Chromosome Study of Yulong Vole (*Eothenomys proditor*)

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Summary A first description of the karyotype of Yulong vole (*Eothenomys proditor*) is presented. Karyotype studies were carried out using conventional staining, G-banding, C-banding and silver-staining. Yulong vole was sharp different from other allied voles on karyotype. The diploid number is $2n=32$, with the fundamental number of 56. The autosomes consist of 13 pairs of meta-/submetacentric elements and two pairs of acrocentric elements. The X chromosome is medium sized and submetacentric, whereas the Y chromosome is small and subacrocentric. The G-banding allows to identify chromosome pairing and to detect the chromosome rearrangements intervening in karyotypic differentiation. In the C-banded karyotype, the centromeric heterochromatin is observed in Nos. 2, 4 and 12 chromosomes, the terminal C-band is found in No. 13 and X chromosomes. Y chromosome is completely C-band-positive, moreover, the interstitial distribution of C-bands has been demonstrated on the biarm of No. 2 chromosome pair and the long arm of Nos. 2, 9 and 10 chromosomes respectively. Nucleolus organizer regions (NORs) appeared in the long arm of Nos. 1, 2 and 4 chromosomes and short arm of No. 11 chromosome.

The genus *Eothenomys* is mainly distributed in Transhimalayan mountains, the southwestern China. Based mainly on morphology, from 9–13 nominal forms have been assigned to the genus which includes 2–3 subgenera (Allen 1940, Ellerman and Morrison-Scott 1951, Corbet 1978, Nowak and Paradiso 1983, Corbet and Hill 1991). However, the taxonomic status of most of these species is still confusing and need of review. Yulong vole (*Eothenomys proditor*) is an endemic species living in Lijiang region, Yunnan province of China. It was originally named as a new species of *Eothenomys* by Hinton (1923). Allen (1940) changed it under *Antelionomys*, and his point has been acknowledged by most taxonomists. Nevertheless, the status of *Antelionomys* is disputable. Cytogenetics is a powerful tool to investigate classification and evolution. However, the chromosome data of *Eothenomys* was scant. Until now, only *E. melanogaster* and *E. miletus* have been karyotyped by conventional and/or differential staining (Wu *et al.* 1989, Chen *et al.* 1994, Harada *et al.* 1991). In this paper, the conventional and banded karyotypes of Yulong vole (*E. proditor*) were first described, and we preliminarily discussed the karyotype evolution and taxonomic situation of this species. We hope this study can provide new information about phylogeny of this genus and its related genera.

Materials and methods

Four animals (two females, two males) were captured alive in Yulong snow mountain, Northern Yunnan province of China.

The chromosomes studied were from fibroblast cells which were obtained from primary lung tissue culture by means of standard air-drying methods. For chromosomal classification, the method

of Leaven *et al.* (1964) was adapted. The procedures for G-banding was the same as the trypsin digestive method of Seabright (1971). The C-banding was produced by a slight modification of Sumner's technique (1972). For the detection of Ag-NORs, the slides were stained with silver (Howell and Black 1980).

Result

The diploid chromosome number of Yulong vole was found to be 32 ($2n=32$) based on the data from a total of 100 metaphases, and the fundamental arm number is 54 ($FN=56$). The autosomes of Yulong vole consisted 7 metacentric pairs (M), 6 submetacentric pairs (SM) and 2 acrocentric pairs (A). The X chromosome was a medium M element while the Y chromosome was a small A element (Fig. 1A). So the karyotype formula of Yulong vole was $32, XY(M, A)+14M+12SM+4A$ (Fig. 1A, Table 1).

The G-bands of the most autosomes and X chromosome were segmented, but Nos. 15 and Y chromosomes were indistinct (Fig. 1B). Based on the G-band pattern, we can identify the homologous chromosome pairs which are equivocal in conventional karyotype, and demonstrate the similarity of karyotype in different species.

The Yulong vole contains a small amount of heterochromatin (Fig. 1C). The pericentromeric regions of Nos. 2, 4 and 12 chromosomes; the terminal of short arm of X and No. 13 chromosome pair, and the entire length of Y chromosome were all stained positively. Moreover we found interstitial heterochromatin on Nos. 2, 9 and 10 chromosomes, however, it was very faint on the long arms of Nos. 2 and 12 chromosomes (Fig. 1C, shown by arrows). Slight C-bands were also found on the two terminals of No. 1 chromosomes (not shown).

Silver-staining karyotype is shown in Fig. 1D. Four pairs of Ag-NORs were observed, and they

Fig. 1. A) The conventional karyotype of Yulong vole. B) The G-banded karyotype of Yulong vole. C) The C-banded karyotype of Yulong vole (arrows show the interstitial heterochromatin). D) The silver stained karyotype of Yulong vole (arrows show the NORs).

Table 1. The measurement of the chromosomes of Yulong vole

Nos.	Position of centromere	Length	Relative length	Arm ratio
1	M	13.94±0.22	10.35±0.16	1.25
2	M	10.22±0.42	7.57±0.37	1.09
3	M	9.85±0.29	7.32±0.18	1.59
4	M	8.37±0.32	6.21±0.22	1.21
5	M	7.13±0.15	5.39±0.11	1.23
6	M	7.16±0.20	5.30±0.17	1.59
7	M	6.95±0.18	5.09±0.13	1.27
8	SM	14.13±0.39	10.51±0.23	2.22
9	SM	12.77±0.26	9.49±0.15	1.73
10	SM	11.36±0.42	8.84±0.35	2.12
11	SM	10.46±0.31	7.79±0.19	1.89
12	SM	8.44±0.14	6.28±0.11	1.88
13	SM	5.19±0.26	3.85±0.21	1.72
14	A	5.92±0.08	4.40±0.05	—
15	A	2.69±0.05	2.00±0.03	—
X	M	10.18±0.35	7.51±0.26	1.52
Y	A	5.52±0.12	4.13±0.07	—

M=metacentric, SM=submetacentric, A=acrocentric.

are located in the terminal of Nos. 1, 2, 4 and 11 chromosome pairs respectively (Fig. 1D, shown by arrows). Also, we observed associations of Ag-NORs, however, the frequency of association was very low (about 2%).

Discussion

Genera *Eothenomys*, *Clethrionomys* and *Caryomys* are similar with each other on the basis of morphologic character, so they are placed in the tribe Clethrionomyini of subfamily Arvicolinae (Koenigswald 1980). Also karyotypes in the three genera reported are similar with each other. As show in Table 2, the karyotypes of all specie are $2n=56$ (except *Caryomys inez* and *C. eva*), and include 1 pair of smaller metacentric chromosomes (except *Eothenomys miletus* and *E. eleusis*). $FN=54-60$, variation in FN was very small. In this paper, the karyotype of Yulong vole was first reported, and the result showed that Yulong vole cytogenetically differed sharply from other voles of the tribe Clethrionomyini. The cytogenetic findings suggest that Yulong vole takes an important role in the classification and phylogeny of the tribe Clethrionomyini.

Carleton and Myeris (1979) stated that, in a group of given taxon, a primitive karyotype is most likely with a high $2n$ consisted of mostly acrocentrics while a derived one with a less $2n$ consisted of mostly biarmed chromosomes. The diploid number of Yulong vole is 32, and most chromosomes are biarmed (Fig. 1A, Table 1). According to above point, Yulong vole is a relative derived specie in the tribe Clethrionomyini. Heterochromatin is a genetic structure that may facilitate chromosome rearrangement. Usually, the ancestral C-banded karyotype have two features. (1) all autosomes and X chromosome carried C-bands and the centromeric heterochromatin was abundant; (2) Y chromosome was completely heterochromtic (Sen and Sharma 1983). It is obvious from Fig. 1C that the elements carrying centromeric heterochromatin decreased in the complement of Yulong vole; while the elements carrying terminal heterochromatin and interstitial heterochromatin increased. Moreover, the terminal heterochromatin is demonstrated on the short arm of X chromosomes, this uncommon distribution of C-band had been found in quite a few differed species, such as *Rattus rattus*, *Tragulus javanicus*, *Martes foina*, *Marts flavigula*, *Hylobates hoolock*, *Hylobates leucogenys* and others (Yosida and Sagai 1975, Shi and Chen 1989, Liu *et al.* 1994, Chen *et al.*

Table 2. Karyotype comparison among Yulong vole and species of *Clethrionomys*, *Caryomys*, *Phaulomys* and *Eothenomys*

Species	2n	FN	Chromosome formula	References
<i>C. rutilus</i>	56	56	XY(A, A)+2M+52A	Kral 1971
<i>C. gapperi</i>	56	56 or 58	XY(A, A)+2M+52A or XY(A, SA)+2M+2SA+50A	Hsu <i>et al.</i> 1970, Frechette <i>et al.</i> 1971
<i>C. glareolus</i>	56	56	XY(A, M)+2M+52A	Hsu <i>et al.</i> 1970, Kuliev <i>et al.</i> 1977
<i>C. imaizuni</i>	56	56	XY(SA, SA)+2M+52A	Mattey 1973
<i>C. niigatae</i>	56	56	XY(A, M)+2M+52A	Hsu <i>et al.</i> 1974
<i>C. rufocanus</i>	56	56	XY(A, A)+2M+52A	Hsu <i>et al.</i> 1969
<i>C. r. shaneius</i>	56	56	XY(M, A)+2M+52A	Jiang <i>et al.</i> 1991
<i>C. rutilus</i>	56	56	XY(A, A)+2M+52A	Kral 1971
<i>C. frater</i>	56	56	XY(A, A)+2M+52A	Jiang <i>et al.</i> 1993
<i>Car. inez</i>	54	60	XY(SM, A)+4M+4SA+44A	Wang <i>et al.</i> 1991
<i>Car. eva</i>	54	56	XY(M, A)+2M+2SA+48A	Ma <i>et al.</i> 1996
<i>Pha. kageus</i>	56	56 60	XY(SM, A)+2M+52A or XY(SA, SA)+2M+4SA+48A	Hsu <i>et al.</i> 1974, Ando <i>et al.</i> 1988
<i>Pha. smithi</i>	56	56	XY(A, A)+2M+52A or XY(SA, SA)+2M+4SA+48A	Hsu <i>et al.</i> 1974, Ando <i>et al.</i> 1988
<i>E. miletus</i>	56	54	XY(A, A)+54A	Wu <i>et al.</i> 1989, Chen <i>et al.</i> 1994
<i>E. melanogaster</i>	56	56	XY(A, A)+2M+52A	Harada <i>et al.</i> 1991
<i>E. eleusis</i>	56	54	XY(A, A)+54A	Yang Unpublished
<i>E. proditor</i>	32	56	XY(SM, A)+14M+12SM+4A	Present paper

C. = *Clethrionomys*, *Car.* = *Caryomys*, *E.* = *Eothenomys*, *Pha.* = *Phaulomys*, M = metacentric chromosome, SM = submetacentric chromosome, SA = subacrocentric chromosome, A = acrocentric chromosome.

1990, Liu *et al.* 1996, Hsu 1978). Hsu *et al.* (1971) interpreted that the appearing of terminal heterochromatin and interstitial heterochromatin is result of pericentric inversions and asymmetrical translocations, and they thought it of one sign of differentiation. Data above-mentioned suggest that Yulong vole might be more differed than other voles of tribe Clethrionomyini.

The chromosome rearrangement is a primary event in initiating divergence of speciation (White 1978). Yosida (1973) stated that the frequency of chromosome evolution is high in rodents, however, it differs by species. In the genus *Eothenomys*, the karyotype evolution of *E. miletus* and *E. eleusis* is slow while of Yulong vole is fast. The reason may be that the former two with 56 acrocentric chromosomes are primitive and from them karyotype evolution occurred. Fossil evidence also showed that *Antelionomys* fauna occurs later than *Eothenomys* fauna (Zheng 1993). For the mode and direction of chromosomal evolution in rodents, Nadler (1969) proposed the fusion hypothesis that centric fusion is the commonest mechanism of structural rearrangement. Most species of the tribe Clethrionomyini have diploid number that vary between 54–56 with FN between 54–60. Although the diploid number of Yulong vole is far from the lower limit of the variation range for this character in those genera, FN is just in the variation range (Table 2). This drastic reduction in the numbers of 2n without affecting the FN suggests that chromosomal differentiation in Yulong vole has occurred basically through centric fusion. However, comparing chromosomes of Yulong vole and other *Eothenomys* known for G-banded karyotype, we found Yulong vole shares little similarities with other *Eothenomys* (Yang, unpublished). This indicates that Robertson fusion is not sufficient to explain the karyotype evolution in here. Several other structural rearrangements have also contributed to the great chromosomal divergence that appeared in Yulong vole karyotype. Considering chromosome morphology, G-banding patterns and C-banding patterns together, we infer that the Yulong vole originated from a *E. miletus*-like ancestor, and the karyotype evolutionary way of Yulong vole was not only Robertsonian fusion but also tandem translocation and pericentric inversion. The ancestor of Yulong vole might has 28 pairs of chromosomes, which were all A elements with centromeric heterochromatin. Under the press of selection, two acrocentric autosomes fused in

the centromeric areas to form a biarmed element; the formation of biarmed autosomes with terminal or interstitial heterochromatin involved in centromere-telomere translocations. Also, changes in the amount of heterochromatin have occurred in the karyotypic evolution. For instance, heterochromatic arm of No. 13 chromosome pair seems to be caused by addition of constitutive heterochromatin, and the presence of terminal heterochromatin in X chromosome may be the result of asymmetrical translocation.

According to Allen's opinion, the genus *Eothenomys* includes three subgenera: *Eothenomys*, *Anteliomys* and *Caryomys*. However the status of *Anteliomys* and *Caryomys* is still in discussion some taxonomists regarded it as an independent genus (Hinton 1926, Repenning *et al.* 1990, Gromov and Polyakov 1992), others thought of it as a subgenus of *Eothenomys* (Ellerman and Morrison-Scott 1951, Corbet 1978, Musser *et al.* 1993). Ma and Jiang (1996) found that the morphological and karyotype characteristics of the two species from *Caryomys* was distinct from *Eothenomys* and *Clethrionomys*, so they reinstated the genus *Caryomys*. At present most taxonomist acknowledged putting Yulong vole under the *Anteliomy*. However, when the skull morphology, dental morphology, comparative karyology and fossil evidence were considered, Yulong vole should be placed in a derived category. Whether separated Yulong vole from *Eothenomys* or refund the genus *Anteliomys* is still an open question because hitherto Karyological information on *Anteliomys* is extremely scanty and there has not been conclusive data. Further studies of other voles of *Anteliomys* is now in progress, and will be reported in the future.

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References

- Allen, G. M. 1940. Mammals of China and Mongolia. Amer. Mus. Nat. Hist., New York. Part II: 820–823.
- Ando, A., Shiraishi, S., Harada, M. and Uchida, T. A. 1988. A karyological study of two intraspecific taxa in Japanese *Eothenomys* (Mammalia: Rodentia). J. Mammal. Soc. Japan. **13** (2): 93–104.
- Carleton, M. D. and Myers, P. 1979. Karyotypes of some harvest mice, genus *Reithrodontomys*. J. Mamm. **60** (2): 307–313.
- Chen, Z. P., Jiang, X. L. and Wang, Y. X. 1994. Studies on the karyotype of Oriental vole (*Eothenomys miletus*). Cytologia. **59**: 289–293.
- , Liu, R. Q. and Wang, Y. X. 1996. Chromosome study of yellow-throated marten (*Martes flavigula*). Acta Theriologica Sinica (in Chinese) **10**(1): 19–22.
- Corbet, G. B. 1978. The Mammals of the Palaearctic Region: A Taxonomic Review. Brit. Mus. Nat. Hist., Cornell Univ. press, London & Ithaca. 101.
- Ellerman, J. R. and Morrison-Scott, T. C. S. 1951. Checklist of Palaearctic and Indian Mammals. Brit. Mus. Nat. Hist., London. 667–669.
- Frechette, A. G. and Jalal, S. M. 1971. Karyological study of two subspecies *C. gapperi gapperi*, *C. g. Loringi* of the red-black vole. Mammal. Chrom. News. **12**: 38.
- Gromov, I. M. and Polyakov, I. Y. 1992. Fauna SSSR, Mlekopitayushchie, tom 3, vyp. 8 [Fauna of USSR, vol. 3, pt. 8, mammals]. Polevki [voles (microtinae)]. E. J. Brill. Leiden-New York-København-Köln. 504.
- Harada, M., Ando, A., Lin, L. K. and Takada, S. 1991. Karyotypes of the Taiwan Vole *Microtus kikuchii* and the Pere David's Vole *Eothenomys melanogaster* from Taiwan. J. Mammal. Soc. Japan. **16** (1): 41–45.
- Hinton, M. A. C. 1926. Monograph of the Voles and lemming (Microtinae), Living and Extinct. Brit. Mus. Nat. Hist., London. 291.

- Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: A 1-step method. *Experientia* **36**: 1014–1015.
- Hsu, T. C. 1978. Human and Mammalian Cytogenetics: An Historical Perspective. Springer-Verlag.
- and Arrighi, F. E. 1975. Distribution of constitutive heterochromatin in mammalian chromosomes. *Chromosoma* **53**: 25–26.
- and Benirschke, K. 1969. An Atlas of Mammalian Chromosomes. vol. 3. Springer, New York.
- and — 1970. An Atlas of Mammalian Chromosomes. vol. 4. Springer, New York.
- and — 1974. An Atlas of Mammalian Chromosomes. vol. 8. Springer, New York.
- Koenigswald, W. V. 1980. Schmelzstruktur und Morphologie in den Molaren der Arvicolidae (Rodentia). *Abhandlungen der Senckenbergischen Naturforschenden. Gesellschaft* **539**: 1–129.
- Kral, B. 1971. Chromosome characteristics of Murridae and Microtidae from Czechoslovakia. *Acta Sci. Brno* **6**: 1–78.
- Leaven, A., Fredga, K. and Sinderg, A. A. 1964. Nomenclature for centromeric position on chromosome. *Hereditas* **52** (2): 201–220.
- Liu, R. Q., Nie, W. H. and Chen, Y. Z. 1994. A stone marten with diploid chromosome number of 38. *Chrom. Infor. Serv.* **56**: 15–16.
- , —, — and Yu, D. H. 1993. Comparative studies of C-band and Ag-NORs on the chromosomes between two species gibbon. *Acta Theriologica Sinica* (in Chinese) **16** (3): 182–187.
- Ma, Y. and Jiang, J. Q. 1996. The reinstatement of the status of genus *Caryomys* (Thomas 1911) (Rodentia: Microtinae). *Acta Zootaxonomica Sinica* (in Chinese) **21** (4): 493–497.
- Musser, G. G. and Carleton, M. D. 1993. Family Microtus. In: Wilson, D. E. and Reeder, D. A. M. (eds.) *Mammal Species of the World. A Taxonomic and Geographic Reference*. Smiths. Inst., Washington and London.
- Nadler, C. F. 1969. Chromosomal evolution in rodents. In: Benirschke, K. (ed.) *Comparative Mammalian Cytogenetics*. Springer-Verlag, Berlin. Heidelberg. New York. 227–309.
- Seabright, M. 1971. A rapid banding technique for human chromosomes. *Lancet* **2**: 971–972.
- Sen, S. and Sharma, T. 1983. Role of constitutive heterochromatin in evolutionary divergence: results of chromosome banding and condensation inhibition studies in *Mus mus musculus*, *Mus booduga* and *Mus dunni*. *Evolution* **37**: 628–636.
- Shi, L. M. and Chen, Y. Z. 1989. The karyotype analysis of Yunnan mouse deer (*Tragulus javanicus williamsoni*). *Acta zoologica Sinica* (in Chinese) **35** (1): 41–44.
- Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **75**: 304–306.
- White, M. J. D. 1978. *Modles of Speciation*. W. H. Freeman and Company, San Francisco.
- Wu, A. G., Wang, G. L. and Zhao, H. 1989. The karyotype of *Eothenomys miletus*. *Acta Theriologica Sinica* **9** (3): 231.
- Yosida, T. H. 1973. Karyotype and evolution. *Iden (The Heredity)* (in Japanese) **27** (7): 60–66.
- and Sagai, T. 1975. Variation of C-band in the chromosomes of several subspecies of *Rattus rattus*. *Chromosoma* **50**: 283–300.
- Zheng, S. H. 1993. Quaternary Rodents of Sichan-Guizhou Area, China. Science Press, Academia Sinica. 219–220.
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