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Karyological Studies on Amphibians in China

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Abstract. - Since 1978, 79 species of anurans have been studied karyotypically using conventional Giemsa staining and various banding techniques. The chromosome numbers are 2n=22-64 and the karyotypes are variable. The homomorphic and heteromorphic sex chromosomes of some species have been identified.The mitotic and meiotic chromosomes of 14 species in two families of Urodela have also been investigated. The family Hynobiidae is karyotypically more primitive than the family Salamandridae.

Key words. - Karyology, amphibian, Anura, Urodela.

Introduction

Karyological studies of amphibians in China were pioneeered by two scholars. In 1952, famous cytologist T. C. Hsu, an American of Chinese origin, developed the hypotonic technique for chromosome separation and observation. In 1956, cytogeneticist J. H. Tjio, a Swede of Chinese origin, reported that the number of human chromosomes is 46, not 48. In China, karyological studies of amphibians began in 1978. Ninety-three species of amphibians have been studied so far, about 42% of the 220 living amphibian species in China (Table 1).

Changes of chromosome numbers. - Kuramoto (1990) summarized the chromosome numbers of 983 species of anurans from 21 families (2n=14-64). Chromosome number changes occurred in 12 of 21 families with polyploids in some species. In China, the chromosome numbers of 79 species from seven families are 22-64 and variations of chromosome numbers were found in four genera, three families (Table 2).

Only two species in the family Discoglossidae have been studied: *Bombina orientalis* and *B. maxima*. Tian and Hu (1985) subdivided the genus *Bombina* into two subgenera, *Bombina* and *Glandula*. *Bombina* (*B*.) *orientalis* has 2n=28 chromosomes (Zhao, 1986), consistent with the chromosome number of *Discoglossus pictus* in Europe (Morescalchi, 1965; Schmid et al., 1987). *Bombina* (*G*.) *maxima* has 2n=24 chromosomes (Jiang et al., 1984), as reported by Okumoto (1974) and Schmid et al. (1987).

The geography of the Hengduan Mountain Ranges greatly influence the evolution of pelobatid frogs, providing refugia for some species as well as discontinuous population distributions that promote allopatric speciation (Yang et al., 1983; Hu et al., 1985). The pelobatids distributed in the Hengduan Mountain region have distinct morphological differences adaptive to the unusual geographic conditions. These species belong to two subfamilies, Megophryinae (*Brachytarsophrys* and *Atympanophrys*) and Oreolalaxinae (*Scutiger*, *Vibrissaphora*, *Leptobrachium*, and *Oreolalax*). Available karyotypical information showed that species studied have 2n=26 chromosomes, but karyotypical differentiations are prominent in the family. The subgenus *Vibrissaphora* is the most specialized, all five species have 2n=26, consisting of six pairs of large chromosomes and seven pairs of small ones, NF=52 and one stable secondary constriction is located in 6q (in the NoRs region; Zhao et al., 1983). The karyotypes of three species in genus *Scutiger* are similar to those of subgenus *Vibrissaphora*, except the secondary constriction located in 2P not in 6q. Polymorphic chromosome number occured in Oreolalax. Two of the three specimens of *O. schmidti* are 2n=28 and one is 2n=26 (Zheng andWu, 1989). A similar phenomenon was found in *O. liangbeiensis* and C-banding showed that the extra pair of small chromosomes is C-band negative and not B chromosome (Li et al., 1990). Polymorphic chromosome numbers were also found in three genera of Megophryinae. Wu (1987) observed one male triploid in *Atympanophrys shapingensis*. Tan et al. (1987) reported the karyotypes of *Brachytarsophrys carinensis*. Among 12 individuals examined, seven males and one female have 2n=26, three males have 2n=27, and the remaining male has 2n=28. The extra chromosomes are metacentric and between No.10 and No.11 in size. In addition, there are four pairs of small chromosomes which are telocentric in the2n=26 karyotype and two pairs of small telocentric chromosomes were observed in *M. omeimontis* (Wu, 1987; Zheng and Wu, 1989).

The karotypes of pelobatids reported by foreign authors are 2n=26, NF=52 with no polymorphic chromosome numbers found, except *Leptolalax pelodytoides* with 2n=24 (Morescalchi, 1973; Morescalchi et al. 1977; Schmid, 1980, 1987). It is interesting that a special karyotype was observed in *Rana phrynoides* distributed in Hengduan Mountains. In this species, 2n=64 consisting of all telocentric chromosomes. Only one homologous pair of NORs being found in interstitial

Table 1. A list of amphibian species studied karyologically.

Famlies	Number of species Known	Number of species studied	Number of species Banded
Anura:			
Discoglossidae	4	2	0
Pleobatidae	46	18	12
Bufonidae	13	4	4
Hylidae	6	4	1
Ranidae	77	35	24
Rhacophoridae	32	7	3
Microhylidae	14	9	4
Total anurans	192	79	48
Caudata:			
Hynobiidae	17	7	0
Salamandridae	17	7	2
Total caudates	34	14	2

segment of No.20 chromosome, i.e, position of the sole secondary constriction, and No.32 being sat-chromosomes (Liu and Zan, 1984; Wu and Zhao, 1984). This karyotype is unique for anurans.

Karyological Studies on wood frogs in China. - In China, wood frogs include five species (*R. altaica*, *R. amurensis*, *R. japonica*, *R. chaochiaoensis*, and *R. chensinensis*; Tian and Jiang, 1986). *Rana chensinensis* had been called *R. temproaria chensinensis* (Pope and Boring, 1940; Liu and Hu, 1961). Wu (1981) reported the karyotype of *R. chensinensis* from Beijing, which has 2n=24 chromosomes, including six pairs of large chromosomes (relative length>7%) and six pairs of small ones (relative length<6%), while *R. temporaria* in Europe has 2n=26 (Guillemin, 1967), including five pairs of large chromosomes and eight pairs of small ones. Consequently, he suggested that R. chensinensis should be a good species, not a subspecies of *R. temporaria*. Wei et al. (1990) compared the C-bands and NORs between *R. chensinensis* from type locality and *R. temporaria* in Europe. As a result, in the former species, centric C-bands located in Nos. 9 and 10 chromosomes and telocentric C-bands in the terminations of a few chromosomes, 28 interstitial C-bands, one pair of standard NORs in 11q, and small additional NORs are found. However, in the latter species, both centric and telocentric C-bands are located in all chromosomes and only three interstitial C-bands and 1 pair of NORs in 10q were developed (Schmid, 1978). Evidently, this comparison supports Wu's suggestion.

Jiang et al. (1984), Luo and Li (1985) and Ma (1987) indicated that *R. chensinensis* from different localities have the same 2n=24 pattern, but the numbers of subtelocentric chromosomes and the positions of secondary constrictionas are locality specific by comparing the karyotypes of *R. chensinensis* from Beijing, Qingdao, Lanzhou, Harbin, Hongyuan and Yanbei. Consequently, it was suggested that *R. chensinensis* might contain different subspecies .

The five species of wood frogs have 2n=24 or 2n=26 chromosomes and may be divided into two groups: *R. japonica*, *R. chaochiaoensis* and *R. amurensis* belong to 2n=26 group and *R. chensinensis* and *R. altaica* to 2n=24 group. The karyotypic differences between two groups are listed in Table 3.

Rana japonica from Hiroshima, Japan has 2n=26 chromosomes, Nos. 8 and 9 are subtelocentric and the secondary constriction is located in 9q; *R. amurensis coreana* from Korea has 2n=26, Nos. 10 and 13 subtelocentric and secondary constriction in 9q; while *R. chensinensis* from Hokkaido, Japan has 2n=24, No.11 is subtelocentric and the secondary constriction in 10q (Nishioka, et al., 1987). Matsui (1991) described *R. chensinensis* from Hokkaido as a new species, *R. pirica*, based on morphometric and electrophoretic studies.

The No.6 chromosome of the species having 2n=24 is nearly the same in relative length as the sum of chromosomes No.11 and No.12 or No.13 of the species with 26 chromosomes, For instance, the average relative length of No.6 chromosomes of *R. chensinensis* and *R. altaica* is 8.19, while the average relative lengths of Nos.11, 12 and 13 chromosomes of the other three species are 4.52,4.31 and 3.82 respectively. In addition, it is clear from Table 3 that there are more subtelocentric chromosomes and secondary constrictions in the species having 26 chromosomes than those in the species having 24 chromosomes, and they are concerned with small chromosomes. Accordingly, it is speculated that all wood frog species would have a common ancestor, from which the species having 26 chromosomes were derived and the species having 24 chromosomes evolved via fusion of two pairs of small chromosomes of the former. Then, the species possessing

Table 2. A summary of chromosome numbers of 79 anuran species.
* = Polymorphic chromosome number occurs in some species.

Taxon	Number of species					
	22	24	26	28	64	Total
Discoglossidae						
Bombina	-	1	-	1	-	2
Pelobatidae						
Atympanophrys	-	-	1	-	-	1
Brachytarsophy	-	-	1	-	-	1
*Megophrys**	-	-	2	-	-	2
*Oreolalax**	-	-	6	-	-	6
Scutiger	-	-	3	-	-	3
Vibrissaphora	-	-	5	-	-	5
Bufonidae						
Bufo	4	-	-	-	-	4
Hylidae						
Hyla	-	4	2	-	-	6
Ranidae						
Rana	1	2	21	-	1	26
Amolops	-	-	7	-	-	7
Ooeidozyga	-	-	2	-	-	2
Altirana	-	-	1	-	-	1
Rhacophoridae						
Rhacophorus	-	-	5	-	-	5
Philautus	-	-	1	-	-	1
Polypedates	-	-	1	-	-	1
Microhylidae						
Microhyla	-	4	1	-	-	5
Kaloula	-	-	-	1	-	1
Kalophrynus	-	-	1	-	-	1
Total	5	11	60	2	1	79

different subtelocentric chromosomes and secondary constriction positions were developed into two groups through inversions and translocations. The high resolution R-bands of *R. japonica* were prepared and analyzed (Heng, 1984), providing a practical technique for studying karyotypic evolution of amphibians.

Sex chromosomes and sex-determining mechanisms.- The first successful demonstration of sex-determining mechanism was made by making use of reversal and breeding experiments (Humphrey, 1942, 1945, 1957). The applications of cytogenetic techniques, such as C-banding, quinacrine mustard staining, Ag-NORs staining and in situ hybridization of nucleic acids, have been helpful to the investigations on sex chromosomes and sex-determining mechanisms in amphibians. So far, eleven species with cytologically detected sex chromosomes, including XY and ZW systems, even an OW/OO system of sex determination and multiple sex chromosomes in one genome(Schmid et al., 1992) were discovered. Few sex-specific chromosome pairs in heterogametic individuals are heteromorphic and most of them are homomorphic.

The homomorphic chromosome pair No.4 in *Rana esculenta* was identified as sex-specific chromosomes of XX/XY type by BrdU replication banding technique. All males have an extremely late-replication band in the long arm of Y, which is lacking in the X (Schempp and Schmid, 1981). The homomorphic chromosome pair No.10 in *Bufo gargarizans* was demonstrated to be sex chromosomes of ZZ/ZW type. The Z chromosomes in all males replicated synchronously, while Z and W chromosomes of females revealed heteromorphic replication bands at the late replication stage. There was a replication band on W chromosome's long arm and Z chromosome lacked the band (Wen et al., 1982;Shang and Deng, 1982). Similarly, the chromosome No.6 pair in *Bufo raddei* was identified as XX/XY sex chromosomes (Deng and Shang, 1984) and the No.9 chromosomes of *Rana nigromaculata* as XX/XY sex chromosomes (Wu and Zhang, 1985). The sex chromosomes of species mentioned above are homomorphic and could only be recognized by BrdU replication banding. So they are at the initial stage of sex chromosome differentiation.

The Y or Z chromosomes of homomorphic sex chromosomes in some anurans heterochromatinized so

Table 3. karyotypic comparison between wood frog groups.

Species	2N	Subtelocentric pair(s)	Position of S.C.
R. japonica	26	No.7 or No.9	2-5p,6-7q
R. chaochiaoensis	26	No.8 Nos.9,10,12,13	5-7p,8q,10p
R. amurensis	26		8q
R. chensinensis (from type locality)	24	No.9	11q
R. altaica	24	--	1q

highly that they could be recognized by C-banding or other specific staining of constitutive heterochromatin, for example, the XX/XY sex chromosomes in genus *Triturus* (Schmid, et al., 1979). Chinese scholars Wu and Chen (unpublished data) determined the homomorphic chromosome pair No.9 as XX/XY sex chromosomes in *Rana margaratae* using C-banding and quinacrine mustard staining. There is one interstitial C-band, i.e, the brightest fluorescense band, on both No.9 chromosomes in females. The interstitial C-band is located only in one No.9 chromosome, while one telocentric C-band in the other No.9 chromosome shows no fluorescense differentiation.

The first-discovered highly heteromorphic ZW type sex chromosomes occured in *Pyxicephalus adspersus* (Schmid, 1980). The W chromosome is much smaller than Z chromosome and its short arm is completely heterochromatic. Wu and Zhao (1984) and Wu et al. (1987) demonstrated that *Amolops mantzorum* has well-differentiated XY type sex chromosomes,the Y chromosome is subtelocentric and mainly composed of euchromatin, but has strong C-band in the middle of long arm and X chromosome is metacentric by conventional Giemsa staining and C-banding methods.

Karyological studied in urodeles. - Only two species were studied by C-banding and the others by conventional Giemsa staining. The karyotypic comparisons are listed in Table 4. The family Hynobiidae has a wide geographical distribution. Twenty-six species in five genera out of more than 34 species in eight genera have been studied karyologically. The chromosome number vary from 40 to 80 . Twelve species in *Hynobius* and one in *Salamandrella* had been studied by C-banding, Ag-NORs staining, and R-banding. The relationships in the two genera were discussed by comparing banded karyotypes and Southern hybridizations. It is suggested that the family is the most primitive living caudate (Morescalchi, 1973; Kohno et al., 1991).The same conclusion is derived from morphological comparisons (Zhao and Hu, 1984).

There are 17 species in seven genera in Hynobiidae known from China. The family can be divided into two groups: *Hynobius* group and *Ranodon* group. The *Ranodon* group evolved by adaptation towards two different life-forms: aquatic and terrestrial. Liua and *Batrachuperus* are aquatic and are closely related. Table 4 shows that seven species have high chromosome numbers: 2n=62-68. Their karyotypes are bimodal and symmetrical, with more microchromosomes (Yang, 1992). *Salamandrella keyserlingii* has 2n=62 chromosomes, the karyotype formula being 4M + 2SM + 10ST + 10T + 36 m (Wang et al., 1983) in accordance with that reported by Morescalchi (1975), Morescalchi et al. (1979), Grafodatsky et al. (1978), Kuro-o(1986), and Ikebe et al. (1990). The bivalent number in cells of male *Batrachuperus pinchonii* in diakinesis is 31. We expect the diploid chromosome number to be 2n=62 (Yang and Zhao, 1984), but this species also has 2n=66 chromosomes (Kuro-o et al., unpublished data). There is little detailed information on the cause of the difference.

The karyotypic differentiation of Hynobiidae is more complex. First, variations in diploid chromosome number occurred not only at the intergeneric level,but also at the intrageneric level. For instance, *Salamandrella* has 2n=62, *Batrachuperus* 2n=62-68, both *Liua* and *Pachyhynobius* 2n=64. Secondly, the numbers of microchromosomes vary from 36 to 46. Finally, the morphology of microchromosomes are variable. The numbers of M, SM, and ST are species-specific. Telocentric macrochromosomes were found in *S. keyserlingii, L. shihi* and *P. shangchengensis*, but not in *Batrachuperus*. The *L. shihi* and *P. shangchengensis* studied were from the type localities (Zhao and Hu, 1983; Fei et al., 1983). Both two species have 2n=64 chromosomes, different from the known genera. However, they are different in morphology of macrochromosome and microchromosome number (Table 4) . Therefore, the cytogenetic data provide evidence supporting those genera and species.

There are four species in *Batrachuperus*. *Batrachuperus karlschmidti* and *B. yenyuanensis* are only found higher than 3000M in the Hengduan Mountains, having 2n=68, no telocentric macromosomes and 44 and 46 microchromosomes respectively. *Batrachuperus pinchonii* and *B. tibetanus* distributed higher than 1600M of Hengduan Mountains and adjacent areas have 2n=62. Obviously, the karyotypic differ-

Table 4. Karyological comparisons of urodela in China. **M**-metacentric macrochromosomes; **SM**-submetacentric macrochromosomes; **ST**-subtelocentric macrochromosomes; **T**-telocentric macrochromosomes; **m**-microchromosomes.

Species	2N	Number of Bivalent	M	SM	ST	T	m	Band
Hynobiidae								
Salamandrella keyserlingii	62		4	2	10	10	36	--
Batrachuperus karlschmidti	68		6	-	18	-	44	--
B. yenyuanensis	68		4	2	16	-	46	--
B. pinchonii	(62)	31						--
B. tibetanus	62							--
Liua shihi	64		6	2	4	10	42	--
Pachyhynobius shangchengensis	64		4	-	2	18	40	--
Salamandridae								
Tylototriton kweichowensis	24	12	16	6	2	-	-	--
T. verrucosus	(24)	12						--
Cynops cyanurus yunnanensis	(24)	12						--
C. orientalis	24	12	16	8	-	-	-	C
Paramesotriton chinensis	(24)	12						--
Pachytriton brevips	24		16	8	-	-	-	C
P. labiatum	(24)							--

entiations in *Batrachuperus* are in conformity with the geographic distribution.

Salamandridae is the advanced family in Caudata either from the viewpoint of karyotypic information or from that of morphological characteristics (Morescalchi, 1973, 1975, 1979; Zhao and Hu, 1984; Yang, 1992).There are five genera of Salamandridae found in China: *Tylototriton*, *Echinotriton*, *Cynops*, *Paramesotriton*, and *Pachytriton*. Of the five genera, *Tylototriton* is the most primitive and *Pachytriton* the most advanced . The seven species studied in five genera have the same chromosome number, 2n=24, without microchromosomes. The karyotypes are unimodal and symmetrical (Table 3). Interspecific differences were found in *Tylototriton*. The karyotypic formula of *T. kweichawensis* is 16M+6SM+2ST, the same as that of *T. verrucosus*. However, Nos. 6, 8 and 11 are submetacentric and no secondary constriction was found in the former (Yang, 1990), while Nos.6, 7 and 11 are submetacentric and secondary constriction were found at every chromosomes except No.12 in the latter (Seto et al., 1982). *Echinotriton andersoni* has 2n=24 chromosomes, 14M + 8SM+ 2ST, one more submetacentric chromosome pair than both *T. kweichowcensis* and *T.verrucosus* (Seto et al., 1982). In addition, the relative length of chromosome No. 1 is the longest and that of No.12 the shortest in *E. andersoni* among the three species mentioned above. The karyotypic formulas of *Pachytriton brevipes* and *Cynops orientalis* are nearly identical and only the differences of C-band patterns are found (Zhu and Wei, 1981).

The predominant mode of karyotypic evolution in Caudata is that the unimodal symmetrical karyotypes with fewer chromosome number are derived from the bimodal and asymmetrical karyotypes with more chromosome number, via Robertsonian centric fusions and pericentric inversions (Morescalchi, 1975). Robertsonian centric fusions, which could occur between telocentric macrochromosomes, between stable microchromosomes, and between telocentric macrochromosomes and stable microchromosomes, cause reduction of the diploid number and the microchromosome number and increase of the metacentric chromosomes. Consequently, the karyotypes tend toward stability. Pericentric inversions do not change the diploid number, but could increase the number of metacentric chromosomes and the stability of karyotypes.

There are some differences in the evolutionary trends of Hynobiidae and Salamandridae. The karyotypic evolution in Hynobiidae involves Robertsonian centric fusion as well as pericentric inversion. However, the phylogeny of the family could not be established on the available data. It is obvious that the karyotypes of Salamandridae are more stable than those of Hynobiidae. Morescalchi (1975) proposed that all species studied possess similar karyotypes that differ very little even at the intergeneric level. The differences between the karyotypes mainly lie in the absolute size of chromosomes and quantity of DNA. Accordingly, the karyotypic diversity among the species has chiefly resulted from pericentric inversions and reciprocal translocations that result in differences between individual chromosomes by changing the telocentric chromosomes into metacentric ones or changing the metacentric chromosomes into submetacentric, subtelocentric and telocentric chromosomes.

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