A Karyotype Study in Chiroptera (Bats)*

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ABSTRACT

The bat (Chiroptera) is the only mammal that is able to fly as birds do and forms a peculiar taxonomic group in that the diploid number of chromosomes seldom are of the same number in the same genus and the different species in contrast to the other eutherian mammals. At the present time, many karyological problems remain unsolved in Korean bats. It is easy enough to imagine that many interesting things have happened in the chromosomes of the Korean bats as well. The present study was designed in order to get karyotypic data on living species of Korean bats (Vespertilio superans THOMAS and Miniopterus schreibersii fuliginosus (HODGSON).

The diploid number of chromosomes of the Vespertilio superans was 38. The autosomes consisted of 6 pairs of the large metacentric, a pair of the small submetacentric and 11 pairs of the small acrocentric chromosomes. The X chromosome was medium sized and metacentric in type and the Y was a small acrocentric type. The fundamental number was 50.

The diploid number of chromosomes of the Miniopterus schreibersii fuliginosus was 46. The autosomes consisted of 8 pairs of the metacentric type including a pair of minute metacentric chromosomes, and 18 pairs of the small acrocentric type chromosomes. The X chromosome was medium-sized and submetacentric, and the Y was a small acrocentric chromosome. The fundamental number was 52.

INTRODUCTION

Jepsen (1970) has described that bats are grouped into a vast number of peculiar taxonomic groupings, and therefore for an understanding of their origins and evolutions, special considerations in the application of primary taxonomic techniques to living as well as extinct forms will be needed. Although morphological characteristics of the eutherian mammals have long been of great use for genetic and phylogenetic studies, the newer karyological technique which has recently been developed is becoming one of the most important and basic tools by means of which many taxonomists are analyzing and identifying various species, including bats.

Relatively few karyological analyses of bats have been performed to data as compared with those of the other eutherian mammals. This probably is due to their peculiar ecology.

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some difficulties in getting specimens, handling them, and so on.

Karyotype analyses of Korean bats are rather rare at the present time, thus leaving us with a number of basic important tasks that remain to be solved in this area. The present study aims to get karyological data of two living species of Korean bats and to discuss their phylogenetic relationships with those obtained by other authors.

MATERIALS AND METHODS

Two species of Korean bats were used in the present study. One was Vespertilio superans THOMAS (Oriental discoloured or particoloured bats, Anzoo-aegoo-bakzui in Korean), and the other was Miniopterus schreibersii fuliginosus (HODGSON) (Japanese long-winged bats, Gin-garac-bakzui in Korean). The Vespertilio superans (V. superans) were captured on July 19, 1975 in the crevices of the tiled-roof of the Euchungboo Middle School, Euchungboo, Gyungi-Do, and the Miniopterus schreibersii fuliginosus (M. schreibersii fuli.) bats were captured on September 29, 1975 in the Baetaeae Cave, Hapchun-Goon, Kyungsangnam-Do.

The taxonomical positions of these are as follows:

Class-Mammalia
Order-Chiroptera
Suborder-Microchiroptera
Family-Vespertilionidae
Subfamily-Vespertilioninae Miniopterinae
Genus-Vespertilio Miniopterus
Species-superans schreibersii
Subspecies fuliginosus

One or two bats of both the male and female group in each species were supplied as materials for karyotype analyses. A modification of Ford and Hamerton’s procedure which was used in the technique of Baker (1970) was applied to the preparation of the chromosomes in this paper. The important procedures are summarized as follows:

1. Weigh the live bat and inject it intraperitoneally with 0.025% colchicine, 0.01 ml per gm. of body weight.
2. Sacrifice the bat 2 hours later and remove the red bone marrow from a chip of the humerus and flush this with a pipette using 3 ml of 1% sodium citrate solution.
3. Incubate the cell suspension at 37°C for 10~15 min.
4. Centrifuge the cell suspension at 1,500 rpm for 5 min.
5. Fix the materials in 3 ml of of Carnoy’s fixative in 4°C for 30 min. Then centrifuge again at 1,500 rpm for 5 min.
6. Resuspend in 0.5 ml of the fixative, and place two drops on clean and wet slides.
7. Pass slides through absolute alcohol promptly.
8. Stain with Giemsa’s stain for 2~3 min.
9. Wash with distilled water 2~3 times and air dry.
10. Observe and photograph with a Nikon binocular microscope fit up with a camera.

The chromosomes prepared in each karyogram were divided into three groups representing the metacentric (M), the submetacentric (SM), and the acrocentric (A) types, respectively, and they in turn are arranged according to the length of their arms and their forms. For the sake of convenience a numbering of the chromosomes was made and therefore all of the numerical notations used in this part of the study are completely arbitrary.

The diploid number (2N) of chromosomes was counted by means of the “most frequent rate of occurrence-method” described by Kang and Kim (1963). The fundamental number
(FN) standardized by Baker (1970) was used in the present study, which is used to represent the total number of the arms of the autosomes.

RESULTS

a) The Karyotype of *Vespertilio superans*

The occurrence of the diploid number and the fundamental number of chromosomes of *V. superans* are shown in Tables 1 and 2, and Figures 1 and 2. In a series of 100 karyotypes of *V. superans*, the diploid number of chromosomes which occurs most frequently (the mode) is 38 and consequently, this value was determined as the diploid number for the species. The range of chromosome numbers was 34 to 42 on a series of 100 bone marrow cells. The autosomes consisted of a series of 6 pairs of large metacentric chromosomes, a pair of small submetacentric chromosomes and 11 pairs of small acrocentric chromosomes including 2 pairs of microchromosomes. As to the sex chromosomes the X chromosome was medium-sized and metacentric, and the Y was a small acrocentric chromosome.

In the male karyotype, (Fig. 1) the first-six pairs of chromosomes were all metacentric type chromosomes and all long and large in size, and there were no remarkable differences between them. Then smaller and acrocentric chromosomes followed in turn to number 13. The number 14 chromosome was characterized by only one submetacentric chromosome pair and showed a remarkable reduction in size, and this was again followed by a
Table 1. Occurrence of Diploid Number (2N) of Chromosomes from Bone Marrow Cells in Two Species of Korean Bats

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>2N</th>
<th>Number of Cells Counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. superans</td>
<td>M</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>M. schreibersii ful.</td>
<td>M</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Occurrence of Chromosome Types and Fundamental Number (FN) of Chromosomes from Bone Marrow Cells in Two Species of Korean Bats

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Type</th>
<th>Autosomes</th>
<th>Sex Chromosomes</th>
<th>2N</th>
<th>FN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>SM</td>
<td>A</td>
<td>Total</td>
</tr>
<tr>
<td>V. superans</td>
<td>M</td>
<td>12</td>
<td>2</td>
<td>22</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>2</td>
<td>22</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>M. schreibersii ful.</td>
<td>M</td>
<td>8</td>
<td>0</td>
<td>36</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8</td>
<td>0</td>
<td>36</td>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

M—Metacentric, SM—Submetacentric, A—Acrocentric, FN—Fundamental Number

small acrocentric, number 15. The remaining chromosomes, numbers 16, 17 and 18 were tiny but distinct in their stainability and were thought to be acrocentric in type. The X chromosome was peculiar in various respects. First, it was more deeply stained by the Giemsa's stain than the autosomes. Secondly, it appeared to occupy a morphological position between the large metacentric group and the small acrocentric groups which comprised the two major chromosome groups of the karyotype. The Y chromosome was also peculiar in that its position in the karyogram would be evident by the fact that it was the last small acrocentric and first microchromosome. The number 7 pair of chromosomes was somewhat strange in that one seems to frequently have a secondary constriction at its tip and the other commonly appeared V-shaped, and thus made the pairing difficult.

Specific differences were not found in the female karyotype (Fig. 2) except that among the sex chromosomes one more X chromosome appeared instead of the Y.

The fundamental number of chromosomes of V. superans was 50. This consisted of 22 arms from the metacentric, 4 from the submetacentric and 24 from the acrocentric chromosomes.

b) The Karyotype of Miniopterus schreibersii fuliginosus

The occurrence of diploid number and fundamental number of M. schreibersii ful. are shown in Tables 1 and 2, and Figures 3 and 4. The diploid number was 46, based on the former criteria, and the range was 43 to 48. The autosomes consisted of a series of 4 pairs of metacentric chromosomes including a minute metacentric pair, number 22 and 18 pairs of acrocentric chromosomes including a pair of microchromosome, number 21. As to the sex chromosomes, the X chromosome was medium-sized and submetacentric, and the Y
was a small acrocentric and the Y was a small acrocentric chromosome.

In the male karyotype, (Fig. 3) the first two pairs of chromosomes were large and metacentric in type; number 11 was also metacentric but much smaller in size, and last metacentric type of chromosome was number 22. This microchromosome was so minute and faintly stained that I originally mistook it for an acrocentric chromosome but later the fundamental number 50 was also corrected to 52 by Uchida (1975). There were 18 pairs of the acrocentric chromosomes (number 3 to number 21) with an interruption of the series by number 11, the small metacentric. There were no troublesome difficulties in arranging the chromosomes in sequence. The X chromosome was placed in the transitional position where the large metacentric series and the small acrocentric series meet. The Y chromosome was placed between number 20 and number 22 where the microchromosomes begin to appear.

The fundamental number of *Miniopterus schreibersii fuliginosus* was 52. This consisted of 16 arms from the metacentric and 36 arms from the acrocentric chromosomes.

Any differences were not found in the female karyotype (Fig. 4) except that among the sex chromosomes one more X chromosome was observed instead of the Y.
DISCUSSION

Ando and Uchida (1974) have recently suggested that, based on their karyotype analysis on phylogenetic relationships in the Genus Rhinolophus (Horseshoe bats), the most primitive karyotype would be one which possessed the highest diploid number and in which all chromosomes were acrocentric. In the present study the diploid number of V. superans was 38, which is in agreement with the results obtained by Sasaki (1968). However, the fundamental number of V. superans was also similar although Sasaki (1968) has only indicated that the range of the fundamental number was between 50~54. This discrepancy seems to be due to some different interpretation of the number of arms of the autosomes. The karyotype features of V. superans in this study correspond well to the values reported by Baker (1970), in which he reports an average Chiroptera diploid number of 36.8 and an average fundamental number of 51.6. It is notable that Baker (1970) describes the ancestral diploid numbers and fundamental numbers and fundamental numbers of the Vespertilionidae as being probably between 44 and 50 with an FN of 50. Further the fundamental number 50 occurs most frequently for the family Vespertilionidae.

The diploid number and fundamental number of M. schreibersii fuliginosus was 46 and 52 respectively. These values are similar to those of species closely related, M. schreibersii (Natter in Kahl) (48 and 50 by Matthews and Bovey, 1948; 46 and 50 by Capanna and Civitelli, 1964b; 46 and 50 by Capanna and Civitelli, 1965). Although the diploid number of M. schreibersii ful. was relatively high with reference to the mean reported Chiroptera diploid number, 36.8, the fundamental number of M. schreibersii ful. was hardly different from the mean value, 51.6. Ando and Uchida (1974) have analysed the karyotypes from bone marrow cells of the Rhinolophus cornutus (Japanese lesser horseshoe bat) and Rhinolophus ferrumequinum nippon (greater Japanese horseshoe bat) and have obtained the results that the mechanism of karyotype evolution is mainly centric fusion of the chromosomes, which leads to a lowering of the diploid number with the concomitant formation of biarmed elements from uniarmed ones. If such a mechanism is true in the V. superans and M. schreibersii as well, it will, therefore, be of great significance to analyse the remaining species in both the V. superans and M. schreibersii ful. in future.

Although modifications of Ford and Hamerton’s procedures seem to work well in the present study, there still remains a technical problem in the interpretation of the fundamental number especially in regard to the microchromosomes such as number twentytwo chromosome of M. schreibersii ful. Even if the banding method were much better than the former method in accurately pairing the troublesome chromosomes, there might be still some difficulties in differentiating the metacentric from the acrocentric type because these microchromosomes are too minute to observe at the light microscope level and are faintly stained.

According to the Illustrated Encyclopedia of Fauna and Flora of Korea, there are 27 living species of bats in Korea, there are 27 living species of bats in Korea. Among these, only four species have been analysed by means of karyotypic evaluation including the two species in this paper. At the present time,
little is known about Korean bats in every respect in contrast to past studies done on foreign bats. More detailed and extensive investigations on the Korean bats should, therefore, be performed first of all on their ecological profiles including their geographic distribution, migration and their mode of life.

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REFERENCES


