Compound Mosaicism, Caused by B Chromosome Variability, in the Chinese Raccoon Dog (*Nyctereutes procyonoides procyonoides*)*

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Accepted September 6, 2005

SZCZERBAL I., KACZMAREK M., SWITONSKI M. 2005. Compound mosaicism, caused by B chromosome variability, in the Chinese raccoon dog (*Nyctereutes procyonoides procyonoides*). Folia biol. (Kraków) **53**: 155-159.

The distribution of B chromosomes in a group of twenty-six farm Chinese raccoon dogs was analysed using Giemsa staining and sequentional Q/C banding techniques. It was shown that three different types of B chromosomes are widely distributed among the studied animals. The variability of the B chromosome number was limited (0-4) and two B chromosomes occurred with the highest frequency. Mosaic karyotypes appeared to be very common in this species. Only two animals had a stable karyotype in terms of the B chromosome number. The mosaicism of the B chromosomes had a compound character, since karyotypes of the studied raccoon dogs differed in terms of the number of Bs as well as the presence of the three morphological types of the Bs.

Key words: B chromosomes, Chinese raccoon dog, mosaic karyotypes, B chromosome variability.

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The occurrence of B chromosomes in karyotypes of mammals is a rather unique phenomenon. These structures were detected only in 1% of cytogenetically investigated mammals but it is estimated that the proportion of species bearing B chromosomes (Bs) is above 3% (VUJOSEVIC & BLAGOJEVIC 2004). Although there are numerous studies concerning the nature, origin and significance of B chromosomes, these structures still remain mysterious elements of the genome (CAMACHO *et al.* 2000). Interesting models for B chromosome studies are some species belonging to the family Canidae. There are two canids with B chromosomes – the red fox (*Vulpes vulpes*) and the raccoon dog (*Nyctereutes procyonoides*) (SWITONSKI *et al.* 2003).

The raccoon dog is considered as the oldest member of the family *Canidae*. This species is widespread in Europe and Asia and is also kept in captivity for the fur trade. Two subspecies with different diploid chromosome numbers have been distinguished – the Chinese raccoon dog, *Nyctereutes procyonoides procyonoides* (2n=54+B) and the Japanese raccoon dog, *Nyctereutes procyonoides viverrinus* (2n=38+B) (MÄKINEN *et al.* 1986). In the karyotype of the Chinese raccoon dog, B chromosomes are of medium size and their number varies from 0 to 4. In the Japanese raccoon dog the Bs are rather small acrocentrics and their number varies from 0 to 7 (YOSIDA *et al.* 1985).

It has been shown that B chromosomes of the raccoon dog are composed of repetitive DNA sequences (TRIFONOV *et al*. 2002). The use of probes representing telomeric (WURSTER-HILL *et al.* 1988) and NOR-like sequences (SZCZERBAL & SWITONSKI 2003) in FISH studies revealed hybridisation signals along the Bs. Recently, for the first time a gene locus on the mammalian B chromosome was reported. GRAPHODATSKY *et al.* (2005) found a locus of the C-KIT gene on B chromosomes of the raccoon dog and red fox.

There are three morphological types of Bs in the Chinese raccoon dog karyotype which differ from each other by size and Q- or G-banding pattern (PIENKOWSKA *et al.* 2002). However, it should be pointed out that Q- or G- banding patterns are not very distinct. It is also known that these structures

*Supported by grant No 88/2004 from Foundation for Polish Science (Young Scientists Fellowship) to I. SZCZERBAL.

have an exceptional C-banding pattern – chromosomes are slightly darker but the centromeric C-band is not visible (MÄKINEN *et al.* 1986). Recognition of the BI chromosome is rather easy due to its size and banding patterns, but it is difficult to distinguish between BII and BIII. In the present study a sequentional Q/C banding was applied and thus identification of all three types became unambiguous. This facilitated, for the first time, a detailed analysis of the distribution of B chromosomes in this species, with respect to their number and morphology.

Material and Methods

Blood samples were collected from twenty six animals kept on a commercial farm. Metaphase spreads were obtained from lymphocyte cultures. The evaluation of the chromosome set was carried out using Giemsa staining and sequentional Q/C banding techniques. Q-banding was performed prior to C-banding in 0.005% quinacrine mustard for 90 s. (CASPERSSON *et al.* 1970). At least twenty Q-banded metaphase spreads were captured and then the C-banding technique (SUMNER 1972) was applied. Chromosome nomenclature for the Chinese raccoon dog proposed by PIENKOWSKA *et al.* (2002) was followed. Metaphase spreads were analyzed under a light/fluorescent microscope Nikon E 600 Eclipse, equipped with a cooled digital CCD camera, driven by computer aided software Lucia.

Results and Discussion

Altogether 724 metaphase spreads, originating from 26 animals, were analysed. It was found that two B chromosomes (2B) occurred most frequently (68.6% of the analysed metaphase spreads). Almost all the animals (92.3%) had a mosaic karyotype and only two of them (no 7 and 19) with a non-mosaic karyotype had two Bs (Table 1).

Table 1

Variability of the B chromosome number in the Chinese raccoon dog

Animal number	No of analysed metaphases	Number of B chromosomes					Bs modal number
		0	1	2	3	4	
1	30	2 (6.7%)	1 (3.3%)	27 (90%)			2
2	26	2 (7.7%)	2 (7.7%)	22 (84.6%)			2
3	20	2 (10%)		14 (70%)	4 (20%)		2
4	61		5 (8.2%)	52 (85.2%)	4 (6.6%)		2
5	29		3 (10.3%)	25 (86.2%)	1 (3.4%)		2
6	29		1 (3.4%)	25 (86.2%)	3 (10.3%)		2
7	20			20 (100%)			2
8	72		5 (6.9%)	61 (84.7%)	5 (6.9%)	1 (1.4%)	2
9	20		5 (25%)	15 (75%)			2
10	20		1 (5%)	19 (95%)			2
11	21	1 (4.8%)	2 (9.5%)	18 (85.7%)			2
12	20		13 (65%)	6 (30%)	1 (5%)		1
13	20	2 (10%)	2 (10%)	11 (55%)	4 (20%)	1 (5%)	2
14	21			1 (4.8%)	13 (61.9%)	7 (33.3%)	3
15	45		2 (4.4%)	13 (28.9%)	30 (66.7%)		3
16	24		3 (12.5%)	18 (75%)	3 (12.5%)		2
17	31		2 (6.5%)	18 (58.1%)	10 (32.3%)	1 (3.2%)	2
18	24		14 (58.3%)	10 (41.7%)			1
19	23			23 (100%)			2
10	29		6 (20.7%)	23 (79.3%)			2
21	33			31 (93.9%)	2 (6.1%)		2
22	35		14 (40%)	19 (54.3%)	2 (5.7%)		2
23	15			7 (46.7%)	8 (53.3%)		3
24	23			3 (13%)	17 (73.9%)	3 (13%)	3
25	12		4 (33.3%)	8 (66.7%)			2
26	21			8 (38.1%)	12 (57.1%)	1 (4.8%)	3
Total	724	9 (1.2%)	85 (11.7%)	497 (68.6%)	119 (16.4%)	14 (1.9%)	2

Sequentional Q/C banding (Fig. 1) of 160 metaphase spreads, obtained from twelve animals, revealed the presence of three types of Bs (Fig. 1, Table 2). The most common type was BII (40%), while two other types occurred with similar frequency. Three morphological types of Bs were present in eleven different mosaic karyotypes and one non-mosaic. It was found that two animals (nos 19 and 21) with the non-mosaic karyotype, in terms of the number of Bs, appeared to be mosaics when the morphology of the Bs was considered. On the other hand, one animal (no. 18) with the mosaic karyotype (1B/2B) was non-mosaic in terms of the Bs morphology, since only BI was present.

It was shown that in the raccoon dog intra- and interindividual variation of Bs is very common. When the variability of the number of Bs is compared with the Japanese raccoon dog, its extent is wider in the Japanese raccoon dog. An analysis of 1372 metaphase spreads derived from 35 animals revealed the presence of 1 to 7 Bs (YOSIDA *et al.* 1984) and spreads with three Bs were found with the highest frequency (36%). Two and four Bs were also quite common and their frequency was 31% and 23%, respectively. All the investigated animals had a mosaic karyotype. In another study performed on a different population of the Japanese raccoon dogs, the B chromosome number varied from 0 to 6 and the most common were two

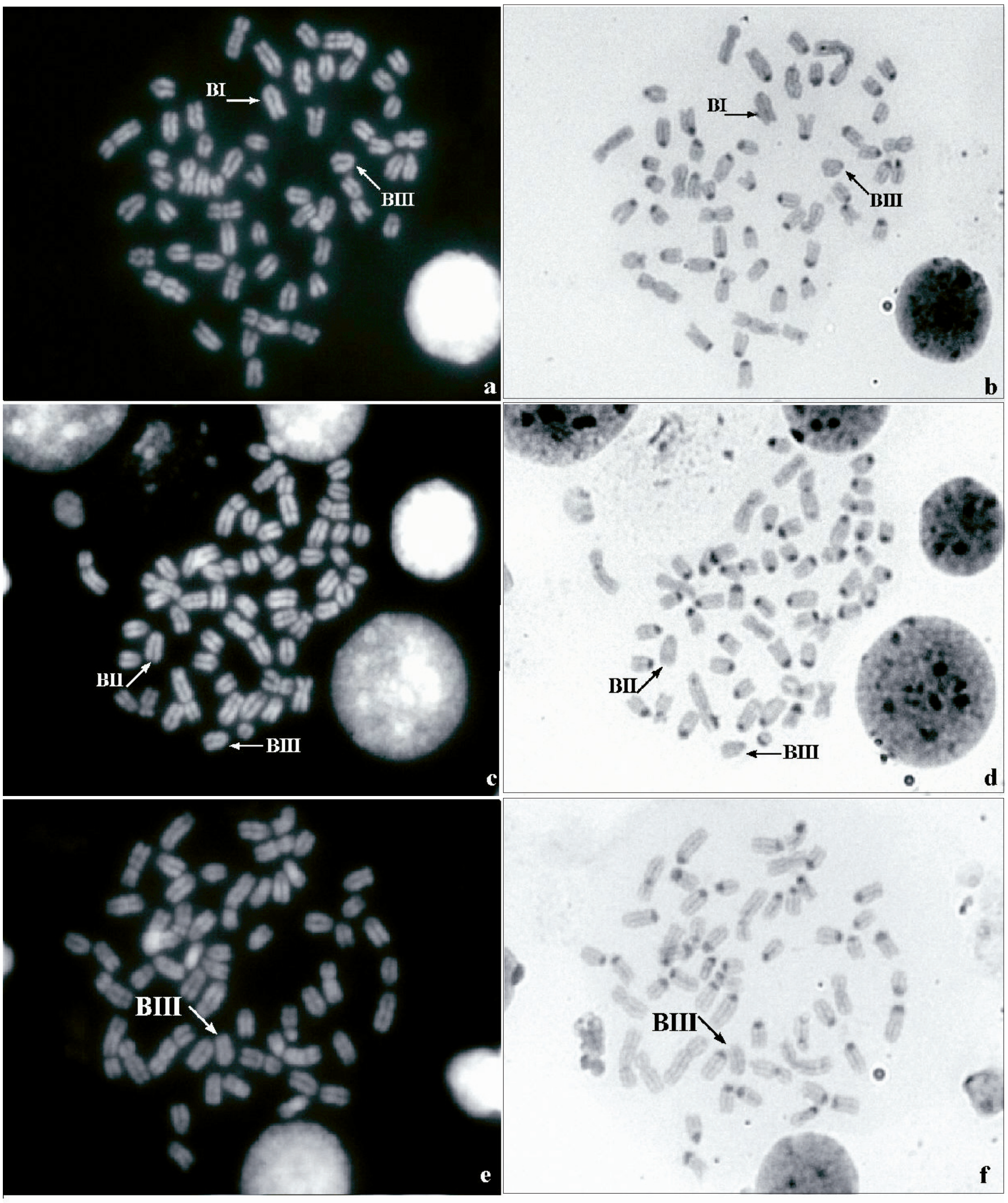

Fig. 1. Q/C sequentional banding of three metaphase spreads of the raccoon dog chromosomes: Q-banding (a, c and e) and C-banding (b, d and f). B chromosomes are indicated by arrows.

Table 2

Distribution of three types of B chromosomes in the Chinese raccoon dog

Animal number	Number of metaphase spread	Number of Bs and their morphology				The most frequent type of Bs
		1B	2B	3B	4B	
15	11	BII	BII+BII BII+BIII	BII+BII+BIII		BII
16	7	BIII	BI+BIII	BI+BIII+BIII		BIII
17	6		BII+BII BII+BIII	BII+BII+BII BII+BII+BIII		BII
18	11	BI	BI+BI			BI
19	20		BI+BIII			BI;BIII
20	27	BII BIII	BII+BII BII+BIII			BII
21	19		BI+BIII			BI;BIII
22	19	BI BII	BI+BI BI+BII			BI
23	11		BI+BI BI+BII	BI+BI+BIII BI+BII+BII BI+BII+BIII		BI
24	7		BII+BIII	BI+BII+BIII	BI+BII+BII+BIII	BII
25	8	BII	BII+BII			BII
26	14		BII+BIII	BII+BII+BIII	BI+BII+BII+BIII	BII
Total	160					

(41%), three (32%) and four Bs (19%) (YOSIDA *et al.* 1985). It was found that among 22 animals eight had a stable chromosome number, but this could be the result of a low number of analyzed metaphase spreads for a single specimen (fewer than 10). It can be assumed that mosaicism, caused be a variable number of B chromosomes, is a common phenomenon in both subspecies of the raccoon dog. A similar situation is present in another canid – the red fox (SWITONSKI *et al.* 2003).

Although the variation in the number of Bs is evident in the group of the investigated raccoon dogs, the most common situation is the presence of a low number of Bs in a cell. It is supposed that some kind of B chromosome number equilibrium is maintained in a population. To explain this mechanism two models have been proposed (for review see: VUJOSEVIC & BLAGOJEVIC 2004). In the parastic model the equilibrium is a result of B chromosome accumulation and selection against individuals with Bs, while in the heterotic model some beneficial effects exist in case of a low number of Bs and harmful ones for a high number of Bs.

Little is know about the phenotypic effect of the Bs. In most cases the detected effect had a quantitative character. A positive correlation was observed in the Japanese raccoon dog between the number of Bs and body weight in males (WURSTER-HILL *et al.* 1986). The same situation was observed in *Apodemus flavicolis*, where the mean number of Bs was related with male body weight (ZIMA *et al.* 2003). It is also suggested that adaptive effects of the Bs, related to unknown ecological factors and/or breeding systems, may exist in other mammalian species (PALESTIS *et al.* 2004). For instance, in the red fox the number of Bs varies from 0 to 8, but the most common numbers are 2 or 3. A comparison of the distribution of Bs in wild and farm populations of foxes showed that in wild animals higher numbers of these structures were present (SWITONSKI *et al.* 2003).

This study revealed that mosaicism of the Bs in the Chinese raccoon dog is a compound phenomenon. One can foresee that a similar situation is also present in the Japanese raccoon dog and the red fox. However, due to the fact that the Bs in these species are smaller than those in the Chinese raccoon dog, identification of different types, with the use of classical cytogenetic techniques, is very difficult or even impossible. It is also rather unlikely that specific genetic markers for different types of Bs will be found, since the molecular nature of the Bs is similar within a species – raccoon dog (WURSTER-HILL *et al.* 1988; TRIFONOV *et al.* 2002; SZCZERBAL & SWITONSKI 2003) and red fox (YANG *et al.* 1999).

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