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A Preliminary Survey of the Chromosomes in Field Mice, *Apodemus* and *Clethrionomys*^{1),2)}

By

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(With 5 Text-figures and 2 Tables)

Subsequent to striking advances of cytogenetic techniques in recent years, our knowledge on mammalian chromosomes has increased to a great extent. A good deal of information is available to date along this line, and consequently, older concepts of the mammalian chromosomes have been revised to some extent.

The chromosomes of *Apodemus* and *Clethrionomys* have been studied by several authors by means of old testis-section methods. (Tateishi 1934, Oguma 1934, 1935, Makino 1951). Tateishi (1934) reported 46 chromosomes in *Apodemus speciosus speciosus*, while Makino (1951) found 50 chromosomes in the same species. In *Apodemus speciosus ainu* Oguma (1934) described 47 chromosomes, and Makino (1951) reported in *Apodemus geisha* (*A. argenteus geisha*) 46 chromosomes. *Eutamias (Clethrionomys) bedfordiae* Thomas was reported to have 55 chromosomes by Oguma (1935).

Recently Yoshida and Kobayashi (1966) found 48 chromosomes in *Apodemus speciosus speciosus* and *Apodemus speciosus ainu*, and 46 in *Apodemus argenteus geisha*, based on modern tissue culture techniques. The present study was undertaken in order to assess the number and the morphology of chromosomes of *Apodemus* and *Clethrionomys* reported by classic workers, by making use of modern techniques with marrow and fetal tissues.

Materials and Methods: Animals were trapped alive and their chromosomes were investigated by means of the routine air-drying method.

From June 1966 to September 1968, the following animals were collected for chromosome study. Twenty-nine specimens of *Apodemus speciosus speciosus* were collected in Iwate, Aomori, Shizuoka, Yamagata and Gifu Prefectures. Fourteen adults and six fetuses

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of *Apodemus speciosus ainu* were obtained in Nopporo, Mt. Taisetsu and Sarobetsu (Hokkaido). Fourteen specimens of *Apodemus argenteus* Temminck were trapped in Nopporo. Nine specimens of *Clethrionomys rutilus mikado* were collected in Tobetsu and Sarobetsu (Hokkaido). Twelve specimens including four fetuses of *Clethrionomys rufocanus bedfordiae* were captured in Nopporo, and Rebun and Daikoku islands (Hokkaido).

Testicular specimens and fetal tissues were cut with scissors into fragments. Marrow cells and fetal tissues received the colchicine treatment in culture medium TC-109 with 15% calf serum at 37°C for 1.5 hours, and the hypotonic treatment with 0.075 M KCl

Table 1. Chromosome counts in 2 species of *Apodemus* from several localities

Locality	Number of specimens	Chromosome count						Total no. of cells
		45	46	47	48	49	4n	
<i>A. speciosus speciosus</i>								
Iwate Pref.	3 ♂				32			32
	3 ♀			1	44			45
Aomori Pref.	4 ♀		1	1	59			61
	1 ♂				8			8
Shizuoka Pref.	2 ♀		2	2	17			21
	5 ♂			1	77			78
Yamagata Pref.	3 ♀			1	42			43
	3 ♂				37			37
Gifu Pref.	2 ♂		35					35
	3 ♀				29			29
	17 ♂		35	2	198			235
	12 ♀		3	4	147			154
Total	29		38	6	345			389
<i>A. speciosus ainu</i>								
Mt. Taisetsu (Hokkaido)	1 ♂				12			12
	2 ♀				26			26
Nopporo (Hokkaido)	6F*		2	5	62	1		70
	6 ♂		2	6	102		2	112
Sarobetsu (Hokkaido)	5 ♀			1	55			56
	7 ♂		2	6	114		2	124
	7 ♀			1	81			82
	6F*		2	5	62	1		70
Total	20		4	12	257	1	2	276
<i>A. argenteus</i> Temminck								
Nopporo (Hokkaido)	7 ♂	1	57					58
	7 ♀	1	96	1	1			99
Total	14	2	153	1	1			157

*: F indicates fetuses derived from a female. Sex was not identified in fetuses.

solution for 20 minutes at room temperature. The testicular tissues were treated with the hypotonic solution without colchicine treatment. Chromosome slides were made according to the routine air-drying method with Giemsa staining.

Table 2. Chromosome counts in 2 species of *Clethrionomys* from several localities

Locality	Number of specimens	Chromosome count					Total no. of cells
		54	55	56	57	58	
<i>C. rutilus mikado</i>							
Tobetsu (Hokkaido)	2 ♂	3	3	46		1	53
	2 ♀	2	1	40	1		44
Sarobetsu (Hokkaido)	2 ♂		2	38			40
	3 ♀	1	4	55			60
	4 ♂	3	5	84		1	93
	5 ♀	3	5	95	1		104
Total	9	6	10	179	1	1	197
<i>C. rufocanus bedfordiae</i>							
Nopporo (Hokkaido)	1 ♂		1	24			25
	2 ♀		2	16			18
Rebun (Hokkaido)	4F*		3	42	1		46
	2 ♀	1	2	32			35
Daikoku (Hokkaido)	2 ♂	1	2	37			40
	1 ♀		1	14			15
	3 ♂	1	3	61			65
	5 ♀	1	5	62			68
	4F		3	42	1		46
Total	12	2	11	165	1		179

*: F indicates fetuses derived from a female. Sex was not identified in fetuses.

Results and Remarks

Results of chromosome study are summarized in Tables 1 and 2. In total, eighty-four specimens were subjected to chromosome analysis.

Apodemus speciosus speciosus and *Apodemus speciosus ainu*

It was shown by the present study that twelve females and fifteen males of *A. speciosus speciosus*, and seven females, seven males and six fetuses of *A. speciosus ainu* were the same in the chromosome number and constitution having 48 chromosomes (Figs. 1 and 2). The complement consisted of 29 pairs of acrocentrics forming a descending seriation in size, and 4 pairs of meta- or submetacentrics. The X chromosome appeared to be the largest acrocentric and the Y the smallest acrocentric. It is thus evident that the two subspecies here dealt with showed the same basic chromosome number as 48.

Two males of *A. speciosus speciosus* collected Ochiai (Gifu Pref.) had 46 chromosomes. The complement showed 18 pairs of acrocentrics forming a gradually diminishing order of size and 5 pairs of meta- or submetacentrics. The X and Y chromosomes appeared to be the largest and the smallest acrocentrics, respectively. A male with 46 elements had 23 bivalents in 12 MI-cells. The variation of the chromosome number is explicable on the basis of centric fusion known as the Robertsonian system: two pairs of acrocentric autosomes of the 48-



Figs. 1 and 2. Two representative karyotypes with 48 chromosomes in two subspecies of *Apodemus speciosus*. 1: from a male specimen of *A. speciosus speciosus*. 2: from a female specimen of *A. speciosus ainu*.

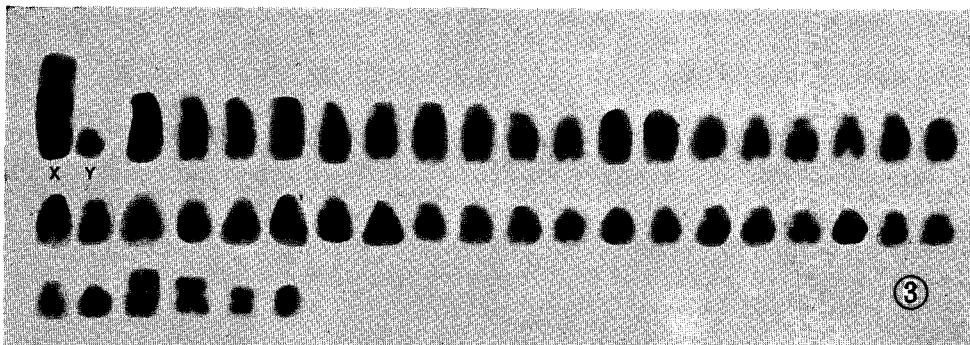


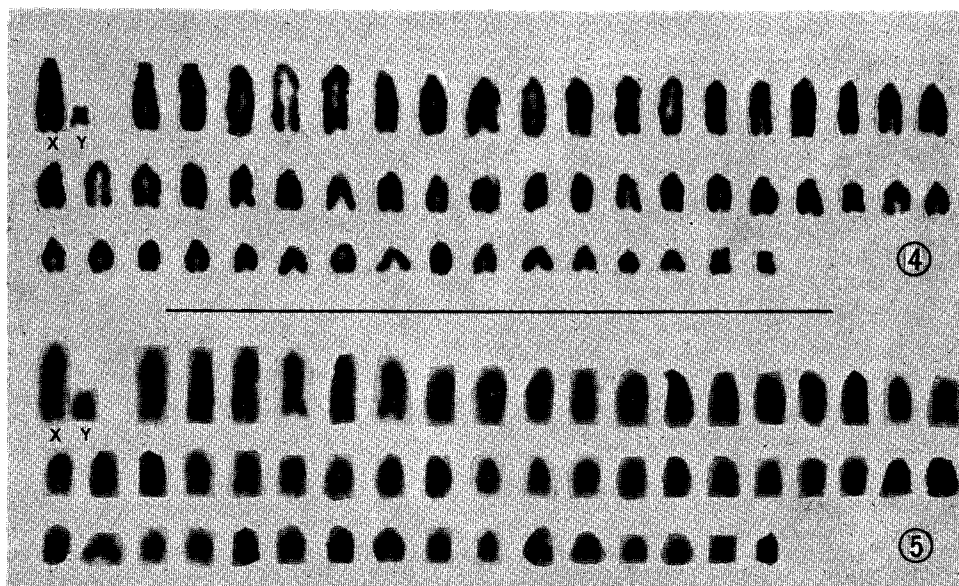
Fig. 3. A representative karyotype with 46 chromosomes in a male specimen of *Apodemus argenteus* Temminck.

specimens were represented as a single pair of metacentrics in the 46-specimens (For detail, see Shimba and Kobayashi 1969).

Apodemus argenteus Temminck: The literature indicates that Makino (1951) described 46 chromosomes in *Apodemus geisha*. Recently Yoshida and Kobayashi (1966) confirmed the same number in this species *Apodemus argenteus geisha* in tissue cultures. We studied the chromosomes of this species in direct bone marrow preparations derived from seven females and seven males. The modal chromosome number was 46, with an XX-XY sex mechanism, consisting of 44 autosomes and the sex-complex, XX in the female and XY in the male (Fig. 3). The autosomes divided into two groups based on their centromere positions: they consisted of 21 pairs of telo- or subtelocentrics indistinguishable from each other, and 2 pairs of metacentrics of small size. The X chromosome was identified as the largest subtelocentric element. The Y appeared as the smallest acrocentric.

Clethrionomys rutilus mikado and *Clethrionomys rufocanus bedfordiae*

Oguma (1935) reported 55 chromosomes in a male of *Evotomys* (*Clethrionomys*) *bedfordiae* Thomas caught in the wood near Sapporo. Four females and 5 males of *C. rutilus mikado*, and 3 females 5 males and 4 fetuses of *C. rufocanus bedfordiae* provided the material for our study to be described here. Air-dried marrow cells from the above-mentioned two species showed no appreciable difference except for



Figs. 4 and 5. Two representative karyotypes with 56 chromosomes in two species of *Clethrionomys*. 4: from a male specimen of *C. rutilus mikado*, having a metacentric Y. 5: from a male specimen of *C. rufocanus bedfordiae*, having an acrocentric Y.

the morphology of the Y chromosome (Figs. 4 and 5). It was found that the autosomes consisted of one large subtelocentric pair, 25 acrocentric pairs showing a gradual seriation, and one pair of small metacentrics. The X chromosome was identified as the largest acrocentric. The Y, on the other hand, differs definitely between two species. The Y chromosome of *C. rutilus mikado* was represented by a small metacentric, similar in size and shape to the smallest metacentric autosomes, whereas the Y chromosome of *C. rufocanus bedfordiae* was characterized by the smallest acrocentric. Chromosomally *C. rufocanus bedfordiae* from two islands, Rebun and Daikoku, was the same without any visible variation.

For the species names of field mice here concerned, we referred to Aoki (1915) and Tokuda (1941).

Summary

A preliminary chromosomal survey of field mice was made in *Apodemus* and *Clethrionomys*, based on direct preparations from bone marrow, fetal and testicular tissues. The chromosome numbers obtained are given in Tables 1 and 2.

A variation in number of the chromosomes was observed in *Apodemus speciosus speciosus*, which are interpreted on the basis of a centric fusion known as the Robertsonian system. A slight difference was noted in the Y chromosome between *Clethrionomys rutilus mikado* and *Clethrionomys rufocanus bedfordiae*.

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