Karyotypic Studies of Nine Species of Chinese Salamanders†

YUHUA YANG[1]

[1]*Department of Bioengineering, Sichuan University, Chengdu 610041, China*

Abstract. -Four species of hynobiid salamanders, from three genera, possess bimodal and asymmetrical karyotypes with 2n=64 or 2n=68 chromosomes. Five species of salamandrids, from four genera, have 2n=24 and unimodal, symmetrical karyotypes with no microchromosomes or telocentric chromosomes. Karyologically, Hynobiidae is the most primitive and the Salamandridae is the most advanced family in the Caudata. The two families conform to different models of chromosome change.

Key words: Amphibia, Caudata, Hynobiidae, Salamandridae, China, karyotype.

TABLE 1. Locality, date of collection, and number of individuals (male, female) for the 9 species used in the karyotypic analyses.

		F	M
Hynobiidae			
Batrachuperus karlschmidti	Sichuan Prov., 1986	2	4
B. yenyuanensis	Mianning Co., Sichuan Prov., July 1986		2
Liua shihi	Wushan Co., Sichuan Prov., Mar. 1986	1	2
Pachyhynobius shangchengensis	Jinzhai Co., Anhui Prov., Apr. 1984	3	
Salamandridae			
Tylototriton kweichowensis	Weining Co., Guizhou Prov., Jun. 1986		3
T. verrucosus	Jingdong Co., Yunnan Prov., Aug. 1984		3
Pachytriton labiatum	He Co., Guangxi Prov., Jun. 1986		1
Paramesotriton chinensis	He Co., Guangxi Prov., Jun. 1986		1
Cynops cyanurus yunnanensis	Jingdong Co., Yunnan Prov., Aug. 1984		1

Introduction

There are 33 species of salamanders known from China, including two suborders, three families and 12 genera (Zhao et al., 1988). Only a few of their karyotypes have previously been reported (Wang et al., 1983; Yang and Zhao, 1984; Yang et al., 1986a, 1986b; Zhu and Wei, 1981). Karyotypic studies among the Caudata (Kuro-o et al., 1987; Makino, 1932; Morescalchi, 1973, 1975; Morescalchi et al., 1977; 1979; Schmid, 1979; Sessions et al., 1982; Seto et al., 1986) have contributed evidence for the phylogeny of salamanders. The present paper reports the karyotypes of nine species belonging to the families Hynobiidae and Salamandridae, thereby providing additional cytogenetic data towards understanding the phylogeny of Chinese salamanders.

Methods

Preparation of Chromosomes

Animals used in this study were collected from Sichuan, Yunnan, Guizhou, Guangxi and Anhui Provinces, China from May 1983 to June 1986 (Table 1). Mitotic chromosomes were prepared using the methods of Kezer and Sessions (1979) with minor modifications. Animals were intraperitoneally injected with 20 mg/ml of colchicine solution about 40 to 60 hours before sacrifice using a dosage of 0.01 ml/g body weight. Liver and intestine were removed, washed with 1% sodium citrate, and sliced. Chromosome preparations were made by air-dry and squash techniques. Chromosomes were also obtained using the methods of Princee and

† This publication was previously published in Chinese by Yang (1990).

Boer (1983) and Wu (1982) with slight modifications (Yang et al., 1986a). Specimens were injected intraperitoneally with PHA solution (5 mg/ml) at a dosage of 0.09 ml/g body weight, administered once every 24 hours for three days. Twenty four hours after the third PHA injection, the specimens received a final injection of colchicine (3 mg/ml) with a dosage of 30 µg/g body weight about 14 hours before they were sacrificed. The spleen was removed, washed with 1% solution of sodium citrate, ground, and put into a hypotonic solution (0.4% KCl). A few drops of the final cell suspensions were used per slide, which was then placed in a large Petri dish. Following hypotonic treatment and fixation in the dish, the air-dried slides were stained with 5% Giemsa PBS (pH 6.8) for 30 minutes.

An intraperitoneal injection of colchicine solution (3 mg/ml) was given using a dosage of 30 µg/g body weight for 14-24 hours before the specimens were sacrificed. The testes were removed, rinsed with 1% sodium citrate, ground, and put into 0.4% KCl solution for 1.0-1.5 hours at room temperature. After centrifugation, the supernatant was discarded and the precipitate was fixed in a 3:1 solution of methanol:acetic acid for three periods of 30 minutes each. Slides were prepared by the air-dry method and stained with 5% Giemsa PBS (pH 6.8) for 1 hour.

Karyotype Analysis

1. Mitotic chromosomes.—Chromosomes were numbered and the spreads which could be used for karyotypes were photographed. The karyotypes were prepared on the basis of relative lengths and arm ratios measured and calculated from the enlarged photographs. The chromosomes in tailed amphibians grade smoothly in size from the largest to the smallest, so that it is impossible to distinguish between the largest microchromosomes and the smallest macrochromosomes. No general criterion of microchromosomes exists to date (Morescalchi, 1973, 1975; Sessions et. al, 1982; Wang et al, 1983). In addition, the morphology of the microchromosomes varies with differences in the preparation techniques and the mitotic chromosomes of the cells analyzed. For the sake of consistency, I have defined the chromosomes less than 3.00% relative length (in percentage of total haploid length, including the microchromosomes) as microchromosomes according to the fact that the minimum value of relative lengths of the smallest chromosomes in Salamandridae, which is the most advanced family without microchromosomes in Caudata, is 3.00 (Table 2). The abreviations of the chromosomes are as follows: M-metacentric; SM-submetacentric; ST-subtelocentric; T-telocentric; and m-microchromosome.

2. Meiotic chromosomes.—Bivalents in diakinesis were numbered and arranged. The relative lengths of the bivalents were calculated according to the principles of ISCN (1978). The arm ratio was not calculated due to the absence of C-band information. The relative chiasma numbers also were calculated (those having joined ends were also considered to be in chiasma) using the method presented by Imai and Moriwaki (1982). For chromosomes in metaphase II, the relative length and arm ratio were measured and calculated. The terminology for centromeric position followed Levan et al. (1964).

Results

Hynobiidae

Batrachuperus.—*B. karlschmidti* (2n=68) has 12 pairs of macrochromosomes and 22 pairs of microchromosomes, which is the highest chromosome number among species of Hynobiidae reported to date except for *Onychodactylus japonicus* (2n=78; Table 3). Nos. 1-3 are metacentric and the rest submetacentric among the macrochromosomes (Fig. 1, Table 4). This is a bimodal and asymmetrical karyotype with a formula of 6M + 18ST + 44M.

B. yenyuanensis (2n = 68), consisting

TABLE 2. Mitotic chromosome data for five species of Salamandridae.

Relative length ($\bar{x}$±S.D.)					
No.	Tk[a]	Tv[b]	Ta[c]	Pb[d]	Co[e]
1	12.24±0.55	12.2	12.8	12.54±0.61	12.17±0.86
2	11.20±0.33	11.5	10.8	11.63±0.41	11.40±0.59
3	10.52±0.53	10.8	11.2	10.52±0.55	10.49±0.58
4	10.13±0.55	10.7	10.1	10.56±0.59	10.13±0.59
5	9.45±0.49	9.5	9.4	9.89±0.42	9.59±0.37
6	8.62±0.73	9.0	8.8	9.17±0.60	9.08±0.57
7	8.07±0.54	7.8	7.7	8.20±0.62	8.27±0.71
8	7.36±0.50	7.6	8.1	7.92±0.73	8.17±0.76
9	6.53±0.58	6.3	6.0	5.89±0.55	6.28±0.48
10	5.81±0.36	5.5	5.6	5.42±0.41	5.63±0.49
11	5.33±0.41	5.4	5.0	4.81±0.34	5.09±0.43
12	4.44±0.58	3.8	3.0	3.21±0.49	3.94±0.52
Arm ratio ($\bar{x}$±S.D.)					
No.	Tk	Tv	Ta	Pb	Co
1	1.2±0.16	1.08	1.13	1.11±0.09	1.07±0.08
2	1.3±0.13	1.22	1.08	1.25±0.16	1.19±0.11
3	1.3±0.32	1.08	1.38	1.62±0.20	1.51±0.18
4	1.3±0.36	1.27	1.08	1.13±0.08	1.11±0.08
5	1.4±0.39	1.08	1.17	1.20±0.12	1.14±0.10
6	2.5±1.20	2.03	2.33	1.42±0.19	1.28±0.14
7	1.4±0.31	2.70	2.85	2.93±0.56	2.35±0.32
8	1.8±0.48	1.33	1.38	1.20±0.15	1.13±0.06
9	1.4±0.08	1.22	1.38	1.67±0.19	1.44±0.17
10	1.5±0.36	1.38	1.70	2.64±0.58	2.57±0.33
11	2.9±1.50	1.70	1.86	2.88±0.44	2.33±0.38
12	3.6±2.30	3.76	3.76	2.00±0±22	2.20±0.31
Centromere position					
No.	Tk	Tv	Ta	Pb	Co
1	M	M	M	M	M
2	M	M	M	M	M
3	M	M	M	M	M
4	M	M	M	M	M
5	M	M	M	M	M
6	SM	SM	SM	M	M
7	M	SM	SM	SM	SM
8	SM	M	M	M	M
9	M	M	M	M	M
10	M	M	SM	SM	SM
11	SM	SM	SM	SM	SM
12	ST	ST	ST	SM	SM

a. Tk = *Tylototriton kweichowensis*, (present paper); b. Tv = *T. verrocosus*, (Seto et al.,1982); c. Ta = *T. andersoni*, (Seto et al., 1982); d. Pb = *Pachytriton brevipes*, (Zhu and Wei, 1981); e. Co = *Cynops orientalis*, (Zhu and Wei, 1981).

of 11 pairs of macrochromosomes and 23 pairs of microchromosomes. Among the macrochromosomes, nos. 1-2 are metacentric, no. 4 is submetacentric, and the rest are subtelocentric (Fig. 1, Table 4). The karyotype is bimodal and asymmetrical, with a formula of 4M + 2SM + 16ST + 46m.

Liua.—L. shihi (2n = 64). The karyotype consists of 11 pairs of macrochromosomes and 21 pairs of microchromosomes, nos. 1-3 being metacentric, no. 7 submetacentric and the

TABLE 3. Chromosome data of some species in Hynobiidae.

Species	2n	Telocentric Macro-chromosomes	Micro-chromosomes	References
Hynobius dunni	56	0	16	Morescalchi et al. 1979
H. retardatus	40	4	0	Morescalchi et al. 1979
Salamandrella keyserlingii	62	24	24	Morescalchi et al., 1979
Ranadon sibericus	66	18	38	Morescalchi et al., 1979
Batrachuperus mustersi	62	10	38	Morescalchi et al., 1979
B. karlschmidti	68	0	44	Present paper
B. yenyuanensis	68	0	46	Present paper
Onychodactylus fischeri	>66	6	>22	Session et al., 1982
O. japonicus	58+2	0	18	Morescalchi et al., 1979
O. japonicus	78	?	?	Yamamoto, 1982
Liua shihi	64	10	42	Present paper
Pachyhynobius shangchengensis	64	18	40	Yang et al., 1986

rest telocentric among the macrochromosomes (Fig. 1, Table 4). The karyotype is bimodal and asymmetrical, with a formula of 6M + 2SM + 4ST + 10T + 42M.

Pachyhynobius.—P. shangchengensis (= *Xenobius melanonychus*), (2n = 64). There are 12 pairs of macrochromosomes and 20 pairs of microchromosomes. Numbers 1 and 5 are metacentric, no. 2 is subtelocentric, and the rest of the macrochromosomes are telocentric (Fig. 1, Table 4). This is a bimodal and asymmetrical karyotype with a formula of 4M + 2ST + 18T + 40M.

Salamandridae

Tylototriton.—T. kweichowensis (2n=24) has 8 pairs of metacentric (nos. 1-5, 7, 9-10), 3 pairs of submetacentric (nos. 6, 8 and 11) and 1 pair of subtelocentric chromosomes (no. 12), without microchromosomes (Fig. 2, Table 2). This is a unimodal and symmetrical karyotype. Twelve chromosomes in metaphase II and twelve bivalents in diakinesis were seen on the meiotic preparations (n=12). The relative lengths of chromosomes in metaphase II are larger than those of chromosomes in mitotic metaphase (Tables 2 and 5). Both nos. 11 and 12 chromosomes in metaphase II are submetacentric, the arm ratios being 1.7 and 2.6 respectively, while no. 11 is submetacentric and no. 12 is subtelocentric for the chromosomes in mitotic metaphase, the arm ratios being 2.6 and 3.6 respectively. The differences might show the different degree of chromosome contraction during meiosis and mitosis (Figs. 2, Tables 2 and 5). The relative lengths and relative chiasma number of the bivalents are larger than those of chromosomes in mitotic and less than those of chromosomes in metaphase II. The relationship of chiasma numbers and lengths of the bivalents in not a straight line, for instance, the relative lengths of

FIG. 1. Karyotypes of four species of hynobiid salamanders. 1) *Batrachuperus karlschmidti*, 2) *B. yenyuanensis*, 3) *Liua shihi*, 4) *Pachyhynobius shangchengensis*.

nos. 1 and 12 are 12.47 and 5.41, while their relative chiasma numbers are 7.96 and 8.47 respectively.

T. verrucosus (2n = 24). The haploid chromosome number is 12. Accordingly, the diploid chromosome number expected is 24, which is consistent with those of *T. andersoni* and *T. verrucosus* from different localities (Ferrier and Beetschen, 1973; Morescalchi, 1973; Seto et al, 1986). The relative lengths of chromosomes in metaphase II are larger than those of bivalents in diakinesis (Tables 5 and 6). The chromosomes in metaphase II are metacentric except nos. 6, 8 and 12 of submetacentric chromosomes and there are no microchromosomes. The karyotype is unimodal and symmetric. 2). The relative chiasma numbers are not proportional to relative lengths, for example, the relative lengths are 9.36 and 5.25 respectively (Table 6).

Pachytriton.—P. labiatum (2n = 24). There are 12 bivalents in diakinetic cells (n=12). Accordingly, the diploid chromosome number should be 24 (Fig. 2), in accord with that of *P. brevies* (Zhu and Wei, 1981). The relative chiasma numbers of nos. 1-6 bivalents vary basically with their relative lengths, while the relative chiasma numbers of nos. 7-12 are more constant (Table 6).

Cynops.—C. cyanurus yunnanensis (2n=24). There are 12 bivalents in diakinetic cells and 12 chromosomes in metaphase II cells (Fig. 2). Consequently, the 2n should be 24. The chromosomes in metaphase II are metacentric except no. 8 (SM), without microchromosomes (Fig.

TABLE 4. Macrochromosome data for four species of Hynobiidae.

Relative length ($\bar{x}$±S.D.)				
No.	Ps[a]	Ls[b]	Bk[c]	By[d]
1	11.95±1.15	13.06±0.76	12.28±1.03	13.87±3.95
2	7.80±0.60	11.19±0.44	9.88±0.79	9.66±0.44
3	7.43±0.55	8.13±1.30	7.51±0.89	8.10±0.82
4	6.94±0.47	7.45±0.35	6.93±0.85	7.52±1.18
5	6.80±0.47	6.87±0.33	6.37±0.79	7.07±0.52
6	5.80±0.33	6.33±0.37	5.24±0.52	6.29±0.43
7	5.27±0.42	5.54±0.55	4.49±0.56	4.78±0.86
8	4.42±0.39	4.93±0.41	3.94±0.51	4.00±0.31
9	3.79±0.25	4.18±0.66	3.56±0.20	3.52±0.16
10	3.62±0.22	3.41±0.47	3.27±0.24	3.36±0.23
11	3.29±0.18	3.18±0.35	3.08±0.16	3.15±0.29
12	3.06±0.19	-	3.00±0.13	-
Arm ratio ($\bar{x}$±S.D.)				
No.	Ps	Ls	Bk	By
1	1.3±0.50	1.3±0.20	1.3±0.14	1.2±0.13
2	3.7±0.75	1.2±0.13	1.3±0.23	1.3±0.17
3	-	1.5±0.22	1.4±0.20	3.0±0.60
4	-	8.0±0.67	4.7±1.50	2.1±1.30
5	1.5±0.07	7.3±1.37	4.5±1.50	3.0±1.30
6	-	6.6±1.09	4.2±1.40	4.2±1.00
7	-	2.4±0.25	4.1±1.24	3.7±0.30
8	-	5.5±1.95	3.1±1.49	3.7±1.60
9	-	-	3.6±0.75	4.1±0.38
10	-	-	3.6±0.78	4.0±2.02
11	-	-	3.6±0.98	4.5±1.56
12	-	-	3.1±0.81	-
Centromere position				
No.	Ps	Ls	Bk	By
1	M	M	M	M
2	ST	M	M	M
3	T	M	M	ST
4	T	T	ST	SM
5	M	T	ST	ST
6	T	ST	ST	ST
7	T	SM	ST	ST
8	T	ST	ST	ST
9	T	T	ST	ST
10	T	T	ST	ST
11	T	T	ST	ST
12	T	-	ST	-

a. Ps=*Pachyhynobius shangchengensis;* b. Ls=*Liua shihi;*
c. Bk=*Batrachuperus karlschmidti;* d. By=*B. yenyuanensis*

2). This is a unimodal and symmetrical karyotype, which is in accord with those of other species in the same genus (Zhu and Wei, 1981). The relative chiasma numbers do not vary with the relative lengths, for instance, the relative lengths of nos. 2 and 5 are 11.30 and 9.42, but their relative chiasma numbers are 9.14 and 9.63 respectively.

Paramesotriton.—P. chinensis (2n=24). Twelve bivalents were seen in the diakinetic cells. The diploid number of 24 (Fig. 2) is the same as that of *P. hongkongensis* (Morescalchi, 1975). The relative lengths and the relative chiasma numbers are

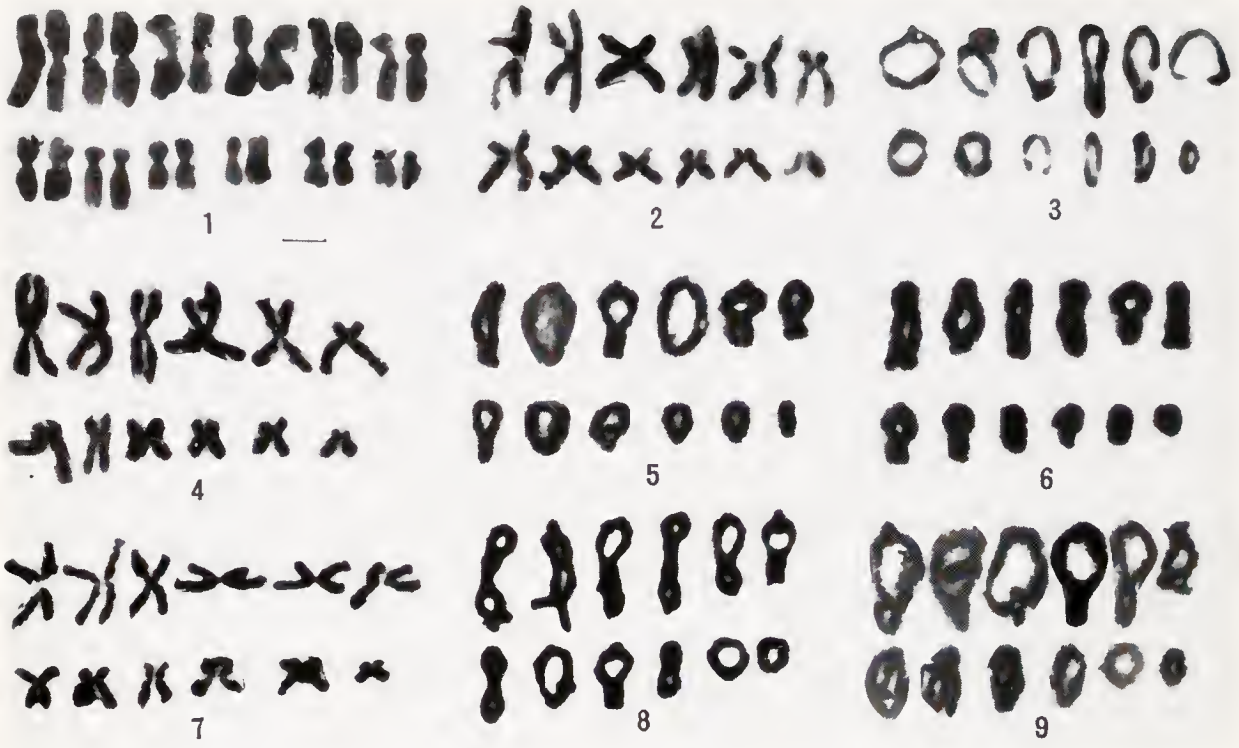

FIG. 2. Karyotypes of five species of salamandrid salamanders. 1-3) *Tylototriton kweichowensis*, mitotic chromosomes (1), chromosomes in metaphase II (2), and meiotic bivalents (3). 4, 5) *T. verrucosus*, chromosomes in metaphase II (4), and meiotic bivalents (5). 6) Meiotic bivalents of *Pachytriton labiatum*. 7, 8) *Cynops cyanurus yunnanensis*, chromosomes in metaphase II (7), and meiotic bivalents (8). 9) Meiotic bivalents of *Paramesotriton chinensis*.

shown in table 6. The relationship of chiasma numbers and the lengths of the bivalents is not a straight line, for example, the relative lengths of the nos. 2 and 12 are 11.89 and 3.55 respectively, while their relative chiasma numbers are the same, 8.10.

Discussion

It has been suggested from karyotypic data that Hynobiidae is the most primitive group of salamanders, while Salamandridae is the most advanced group in Caudata (Morescalchi, 1973, 1975; Morescalchi et al., 1979). The same conclusion is derived from morphological comparisons (Zhao and Hu, 1984). There are some differences in the evolutionary ways of the two groups.

Karyotypic Evolution in Hynobiidae

The bimodal (with macrochromosomes and microchromosomes) and asymmetrical (with metacentric and telocentric chromosomes) karyotype is considered to be primitive (Morescalchi, 1975) in reference to the karyotypic evolution in Caudata. Among some species of Hynobiidae, the diploid numbers are 40-66 and their karyotypes are bimodal and asymmetrical except for *Hynobius retardatus* [=*Satobius retardatus* (Adler and Zhao, 1990)], (Table 3). The karyotypes of the four species (in three genera) I studied here are all bimodal and asymmetrical, with 2n=64 or 68 chromosomes. *Batrachuperus* is one of 2 hynobiid genera in China that are aquatic for their entire lives and different from other genera morphologically. The diploid number of *B. karlschmidti* and *B. yenyuanensis* is 68, the second highest number among species of Caudata studied so far (Table 3). *Onychodactylus* is more derived and allied with *Ranodon* and

TABLE 5. Metaphase II chromosome data for 3 species of Salamandridae.

	Relative length ($\bar{x}$±S.D.)		
Chromosome No.	*T. verrucosus*	*T. kweichowensis*	*C. c. yunnanensis*
1	13.10±1.67	13.14±1.48	12.03±0.75
2	11.39±0.83	12.01±1.63	11.23±0.54
3	10.92±1.05	10.64±1.14	10.24±0.39
4	10.27±0.90	10.08±1.23	10.07±0.39
5	9.56±0.50	9.39±1.24	9.46±0.38
6	7.81±0.91	8.62±1.26	8.91±0.34
7	7.48±0.80	8.54±1.33	8.28±0.41
8	7.33±0.82	7.61±0.90	7.63±0.58
9	6.87±0.77	6.66±0.86	6.66±0.58
10	5.60±0.86	6.24±0.84	5.98±0.60
11	5.23±0.81	5.69±0.72	5.32±0.43
12	4.45±0.90	4.92±0.61	4.28±0.25
	Arm Ratio ($\bar{x}$±S.D.)		
Chromosome No.	*T. verrucosus*	*T. kweichowensis*	*C. c. yunnanensis*
1	1.2±0.15	1.2±0.20	1.3±0.11
2	1.3±0.17	1.3±0.23	1.2±0.14
3	1.5±0.53	1.3±0.28	1.2±0.08
4	1.4±0.10	1.2±0.10	1.3±0.19
5	1.4±0.13	1.4±0.43	1.4±0.32
6	1.7±0.36	2.1±0.55	1.3±0.39
7	1.9±0.76	1.4±0.44	1.5±0.89
8	1.7±0.58	1.8±0.24	2.0±0.26
9	1.6±0.26	1.6±0.24	1.6±0.28
10	1.4±0.26	1.5±0.25	1.6±0.39
11	1.4±0.05	1.7±0.24	1.6±0.38
12	2.5±0.81	2.6±1.11	1.5±0.32
	Centromere Position		
Chromosome No.	*T. verrucosus*	*T. kweichowensis*	*C. c. yunnanensis*
1	M	M	M
2	M	M	M
3	M	M	M
4	M	M	M
5	M	M	M
6	SM	SM	M
7	SM	M	M
8	SM	SM	SM
9	M	M	M
10	M	M	M
11	M	SM	M
12	SM	SM	M

Batrachuperus (Zhao and Hu, 1984), in accordance with the fact that *O. fischeri* has 2n>66 chromosomes (Sessions et al., 1982) and *O. japonicus* has 2n=78 (Yamamoto, 1982). The diploid number of *B. musteri* (Morescalchi et al., 1979), *B. pinchonii* and *B. tibetanus* (Yang and Zhao, 1984) is all 62, distinctly different from those of *B. karlschmidti* and *B. yenyuanensis*. Both *B. karlschmidti* and *B. yenyanensis* have 2n=68 chromosomes, but differ in chromosome component and morphology (Tables 3 and 4). The former has one more macrochromosome pair and one less microchromosome pair than the latter. In addition, the latter has one pair of subtelocentric chromosomes, while the former has none. It is obvious that the karyotypic evolution in *Batrachuperus* is more complex.

Pachyhynobius shangchengensis, which

TABLE 6. Bivalent data for 5 species of Salamandridae.

	Relative length ($\bar{x}$±S.D.)				
No.	Tv[a]	Tk[b]	Pc[c]	Pl[d]	Ccy[e]
1	12.49±0.83	12.47±1.79	13.27±1.25	12.62±1.08	11.92±0.31
2	11.65±0.79	11.33±0.64	11.89±0.82	11.85±0.53	11.30±0.51
3	11.07±0.62	10.53±0.69	11.01±0.45	11.09±0.29	10.58±0.37
4	10.24±0.54	9.99±0.76	10.10±0.68	10.25±0.50	10.10±0.45
5	9.36±0.52	9.32±0.56	9.60±0.88	9.57±0.47	9.42±0.29
6	8.66±0.50	8.71±0.35	8.67±0.64	9.11±0.48	8.83±0.31
7	7.80±0.62	7.81±0.55	7.95±0.67	8.02±0.49	8.16±0.25
8	7.23±0.57	7.39±0.72	7.30±0.60	7.49±0.54	7.46±0.47
9	6.28±0.48	6.50±0.72	6.35±0.33	5.97±0.41	6.60±0.24
10	5.77±0.39	5.86±0.63	5.65±0.43	5.36±0.49	5.86±0.27
11	5.25±0.38	5.41±0.80	4.66±0.54	4.85±0.49	5.42±0.32
12	4.21±0.45	4.67±0.66	3.55±0.64	3.79±0.36	4.37±0.47
	Relative chiasma number ($\bar{x}$±S.D.)				
No.	Tv	Tk	Pc	Pl	Ccy
1	10.29±1.62	7.96±1.32	10.94±1.67	11.20±2.13	11.65±1.33
2	8.29±2.11	8.91±1.22	8.10±0.32	10.00±1.73	9.14±2.21
3	9.36±1.93	8.47±0.41	8.65±1.30	9.23±1.73	9.16±2.36
4	8.81±1.23	8.47±0.41	8.67±1.50	8.53±1.86	9.77±2.05
5	7.92±0.57	8.91±1.22	8.10±0.32	7.82±0.22	9.63±2.10
6	8.44±1.73	8.47±0.41	8.10±0.32	8.12±1.24	7.82±0.90
7	7.38±1.31	8.47±0.41	7.48±1.39	7.73±0.30	7.92±1.70
8	7.92±0.57	7.98±1.42	7.53±1.59	7.73±0.30	7.23±1.01
9	8.89±1.90	8.47±0.41	8.10±0.32	7.32±1.13	6.92±0.72
10	7.38±1.31	8.47±0.41	8.10±0.32	7.32±1.13	7.21±0.75
11	7.92±0.57	8.47±0.41	8.10±0.32	7.73±0.30	6.92±0.72
12	7.40±1.42	6.95±2.89	8.10±0.32	7.73±0.30	6.92±0.72

a. Tv = *Tylototriton verrucosus*; b. Tk = *T. kweichowensis*; c. Pc = *Paramesotriton chinensis*; d. Pl = *Pachytroton labiatum;* e. Ccy = *Cynops cyanurus yunnansis.*

is a genus and species of Hynobiidae described on morphological characteristics (Fei and Ye, 1983), has 2n=64. The karyotype differs from those of other species in Hynobiidae, providing the cytogenetic evidence for establishing the new genus and species.

Liua is a genus established by Zhao and Hu (1983), based on morphological characteristics. *Liua shihii,* which is the only species, has 2n=64, the same as *P. shangchengensis*. However, there are some differences between them in chromosome component and morphology. *Liua shihii* has 11 pairs of macrochromosomes, including 3 pairs of metacentric, 1 pair of submetacentric, 2 pairs of subtelocentric, and 5 pairs of telocentric chromosomes, while *P. shangchengensis* has 12 pairs of macrochromosomes, consisting of 2 pairs of metacentric, 1 pair of subtelocentric and 9 pairs of telocentric chromosomes (Fig. 1 and Table 4).

The predominant mode of karyotypic evolution in Caudata is that the unimodal symmetrical karyotypes with low chromosome number are derived from the bimodal and asymmetrical karyotypes with high chromosome number, through 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, reduce the diploid number and/or the microchromosome number and increase 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.

B. karlschmidti and *B. yenyuanensis* possess 22 and 23 pairs of microchromosomes respectively and lack telocentric macrochromosomes. Contrastingly, *P. shangchengensis* and *L. shihii* have 21 and 20 pairs of microchromosomes and 5 and 9 pairs of telocentric macrochromosomes. It is concluded that the karyotypic evolution of the 4 species above has involved Robertsonian centric fusion as well as pericentric inversion. However, the phylogeny of the 4 species could not be established based on the present data. It is necessary to have information from chromosome banding and biochemistry in order to define the structures and functions of microchromosomes and telocentric chromosomes.

Karyotypic Evolution in Salamandridae

The 5 salamandrid species studied here all have 2n=24 chromosomes, lack microchromosomes, and possess unimodal and symmetrical karyotypes (Fig. 2, Tables 2 and 5) as consistent (Ferrier and Beetschen, 1973; Seto et al., 1986; Zhu and Wei, 1981). Morescalchi (1975) suggested that all species studied possess similar karyotypes that differ very little even at the intergeneric level. The differences between these karyotypes predominantly concern the absolute size of chromosomes and quantity of DNA. Accordingly, the karyotypic diversity among the species has chiefly resulted from pericentric inversions 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. The difference, which occured not only at the intergeneric level, but also at the intrageneric level, are as follows: 1. The data of chromosomes in mitotic metaphase: as shown in table 2, the no. 12 chromosomes of 3 species in *Tylototriton* are all subtelocentric, while the karyotypes of *C. orientalis* and *P. brevipes* have no subtelocentric chromosomes, only metacentric and submetacentric chromosomes. In addition, the chromosome differences in morphology were seen among 3 species in *Tylototriton*, 4 pairs of submetacentric chromosomes in *T. andersoni*, 3 pairs in *T. kweichowensis* and 2 pairs in *T. verrucosus*. 2. The data of chromosomes in meiotic metaphase II (Table 5): *C. cyanurus yunnanensis* has only 1 pair of submetacentric chromosomes, while there are 4 pairs in both *T. verrucosus* and *T. kweichowensis*. Numbers 6, 7, 8, and 12 are submetacentric chromosomes in *T. verrucosus* and nos. 6, 8, 11, and 12 in *T. kweichowensis*. 3. The data of bivalents in diakinesis (Table 6): the relative lengths of no. 1 bivalents of *T. verrucosus* and *T. kweichowensis* are similar, 12.49 and 12.47 respectively, but 13.27, 12.62 and 11.92 in *P. chinensis, P. labiatum* and *C cyanurus yunnanensis* individually. The relative chiasma numbers of no. 1 bivalents reveal the intergeneric and intrageneric variations. It is noteworthy that the interspecific and intraspecific variations of individual bivalents on relative chiasma number in 2 species of *Tylototriton* are more distinctive than those in other genera and species. The relative chiasma numbers are apparently not directly proportional to the relative lengths.

Tylototriton has karyotypically been considered to be the most primitive genus in Salamandridae, based on the fact that there are more subtelocentric chromosomes in mitotic metaphase, more submetacentric chromosomes in meiotic metaphase II, and more variations of relative chiasma numbers in diakinesis. The same conclusion was reached based on morphological comparisons (Zhao and Hu, 1984). However, as shown in table 2, *T. andersoni* has 2 more submetacentric chromosomes pairs (nos. 10-11) than *T. kweichowensis* and 1 more submetacentric chromosomes pair (no. 10) than *T. verrucosus*. The relative length of chromosome no. 1 is largest and the relative length of no. 12 is the shortest in *T.*

andersoni among the 3 species above. These data could provide cytogenetic evidence for reestablishing *Echinotoriton*. The mitotic chromosome number and morphology of *C. orientalis* are the same as those of *P. brevipes*, but the color, size and distribution of C-bands are different from each other. Consequently, the authors proposed that the differences of heterochromatic components and distributions on chromosomes of different genera could be the block of interspecific fertilization (Zhu and Wei, 1981).

Literature Cited

ADLER, K., AND E. ZHAO. 1990. Studies on hynobiid salamanders, with description of a new genus. Asiatic Herpetological Research 3:37-45.

FEI, L., AND C. YE. 1983. [A new genus and species of Hynobiidae from Henan, China.] Amphibian Research 1:1 (In Chinese).

FERRIER, V., AND J. C. BEETSCHEN. 1973. Étude des chromosomes de *Tylototriton verrucosus* Anderson et de l'hybride viable *Pleurodeles waltii* x *Tylototriton verrucosus* (Amphibiens Urodèles, Salamandridae). Chromosoma 42:57-69.

IMAI, H. T., AND K. MORIWAKI. 1982. A re-examination of chiasma terminalization and chiasma frequency in male mice. Chromosoma 85:439-452.

KEZER, J., AND S. K. SESSIONS. 1979. Chromosome variation in the plethodontid salamander, *Aneides ferreus*. Chromosoma 71:65-80.

KURO-O, M., C. IKEBE, AND S. KOHNO. 1987. Cytogenetic studies of Hynobiidae (Urodela) VI. R-banding patterns in five pond-type *Hynobius* from Korea and Japan. Cytogenetics and Cell Genetics 44:69-75.

LEVAN, A., K. FREGDA, AND A. A. SANDBERG. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201-222.

MAKINO, S. 1932. The chromosome number in some salamanders from Northern Japan. Journal of the Faculty of Science, Hokkaido Imperial University ser.6 (Zool.) 2:97-108.

MORESCALCHI, A. 1973. Pp. 233-259. *In* A. B. Chirelli and E. Capama (eds.), Cytotaxonomy and vertebrate evolution. Academic Press, New York.

MORESCALCHI, A. 1975. Chromosome evolution in the caudata Amphibia. Pp. 339-387. *In* T. Dobzhansky et al. (eds), Evolutionary Biology, vol. 8. Plenum Press, New York.

MORESCALCHI, A., G. ODIERNA, AND E. OLMO. 1977. Karyological relationships between the cryptobranchid salamanders. Experientia 33:1579-1581.

MORESCALCHI, A., G. ODIERNA, AND E. OLMO. 1979. Karyology of primitive salamanders, family Hynobiidae. Experientia 35:1434-1436.

PRINCEE, F. P. G., AND L. E. M. BOER. 1983. A new technique for obtaining chromosome preparations of small reptiles. Chromosome Information Service 34:3-5.

SCHMID, M. 1979. Chromosome banding in Amphibia. III. Sex chromosomes in *Triturus*. Chromosoma 71:29-55.

SESSIONS, S.K., P.E. LEON, AND J. KEZER. 1982. Cytogenetics of the Chinese giant salamander *Andrias davidianus* (Blanchard): The evolutionary significance of cryptobranchoid karyotypes. Chromosoma 86:341-357.

SETO, T., M. MATSUI, AND E. KAWAKAMI. 1986. Karyotypes and banding patterns of *Tylototriton andersoni* Boulenger, a newt endemic to the Ryukyu islands. The Japanese Journal of Herpetology 11:137-144.

WANG, X., J. FANG, AND X. TANG. 1983. [Preliminary observations on karyotype of *Salamandrella keyserlingii*]. Acta Herpetologica Sinica 2(2):19-22. (In Chinese).

WU, Z. 1982. A simple method for chromosome preparations from amphibian bone marrow. Hereditas (Beijing) 4(1):38-39. (In Chinese).

YAMAMOTO, T. 1982. Karyotypes of amphibians in Japan. Nippon Herpetological Journal 23:45.

YANG, Y., AND E. ZHAO. 1984. [Meiotic chromosomes and chromosome sets in male *Batrachuperus pinchonii* and *B. tibetanus*]. Pp. 59-65 *In* E. Zhao and Q. Hu (eds), Studies on

Chinese Tailed Amphibians. Sichuan Scientific and Technical Publishing House, Chengdu. (In Chinese).

YANG, Y., Q. HU, AND E. ZHAO. 1986a. [The cytotaxonomy and evolution of Salamandridae of China. I. The meiosis of *Tylototriton (T.) verrucosus* and *Cynops cyanurus yunnanensis*] Acta Herpetologica Sinica 5(2):90-93. (In Chinese).

YANG, Y., Q. HU, AND E. ZHAO. 1986b. [Studies on the karyotype of *Xenobius melanonychus* and its phylogenetic significance]. Acta Herpetologica Sinica 5(2):94-97. (In Chinese).

YANG, Y. 1990. [Karyotypic studies on nine species of Chinese salamanders]. Pp. 150-158. *In* E. Zhao (ed.) From water onto land. China Forestry Press, Beijing. (In Chinese).

ZHAO, E., AND Q. HU. 1983. [Taxonomy and evolution of Hynobiidae in western China, with a description of a new genus]. Acta Herpetologica Sinica 2(2):29-35. (In Chinese).

ZHAO, E., AND Q. HU. 1984. [Studies on Chinese tailed amphibians]. Sichuan Scientific and Technical Publishing House, Chengdu. 68 pp. (In Chinese).

ZHAO, E., Q. HU, Y. JIANG, AND Y. YANG. 1988. Studies on Chinese salamanders. Society for the Study of Amphibians and Reptiles, Contributions to Herpetology 4:1x, 1-67.

ZHAO, E., AND K. ADLER. 1989. [*Hynobius chinensis*, a re-description on its 100th anniversary]. Sichuan Journal of Zoology 8(2):18-20. (In Chinese).

ZHU, J., AND L. WEI. 1981. [The karyotypes, C-banding and indirect immunoperoxidase staining in *Cynops orientalis* and *Pachytriton brevipes*]. Acta Biologiae Experimentalis Sinica 14(3):281-191. (In Chinese).