguchi-ken; 5 specimens (nos. 1673-1676 and 1740) from the Tennoji Zoo, where the animals were protected, which were originally collected in the suburb of Osaka-city; and 3 specimens (nos. 1868 to 1870) from the Wakayama Municipal Zoo, where the animals were protected from the wild capture in Wakayama-ken (Table I). A small piece of the skin was obtained from these specimens, cultured *in vitro* and then the slides for the chromosome observations were made. The conventional Giemsa and the G-banding stainings were used with our routine procedures.

From the metaphase chromosomes obtained by the conventional staining, the number of B's and the Robertsonian fission was determined by the following formulae; $nF = M - Z$ (nF =number of Robertsonian fission; $M=28$ which are the basic chromosome number of the metacentrics; Z =total number of metacentrics observed). $nB = W - A - 2nF$ (nB =number of B's; W =total number of acrocentrics observed; $A=10$, which are the basic number of acrocentrics; nF =number of the Robertsonian fission).

Results and discussion. The basic karyotype of all the 22 Japanese raccoon dogs examined in the present study was shown to be $2n=38$ (18 autosome pairs and XY elements). Among them, 13 autosome pairs (nos. 1 to 13) were characterized by the metacentric or submetacentric type, 5 autosome pairs (nos. 14 to 18) were by the acrocentric one and the X and Y were of the metacentric and

^{†)} Contribution no. 1611 from the National Institute of Genetics, Japan.

^{*)} National Institute of Genetics, Misima, Shizuoka-ken.

^{**)} Osaka City University, Medical School, Osaka.

^{***)} Amagi Wild Boar Park, Amagi-yugashima, Shizuoka-ken.

Table I. Distribution of the B-chromosomes and the Robertsonian fission in 22 Japanese raccoon dogs collected in four localities of Japan

Local collect.	No. of specimens	Sex	No. of cells analysed	Chromosome numbers	No. of B's							Robert. fission
					0	1	2	3	4	5	6	
Ishikawa pref.	1827	Female	3	42(3)*					3*			Non
	1829	Male	5	41(3), 42(2)				3	2			"
	1830	"	2	39(1), 42(1)	1			1				1
	1840	Female	2	40(1), 42(1)			1		1			Non
	1841	"	2	42(1), 43(1)					1	1		"
	1842	"	2	41(1), 42(1)			1	1				1
	1843	"	1	40(1)				1				Non
	1844	"	2	41(2)				2				"
Yamaguchi pref.	1984	Male	3	43(2), 44(1)						2	1	Non
	1985	"	3	42(3)					3			"
	1986	Female	5	41(4), 42(1)				4	1			"
	1987	Male	2	41(2)				2				"
	1988	"	1	41(1)				1				"
	1989	"	2	42(2)					2			"
Osaka pref.	1673	Female	2	40(1), 41(1)			1	1				Non
	1674	"	5	39(2), 40(3)		2	3					"
	1675	Male	8	39(1), 40(7)		1	7					"
	1676	Female	13	39(1), 40(12)		1	12					"
	1749	"	3	40(2), 41(1)			2	1				"
Wakayama pref.	1868	Female	1	42(1)					1			Non
	1869	"	4	40(1), 41(3)			1	3				"
	1870	"	4	41(3), 44(1)				3		1		1
Total	22		75		1	4	31	24	14	4	1	3

* Number of cells observed.

the small acrocentric types respectively. In all the specimens, however, the chromosomes increased in number more than the basic one due to the presence of the B-chromosomes and also by the Robertsonian fission (Table I). The morphology of all B's in the present materials was the median sized acrocentrics similar to those of the other specimens already observed by us (Yosida *et al.* 1983, 1984; Yosida and Wada 1984a-d).

1) Specimens from Ishikawa prefecture: All 8 specimens (6 females and 2 males) had 2 to 5 B's. Three cells of one specimen (no. 1827♀) had 42 chromosomes including 4 B's (Fig. 1), but 5 cells in the other one (no. 1829♂) had 41 (3 cells) and 42 chromosomes (2 cells), the former having 3 B's and the latter having 4 B's. In the specimen no. 1830(♂), two cells were analysed, among them one cell had 3 B's, but in the other one the B-element was not observed. At the present it is questionable whether the B-element was not included in the cell or lost from the metaphase by the technical error, because the cells without B's were never observed in 1,372 cells from 13 specimens (Yosida and Wada 1984d). In this specimen the Robertsonian fission of one metacentric chromosome was observed. In the other one specimen (no. 1842♀) the Robertsonian fission was also observed in one metacentric. In this specimen two cells had 2 and 3 B's, respectively. The remaining 4 females (nos. 1840, 1841, 1843 and 1844) had 2

Fig. 1. Karyotype of a female Japanese raccoon dog (specimen no. 1827) collected in Yamaguchi prefecture. Chromosome number is $2n=42$ including 4 B-chromosomes. The X is not identified from the biarmed autosomes.

to 5 B's. One specimen (no. 1840) had 2 and 4 B's, but the specimen no. 1841 had 4 and 5 B's. The other two (nos. 1843 and 1844) had 3 B's.

2) Specimens from Yamaguchi prefecture: Any Robertsonian fission was not observed in all 6 specimens collected in Yamaguchi prefecture. It is interesting that a considerable high number of B's were observed in these specimens. The specimen no. 1984(♂) had 5 B's (2 cells) and 6 B's (one cell) and in nos. 1985(♂) and 1989(♂), 5 B's were observed in all three and two cells, respectively. The remaining three specimens (nos. 1986♀, 1987♂ and 1988♂) had 3 B's, although one cell had 4 B's in the specimen no. 1986.

3) Specimens from Osaka prefecture: Five specimens collected in Osaka had not any Robertsonian fission, but two B's were observed in the most cells. Two cells of the specimen no. 1673(♀) showed each one of 2 B's and 3 B's. Five cells of the specimen no. 1674(♀) had one B (2 cells) and 3 B's (3 cells). Among 8 cells from the specimen no. 1675(♂), 7 cells had 2 B's and the remaining one had one B. Similarly, among 13 cells in the specimen no. 1676(♀), 12 cells had 2 B's, but one cell had one B. The last one specimen (no. 1749♀) had 2 B's (2 cells) and 3 B's (one cell).

4) Specimens from Wakayama prefecture: Among three specimens, one (no. 1870♀) had the Robertsonian fission of one metacentric, but the other two (nos. 1868♀ and 1869♀) had not any fission. One cell from the specimen no. 1868 showed 4 B's, but 4 cells in the specimen no. 1869 showed 2 B's (one cell) and 3 B's (3 cells). The remaining one specimen (no. 1870) with the fission had 2 B's (3 cell) and 5 B's (one cell).

Although the number of cells examined was few in each specimen, it has been clearly shown that all present specimens examined had the B-chromosomes and the somatic variation of the B's was also observed in the most specimens as observed in the previous materials (Yosida and Wada 1984d). In the present materials 75 cells in total were analysed (Table I). Among them 69 cells (92.0%) had any one of 2 B's (41.3%), 3 B's (32.3%) or 4 B's (18.7%). This frequency was also similar to our previous study. On the Robertsonian fission, 5 (38.5%) among 13 specimens had the fission in the previous materials, but in the present

materials only 3 (13.6%) among 22 specimens had one Robertsonian fission. This was a large difference between our present and the previous materials. In the previous materials, the Robertsonian fission was observed in one of the no. 8 chromosomes (3 specimens), in one of the no. 6 chromosomes (1 specimen) and in each one of 3 chromosomes (nos. 5, 8 and 11) (1 specimen). In the present materials, although 3 specimens had the Robertsonian fission, it was not determined at present in which chromosome the fission was taken place. The details of the chromosome analysis in the present materials will be reported elsewhere.

In the total we observed 35 specimens of the Japanese raccoon dogs including those of the previous studies. All these materials had the B-chromosome, although its number was highly variable, but any specimen without the B's was not observed. As we already discussed, the B-chromosome would have some selective advantage for the development or the evolution of this animal species. The rate of the Robertsonian fission was counted as 0.23 in total of 35 specimens. This rate seemed to be considerably high in the natural population, because such fission seldom occurs in the mammalian species. Thus, the Robertsonian fission would have also some selective advantage in the karyotype evolution of this species.

Summary. The present paper deals with the karyotype analysis of 22 Japanese raccoon dogs collected in Ishikawa, Yamaguchi, Osaka and Wakayama prefectures, Japan. The basic chromosome number of these specimens is $2n=38$, but the higher number of the chromosomes than the basic one has been observed in all specimens, because the B-chromosomes are included in all of them. Among the 22 specimens, the Robertsonian fission of one metacentric element has been observed in 3 specimens.

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