

# Karyological Identity of Anderson's Red-backed Voles from the Kii Peninsula and Central Honshu in Japan

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**Summary :** To reexamine the taxonomic status of the Anderson's red-backed voles from the Kii Peninsula population, we compared the karyotype with that of voles from the central Honshu population. As a result, it was revealed that the karyotypes of specimens from both populations were almost identical ( $2n=56$ , FN=60). Namely, the karyotypes of both populations were composed of 2 pairs of subtelocentric, one pair of metacentric, 24 pairs of acrocentric, X chromosome of subtelocentric, and Y chromosome of submetacentric elements. Further, in comparison of karyotypes of Anderson's red-backed voles used here with those of *Clethrionomys rufocanus* and *Eothenomys smithii*, the present voles were recognized as being closer to *E. smithii* than *C. r. bedfordiae*. Accordingly, it is concluded that the voles from both populations belong to the same species, and the authors support the opinion of AIMI (1980), who has regarded the voles constituting both populations as *Eothenomys andersoni*.

## Introduction

Anderson's red-backed voles are composed of populations distributed from the Tohoku to Chubu districts and isolated in the Kii Peninsula. Several taxonomic investigations have been done on these populations from the viewpoint of distributive characteristics and morphological variations, as follows. They have been regarded as three species of *Aschizomys*, i. e., *A. andersoni* in the Tohoku district, *A. niigatae* in the Chubu district and *A. imaizumii* in the Kii Peninsula (JAMESON, 1961; IMAIZUMI, 1979), or have been considered as *Eothenomys andersoni* (AIMI, 1980), *Clethrionomys andersoni* (CORBET and HILL, 1986) or *Phaulomys andersoni* (KAWAMURA, 1988). However, these taxonomic investigations have dealt with only morphological aspects using a few specimens, consequently there remains a need for further research. From the viewpoint of geographical distribution, the Anderson's red-backed vole from the Kii Peninsula is a completely isolated population and requires further taxonomic investigation.

The purpose of this study is to describe and compare karyotypes of Anderson's red-backed voles from the Kii Peninsula and central Honshu whose distributions are remote, and to discuss their taxonomic status from a chromosomal perspective.

## Materials and Methods

A total of 10 Anderson's red-backed voles were used in this study. Out of these, the 5 voles from the Kii Peninsula consisted of 3 males and 1 female caught in and around the Taikoh Mountain Range (Kami and Shimokitayama Villages, Nara Prefecture and Owase City, Mie Prefecture) in December 1988, and 1 female from Nachi of Wakayama Prefecture in March 1989. The 5 voles from central Honshu were composed of 1 male from Mt. Ontake (Nagano Prefecture)

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in May 1982, 1 male and female from Mt. Yatsugatake (Nagano Prefecture) in September 1990, and 1 male and female from Mt. Tateyama (Toyama Prefecture) in November 1988.

Chromosomal preparations were made from primary lung or tail tissue culture using the standard air drying method (HARADA and YOSIDA, 1978). The culture medium was Eagle's MEM supplemented with 12% calf serum and 3% fetal calf serum. The G- and C-band techniques of SEABRIGHT (1971) and YOSIDA and SAGAI (1975) were applied, respectively, and chromosomes were arranged and compared with each other according to ANDO *et al.* (1988). Moreover, chromosomes were classified into acrocentrics, subtelocentrics, submetacentrics and metacentrics following PATTON (1967). The final determination of karyotypes was performed on about 50 good metaphase plates for each population.

## Results

### 1) Conventional staining

The karyotypes of Anderson's red-backed voles from the Kii Peninsula and central Honshu were identical with each other in the diploid number ( $2n=56$ ) and the fundamental number (FN = 60) (Fig. 1). In the specimens from both populations, the autosomes were composed of two pairs of large and medium-sized subtelocentric (ST) elements (nos. 1 and 2), one pair of small-sized metacentric (M) elements (no. 3) and 24 pairs of large to small acrocentric (A) elements (nos. 4-27). In reference to the sex chromosomes, the X chromosomes were large-sized subtelocentric (ST) elements in both specimens and the Y chromosomes were small submetacentric (SM) elements, also in both. There were scarcely any differences between both karyotypes, but the short arm of the X chromosome was more deeply stained in the Kii Peninsula specimens than in the central Honshu specimens.

### 2) G-band staining

The G-bands were well segmented on the autosomal elements of both specimens, in which there were enough differences to allow easy identification of each autosome (Fig. 2). In both specimens, the X chromosomes were also well segmented, while the Y chromosomes were deeply stained on the whole, but not segmented.

### 3) C-band staining

No differences were detected in the C-banded karyotypes between the Kii peninsula and central Honshu specimens (Fig. 3). By the centric region consisting of constitutive heterochromatin, it was confirmed that the chromosomes nos. 1 and 2 and the X chromosome are subtelocentric, and the chromosome no. 3 is metacentric. Both Y chromosomes were deeply stained, and were entirely composed of constitutive heterochromatin.

## Discussion

### 1) Karyological identity between the Kii Peninsula and central Honshu specimens

Although there have been a few reports on karyotypes of the Anderson's red-backed vole from Honshu (TSUCHIYA, 1977, 1981; YOSHIDA *et al.*, 1989), our knowledge of the Kii Peninsula specimens is particularly scarce. This is the first report that demonstrates a karyological identity between the Kii Peninsula and central Honshu specimens. Both specimens possessed almost the same karyotype ( $2n=56$ , FN=60), except for the faintly stained short arm of the X chromosome in the conventional pattern of the central Honshu specimens, and were also identical

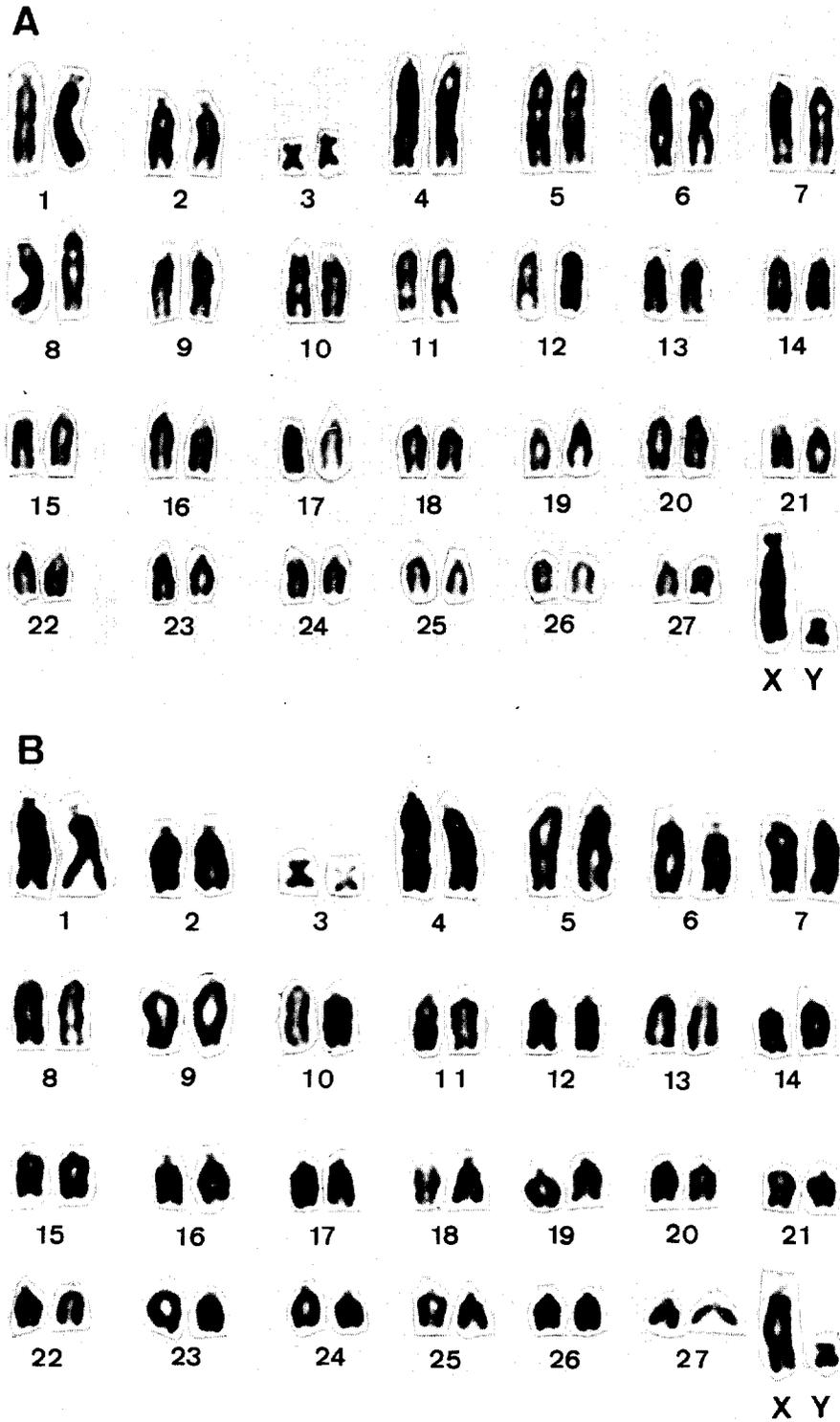


Fig. 1 Conventional karyotypes of Anderson's red-backed voles from the Kii Peninsula (A) and central Honshu (B)

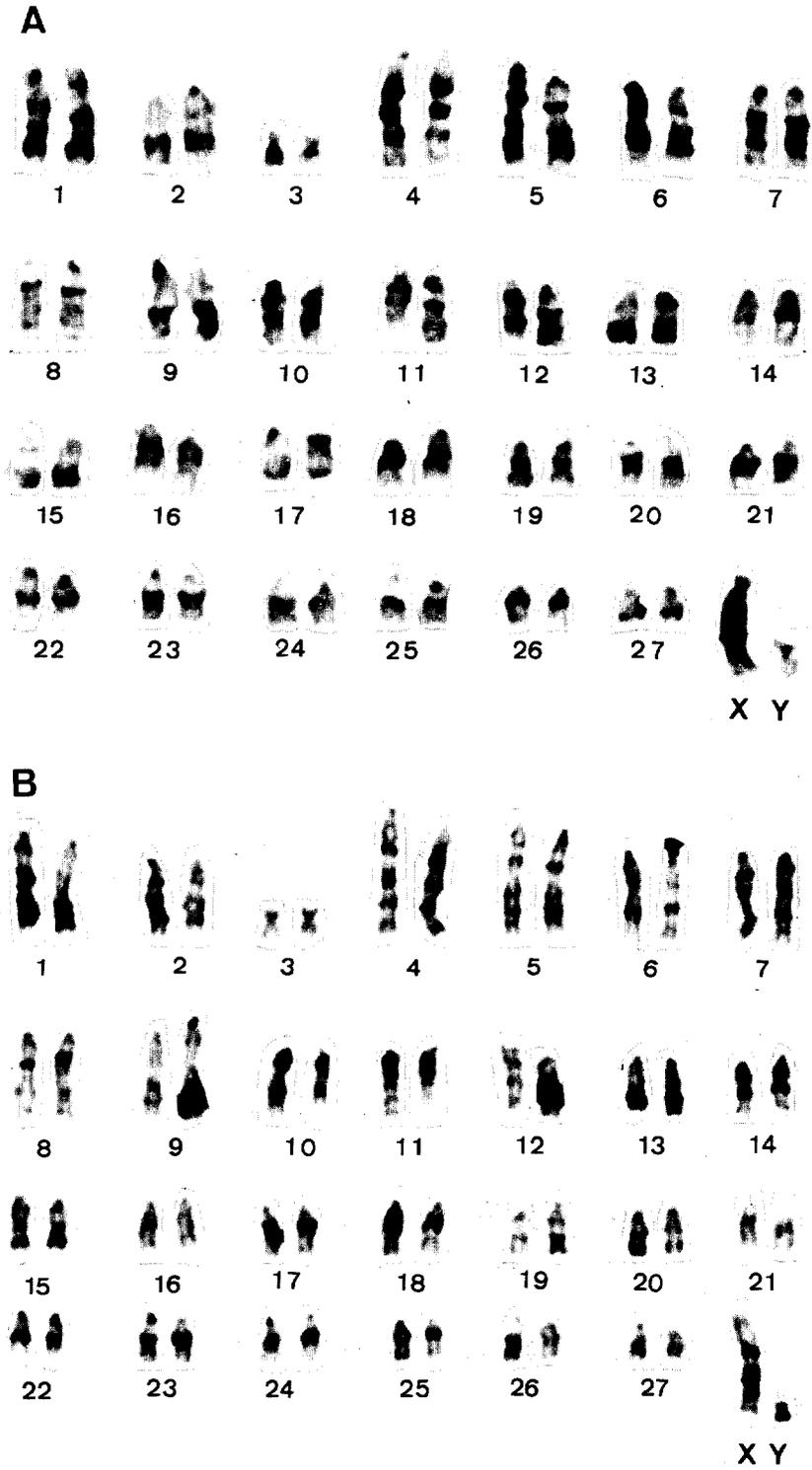


Fig. 2 G-banded karyotypes of Anderson's red-backed voles from the Kii Peninsula (A) and central Honshu (B)

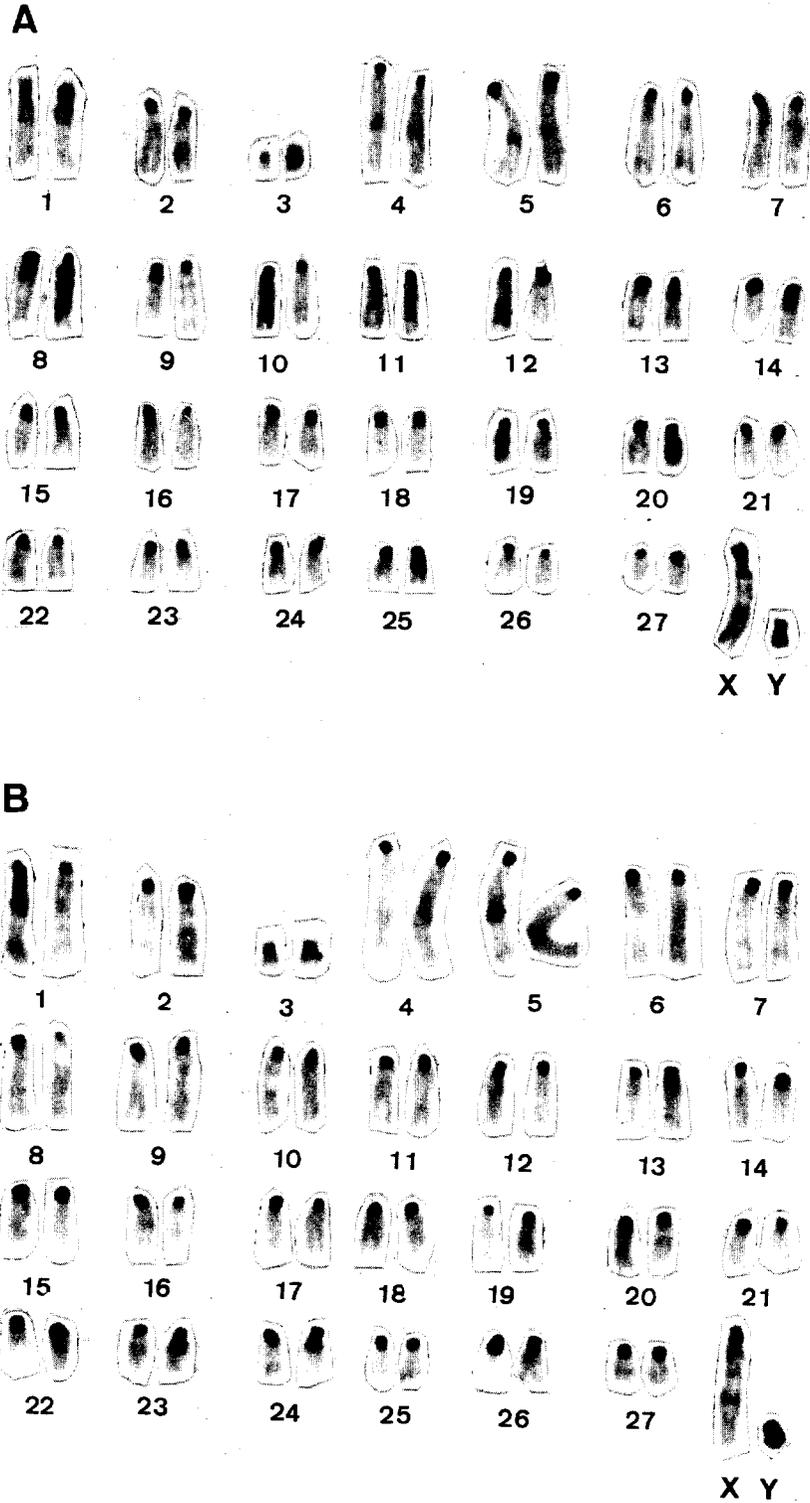


Fig. 3 C-banded karyotypes of Anderson's red-backed voles from the Kii Peninsula (A) and central Honshu (B)

in the G- and C-band patterns.

Concerning autosomes, according to TSUCHIYA (1977, 1981), both *Aschizomys imaizumii* [*Eothenomys andersoni*] from the Kii Peninsula and *A. niigatae* [*E. andersoni*] from central Honshu possess the same autosomal chromosomes of one pair of large subtelocentric and small metacentric, and 25 pairs of acrocentric elements. On the other hand, YOSHIDA *et al.* (1989) have demonstrated that autosomal chromosomes of *Clethrionomys andersoni andersoni* [*E. andersoni*] from Saitama Prefecture, central Honshu are composed of one pair of small metacentric and 26 pairs of large and small acrocentric elements. The reason is that the short arms of chromosome no. 3, which is regarded as subtelocentric elements by TSUCHIYA (1981), are extremely small, and are consequently considered to be acrocentrics. According to PATTON (1967), however, chromosome no. 3 is unquestionably subtelocentric. Accordingly, chromosome no. 3 is apparently identical with the subtelocentric chromosome no. 1 in both the Kii Peninsula and central Honshu specimens used in this study. Unlike these two reports, however, our results showed that Anderson's red-backed voles from the Kii Peninsula and central Honshu possessed 2 pairs of subtelocentric elements.

Regarding the X chromosomes, TSUCHIYA (1977, 1981) has recognized a subtelocentric X in *A. imaizumii* and an acrocentric X in *A. niigatae*, and consequently a remarkable difference between them. Further, YOSHIDA *et al.* (1989) have regarded all X chromosomes as acrocentric elements in Anderson's red-backed voles from Honshu (*Clethrionomys andersoni andersoni* from the Tohoku district and *C. a. niigatae* from central Honshu), but the results of this study (ST) in both the Kii Peninsula and central Honshu specimens were different from those of YOSHIDA *et al.* (1989). These findings suggest the possibility of polymorphism in X chromosomes of Anderson's red-backed voles from Honshu. In this study, the short arms of X chromosomes in the central Honshu specimens were faintly stained compared with the Kii Peninsula specimens, and this fact may relate to the results of YOSHIDA *et al.* (1989), who judged the X chromosome to be acrocentric in the central Honshu specimens. Such variations in X chromosomes suggest a certain outcome of geographic isolation as the biological implication. However, it remains an intraspecific variation at the most, and does not support the idea that they are different species. In this respect, the variation of the X chromosome caused by pericentric inversion and subsequent addition of constitutive heterochromatin has also been reported in the local population of *Eothenomys smithii* from Toyama Prefecture (ANDO *et al.*, 1991).

As for the Y chromosome, TSUCHIYA (1977, 1981) has regarded it as submetacentric in *A. imaizumii* and acrocentric in *A. niigatae*; on the other hand, YOSHIDA *et al.* (1989) have considered it as metacentric in *C. a. niigatae*. In the present study, however, the Y chromosome was identified as submetacentric in both the Kii Peninsula and the central Honshu specimens, which was different from the above findings in the central Honshu specimens. There may be polymorphism of the Y chromosomes also in Anderson's red-backed voles as in *Clethrionomys rufocanus* (VORONTSOV *et al.*, 1978) and *C. glareolus* (GAMPERL, 1982). In this respect, the small submetacentric Y of the present Anderson's red-backed voles and the medium-sized subtelocentric Y of *E. smithii* seem to have differentiated from the small acrocentric or metacentric Y in *Clethrionomys* by pericentric inversion and the addition of constitutive heterochromatin.

Thus, the present results strongly suggest that the Kii Peninsula and central Honshu populations are karyologically identical.

2) Relationships between Anderson's red-backed voles, *Clethrionomys* and *E. smithii* from the view of chromosomal evolution

Three hypotheses have been proposed for the mode and direction of chromosomal evolution in mammals: 1) the fusion hypothesis (OHNO, 1969), 2) the fission hypothesis (TODD, 1970; IMAI and CROSIER, 1980) and 3) the modal hypothesis that ancestral mammals would have had a diploid number near the present mode of frequency distribution of diploid numbers (MATTHEY, 1973). In mammals, however, the mechanism of centric fusion is the commonest rearrangement and consequently,  $2n$  is in the evolutionary direction of decreasing (NADLER, 1969). Diploid numbers in *Microtus*, which is thought to be the most advanced in the herbivorous direction, are broadly spread from 30 in *M. montebelli* (UTAKOJI, 1967; TSUCHIYA, 1981; YAMAKAGE *et al.*, 1985) to 46 in *M. arvalis* (ADAMCZEWSKA-ANDRZEJEWSKA *et al.*, 1989), but these numbers are probably smaller than those of other rodents. On the other hand, the standard karyotype of *Clethrionomys* shows  $2n=56$ , FN=56 or 58 composed of 26 pairs of A-autosomes (one pair is occasionally regarded as ST-elements), one pair of M-autosomes, A-X chromosomes and A to M-Y chromosomes (SHIMBA *et al.*, 1969 for *C. rutilus mikado* and *C. rufocanus bedfordiae*; MASCARELLO *et al.*, 1974 for *C. rufocanus bedfordiae*; GAMPERL, 1982 for *C. rufocanus* and *C. glareolus*; YOSHIDA *et al.*, 1989 for 6 taxa of *Clethrionomys*). They are strikingly similar to our results ( $2n=56$ , FN=60) obtained from Anderson's red-backed voles of the Kii Peninsula and central Honshu. Moreover, since autosomes of specimens from both districts were mainly composed of acrocentric elements, these voles are likely of a more primitive member of Arvicolinae [Microtinae] according to MATTHEY (1973).

Comparing the autosomal elements of the karyotypes between the present Anderson's red-backed vole and *Clethrionomys* shows the present vole has two more pairs of large and medium-sized ST-elements and two fewer pairs of large and medium-sized A-elements than *Clethrionomys*, and the X chromosomes are subtelocentric in the present vole, but acrocentric in *Clethrionomys*. Accordingly, the karyotype of the present vole may have originated from a *Clethrionomys*-like karyotype by pericentric inversion in two pairs of unpaired autosomes and in the A-X chromosomes, just as was pointed out in *E. smithii* by ANDO *et al.* (1988).

Furthermore, the present Anderson's red-backed voles and *Eothenomys smithii* (ANDO *et al.*, 1988) both showed the same karyotype ( $2n=56$ , FN=60), in which autosomes consisted of 2 pairs of ST-autosomes, one pair of M-autosomes and 24 pairs of A-autosomes, and the G- and C-banded autosomal patterns were identical. Moreover, large ST-X chromosomes of both species were basically the same, though they indicated polymorphism. On the other hand, Y chromosomes were small SM-elements in the present voles and medium-sized ST-elements in *E. smithii*, i. e., there is a difference between the two. This is considered to be a result of the addition of constitutive heterochromatin in *E. smithii* (ANDO *et al.*, 1988). Further, a close affinity of Anderson's red-backed voles from Honshu with *E. smithii* is also strongly suggested by the estimated values of RFLP (restriction fragment length polymorphism) of mitochondrial DNA in both (WAKANA, 1992). Both species were the same in the relative growth coefficients ( $\alpha$ ) of the nasal length, frontal length, occipital length, length of upper molar series, auditory bulla length and cranial width against the condylobasal length, and the character of occlusal enamel patterns in the 3rd upper molar (KITAHARA, 1995). According to MIYAO's (1967) conception, in which the number of mammae has a tendency to decrease from 4 to 2 pairs in *E. smithii*, Anderson's red-backed voles with 4 pairs of mammae may be regarded as an ancestor of *E. smithii*.

Thus, since both populations of Anderson's red-backed voles from the Kii Peninsula and central Honshu showed the same karyotype, it is concluded that they are in the same species taxonomically, and the present Anderson's voles are closer to *E. smithii* than *C. r. bedfordiae*. Furthermore, if we consider that both populations should be allocated to *Eothenomys*, not to *Clethrionomys*, because of their unclosed molar roots (KITAHARA, 1995), and that hybrids produced by crossbreeding between the two populations possess normal breeding ability (KITAHARA and KIMURA, 1995), the results obtained in this study could support the opinion of AIMI (1980), who has regarded the voles of both populations as *Eothenomys andersoni*.

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## 紀伊半島産と本州産ヤチネズミの核型的一致

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## 摘 要

紀伊半島産ヤチネズミ個体群の分類学的位置を調べるため、その染色体を本州中部産ヤチネズミと比較した。その結果、紀伊半島産と本州中部産ヤチネズミの核型 ( $2n=56$ , FN=60) はほとんど同一であることが明らかとなった。すなわち、ヤチネズミ両個体群の染色体はともに2対のサブテロセントリック、1対のメタセントリック、24対のアクロセントリック、サブテロセントリックのX染色体とサブメタセントリックのY染色体からなっていた。また、供試ヤチネズミの染色体を *Clethrionomys rufocanus* 及び *Eothenomys smithii* と比較することにより、本ヤチネズミは *C. rufocanus* よりも *E. smithii* に近縁であることが分かった。従って、ヤチネズミ両個体群は分類学的に同一種に属することが結論され、両者を一括して *Eothenomys andersoni* とする AIMI (1980) の考えを一層強く支持した。

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