KARYOTYPES AND B CHROMOSOMES OF APODEMUS PENINSULAE (RODENTIA, MAMMALIA) *

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Abstract: The mitotic and meiotic chromosomes of *Apodemus peninsulae* from Mt. Changbai, Jilin Province (*A. p. preator*) and Mt. Tai, Shandong Province (*A. p. sowerbyi*), and Mt. Qinling, Shaanxi Province (*A. p. sowerbyi*), China are described. The A chromosomes of two subspecies *A. peninsulae* are identical, i. e. 48 telocentric chromosomes. There are differences in the number and types of B chromosomes between three populations. One to three medium size metacentric B chromosomes appeared in *A. p. preator*, and five to fourteen (two types - one medium size metacentric and four to thirteen dot-like B chromosomes) in *A. p. sowerbyi* from Mt. Tai and 0～1 (telocentric) in *A. p. sowerbyi* from Mt. Qinling. Two levels of B chromosome numerical variation, inter- and intraindividual, were found in two subspecies.

Key words: *Apodemus peninsulae*; Karyotype; Banding patterns; B chromosomes; Numerical variation

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B chromosomes are dispensable, supernumerary chromosomes that do not recombine with the A chromosomes and follow their own evolutionary pathway[1]. These supernumerary chromosomes are present in the genomes of a large proportion of individuals in wild populations of many plant and animal species. It has been concluded that they probably occur in all taxa over a wide geographical distribution[1,2]. At present, about 30 species of mammals have been reported to have B chromosomes in their karyotypes[3,4]. B chromosomes are characterized by certain peculiarities that differentiated them from A chromosomes. The number of B chromosomes can vary between individuals and, in mosaic individuals, between cells of a single tissue[4].

Korean field mice, *Apodemus peninsulae*, are distributed over much of Siberia, China, Korea and Hokkaido[5,6]. *A. peninsulae* was considered as a subspecies of *A. speciosus*[7～9]. Vorontsov et al.[10] claimed on the basis of karyological and morphologi-

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cal analyses with samples of boreal regions of East Asia that all the Eastern Asian forms of *A. speciosus should be transferred to the species, A. peninsulae*. The karyological characteristic of the species is that all samples in the regions have B chromosomes. The karyotype and B-chromosomes of *A. peninsulae* in China were first investigated and characterized in this paper.

1 Materials and Methods

Seventeen samples of Korean field mice, *Apodemus peninsulae*, from 3 regions (Mt. changbai, Jilin province; Mt. Tai, Shandong Province, Mt. Qinling, Shaanxi Province, China), representing 2 subspecies (*preator* and *sowerbyi*) were used for karyological study.

The bone-marrow in vivo method was used for the preparation of slides; chromosome classification followed Levan et al.[11]; Seabright's trypsin G-banding method to obtained G-banded chromosomes[12]; Sumner's method to get C-banded chromosomes[13]; and Howell and Black's silver-staining method to obtained silver-stained chromosomes[14].

According to former research, the A chromosomes of *A. peninsulae* are 48 telocentrics. There are several types of B chromosomes in the species, such as large, medium, and small metacentrics, telocentric and minute dot-like chromosomes[4,15]. So we can easily distinguish metacentric and dotlike B chromosomes in the metaphase with conventional staining. Under the help of C-banding staining, we can identify telocentric B chromosome.

2 Results

2.1 Diploid chromosome number and number of B chromosomes

Diploid chromosome number and chromosome frequency of 17 samples of *Apodemus peninsulae* from three localities are shown in Table 1. The A chromosomes of the species is identical in three populations, i. e. 48 telocentric chromosomes. The chromosome number varied from 48～51 in the samples of Mt. Changbai, Jilin Province (subspecies *preator*), China, indicating that B chromosome varied from 0～3. Diploid chromosome number varied from 53～62 and 48～49 in the samples of Mt. Tai, Shandong Province and of Mt. Qinling, Shaanxi Province, China respectively (subspecies *sowerbyi*), indicating that B chromosome in the samples of Mt. Tai and Mt. Qinling varied from 5～14 and 0～1 respectively.

The samples studied showed both inter- and intraindividual variations (mosaicism) in number of B-chromosomes present (Table 1). Samples from Mt. Changbai have 1 to 3 metacentric B chromosome, and samples from Mt. Tai have 1 metacentric and 4～13 dotlike B chromosomes. The mosaicism for B's number was found in bone marrow cells in two subspecies. Mosaicism was estimated by the number of cellular clones, using published criteria[16]. If the number of hypoploid cells is (10 % and that of hyperploid cells is ≥5 %, such deviation was regarded as cell clones. The karyotype of B chromosomes appeared to be mitotically unstable in *A. peninsulae*. The number of cellular clones varied from 1 to 2 in the samples of Mt. Changbai, 1 to 3 in the samples of Mt. Tai and only one in the samples of

Mt. Qinling.

Table 1　Summary of chromosome counted in 17 specimens of *Apodemus peninsulae* from three localities

Locality (subspecies)	No. of species	Sex	Chromosome frequency																	Total cells observed	B chromosome number	No. of cell clones
			48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63				
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
Mt. Changbai (*preator*)	C1	F	2	35	3															40	0～2	1
	C2	M	1	2	37	2														42	0～2	1
	C3	F	3	38	1															42	0	1
	C4	F	2	7	41	3												53		0～2	2	
	C5	M		1	5	35	2													43	0～3	2
Mt. Tai (*sowerbyi*)	T1	F						1	37	2										42	5～7	1
	T3	F						5	30	3	1									39	5～8	2
	T4	M							1	8	28	1								38	6～9	2
	T5	F										1	28	2						31	9～11	1
	T6	M												3	9	22	15	1		50	11～14	3
	T7	M									17	26	2							45	8～10	2
	T8	F							1	23	4									28	6～8	2
Mt. Qinling (*sowerbyi*)	Q13	M	30																	30	0	
	Q14	M	42																	42	0	
	Q15	M	50																	50	0	
	Q18	M	3	30																33	0～1	1
	Q67	F	5	25																30	0～1	1

Numerator: diploid number, denominator: Number of B-chromosomes

2.2　**Karyotype of** A. peninsulae

Eight to ten metaphases of different populations were analyzed respectively. The chromosomes of the standard karyotype of *Apodemus peninsulae* comprise 24 pairs of telocentric A chromosomes. The X chromosome is one of the larger acrocentrics and the Y of the smaller. Zero to fourteen B chromosomes are present, including medium sized metacentrics and small telometrics and dotlike B chromosomes. Consequently, all of the B chromosomes examined are easy to distinguish from the A chromosomes of the standard karyotype by their morphology and heterochromatin character (Plate Ⅱ). For example, we mentioned above, A Chromosomes of all samples of the species (including European and Asian samples) are telocentrics. So in the samples of Mt. Changbai metacentric chromosomes are Bs. Samples from three localities showed significant differences in B chromosome number and types (Plate Ⅰ and Ⅱ).

2.3　**G-banding karyotype**

Fifteen G-banding metaphases were analyzed. The G-banding patterns permitted the identification of all chromosome pairs of a set. The X chromosome has 2 interstitial bands and a band at pericentric and telocentric sites respectively. Y chromosome has two bands, one band on the pericentric and one on the telomeric. Almost no bands on the B chromosomes (Plate Ⅰ, 4).

2.4　**C-banding patterns**

With C-banding all autosomes show heterochromatin near the centromeric regions in A chromosomes. X chromosome shows a positive band near the centromeric region. Y chromosome is whole heterochromatin. B-chromosomes (metacentric chromosomes) in the Mt. Changbai samples appeared to be euchromatin (C-negative), and Bs in Mt. Tai samples have two cases: it is C-negative for the medium size metacentric chromosomes and positive C-band for the dotlike chromosomes. The telocentric B chromosome of samples in Mt. Qinling is also composed of heterochromatin. More than ten C-banding metaphases were observed for each population. (Plate Ⅰ, 5 ～ 6; Plate Ⅱ).

2.5 Silver-stained karyotype

The NORs are located on autosome pairs 7 and 8. There is no variation among three populations (Plate Ⅲ, 1).

2.6 B chromosome behavior in meiosis

The B chromosomes at diakinesis/ metaphase I of meiosis in testis were observed under the light microscopy. The Bs appeared in bivalants and univalents (Plate Ⅲ, 2～4). We can see more than 24 bivalents in one single cell. This means that some Bs formed bivalents.

3 Discussion

3.1 Numerical variation in different subspecies of A. peninsulae

Korean field mice, *Apodemus peninsulae*, are distributed in vast range. A characteristic B-chromosome system with individual variants composed of various combinations of five B-chromosome classes (large, medium, and small metacentrics, small telocentric and minute dotlike B-chromosomes)[15]. The number of B-chromosomes in different animals of this species ranges from 0～24[3,17]. Table 2 shows the numerical variation of B chromosomes in different subspecies. The types and number of B chromosomes of *A. p. preator* in China are similar with that of B chromosomes of *A. p. peninsulae* in Korea, and that of B chromosomes of *A. p. sowerbyi* from Mt Tai and Mt. Qinling are much different in number and types.

Table 2 Numerical variation at B chromosome in A. peninsula

Subspecies	A chromosome	B chromosome	Authors
Major	48	1～3	Kral, 1971[18], Bekasova, 1978[19]
Preator	48	1～13	Kobayashi and Hayate, 1979[20], Bekasova and Vorontsov, 1975[21]
Preator	48	0～24	Volobujev, 1979[17]
Peninsulae	48	1～3	Koh, 1988[22]
Preator Mt. Changbai	48	0～3	This paper
Sowerbyi Mt. Tai	48	5～14	This paper
Sowerbyi Mt. Qinling	48	0～1	This paper

3.2 **Intraindividual variation of Bs in mosaic individuals of** A. peninsulae

Two levels of B chromosome numerical variation, inter- and intraindividual, were found in the samples studied. Because of intraindividual variation, two or more cellular clones were observed in a mosais individual. These clones were likely to be caused by irregular disjunction of the B chromosomes or resulted from elimination of dotlike B chromosomes in mitosis.

3.3 **Characteristics of B chromosomes in different subspecies of** A peninsulae.

There are five types of polymorphic B chromosomes in *A. peninsulae*. It can only be found 1 - 2 types in an individual. For example, one type of B chromosome (medium size metacentric chromosome) appeared in the *A. p. peninsulae* in Korea[22] and *A. p. preator* (Mt. Changbai, China), all of them showed euchromatic (C-negative), that means they are similar in the B chromosome number and characteristics. Two types of Bs in the *A. p. sowerbyi* (Mt. Tai, China). The character of type Ⅰ (medium sized metacentric chromosomes) shows no heterochromatin (C-negative) and type Ⅱ (dot-like chromosomes) shows heterochromatin. However, Bekasova et al.[23] reported that all B chromosomes including metacentrics of *A. peninsulae* from Siberia were heterochromatin. Volubujev[4] reported that in *A. peninsulae* the small and medium size B possess small bands of centromeric heterochromatin which are equal in the size to the segments of the As. Only large B chromosome is stained almost totally. We know Bs are fully or partly composed of the heterochromatin. One of the characteristics of the heterochromatin is its late replication. This character can be examined autoradiographically. The replication pattern of Bs in *Ruttus ruttus* were studied by Raman and Sharma[24]. They are the last to finish the replication. But they are stained significantly fainter than the C-positive bands of the As in C-banding method. Same situation was found in silver fox, Vulpes vulpes[4], and in some plants, for example, Brachycome dichromosomatica[25,26]. This means that B chromosomes can hardly be regarded as composed only of the structural heterochromatin, and A and B heterochromatin may be not identical in its make-up.

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 25S RNA genes, have been identified on Bs in about 20 species[27]. In some cases, the NORs were identified by silver staining[28] and this method is frequently assumed to reflect transcriptional activity of the rRNA genes. Donald et al.[29] also found that the regular attachment of the B chromosome to a nucleolus in B. dichromosomatica. This suggests that these ribosomal RNA genes are transcribed.

3.4 **Accumulation mechanisms of Bs in** A. peninsulae

Comparative analysis of the B-chromosome number in somatic cells and in spermatocytes at diakinesis/ metaphase Ⅰ revealed a difference in their distribution. Spermatocytes at the stage tended to have higher number of B chromosomes (the highest number of Bs is 10 of univalents and 3 bivalents in Plate Ⅲ, 3). Most Bs are present in univalents and some in bi-

valents. A variety of mechanisms of B chromosome accumulation has been described in plant and animals[1]. B chromosome accumulation in the reproductive tissue may be attributed to their irregular disjunctions and B-chromosome selection.

3.5 The origin of B chromosome in A. peninsulae

B chromosomes have been regarded as having originated from the A chromosomes, even though they lack sufficient homology with the A chromosomes (to pair at meiosis). According to the view of Volobujev[4], the B's system of *A. peninsulae* arose at several stages. At the first one the polyploidy for one or several of the small autosomes and Y chromosomes result in formation of the supernumerary chromosomes which are subjected to genetic inactivation. Subsequently the middle sized mertacentric Bs are formed due to Robertson's translocations of the B^A chromosome the dotlike Bs appear as the side translocation products. Therefore, B chromosomes of *A. p. preator* in Mt. Changbai originated from Robertson's translocations of B^A chromosomes; and that of *A. p. sowerbyi* in Mt. Tai from Robertson's translocations of B^A chromosomes and the side translocation products. B chromosome of *A. p. sowerbyi* from Mt. Qinling originated from polyploid of small autosomes.

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中　文　摘　要

大林姬鼠的核型与 B 染色体研究

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采用骨髓染色体制片法, 对分布于吉林长白山、山东泰山和陕西秦岭的大林姬鼠的染色体组型、C - 带、G - 带和减数分裂的染色体行为进行了观察分析。发现 3 个地区标本的染色体数目存在着显

著差异。东北标本的 2n = 48～51，A 组染色体为 48 条，均由端着丝粒染色体组成，同时具有 1～3 条 B 染色体，其形态为中着丝粒染色体；山东标本的 2n = 53～62，A 染色体同样为 48 条端着丝粒染色体组成，具 5～14 条 B 染色体，其中 1 条为较大的中着丝粒染色体，其余为小的中着丝粒和点状染色体。秦岭标本 2n = 48～49，A 染色体为 48 条端着丝粒染色体，具 1 条形态很小的端着丝粒 B 染色体。3 地标本的 B 染色体均存在个体间和个体内变异。长白山标本 B 染色体的细胞克隆数目为 1～2，泰山标本为 1～3。在 3 地标本中，中着丝粒 B 染色体呈现 C - 带阴性，点状 B 染色体呈中度深染。通过对减数分裂的观察，多数 B 染色体是以单价体的形式存在。中国长白山种群的 B 染色体数目和形态与朝鲜种群相似。与欧洲种群存在着显著差异。泰山种群的 B 染色体数目和形态与朝鲜种群及欧洲种群均存在显著差异。泰山种群与秦岭标本同属华北亚种，但它们的 B 染色体形态和数目差别很大。

关键词：大林姬鼠；核型；带型；B 染色体；数目变异

欢迎订阅 2001 年《生物多样性》期刊

《生物多样性》期刊由中国科学院生物多样性委员会主办，中国科学院植物研究所、动物研究所和微生物研究所共同承办，创办于 1993 年 10 月。据中国科学院引文数据库 1998 年数据统计，《生物多样性》期刊为“被引频次最高的中国科技期刊前 500 名”之一，并且在中国生物类科技期刊影响因子排行表中名列第 4。据中国科技信息研究所数据统计，《生物多样性》期刊在中国生物类科技期刊影响因子排行表中名列第 6。

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本刊承诺对生物多样性学科领域有创新性的基础及应用研究的研究报告、高质量的综述予以优先发表，优秀论文在半年内发表，对于代表学科前沿、得到审者及专家高度评价的优秀论文，还将给予作者减收甚至免收版面费的特殊优惠。

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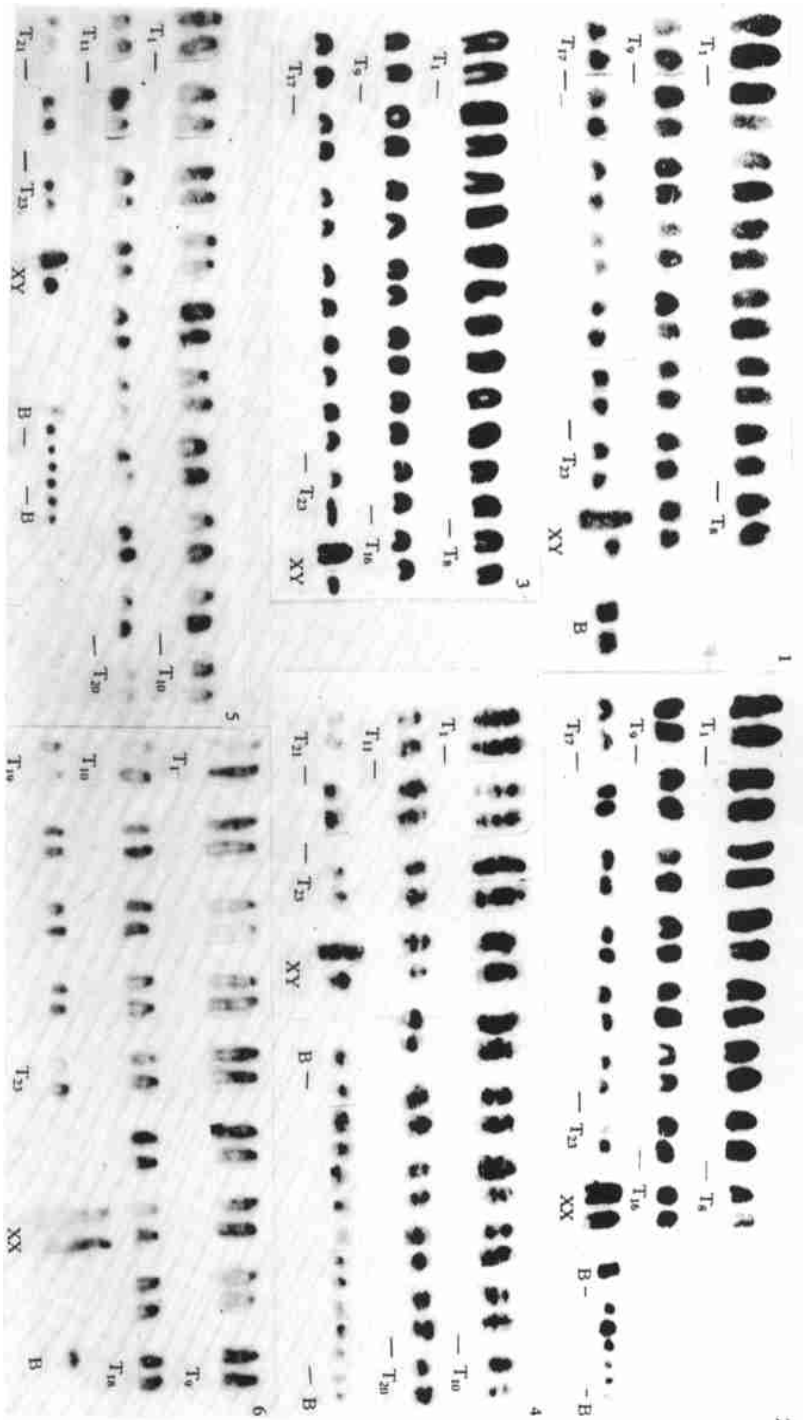
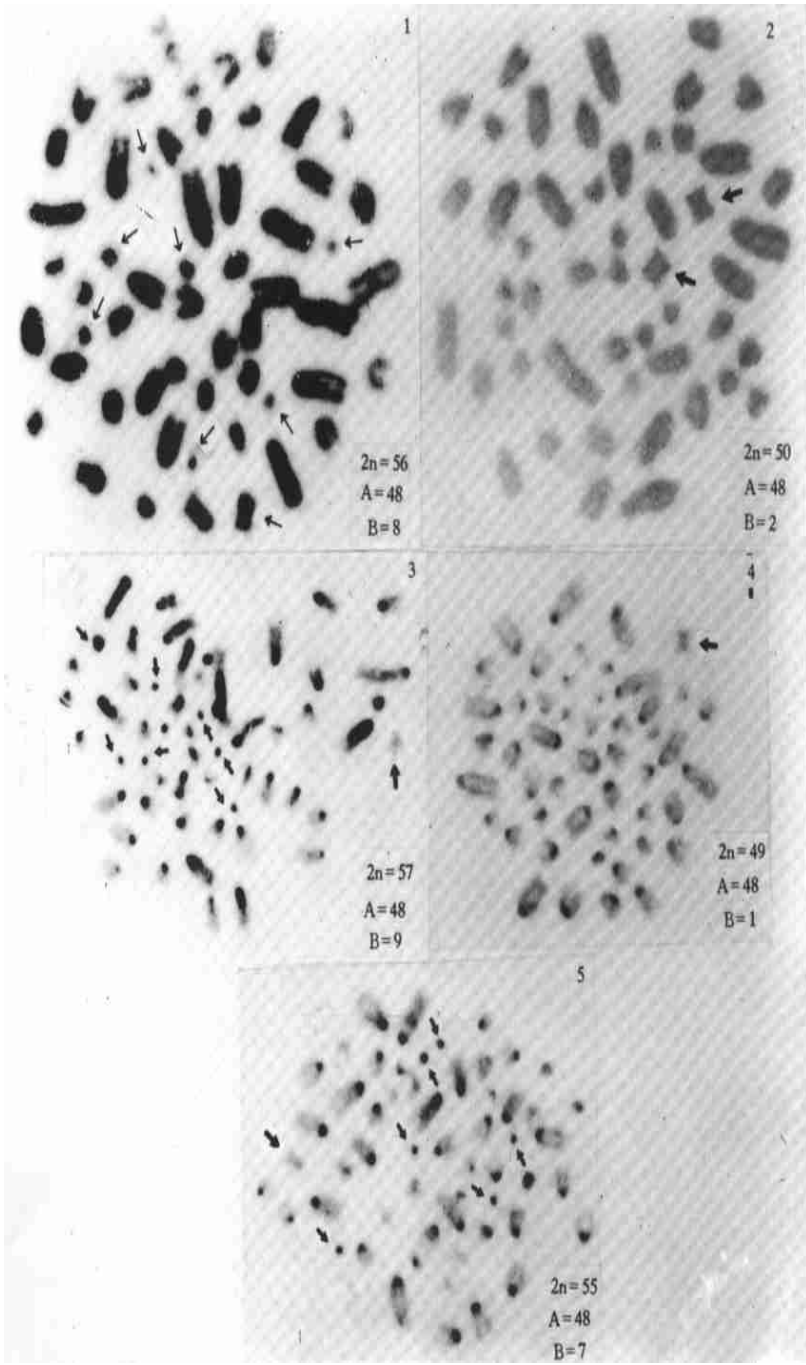
王金星等：大林姬鼠的核型与 *B* 染色体研究 图版 I

WANG Jinxing et al: Karyotypes and B chromosomes of *Apodemus peninsulae* (Rodentia, Mammalia) Plate I



图版说明见第 320 页

Explanation of the plates see page



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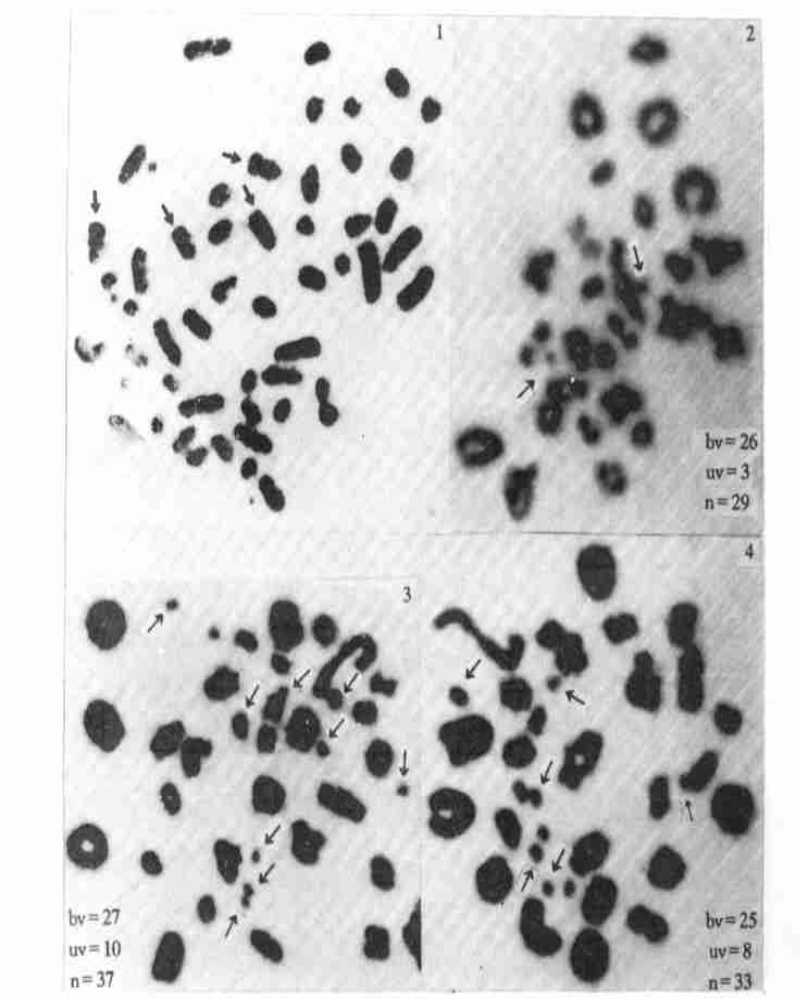

图版说明

Explanation of the plates

Plate I:

1. Karyotype of *A. p. preator* from Mt. Changbai (show two metacentric B chromosomes in samples C2); 2. Karyotype of *A. p. sowerbyi* from Mt. Tai (show 8 Bs in samples T4, Two types of Bs - metacentric and dotlike - present in these samples); 3. Karyotype of *A. p. sowerbyi* from Mt. Qingling; 4. G-banded karyotype of *A. p. sowerbyi* from Mt. Tai; 5 and 6. C-banded karyotype of *A. p. sowerbyi* from Mt. Tai and Mt. Qinling.

Plate II:

1. Metaphases of *A. peninsulae* from Mt Tai and 2 from Mt Changbai (Giemsa staining); 3 and 5. Metaphases of *A. peninsulae* from Mt Tai (C-banding); 4. A metaphase of samples from Mt Changbai (C-banding). Large arrows indicate metacentric B chromosomes, they are C-negative, and small arrows dot-like B chromosomes which are C-positive.

Plate III:

1. Silver-stained Karyotype of *A. p. sowerbyi* from Mt. Tai; 2, 3 and 4. Meiotic metaphases from samples of Mt Changbai (2) and Mt. Tai (3, 4). Most of Bs are present in univalents and some in bivalents (the arrows show univalents)