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Cytotaxonomic Studies on Chinese Pelobatids VI. The Karyotypes, C-bands and Ag-NORs of *Megophrys minor* and *Oreolalax major*

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Abstract: -Chromosome preparations were successfully stained for C-bands and Ag-NORs in two Chinese pelobatids, *Megophrys minor* and *Oreolalax major*. The results were analyzed and compared. The karyotype formula of *O. major* was 6+7 like most species of Chinese Oreolalaxinae whereas *M. minor* was 5+8 just as in most species of Chinese Megophryinae. The SC on the long arm of chromosome No. 6 associated with the C-band-positive was speculated the Standard NORs of genus *Oreolalax*. The NOR just in the conspicuous SC was not emerged firmly in the genus *Megophrys*.

Key words: Anura, Pelobatidae, *Megophrys*, *Oreolalax*, cytotaxonomy, China

Introduction

Chinese pelobatids are attributed to two subfamilies with about 50 species (Tian et al., 1986). The karotypes of 15 species among those 50 species, with the majority distributed in the Heng Duan Mountains region where the karyotypic characteristics of some anurans are unusual (Zeng and Wu, 1989), were reported. In this paper, two other species, *Oreolalax major* and *Megophrys minor* were analyzed by means of C-banding and silver-staining NORs techniques.

Materials and Methods

Megophrys minor (3 males and 1 female) were collected on Mt. Emei, Sichuan Province, China and in Maowen County, Sichuan Province, China, respectively. *Oreolalax major* (3 males) were captured from Mt. Emei in 1989-90. Chromosome preparations were performed by centrifugal air-drying method (Wu et al., 1981) using 0.4 M KCl as hypotonic solution for 40 minutes. C-banding was made following Sumner (1972), for 5 minutes with Barium Hydroxide treatment at 54° C. Silver-staining NORs was prepared following Tan et al. (1986), $AgNO_3$ acting time for about 5 minutes at about 55° C.

Results

The measurements of chromosomes for the two species are shown in Table 1.

All specimens of *M. minor* from two places, Mt. Emei and Maowen had 2n=26 and the complement included five pairs of large (Nos. 1-5) and eight pairs of small (Nos. 6-13) chromosomes. Nos. 1, 4-5 and 8-12 were metacentric (m), whereas Nos. 2-3 and 6-7 were submetacentric (sm) chromosomes. The last pair was telocentric (t). The highly differentiated heteromorphic sex chromosomes as seen in *Pyxicephalus adspersus* (Schmidt, 1980a) were not found when males and females were compared.

Three males of *O. major* also had 2n=26 with the complement of six pairs of large (Nos. 1-6) and seven pairs of small (Nos. 7-13) chromosomes. The chromosomes were metacentric except for Nos. 3-5 and 9 with submetacentric and No. 6 with sub- or metacentric. Whether or not highly differentiated heteromorphic sex chromosomes are present is not known, due to the lack of female animals.

The conspicuous secondary constriction was found in a pericentric position on the long arm of chromosome No. 6 of *O. major* whereas the small inconspicuous one can be seen in a proximal position on the short arm of chromosome No. 6 of *M. minor* (Figures 1 and 2).

The result of silver-staining NORs revealed that Ag-NORs were present on chromosome No. 6 associated with the secondary constriction in both species (Figs. 1, 2). The strongly C-band-positive was in a

TABLE 1. The arm ratio and relative length of *Megophrys minor* and *Oreolalax major*.

	Megophrys minor				*Oreolalax major*		
	Arm Ratio	Relative length	Type		Arm Ratio	Relative length	Type
1	1.32±0.10	17.91±1.39	m	1	1.35±0.12	16.75±0.77	m
2	1.74±0.07	14.10±0.90	sm	2	1.54±0.15	13.53±1.28	m
3	2.12±0.20	12.52±0.52	sm	3	2.09±0.24	11.90±0.84	sm
4	1.65±0.05	11.41±0.36	m	4	1.98±0.25	11.11±0.48	sm
5	1.53±0.10	9.89±0.49	m	5	1.77±0.08	9.38±0.50	sm
6	1.72±0.03	5.81±0.48	sm	6	1.61±0.21	7.63±0.70	m/sm
7	2.24±0.31	5.13±0.35	sm	7	1.38±0.21	5.52±0.63	m
8	1.51±0.15	4.72±0.41	m	8	1.44±0.14	5.03±0.35	m
9	1.31±0.15	4.35±0.44	m	9	1.87±0.22	4.75±0.39	sm
10	1.35±0.15	3.93±0.30	m	10	1.41±0.19	4.18±0.53	m
11	1.50±0.19	3.71±0.40	m	11	1.36±0.18	3.97±0.55	m
12	1.33±0.19	3.47±0.34	m	12	1.10±0.12	3.60±0.63	m
13	*	3.06±0.39	t	13	1.28±0.17	3.17±0.39	m

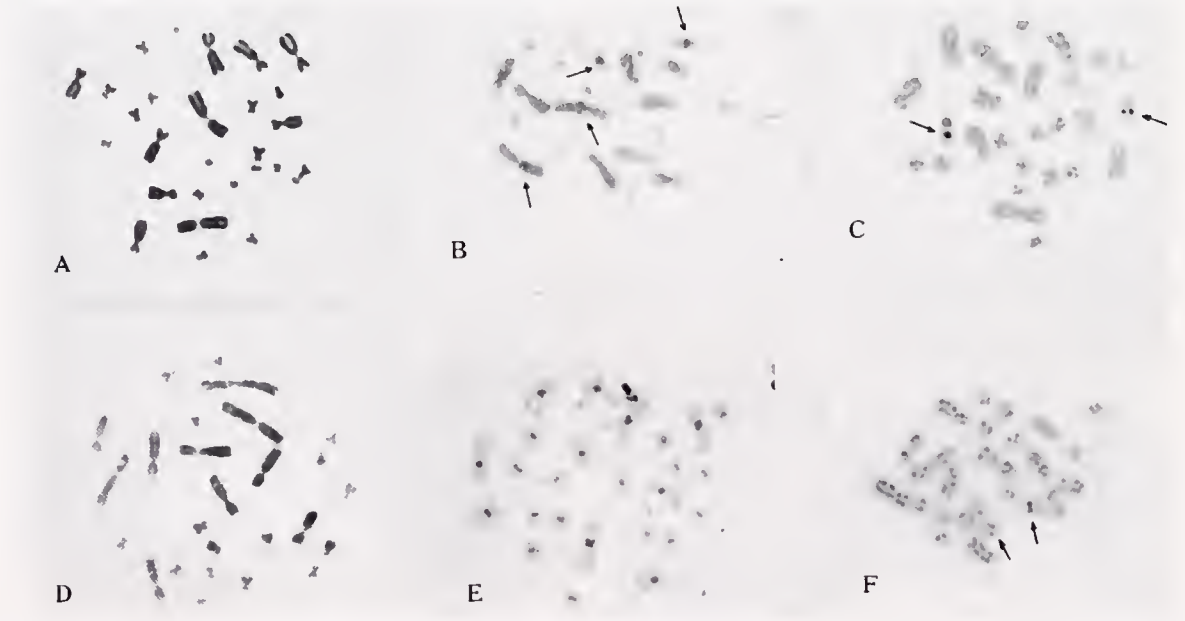

FIG. 1

centric position on each chromosome pair of *M. minor*, and only two weakly positive C-bands were discovered in *O. major*. One was associated with the secondary constriction of chromosome No. 6, and another was in the proximal position of the long arm of chromosome No. 1 which was not related to the secondary constriction (Figs. 1, 2).

Unfortunately, we cannot get the C-bands from females from the two species. Moreover, it is also impossible to know whether the early stage of ZW/ZZ sex chromosomes differentiation like *Poecilia shenops var. melanistica* (Haaf and Schmidt, 1984) and *Leiopelma hamiltoni* (Green, 1988) exists or not.

Discussion

Karyotypes

There is no doubt that *M. minor* has the second karyotype formula (5+8) of Morescalchi (1973) as shown in Table 1.

FIG. 2

TABLE 2. The karyotypes of three *Megophrys* species and six *Oreolalax* species.

Species	Centric Type												
	1	2	3	4	5	6	7	8	9	10	11	12	13
M. lateralis	m	m	sm	m/sm	m	m	m	st	m	m	m	m	st
M. omeimontis	m	m/sm	sm	m	m	m	sm	m	t	m	m	sm	t
M. minor	m	sm	sm	m	m	sm	sm	m	m	m	m	m	t
O. omeimontis	m	m/sm	sm	sm	m	m	m	m/sm	m/sm	m	m	m	m
O. pingii	m	m	sm	sm	m/sm	m	m	sm	m	m	m	sm	m
O. popei	m	sm	sm	m/sm	m	m	m	m	m	m	m	m	m
O. rugosa	m	m/sm	sm	sm	sm	sm	m/sm	m/sm	m/sm	m	m	m	m
O. schmidti	m	m	sm	m	m	m	m	m	m	m	m	m	t
O. major	m	m	sm	sm	sm	m/sm	m/sm	m	m	m	m	m	m

This result corresponded to that of most species of Chinese Megophryinae. The karyotype of *M. minor* was roughly similar to those of the other two species of *Megophrys*, *M. lateralis* (Wu, 1987) and *M. omeimontis* (Zeng and Wu, 1989). All of them had several pairs of sm, st and t chromosomes, and chromosome No. 3 was sm (Table 2). The difference among species of the genus were mainly shown on the position of secondary constriction (SC): on the short arm and long arm of chromosome No. 6 for *M. minor* and *M. omeimotis*; on the short arm of chromosome No. 5 for *M. lateralis*. Besides, these three species can be distinguished from each other by the number of sm, st and t chromosome pairs.

As shown in Table 1, the karyotype of *O. major* was classified into the first formula (6+7) of Morescalchi. The result was the same as the other 5 species of genus *Oreolalax*, *O. pingii*, *O. rugosa*, *O. popei*, *O. omeimontis* (Wu, 1988) and *O. schmidti* (Zeng and Wu, 1989). The karyotype of *O. major* only consists of m and sm chromosomes like the other 5 species of the same genus (Table 2). Moreover, 6 *Oreolalax* species had a conspicuous SC present on the long arm of chromosome No. 6. The differences among the *Oreolalax*

species were only reflected on the arrangement of m and sm chromosomes.

Compared with the genus *Megophrys*, the *Oreolalax* species had no st chromosomes and all chromosomes in the 6 known species were m or sm except chromosome No. 13 of *O. schmidti* (Table 2) which was t chromosomes. That their SC always appeared firmly on the long arm of chromosome No. 6 was different from *Megophrys*, in which 3 species had 3 different positions of SC. Furthermore, the number of the large sm chromosome pairs of the *Oreolalax* species were much more than that of *Megophrys*. *Oreolalax* was a subgenus of the *Scutiger* (Duellman, 1985) and it was considered as a genus (Myers and Leviton, 1962) which was attributed to Megophryniae. The differences of the two subfamilies (most species mentioned above) were present on two different karyotype formulas: the former was 6+7, whereas the latter was 5+8. It was thought that the more primitive karyotypic characteristics the karyotype of pelobatids had, the more t, st and sm pairs of chromosomes in comparison with that of the higher Anura (Duellman, 1985; Morescalchi, 1973). This position is still similar to the point of view on morphological taxonomy.

C-bands and NORs

C-bands in pelobatids were found more weakly and in less number than those in higher Anura (Bufonidae, Ranidae, and Hylidae). The C-banding of *M. minor* was very similar to those of the other two species of *Megophyrs*, *M. nasuta* (Schmidt, 1980b) and *M. omeimontis*. The constitutive heterochromatin emerged on the procentric area of each pair of chromosomes. The C-band associated with the SC on chromosome No. 6 *(M. nasuta, M. omeimontis)* and No. 5 *(M. minor)* was not enhanced to be particularly distinguished with centric C-band. The result of the C-banding treatment to *Oreolalax* species were less active than that to *Megophrys*. The centric C-bands were always weak *(O. omeimontis, O. pingii, and O. rugosa)* or invisible *(O. schmidti, O. major*, and this paper). Most of them had no centric C-bands and only had one interstitial C-band positive on chromosome No. 6 which was just associated with the position of the SC. Apart from one on chromosome No. 6, *O. major* had the other interstitial C-band on chromosome No. 1. This is different from the other species of this genus. If the interstitial C-bands revealed the relics of chromosome rearrangement (Schmidt, 1978a; King, 1980), it should be possible that the karyotype of *O. major* shows more higher evolutionary level in the *Oreolalax*.

It is said that stable and conspicuous SC is always the location of NORs. In respect to the genus *Rana*, Schmidt (1978b) concluded that the Standard NORs were always emerged in this SC on the long arm of chromosome No. 10 and thought it as a sign of *Rana*. The result of silver staining in *O. major* and *O. schmidti* proved that NOR was just in the SC on the long arm of chromosome No. 6. In terms of the other four species of this genus, *Oreolalax* with the same SC, it is speculated that the SC region on chromosome No. 6 should be in the location of the standard NORs of this genus. Furthermore, it is possible the close relationship between species in this genus was shown due to them having the same NORs (Tymoska, 1977). The NOR of *M. minor* and *M. omeimontis* emerged on the place associated with themselves SC. Compared with *Oreolalax*, they did not have the same SC associated with NOR, thus species showing higher interspecific differentiation.

Literature Cited

DUELLMAN, W. E. AND LINDA TRUEB. 1985. Biology of Amphibians. McGraw-Hill, New York. 670 pp.

GREEN, D. M. 1988. Heteromorphic sex chromosomes in the rare and primitive frog *Leiopelma hamiltoni* from New Zealand. Journal of Heredity 79:165-169.

HAAF, T. AND M. SCHMIDT. 1984. An early stage of ZW/ZZ sex chromosome differentiation in *Peocilia sphenops var. melanistica* (Poeciliidae, Cyprinosontiformes). Chromosoma 89:37-41.

KING, M. 1980. C-banding studies on Australian Hylid frogs: Secondary constriction structure and the concept of Euchromatin Transformation. Chromosoma 80:191-217.

MYERS, G. S. AND A. E. LEVITON. 1962. Generic classification of the high-altitude pelobatid toads of Asia *(Scutiger, aelurophryne,* and *Oreolalax).* Copeia 1962(2):287-291.

MORESCALCHI, A. 1973. Amphibia. Pp. 233-248 *In* A. B. Chiarelli and C. Capanna (eds.), Cytotaxonomy and vertebrate evolution. Academic Press. London-New York.

SCHMIDT, M. 1978a. Chromosome banding in Amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla.* Chromosoma 66:361-388.

SCHMIDT, M. 1978b. Chromosome banding in Amphibia. II. Constitutive heterochromatin and nucleolus organizer regions in Ranidae, Microhylidae and Rhacophoridae. Chromosoma 68:131-148.

SCHMIDT, M. 1980a. Chromosome banding in Amphibia. V. Highly differentiated ZW/ZZ sex chromosomes and exceptional genome size in *Pyxicephalus adspersus* (Anura, Ranidae). Chromosoma 80:69-96.

SCHMIDT, M. 1980b. Chromosome banding in Amphibia. IV. Differentiation of GC- and AT- rich chromosome regions in Anura. Chromosoma 77:83-103.

SUMNER, A. T. 1972. A simple technique for demonstrating centrometric heterochromatin. Experimental Cell Ressearch 75:304-306.

TAN, A. M. , Z. G. WU, E. M. ZHAO, AND H. X. OUYANG. 1986. A handy one-step method for silver-staining NORs. Acta Herpetologica Sinica 5:72-74. (In Chinese).

TIAN, W. S. AND Y. M. JIANG (EDITORS), ASSISTED BY G. F. WU, Q. X. HU, E. M. ZHAO, AND Q. Y. HUANG. 1986. Identification manual for Chinese species of amphibians and reptiles. Science Press, Beijing. 164 pp. (In Chinese).

TYMOWSKA, J. 1977. A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing a 36-chromosome complement. Cytogenetics and Cell Genetics 18: 165-181.

WU, G. F., W. M. YANG, AND E. M. ZHAO. 1981. Studies on genus *Vibrissaphora* . 3. A preliminary observation on karyotype of *Vibrissaphora liui.* Acta Herpetologica Sinica. 5: 139-142. (In Chinese).

WU, G. F. 1987. Cytotaxonomical studies on Chinese Pelobatids III. The analysis of the Karyotypes of *Megophrys lateralis* and *Atympanophyrs shapingensis.* Acta Herpetologica Sinica. 6: 45-48. (In Chinese).

WU, G. F. 1988. Cytotaxonomical studies on Chinese Pelobatids IV. The Karyotypes and C-bands of four species in the genus *Oreolalax.* Acta Herpetologica Sinica. 1: 1-4. (In Chinese).

ZENG, X. M. AND G. F. WU. 1989. Cytotaxonomical studies on Chinese Pelobatids V. The Karyotypes, C-bands and AgNORs of *Megophrys omeitmontis Oreolalax schmidt.* Chinese Herpetological Research 2: 37-45.