

## KARYOTYPES AND B CHROMOSOME OF GREATER LONG-TAILED HAMSTER (CRICETULUS TRITON)\*

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**Abstract:** The karyotypes of greater long-tailed hamster (*Cricetulus triton*) from Jinan, Mt. Tai, Shandong Province, Mts. Changbai, Jilin Province and Xi'an, Shaanxi Province in China were observed by conventional and banding technique. The diploid chromosome number and karyotype are identical in the samples of Jinan, Mts. Changbai and Xi'an, i. e.,  $2n = 28$ , and the karyotype consists of 11 pairs of telocentric and 2 pairs of metacentric chromosomes, X chromosome is subtelocentric and Y metacentric. Diploid number of the sample from Mt. Tai is  $28 \sim 29$ , one extra unpaired chromosome existed in the observed metaphases by 67.5%. It is B chromosome which is the smallest telocentric in the complement. A chromosomes of the sample were identical with that of samples from Jinan, Xi'an and Mts. Changbai. The G-banded, C-banded, and silver stained karyotypes have been observed, no difference was found among samples from four localities. The origin of B chromosome was discussed in the paper.

**Key words:** *Cricetulus triton*; Karyotype; Banding patterns of chromosome; B chromosome

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B chromosomes have been identified in number of some populations of nearly 500 animal species and more than 1 300 plant species<sup>[1,2]</sup>. The standard chromosomes of an organism are A chromosomes. B chromosomes are extra to this normal complement. In the 1st B-chromosome conference held in Spain in 1993, B chromosome is defined as a dispensable supernumerary chromosome that does not recombine with the A chromosomes and follow its own evolution<sup>[3]</sup>. In mammals Bs have been known since 1965, when they were first described for *Shoinobates volans* (Marsupialia). Up to now, these structures have been described for nearly 30 mammalian species, more than half of them are rodent species.

Greater long-tailed hamsters (*Cricetulus triton*), are distributed from Gansu and Jiangsu to N. E. China, Korea and upper Ussuri of Siberia<sup>[4,5]</sup>. There are four

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subspecies in China (triton, fuscipes, collinus and incanus)<sup>[5]</sup>. The B chromosomes in *Tscherskia triton* (= *C. triton*) from former USSR was first described by Borisov<sup>[6]</sup>. Xu and Huang<sup>[7]</sup> reported the karyotype of the species, and no B chromosome was found in it.

## 1 Materials and Methods

Eight samples of greater long-tailed hamster (*Cricetulus triton*) from 4 regions (Mts. Changbai, Jilin Province, subspecies *fuscipes*; Jinan and Mt. Tai, Shandong Province, subspecies *triton* and Xi'an, Shaanxi Province, subspecies *collinus*) representing 3 subspecies were used for karyological study.

The bone-marrow in vivo method was used for the chromosome preparation; Classification of chromosomes was based upon the study of Levan et al.<sup>[8]</sup>. Seabright's<sup>[9]</sup> trypsin G-banding method to obtained G-banded chromosomes; Sumner's<sup>[10]</sup> method to get C-banded chromosomes; and Howell and Black's silver-staining method<sup>[11]</sup> to obtained silver-stained chromosomes.

## 2 Results

### 2.1 Diploid chromosome number

Most of metaphase cells in the preparations were diploid and normal. The chromosome counts for the samples from four localities are shown in Table 1.

Table 1 The counts of diploid chromosome number in *Cricetulus triton*

Locality (Subspecies)		Chromosome frequency					Total cells observed
		26	27	28	29	30	
Jinan (triton)	Cell numbers	3	8	128	1	0	140
	Percentage	2.2	5.7	91.4	0.7	0	100
Mt. Tai (triton)	Cell numbers	2	6	54	135	3	200
	Percentage	1	3	27	67.5	1.5	100
Mts. Changbai (fuscipes)	Cell numbers	1	7	92	0	0	100
	Percentage	1	7	92	0	0	100
Xi'an (collinus)	Cell number	0	2	78	0	0	80
	Percentage	0	2.5	97.5	0	0	100

According to Table 1, diploid chromosome number of samples in Jinan, Xi'an and Mts. Changbai is  $2n=28$ ; but that of the samples from Mt. Tai varies from 28 to 29. Among the metaphases observed, the chromosome number in 67.5 % of cells is 29.

### 2.2 Karyotype analysis

Seven to ten metaphases of the species from four localities were photographed and used for karyotypic analysis respectively. The model karyotype of Jinan, Xi'an and Mts. Changbai is  $22t + 4m + XY$  (st, m) (Table 2 and Plate I —1, Xi'an sample).

11 pairs of autosomes in their complement are uniarmed chromosomes, 2 pairs of small biarmed chromosomes; X chromosome is subtelocentric (uniarmed) and Y metacentric (biarmed) chromosome.

Table 2 The measurements and classification of chromosomes in *C. triton*

Chromosome number	Relative length	Arm ratio	Classification
1	106.89 $\pm$ 3.97		t
2	102.73 $\pm$ 2.50		t
3	99.03 $\pm$ 5.88		t
4	87.92 $\pm$ 4.33		t
5	86.92 $\pm$ 4.66		t
6	83.29 $\pm$ 4.19		t
7	81.91 $\pm$ 4.38		t
8	73.11 $\pm$ 2.10		t
9	70.34 $\pm$ 3.80		t
10	64.78 $\pm$ 4.19		t
11	62.00 $\pm$ 3.35		t
12	43.04 $\pm$ 3.61	1.04 $\pm$ 0.11	m
13	37.02 $\pm$ 4.94	1.39 $\pm$ 0.29	m
X	89.77 $\pm$ 8.12	3.08 $\pm$ 0.05	st
Y	60.16 $\pm$ 3.29	1.04 $\pm$ 0.09	m

For the karyotype of the samples from Mt. Tai, there are two difference from the karyotypes of Jinan, Xi'an and Mts. Changbai samples. (1) Except 11 pairs of telocentric and 2 pairs of metacentric chromosomes, there is an additional small unpaired telocentric chromosome. It causes chromosome number variation in the same tissue-bone marrow cells. So we think this accessory chromosome is B chromosome. (Plate I—2). (2) the length of two X chromosomes exhibited significant difference by measurement and statistic analysis of 50 metaphases ( $P < 0.01$ ) (Plate I—6).

### 2.3 C-banded karyotype

C-banding patterns are similar in the samples from four localities. The representative C-banded karyotype of the sample from Mt. Tai is shown in Plate I—3. C-positive regions near centromere are recognized in eleven acrocentric pairs (nos. 1~11). A large C-positive block appears around centromere of X. Whole Y chromosome is deeply stained. No C-band was revealed in two metacentric pairs (nos. 12, 13), and in B chromosome.

### 2.4 G-banding patterns

G-banding patterns of *C. triton* from four localities are easily matched and no difference in them. Each chromosome pair has its own distinct banding pattern. The representative G-banded karyotype of the Mt. Tai sample is shown in Plate I—4. No G-bands appeared in B chromosomes. One more dark stained band in the telomeric region of long arm in the longer X chromosome.

### 2.5 Silver-stained karyotype

The number of NORs varied from four to five pairs, both among animals and within individuals from four localities. When the number of NORs is maximal (i. e., 5 pairs), they are located on autosome pairs 2, 4, 8, 9 and 13. (Plate I—5). Moreover, three kinds of associations of NORs have been observed in *C. triton* (Plate I—7A, B, C). No NOR appeared on B chromosome.

## 3 Discussion

### 3.1 Character and origin of B chromosome in *Cricetulus triton*

B chromosomes are characterized by certain peculiarities that differentiate them from chromosomes of basic set. For example, they are morphologically different from As (usually smaller), and often display nondisjunction at anaphase of mitosis resulting in frequencies varying between individuals and mosaic individuals, between cells of a single tissue, and fully or in their most part composed of the heterochromatin. The B chromosome in *C. triton* from Mt. Tai is the smallest telocentric. There is 0 ~ 1 B in bone marrow cells. But B in the sample was not stained by C-banding method. Same situation was found in some other species, *Apodemus peninsulae* <sup>[12]</sup>, silver fox (*Vulpes vulpes*) <sup>[13]</sup>, *Rattus rattus* <sup>[14]</sup> and *Dicrostonyx torquatus* <sup>[15]</sup>. But when the Bs of *R. rattus* and *V. vulpes* were examined autoradiographically, they were shown late replication <sup>[14,16]</sup>. We know one of the characteristics of the heterochromatin is its late replication. These data indicated that B heterochromatin is different from A's in make up and regulation. They can hardly be regarded as composed only of the structural heterochromatin. More and more evidences show that Bs are not genetically inert and it is known that some carry functional genes. For example, nucleolar organizer regions (NORs), the sites of ribosomal 18S and 28S RNA genes, have been identified on Bs in about 20 species <sup>[17]</sup>. In some cases, the NORs were identified by silver staining <sup>[18]</sup> and this method is frequently assumed to reflect transcriptional activity of the rRNA genes. Donald et al. <sup>[19]</sup> also found that the regular attachment of the B chromosome to a nucleolus in *B. dichromosomata*. This suggests that these ribosomal RNA genes are transcribed.

Two hypotheses exist concerning the origin of the mammalian B chromosomes. The majority of the authors is inclined to considered them to be the remnants of the

structural rearrangements taking place in the karyotypic evolution of the ancestral forms. The translocations of Robertson's type are considered more often than the others. Another way of the B's origin may result from the nondisjunction of the autosomes or sex chromosomes which are subjected to genetic inactivation afterwards and then pass into the B's class with the all consequence characteristics of the latter.

Borisov et al.<sup>[6]</sup> first reported the B chromosomes in *C. triton* from former USSR. The number of Bs is 1~2. Xu and Huang<sup>[7]</sup> reported conventional and G-banded karyotypes of the species from northern Jiangsu Province, China. They found no B chromosomes in it. Kang and Koh<sup>[20]</sup> reported the karyotype of *C. triton* from Korea, and also no B chromosome in the samples. It is the first report for *C. triton* from Mt. Tai having B chromosome. B chromosome was the smallest telocentric and was easily recognized only by its morphology in the complement of the species. The length of two X chromosomes in 50 metaphases of a female sample from Mt. Tai revealed the significant difference ( $P < 0.01$ ); we can also find the difference between two X chromosomes in G-banding. Therefore, we presumed that the B chromosome of the sample from Mt. Tai originated from X chromosome.

### 3.2 Ag-NORs

It has been demonstrated that the substance stained with the Ag procedure seems to be an acidic protein located on sites of ribosomal gene accumulation, i. e., on NOR's. NOR associations have been described in mammal species as occurring randomly among both homologous and heterologous and less frequently involving more than two chromosomes at same time. In our case, three kinds of associations were found. (1) Associations of two homologous chromosomes at telomeric regions of four chromatids (Plate I—7A). (2) Associations of two chromosomes at telomeric regions of two chromatids in different chromosomes (Plate I—7B). (3) Associations of three chromosomes at telomeric regions of four chromatids in different three chromosomes (Plate I—7C). The mechanism of NOR associations is still not clear. However, Thode et al.<sup>[21]</sup> suggested that the NORs that are associated together in metaphase plates can be considered to have attached the same nucleolus in the preceding prophase and interphase.

### 3.3 Comparison of karyotypes in different subspecies

Xu and Huang<sup>[7]</sup> reported the karyotype of *C. triton* from northern Jiangsu, China is 22 telocentrics and 4 metacentrics with larger subtelocentric X and small metacentric Y chromosome. Kang and Koh<sup>[20]</sup> reported that the karyotype of *C. triton* from Korea is diploid number of 28, i. e., 22 telocentric autosomes and 4 small metacentric autosomes, large subtelocentric X and small metacentric Y chromosome. Comparing the karyotypes of samples from northern Jiangsu, Jinan, Mts. Changbai,

China and Korea, we can concluded that the karyotypes of above four populations are identical.

Borisov et al.<sup>[6]</sup> reported that the karyotype of *C. triton* from former USSR is 26 acrocentrics with metacentric X and subtelocentric Y chromosome and also 1~2 B chromosomes. The karyotype of the sample from Mt. Tai is 11 pairs of telocentric and 2 pairs of metacentric, subtelocentric X and metacentric Y chromosomes with an additional B chromosome. Although similar in having B chromosome, they are much different in A chromosomes. This may be the chromosomal polymorphism in different populations or subspecies.

Because of the relatively smaller diploid number and easily distinguished B chromosome in *C. triton*, we can use this species as model animal to study the origin of B chromosome, the relationship between A and B chromosome and the genetic effect of B chromosomes.

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## 中 文 摘 要

# 大仓鼠的核型与 B 染色体研究

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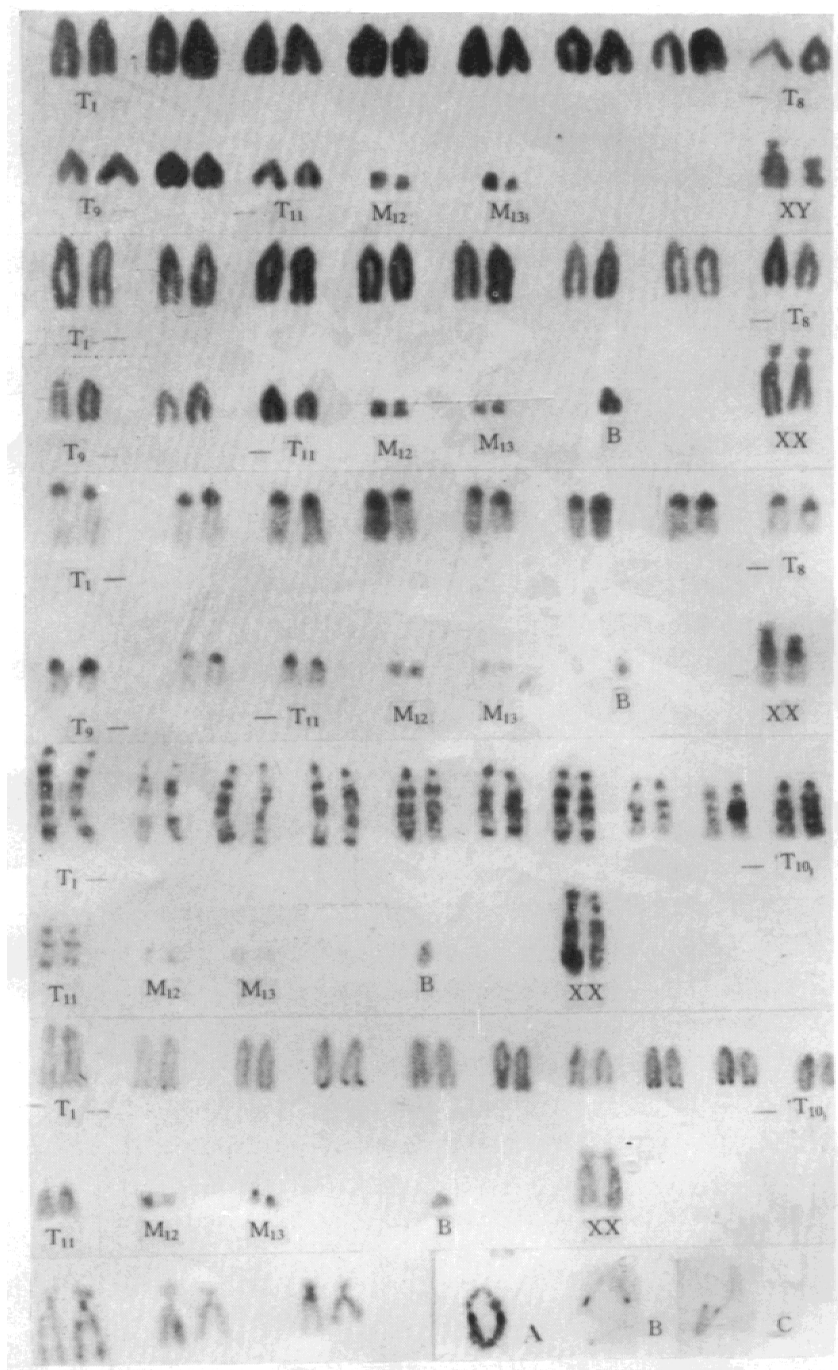
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采用骨髓细胞染色体制片法, 对分布于山东济南、泰山、东北长白山和陕西西安的大仓鼠的染色体组型、G-带、C-带和银染核型进行了分析研究。济南、西安和长白山的标本的二倍体数目和核型相似,  $2n = 28, 22t + 4m + XY (st, m)$ 。泰山标本的二倍体数目为  $2n = 28 \sim 29$ , 即在 67.5% 的中期相中多出了一条形态最小的端着丝粒染色体, 这条染色体为 B 染色体, 可能起源于 X 染色体。泰山标本的 A 染色体组与上述 3 地标本相同。4 地标本的 G-带、C-带和银染核型相似。除 B 染色体外, 每个端着丝粒染色体都具有着丝粒异染色质, Ag-NORs 较恒定地出现在 Nos. 2, 4, 8, 9, 13 染色体上。也就是说大仓鼠的 B 染色体为 C-带阴性, 不携带核仁组织者。这种 B 染色体 C-带阴性的特征在赤狐、黑家鼠和大林姬鼠朝鲜亚种中亦有报道。

关键词: 大仓鼠; 核型; 带型; B 染色体



图版说明

- 1, 2. Karyotype of *C. Triton* (male and female); 3. C-banded karyotype; 4. C-banded karyotype;  
5. Silver-stained karyotype; 6. X chromosomes of same metaphase; 7. Association of NORs.