Studies on the Karyotype of Oriental Vole (*Eothenomys miletus*)

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Oriental vole (*Eothenomys miletus*), in the subfamily Microtinae, is a large genus composed of about 11 species which are distributed in the East Asia (Corbet and Hill 1991). The classification of *E. miletus* is still a matter of considerable controversy. Based on their morphological features, Oriental vole has been identified as a valid species (Allen 1940, Wang 1984) or a subspecies of *E. melanogaster* (Ellerman and Morrison-Scott 1951, Corbet 1978). Therefore, genetics studies are of interest in understanding the classification and evolution of *E. miletus*. As far as we know, the chromosomes of Oriental vole have so far been studied only by the conventional staining (Wu *et al.* 1989), and the cytogenetics information of genus *Eothenomys* is also quite scanty. To our knowledge, no banding study of genus *Eothenomys* species has been published up to now.

The report describes results of conventional karyotype, C-banding patterns and Ag-NORs (Ag-stained Nucleolar Organizer Regions) of Oriental vole from China with a hope of presenting further evidence from chromosomes that might be useful in elucidating the classification of this animal, and provides new information about chromosome evolution in this subfamily.

Materials and methods

All experiment animals (three males, three females) used were collected from Dali and Kunming, Yunnan, China.

To make slides for chromosome analysis, short term cultures of bone marrow cells was carried by means of the routine air-drying method. The marrow cells from the femur were colchicized for 1.5 hr (final concentration 0.5 µg/ml), treated with hypotonic KCl solution for 15 min at 37°C, fixed in methanol-acetic acid (3:1), air-dried on slides and stained with Giemsa. C-bands were obtained with the methods of Summer (1972). For the detection of Ag-NORs (Ag-Stained Nucleolar Organizer Regions), slides were stained according to the technique of Howell and Black (1980). The categorisation of chromosomes was done following the criteria of Levan *et al.* (1964), the chromosomes of *E. miletus* were arranged in order of decreasing size.

Result

Most of the metaphase cells in the preparations were diploid and normal. One hundred cells were counted in each specimen and the diploid chromosome number (2n) of *E. miletus* was found to be 56. The X chromosome is a medium-sized acrocentric almost equals in length between No. 2 and No. 3. The Y chromosome is the smallest acrocentric (Fig. 1). The measuring data of its karyotype are shown in Table 1.

Centromeric C-bands of all chromosomes in the complement are positive, and the

Fig. 1. The conventional karyotype of Oriental vole (*E. miletus*).Table 1. The karyological data of Oriental vole (*E. miletus*)

No. of chromosome	Total length	Relative length	Chromosome type
1	13.30±0.42	8.19±0.36	A
2	11.37±0.33	7.00±0.31	A
3	8.14±0.24	5.01±0.21	A
4	7.89±0.27	4.86±0.25	A
5	7.58±0.26	4.67±0.21	A
6	6.81±0.22	4.20±0.18	A
7	6.46±0.24	3.98±0.21	A
8	6.28±0.21	3.87±0.14	A
9	6.10±0.25	3.75±0.18	A
10	5.58±0.22	3.43±0.16	A
11	5.27±0.18	3.24±0.18	A
12	5.03±0.21	3.10±0.16	A
13	4.91±0.14	3.02±0.11	A
14	4.75±0.17	2.92±0.13	A
15	4.68±0.13	2.88±0.11	A
16	4.47±0.15	2.75±0.11	A
17	4.39±0.14	2.70±0.10	A
18	4.08±0.12	2.51±0.10	A
19	3.96±0.11	2.44±0.08	A
20	3.89±0.13	2.39±0.10	A
21	3.83±0.12	2.36±0.09	A
22	3.80±0.08	2.34±0.07	A
23	3.75±0.11	2.31±0.09	A
24	3.51±0.11	2.16±0.08	A
25	3.25±0.12	2.00±0.09	A
26	3.16±0.09	1.95±0.06	A
27	2.94±0.07	1.81±0.05	A
X	10.38±0.38	6.39±0.26	A
Y	2.89±0.08	1.78±0.05	A

heterochromatin is abundant. However, their relative size are different, and the stained blocks of Nos. 1, 2, 5 and X chromosome are very large. The Y chromosome is completely heterochromatin. Neither interstitial nor terminal C-bands were detected (Fig. 2).

Silver-stained karyotype is shown in Fig. 3, 5 pairs of Ag-NORs were observed, and they are located in the terminal of Nos. 1, 2, 6, 14, 27, respectively. The Ag-NORs of Nos. 2, 6 are heteromorphic, only one member of these homologous pairs exhibited a NOR in most cells

Fig. 2. The C-banded karyotype of Oriental vole (*E. miletus*).

Fig. 3. The silver stained karyotype of Oriental vole (*E. miletus*).

observed, this may indicate a difference in either quantity or expression of rDNA genes. Moreover, the associations of Ag-NORs were observed, but the frequency of association was very low (roughly 2%).

Discussion

The conventional karyotype of *E. miletus* is according with the report of Wu *et al.* (1989), and all chromosomes in the complement are acrocentrics. On the basis of morphologic features, the genus *Eothenomys* is similar to *Clethrionomys*. All species reported their karyotypes in the two genera listed in Table 2. As shown in Table 2, the karyotypes of all species include 1 pair smaller metacentric chromosomes except Oriental vole, while all chromosomes in the complement of oriental vole are acrocentrics. So the karyotype of *E. miletus* is specific. Unfortunately, no banding patterns of most of these animal are published up to now. However, we believe that the cytogenetic studies is very useful information for the classification of Oriental vole, and suggest that *E. miletus* should be a separate species.

Usually in a given taxon, a primitive karyotype is most likely one with a high $2n$ consisting

Table 2. The karyotypes of Clethrionomys and Eothenomys

Species	2n	Chromosome formula		References
<i>C. r. rutilus</i>	56	2M+52A	X, Y (A, A)	Kral 1971
<i>C. gapperi</i>	56	2M+52A	X, Y (A, A)	Hsu, <i>et al.</i> 1970
<i>C. g. gapperi</i>	56	2M+2SA+50A	X, Y (A, SA)	Frechette, <i>et al.</i> 1971
<i>C. g. loringi</i>	56	2M+2SA+50A	X, Y (A, SA)	Frechette, <i>et al.</i> 1971
<i>C. glareolus</i>	56	2M+52A	X, Y (A, M)	Hsu, <i>et al.</i> 1970
<i>C. imauzumi</i>	56	2M+52A	X, Y (SA, SA)	Matthey 1973
<i>C. niigatae</i>	56	2M+52A	X, Y (A, M)	Hsu, <i>et al.</i> 1974
<i>C. rufocanus</i>	56	2M+52A	X, Y (A, A)	Hsu, <i>et al.</i> 1969
<i>C. rutilus</i>	56	2M+52A	X, Y (A, A)	Kral 1971
<i>E. kageus</i>	56	2M+52A	X, Y (A, A)	Hsu, <i>et al.</i> 1974
<i>E. smithi</i>	56	2M+52A	X, Y (A, A)	Hsu, <i>et al.</i> 1974
<i>E. miletus</i>	56	54A	X, Y (A, A)	Present paper

of mostly acrocentrics, and a derived one with a less 2n consisting of mostly biarmed chromosomes (Carleton and Myers 1979). Modi (1987) summarized the evolution of heterochromatin among arvicolid rodents and provided three categories of ancestral C-banded karyotype: (a) all autosomes carried C-bands and the centromeric heterochromatin was abundant, (b) X chromosome was C-band positive, and (c) whole Y chromosome was completely heterochromatin. Chen *et al.* (1992) suggested the following factors may be facilitate the C-banded karyotype evolution in *Rattus*: (1) Loss or absent of centromeric heterochromatin in the complement, (2) appearance or increases of interstitial heterochromatin, terminal heterochromatin and heterochromatin arms. As clearly shown in Table 2 and Fig. 1, the diploid chromosome number of *E. miletus* is 56, and centromeric C-bands of all chromosomes are positive. The Y chromosome is completely heterochromatin, and no terminal and interstitial heterochromatin was found. On the basis of the above-mentioned hypotheses, *E. miletus* is a relative primitive species, and this is consistent with the primitive morphological feature of its upper first Molar type. According to the data of Table 2 and Fig. 1, we infer that one of the karyotype evolutionary ways of Oriental vole group may be pericentric inversion, but require a more accurate comparison of G-, C-banding patterns among genus *Eothenomys* species.

Summary

The diploid chromosome number of Oriental vole is 56, and all chromosome in the complement are acrocentrics. The Y chromosome is the smallest one.

The C-banded karyotype reveals that the centromeric C-bands are abundant, and all chromosomes are C-bands positive, and Y chromosome is completely heterochromatin.

The silver-stained karyotype has been studied. Five pairs of Ag-NORs were found in the terminal of Nos. 1, 2, 6, 14, 27, respectively, and the heteromorphism and association of Ag-NORs were also observed.

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