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A Karyotypic Study on Six Korean Vespertilionid Bats

***Dong Ho Yoo and Myung Hee Yoon**

Department of Biology, Kyung Sung University, Pusan 608-736, Korea;
**Kyungnam Girls' Commercial School, Pusan 607-071, Korea*

The karyotypes of 4 Korean *Myotis* species (*M. mystacinus gracilis, M. formous tsuensis, M. daubentonii ussuriensis* and *M. macrodactylus*) and 2 *Pipistrellus* species (*P. coreensis* and *P. abramus*) belonging to the Vespertilionidae were examined. The 4 *Myotis* species had the karyotypes of 2n = 44 with FN = 50 (*M. m. gracilis* and *M. f. tsuensis*) or 52 (*M. d. ussuriensis* and *M. macrodactylus*). Furthermore the karyotype of *P. abramus* (2n = 26, FN = 44) seemed advanced compared with that of *P. coreensis* (2n = 44, FN = 50) which is similar to the original karyotype of *Myotis* species (2n = 44, FN = 50).

KEY WORDS: Karyotype, *Myotis, Pipistrellus,* Vespetilionids

As suggested by many authors (Capanna, 1968; Capanna and Civitelli, 1970; Baker *et al.*, 1974; Andō *et al.*, 1977, 1980; Bickham, 1979a, b; Andō, 1982; Harada and Uchida, 1982; Harada *et al.*, 1982), karyology would be regarded as a good taxonomic tool for better understanding of phylogenetic relationships, because of the conservatism of karyotypes against environmental factors and gradual transitions of chromosomal changes. However, karyotypic information on members of the Korean Chiroptera consisting of 21 species with 4 subspecies belonging to the 2 families, Rhinolophidae and Vespertilionidae (Yoon and Son, 1989) has been scanty; namely, only 5 species such as *Rhinolophus ferrum-equinum korai* (Lee and Son, 1988), *Myotis macrodactylus, Pipistrellus coreensis* [*P. savii coreensis*] (Park and Won, 1978), *Vespertilio superans* and *Miniopterus shcreibersii fuliginosus* (Oh, 1975) have been karyotyped.

The aim of the present study was to analyze the karyotypes of 4 Korean vespertilionid bats, and to discuss their phylogenetic relationships on the basis of the karyotypes as well as the conventional taxonomy studied by Tate (1942). Of the bats examined, *Myotis formosus tsuensis* was karyotyped for the first time, and *Myotis mystacinus gracilis, M. daubentonii ussuriensis* and *Pipistrellus abramus* had not been previously recorded in Korea. For the purpose of comparison, the karyotypes of *Myotis macrodactylus* and *Pipistrellus coreensis* also were analyzed.

Materials and Methods

Materials

A total of 19 male bats of 4 *Myotis* species and 2 *Pipistrellus* species were captured by mistnets. The scientific names of the materials examined and their localities are listed in Table 1. The specimens were identified by the aid of the descriptions of Ognev (1928), Corbet (1978), Yoshiyuki (1989) and Yoon and Son (1989). All the vòucher specimens were kept in the department of Biology, Kyung Sung University.

Chromosomal analyses

Metaphase chromosomes from bone marrow cells were prepared for analysis by a modification of the technique described by Tsuchiya (1974).

Before bone marrow cells from humeri were washed with 2 ml of isotonic NaCl solution, live bats were injected intraperitoneally with 0.01 ml of 0.1% colchicine per grm of body weight and kept for 3 hours. The cell suspension was centrifuged at 3,000 rpm for 5 minutes and the supernatant was discarded. Then, 5 ml of isotonic NaCl solution were added and the cell suspension was centrifuged at 2,000 rpm for 3 minutes. After supernatant was removed, 2 ml of 1% sodium citrate solution were added, and then the cell suspension was incubated at 37°C for 15 minutes. After 2 ml of a fixative (acetic acid: absolute alc. = 1:1) were added, the cell suspension was allowed to fix for 30 minutes at room temperature and centrifuged as before. Again, the supernatant was poured off and 4 ml of the same fixative were added. The fixation-centrifuge sequence was repeated twice, and then cells were resuspended in 0.1 ml of the same fixative. The blaze-drying method of Scherz (1962) was used for chromosomal preparation and slides were stained with 2% Giemsa in phosphate buffer at pH 7.0 for 10 minutes.

The chromosomes, in accordance with Patton (1967), were grouped into metacentrics or submetacentrics (M • SM), subtelocentrics (ST) and acrocentrics (A), and subsequently arranged in descending order of size. The determination of the diploid number (2n) was performed on about 22 metaphase cells in each species, and the fundamental number (FN) was defined as the total number of autosomal arms. Since *M. f. tsuensis* was karyotyped for the first time, the relative length of each chromosome was measured by the method of Árnason (1974).

Results

The karyotypes of 6 Korean vespertilionid bats are shown in Table 2. The detailed karyotypic pattern of each species is given below.

***Myotis mystacinus gracilis* (2n = 44, FN = 50)**

The autosomes consist of 3 pairs of large, 1 pair of small M • SM-elements and 17 pairs of medium to small A-elements (Fig. 1). The X chromosome is a medium-sized M • SM-element and the Y is a dot-like minute A-element.

***Myotis formosus tsuensis* (2n = 44, FN = 50)**

The karyotype of this species is identical with that of *M. m. gracilis* (Fig. 2). The relative length of chromosomes based on well dispersed 14 metaphase plates is shown in Table 3. In this Table, the M • SM-X chromosome is considered the fourth from the largest chromosome and the Y is the smallest element in the complement.

***Myotis daubentonii ussuriensis* (2n = 44, FN = 52)**

The autosomes consist of 3 large and 2 small pairs of M • SM-elements, and 16 pairs of

Table 1. Species, number examined and locality of 6 Korean vespertilionids (♂).

Species	N	Locality
Myotis mystacinus gracilis	2	Kwangsan-gun, Jeonranam-do
Myotis formosus tsuensis	3	Kwangsan-gun, Jeonranam-do
	2	Haenam-gun, Jeonranam-do
	1	Namhae-gun, Kyungsangnam-do
Myotis daubentonii ussuriensis	2	Yeongdeok-gun, Kyungsangbuk-do
Myotis macrodactylus	3	Haenam-gun, Jeonranam-do
	2	Seungju-gun, Jeonranam-do
Pipistrellus coreensis	2	Seungju-gun, Jeonranam-do
	1	Geochang-gun, Kyungsangnam-do
Pipistrellus abramus	1	Geochang-gun, Kyungsangnam-do

Table 2. Karyotype data on 6 species of Korean vespertilionids.

Species	2n	FN	Autosomes			Sex chromosomes	
			M • SM	ST	A	X	Y
Myotis mystacinus gracilis	44	50	4	0	17	M • SM	A
Myotis formosus tsuensis	44	50	4	0	17	M • SM	A
Myotis daubentonii ussuriensis	44	52	5	0	16	M • SM	A
Myotis macrodacylus	44	52	5	0	16	M • SM	M • SM
Pipistrellus coreensis	44	50	4	0	17	M • SM	A
Pipistrellus abramus	26	44	8	2	2	A	A

M • SM, metacentrics or submetacentrics; ST, subtelocentrics; A, acrocentrics.

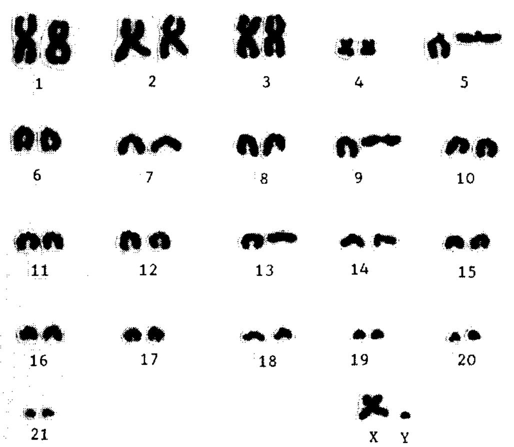

Fig. 1. Male karyotype of *Myotis mystacinus gracilis.*

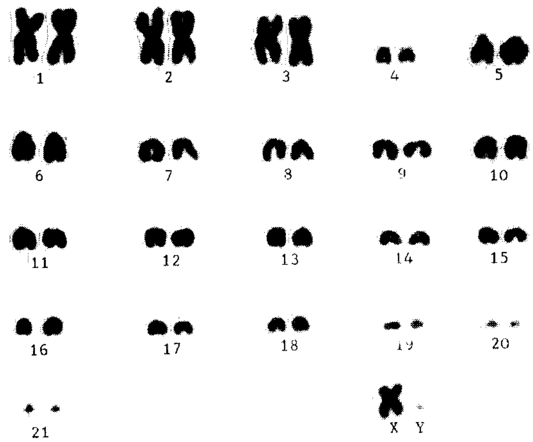

Fig. 2. Male karyotype of *Myotis formosus tsuensis.*

Table 3. Relative length of chromosomes in *Myotis formosus tsuensis* based on 14 metaphase plates.

Chromosome	Relative length		Chromosome	Relative length	
	Mean	SD		Mean	SD
M · SM-1	11.11	0.51	A-8	3.79	0.09
M · SM-2	10.30	0.37	A-9	3.55	0.10
M · SM-3	9.66	0.44	A-10	3.26	0.15
M · SM-4	3.32	0.16	A-11	3.06	0.17
A-1	5.93	0.30	A-12	2.89	0.20
A-2	5.49	0.33	A-13	2.72	0.24
A-3	4.96	0.15	A-14	2.48	0.23
A-4	4.61	0.12	A-15	1.81	0.24
A-5	4.35	0.13	A-16	1.46	0.20
A-6	4.18	0.11	A-17	1.31	0.23
A-7	3.99	0.15	M · SM-X	6.07	0.25
			A-Y	1.09	0.22

medium to small A-elements (Fig. 3). The X chromosome is a medium-sized M · SM-element and the Y is a small A-chromosome.

Myotis macrodactylus (2n = 44, FN = 52)

The karyotype is similar to that of *M. d. ussuriensis* in morphology except for the larger M · SM-Y chromosome (Fig. 4).

Pipistrellus coreensis (2n = 44, FN = 50)

The autosomes consist of 3 large and 1 small pairs of M · SM-elements, and 17 pairs of A-elements graded in size from medium to small (Fig. 5). The X chromosome is a medium-sized M · SM-element and the Y is a minute A-element. One pair of A-elements (no.11) is characterized by an achromatic region (secondary constriction) adjacent to the centromere (marker chromosomes).

Pipistrellus abramus (2n = 26, FN = 44)

The autosomes consist of 8 pairs of large to medium M · SM-elements, 2 pairs of large ST-elements and 2 pairs of A-elements (Fig. 6). The X chromosome is a medium-sized A-element and the Y is a minute one.

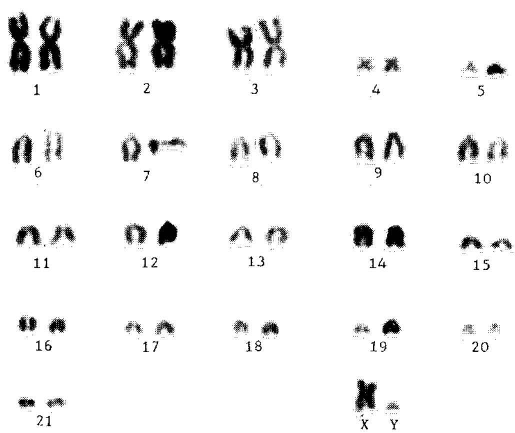

Fig. 3. Male karyotype of *Myotis daubentonii ussuriensis.*

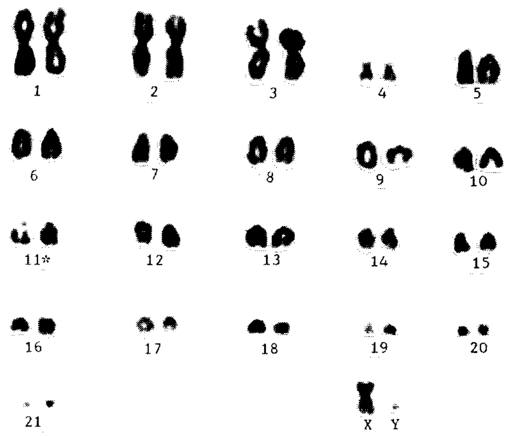

Fig. 5. Male karyotype of *Pipistrellus coreensis.*
* = marker chromosome.

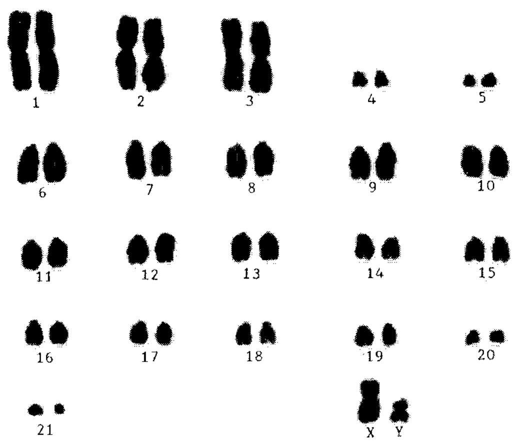

Fig. 4. Male karyotype of *Myotis macrodactylus.*

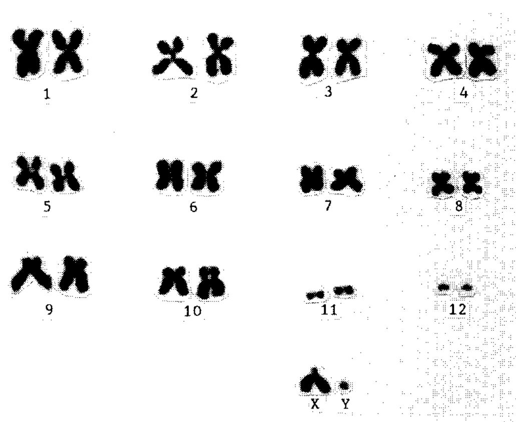

Fig. 6. Male karyotype of *Pipistrellus abramus.*

Discussion

It has been assumed that various karyotypes found in many species of the Vespertilioninae are mainly derived from that of a *Myotis*-like bat (2n = 44, FN = 50) by Robertsonian translocation (centric fusion or fission) (Baker and Patton, 1967; Capanna, 1968; Baker, 1970; Andō *et al.*, 1977; Bickham, 1979a, b). The karyotype of the genus *Myotis*, composed of more than 69 species with few interspecific differences in external morphology (cf. Koopman and Jones, 1970; Yoshiyuki, 1971), is extremely uniform. That is, all the 28 species examined, so far as we know, have 2n = 44 with FN = 50 or 52, and are generally characterized by having 3 pairs of large M · SM-elements (nos. 1-3), one or 2 pairs of small M • SM-elements (no. 4 or nos.4 and 5), 17 or 16 pairs of A-elements (nos. 5-21 or nos. 6-21) ranging from medium to small, a medium sized M · SM-X and a small A-Y or M · SM-Y chromosome.

Ten species including Russian *M. daubentonii* (Strelkov and Volobuev, 1969), Czechoslovakian *M. myotis* and *M. blythi* (Baker, 1970), Japanese *M. hosonoi* and *M. frater kaguyae* (Harada and Yosida, 1978), *M. macrodactylus* (Obara *et al.*, 1976a; Harada and Yosida, 1978; Andō, 1982) and *M. pruinosus* (Harada and Uchida, 1982), American *M. evotis*, *M. thysanodes* and *M. auriculus* (Bickham, 1979a), and Korean *M. daubentonii ussuriensis* and *M. macrodactylus* (this paper) have the karyotype with FN = 52. In some species such as *M. hosonoi*, *M. frater kaguyae* and *M. macrodactylus*, it is assumed that constitutive heterochromatin might has increased on the pair no. 5 of the original karotype (Harada and Yosida, 1978). According to Andō (1982), such an increase of constitutive heterochromatin on no. 5 chromosome in *Myotis* might occur in each subgenus and species, and the difference in size of this chromosome seems due to the difference in quantity of constitutive heterochromatin.

Only *M. macrodactylus* (2n = 44, FN = 50) has been karyotyped by Park and Won (1978), although 7 species of *Myotis* have been hitherto known in Korea (Yoon and Son, 1989; Yoon, 1990). According to Tate (1941), the 4 species of *Myotis* examined in this study belong to 3 subgenera, and their phylogenetic sequence in each subgenus might be proposed on the basis of chromosomal morphology as follows. As for members of the subgenus *Selysius*, the karyotype of *M. mystacinus gracilis*, which is the same with that of *M. mystacinus muricolor* (FN = 50, Andō, 1982), seems primitive as compared with the karyotypes of *M. hosonoi* (FN = 52) possessing tiny M · SM-5 and *M. frater* (FN = 52) having small M · SM-5 which is the same size as M · SM-4 (Harada and Yosida, 1978). In this connection, it is of interest that *M. frater* has some advanced characters in skeletal and dental morphology (cf. Ognev, 1928; Yoshiyuki, 1989). Furthermore, also in humeral morphology, *M. hosonoi* and *M. frater* are advanced in the order given, compared with *M. mystacinus* (Yoon *et al.*, 1984).

M. formosus tsuensis, belonging to the subgenus *Chrysopteron*, also had the primitive karyotype (2n = 44 and FN = 50), as contrasted with rather specialized external morphology such as a peculiar dichromatic wing-pattern and orange body hairs. This fact suggests that the karyotype is extremely stable, whereas the external characters are unstable.

As regards karyotypes of members belonging to the subgenus *Leuconoe* characterized by the large hind foot and distinctive protoconules on the upper molars, *M. d. ussuriensis* (FN = 52) was similar to *M. macrodactylus* (FN = 52) except for the shape of the Y chromosome. The M • SM-Y chromosome in *M. macrodactylus* is due to addition of C-band material to an A-Y chromosome as seen in *M. daubentonii ussuriensis* (Andō, 1982); thus, *M. macrodactylus* seems to be advanced in karyotype as compared with *M. d. ussuriensis*. In connection with the karyotype of *M. macrodactylus*, the fundamental number has been known as 52 by many authors (Sasaki and Hattori, 1970; Obara *et al.*, 1976a; Harada and Yosida, 1978; Andō, 1982) as well as in this study, except for the report of Park and Won (FN = 50, 1978). The detailed review is demanded for this species.

Pipistrellus, which consists of 53 species (Koopman and Jones, 1970) and widely distributes in both the New World and the Old World, has a great diversity of karyotypes. Five species such as *P. kuhli* (Capanna and Civitelli, 1966; Baker *et al.*, 1974), *P. savii* (Capanna and Civitelli, 1967), *P. coreensis* (Park and Won, 1978; this paper), *P. pipistrellus* and *P. nathusii* (Fedyk and Ruprecht, 1976) have the same karyotype (2n = 44 and FN = 50) as the primitive karyotype of *Myotis*, except for 1 pair of marker chromosomes. However, 2n values for 8 other species range from 26 (*P. abramus*) to 38 (*P. mimus*) with FN values of 44 (*P. abramus*) to 56 (*P. subflavus*) (Takayama, 1959; Baker and Patton, 1967; Pathak and Sharma, 1969; Peterson and Nagorsen, 1975; Obara *et al.*, 1976a, b; Ando *et al.*, 1977; this paper).

Accoriding to Tate (1941), the *Pipistrellus abramus* group (= *P. javanicus* group, Andō, 1982) consisting of 14 species is regarded as the most primitive group in the genus. However, his classification has been denied by the karyological study of Andō (1982). That is, members of the *javanicus* group, i.e. *P. endoi*, *P. javanicus* and *P. abramus*, characterized by bearing an A-X chromosome, have more advanced karyotype than the *P. pipistrellus* group, i.e. *P. p. pipistrellus* with 2n = 44 and FN = 50 (Fedyk and Ruprecht, 1976), *P. p. bactrianus* with 2n = 42 and FN = 48 (Vorontsov *et al.*, 1969) and *P. nathusii* with 2n = 44 and FN = 50 (Fedyk and Ruprecht, 1976), the *P. kuhli* group, i.e. *P. kuhli* with 2n = 44 and FN = 50 (Capanna and Civitelli, 1967) and the *P. savii* group, i.e. *P. savii* with 2n = 44 and FN = 50 (Capanna and Civitelli, 1967) and *P. coreensis* with 2n = 44 and FN = 50 (Park and Won, 1978; this paper). Namely, *P. abramus* (*javanicus* group) seems to have the advanced karyotype compared with that of *P. coreensis* (*savii* group), similar to the original karyotype of *Myotis* species with 2n = 44 and FN = 50.

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*유동호 • 윤명희(경성대 이과대 생물학과; *경남여상)

애기박쥐과에 속하는 한국산 박쥐류 *Myotis* 4종(*M. mystacinus gracilis*, *M. formosus tsuensis*, *M. daubentonii ussuriensis*, *M. macrodactylus*)과 *Pipistrellus* 2종(*P. coreensis*, *P. abramus*)의 핵형을 분석하였다. 조사한 *Myotis* 4종의 핵형은 2n = 44, FN = 50(*M. m. gracilis*, *M. f. tsuensis*) 또는 52(*M. d. ussuriensis*, *M. macrodactylus*)이었다. 또한, *P. abramus*의 핵형은 2n = 26, FN = 44였으며, *P. coreensis*(2n = 44, FN = 50)의 핵형과 비교해 볼 때, 후자의 핵형이 *Myotis*의 원시핵형(2n = 44, FN = 50)과 유사한 점으로 미루어, 전자가 후자보다 진화된 종으로 생각되었다.