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A preliminary note on banded karyotypes of the short-tailed shrew *Blarina brevicauda* (Mammalia, Insectivora)

TATSUO OSHIDA[1, *], SATOSHI D. OHDACHI[2] and RYUICHI MASUDA[3]

[1] Laboratory of Molecular Ecology, Department of Biology, Tunghai University, Taichung Taiwan 407, R.O.C.
[2] The Institute of Low Temperature Science, Hokkaido University, Sapporo 060-0819, Japan.
[3] Laboratory of Genetic Diversity, Center for Advanced Science and Technology, Hokkaido University, Sapporo 060-0810, Japan.

Abstract - Chromosomes of the short-tailed shrew *Blarina brevicauda*, which display the numerical polymorphism arisen from Robertsonian rearrangements, were analyzed with conventional and silver staining and G- and C-banding techniques. With respect to all specimens examined in the present study, the diploid chromosome number (2n) and fundamental autosomal arm number (FN) were 50 and 48, respectively. The karyotype consisted of 24 pairs of acrocentric autosomes, a large-sized metacentric X chromosome, and a small-sized submetacentric Y chromosome. The comparison with previous findings suggested the geographic polymorphism of Y chromosome in this species. All autosomes and the X chromosome carried slight centromeric constitutive heterochromatin, whereas the Y chromosome was entirely heterochromatic. On the satellites of short arms of two autosomal pairs, the nucleolus organizer regions (NORs) were recognized. The G- and C-banded and Ag-NOR-stained karyotypes presented in the present study could be useful cytogenetic characteristics for specification of chromosomes participating in Robertsonian rearrangements within this species and for karyo-systematic study of genus *Blarina*.

Key words: short-tailed shrew; *Blarina brevicauda*; C-banding; G-banding; nucleolus organizer region; Y chromosome.

INTRODUCTION

The North American short-tailed shrews (genus *Blarina*), which are widely distributed throughout the North American Continent, are classified into three species: *B. brevicauda*, *B. carolinensis*, and *B. hylophaga* (HALL 1981; NOWAK 1991; WILSON and REEDER 1992). The conventionally stained chromosomes of *Blarina* have been reported heretofore (e.g., GEORGE *et al.* 1981; MEYLAN 1967; MONCRIEF *et al.* 1982). However, the banded karyotypes of *Blarina* have not been established so far. Chromosomal polymorphism caused by Robertosonian rearrangements or non-Robertsonian mechanisms is widespread in genus *Blarina* (ZIMA *et al.* 1998). *Blarina brevicauda* has the diploid number (2*n*) ranging from 48 to 50 (MEYLAN 1967; ZIMA *et al.* 1998). Diploid chromosomal numbers of *B. carolinensis* ranging from 37 to 52 are more variable than that of *B. brevicauda* (GEORGE *et al.* 1982; ZIMA *et al.* 1998). Although *B. hylophaga* does not have numerical chromosomal polymorphism, it exhibits a structural polymorphism that resulted in fundamental numbers of 60 or 61 (GEORGE *et al.* 1981). Therefore, for analysis of polymorphic chromosomes in karyotypes of *Blarina* species, the precise identification of each chromosome with differential staining techniques is of importance. In the present study, we prelimi-

* Corresponding author: fax: +886-4-23507025; e-mail: oshidata@mail.thu.edu.tw

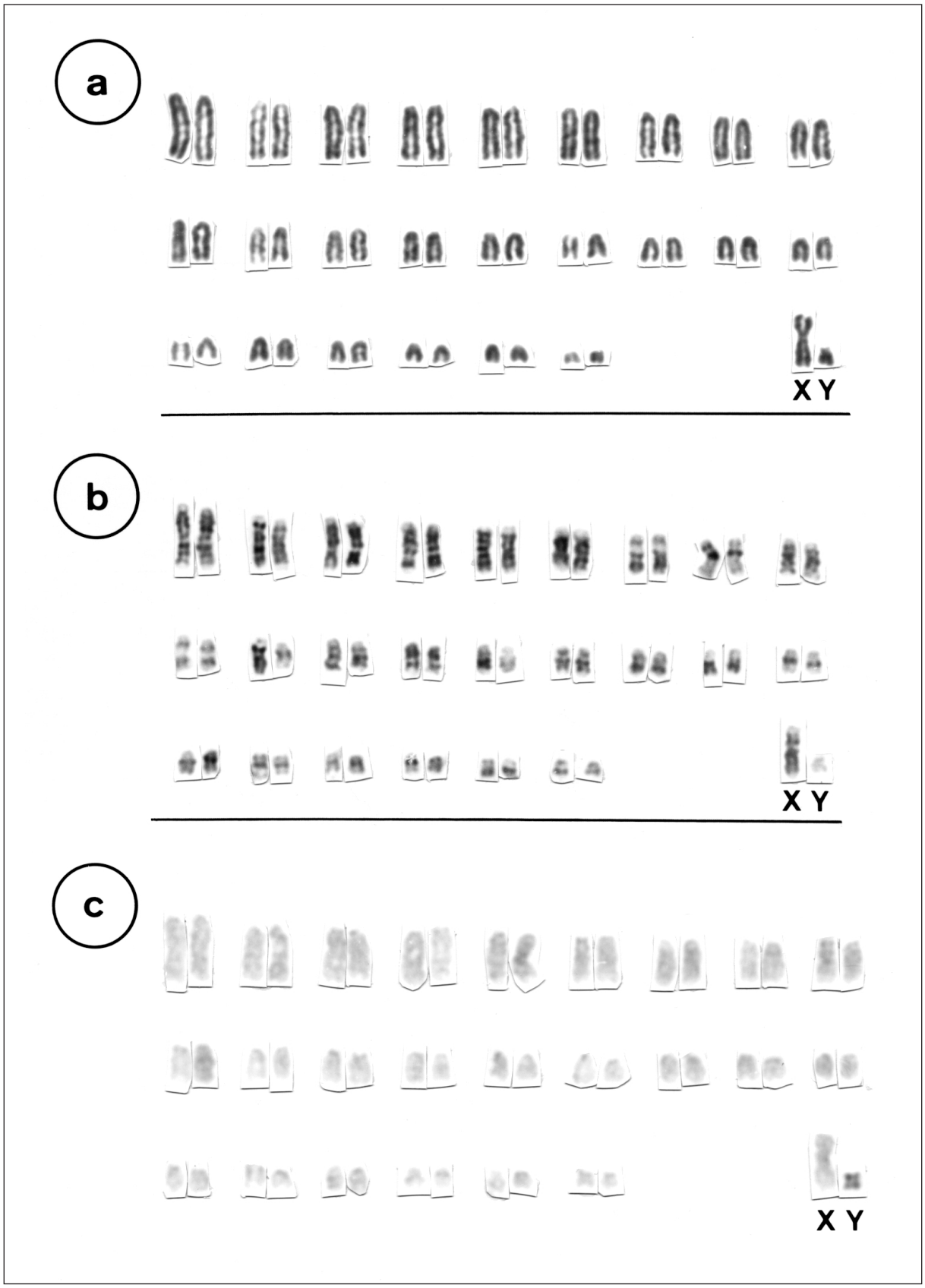

Fig. 1 – Conventional (a), G-banded (b), and C-banded (c) karyotypes of a male *Blarina brevicauda* from Michigan, U.S.A.

Table 1 – Numbers of metaphase plates of *Blarina brevicauda* examined in the present study.

Identity number	Sex	Conv.	G	C	Ag
990917-1	♀	6 (3)	–	–	–
990917-2	♀	10 (9)	18 (10)	12	15 (6)
990917-4	♂	15 (6)	–	–	–
990918-3	♀	7 (4)	–	–	–
990920-1	♀	2 (2)	–	–	–
990920-2	♂	2 (2)	–	–	–
990921-2	♂	10 (5)	12 (10)	31	6 (3)

Conv. conventionale staining; G, GTG staining; C, CBG staining; Ag, Ag-NOR staining. Numbers of karyotyped plates out of observed plates are shown in parentheses.

nary present and discuss the G- and C-banded and Ag-NOR stained karyotypes of *B. brevicauda*.

MATERIALS AND METHODS

Three males and four females of northern short-tailed shrew (*Blarina brevicauda* Say, 1823) were collected on 17-21, September, 1999, in Livingstone County, Michigan, U.S.A. Chromosomal preparations were made from skin fibroblasts according to our routine air-drying method. For these preparations, the conventional Giemsa staining was carried out. To characterize each chromosome, GTG, CBG, and Ag-NOR staining were performed with routine cytogenetical techniques (SEABRIGHT 1971; SUMNER 1972; HOWELL and BLACK 1980).

RESULTS AND DISCUSSION

The number of metaphase plates of *Blarina brevicauda* examined in the present study is shown in Table 1. All seven specimens had the 2*n* of 50 and FN of 48. The autosomal complement in the karyotype comprised 24 pairs of acrocentrics. The X was a large-sized metacentric chromosome, and the Y was a small sub-

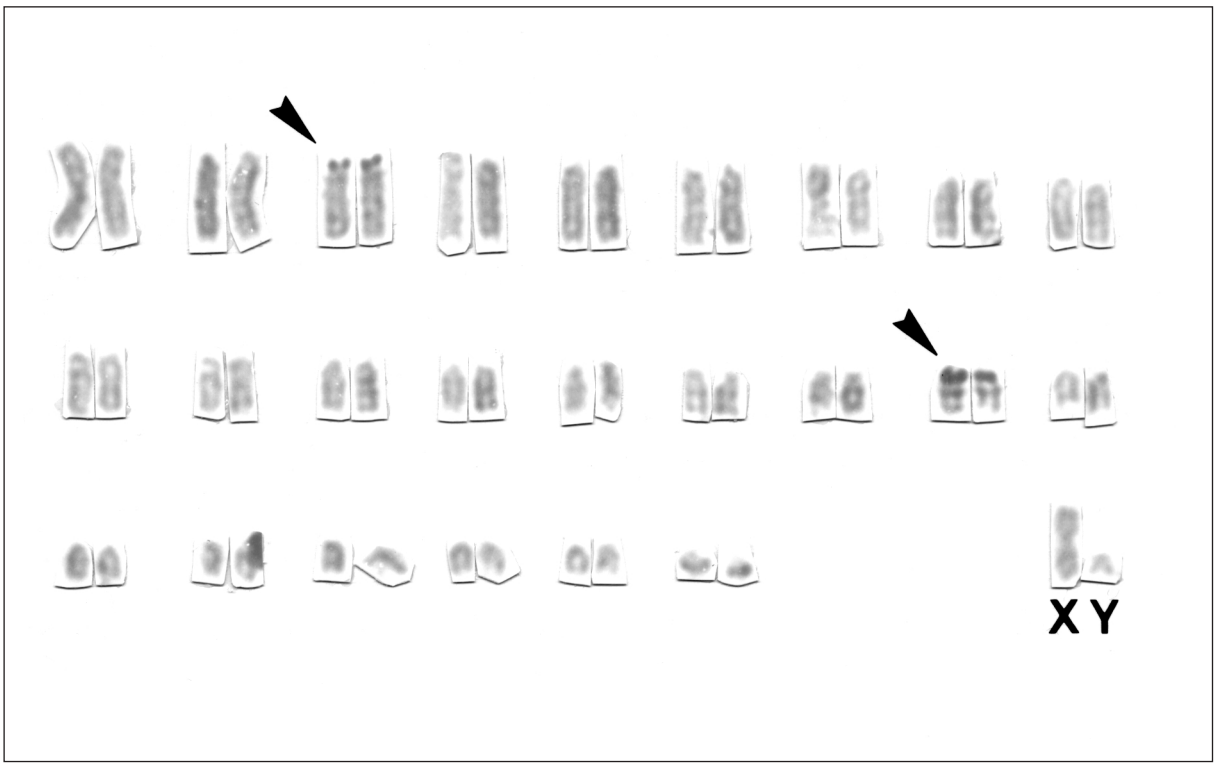

Fig. 2 – A silver-stained karyotype of *Blarina brevicauda* from Michigan, U.S.A. Arrowheads indicate Ag-NORs.

Table 2 – Numbers of *Blarina brevicauda* reported diploid chromosome number (2*n*) in the previous and present studies.

Sampling localities	2*n*			References
	48	49	50	
Barrow Co., Georgia			2	GEORGE *et al.* (1982)
Roan Mountain, North Carolina and Tennessee		3	2	GEORGE *et al.* (1982)
Marshall Co., Tennessee			1	GEORGE *et al.* (1982)
Buffalo, Cass, and Sarpy Co., Nebraska and Wesrmoreland Co., Pennsylvania		4	6	GENOWAYS *et al.* (1977)
Wesrmoreland Co., Pennsylvania		3	4	GEORGE *et al.* (1982)
Ontario		5	16	MEYLAN (1967)
Livingstone Co., Michigan			7	present study
Central Illinois	1	6	46	LEE and ZIMMERMAN (1969)
Douglas Co., Kansas			1	GEORGE *et al.* (1982)
Cheshire Co., New Hampshire		2	4	GEORGE *et al.* (1982)
Aroostook Co., Maine		1	4	GEORGE *et al.* (1982)
Bonaventure Co., Quebec		1		GEORGE *et al.* (1982)

metacentric chromosome (Fig. 1a). *Blarina brevicauda* occurs throughout the central to northern ranges of the North American Continent across eastern to western margins. GEORGE *et al.* (1982) reported that numerical chromosome polymorphism ranging from 48 to 50 in this species, resulting from Robertsonian rearrangements between large- and small-sized acrocentric chromosomes, and that the 2*n* of 50 was the most dominant complement. Chromosomal polymorphism was summarized in Table 2. In the present study, seven specimens from Michigan, U.S.A., possessed 50 chromosomes, and did not demonstrate chromosomal polymorphism (Fig. 1a). Although, owing to the absence of numerical polymorphism in the present samples, chromosomes taken part in Robertsonian events could not be specified, all chromosomes were precisely identified on the basis of their unique G-banding patterns (Fig. 1b). So, in the further study, chromosomes related with Robertsonian rearrangements would be found out with the unique G-banding patterns established here.

The Ag-NORs were successively detected on terminal regions of short arms of two autosomal pairs (Fig. 2), showing the existence of satellites on short arms of these chromosome. In metaphases, satellite associations are often observed, namely, the short arms of multiple (about 2-10) acrocentric chromosomes are touching or very close to one another (MILLER *et al.* 1977; MILLER and THERMAN 2001). As *B. brevicauda* carried all acrocentric autosomes, the high frequency of satellite association had been expected, however, it was not notable for all seven specimens. Although the Ag-NORs of other *Blarina* species have not been reported so far, it may be one of useful markers for karyo-taxonomy of this genus.

The constitutive heterochromatin was slightly presented on centromeres of all autosomes and X chromosome (Fig. 1c). On the other hand, the Y chromosome appeared to be entirely heterochromatic (Fig. 1c), as in those of human and mouse. MEYLAN (1967) described the Y chromosome of *B. brevicauda* from Ontario, Canada, as being a very small metacentric chromosome, whereas in specimens from Nebraska and Pennsylvania, U.S.A., GENOWAYS *et al.* (1977) found it to be a small acrocentric. However, in the present study, three male specimens from Michigan possessed submetacentric Y chromosomes (Fig. 1a). From these findings, this animal seems to have geographically polymorphic Y chromosome, which may have been arisen from duplication or deletion of constitutive heterochromatin. As the illustration of Y chromosomes were not presented in the previous reports (GENOWAYS *et al.* 1977; MEYLAN 1967), it is difficult to argue here the matter with regard to the shape of Y chromosome of *B. brevicauda* in detail. So, we need further investigations for geographic polymorphism of Y chromosome of *B. brevicauda*.

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