Genetica Vol. 48,1: 1-3, 1978

A CHROMOSOMAL BANDING STUDY OF THREE SPECIES OF VESPERTILIONID BATS FROM YUGOSLAVIA

John W. BICKHAM[1] & John C. HAFNER[2]

Department of Biological Sciences and The Museum, Texas Tech University, Lubbock, Texas 79409, USA

Received May 24, 1977 / Accepted June 1, 1977

G-band and C-band patterns were studied for three species of vespertilionid bats (*Myotis myotis, M. oxygnathus*, and *Miniopterus schreibersi*) from Yugoslavia. Banding patterns of the two species of *Myotis* were identical. The major differences between the karyotypes of *Myotis* and *Miniopterus* can be explained by pericentric inversions in two chromosomes and a centric fission (or fusion) in one chromosome. No differences were found in constitutive heterochromatin.

Introduction

The study of vertebrate cytogenetics was greatly advanced by the discovery of Caspersson et al. (1969) that banding patterns could be produced on eukaryotic metaphase chromosomes. This allows homologous chromosomes to be identified by their unique banding pattern. Banding techniques are now important tools for biologists studying many aspects of cytogenetics including systematics and evolution (Bickham & Baker, 1976).

Studies of chromosomes have led to a better understanding of systematic and phylogenetic relationships of bats and other groups (Baker, 1970; Capanna & Civitelli, 1973) but only a few studies have made use of banding techniques (Bickham, 1976; Mascarello et al., 1974; Pathak et al., 1973; Stock, 1975). Chromosome banding data are presented for *Myotis myotis* Kaup, *M. oxygnathus* Monticelli, and *Miniopterus schreibersi* (Bonaparte) from Yugoslavia. Taxonomic and phylogenetic implications are discussed.

Material and methods

A single male of each species was studied. The bats were collected from natural populations and tissue biopsies were taken in the field. The biopsied tissues were returned to Texas Tech University where fibroblast cultures were established and grown in Ham's F-10 medium, fortified with 10% fetal calf serum. Karyotypes were prepared by using Velban (Lilly) as a mitotic inhibitor at a rate of 0.075 mg Velban per ml growth medium for 15 minutes. The cells were treated in hypotonic solution (1 part growth medium; 2 parts water) for 15 minutes, and fixed in 3:1 (methanol:acetic acid) fixative. Air dried slides were prepared.

The G-band technique is that of Seabright (1971) and utilized fresh slides which had been warmed on a slide warmer (60°C) for 2 hours. The C-band technique is a modification of Sumner 's (1972) technique. Slides were allowed to set overnight at room temperature before being treated with 5% $Ba(OH)_2$ for 4 minutes at room temperature, rinsed, and placed in 1 x SSC (60°C) for 30 minutes. The slides were then stained with Giemsa (2%) in phosphate buffer pH7.

Results

Two species of *Myotis* (*M. myotis*, and *M. oxygna-*

[1] Present address: Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843.
[2] Present address: Museum of Vertebrate Zoology, University of California, Berkeley, California 94720.

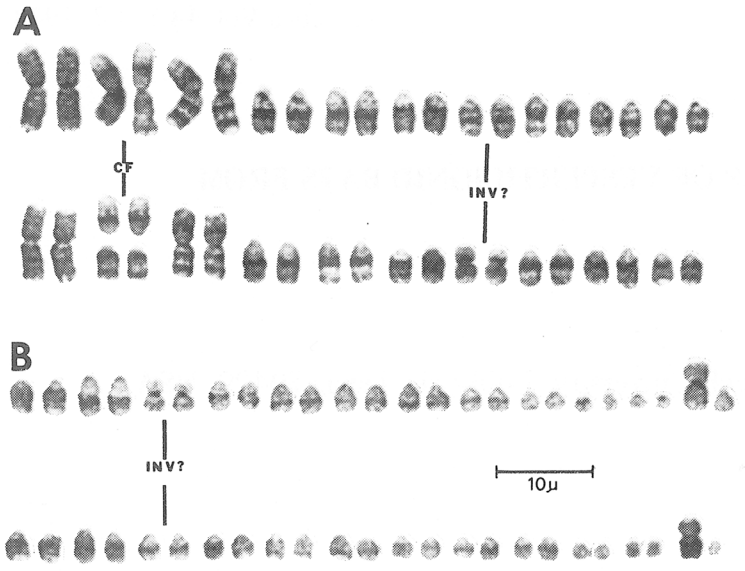

Fig. 1. G-band karyotypes of *Myotis myotis* and *Miniopterus schreibersi*. CF means centric fission or centric fusion, INV means inversion. A is the largest 10 pair of autosomes of *M. myotis* (top row) and their presumed homologues of *Miniopterus* placed beneath. B is the 11 smallest pairs of autosomes of *M. myotis* (top row) and the presumed homologues of *Miniopterus* placed beneath. The sex chromosomes are placed at the end of the karyotypes. X1000.

thus) were studied. G- and C-band patterns for these two species are identical. Both species possess 2n = 44 chromosomes. There are three pairs of large and one of small biarmed autosomes, and 17 pairs of acrocentric autosomes. The X is submetacentric, the Y is acrocentric and the same size as the third smallest autosome. Each chromosome pair is identifiable by its unique G-band pattern (Fig. 1). Constitutive heterochromatin, as identified by the C-band technique, is restricted to the centromeric regions of the autosomes and X, the Y is mostly heterochromatin.

Miniopterus schreibersi possesses 2n = 46 chromosomes. There are two large, one medium, and one small pair of biarmed autosomes and 18 pairs of acrocentric autosomes. The sex chromosomes are a submetacentric X and an acrocentric Y the size of the third smallest autosome. The C-band pattern of *Miniopterus* is identical to that of Myotis described previously. G-band karyotypes are shown for *Myotis myotis* and *Miniopterus schreibersi* in Fig. 1.

Discussion

The two genera of vespertilionids studied belong to different subfamilies. *Myotis* is in the subfamily Vespertilioninae and *Miniopterus* is in the subfamily Miniopterinae (Koopman & Jones, 1970). Despite the distant taxonomic relationship between these two genera, there is a remarkable similarity in the banding patterns of the chromosomes. In both genera C-band heterochromatin is restricted to the centromeric regions of the autosomes and the X, the Y is mostly heterochromatic. The G-band patterns indicate that a single centric fission (or fusion) and two pericentric inversions are the major differences between the karyotypes of the two genera. The sex chromosomes and 18 pairs of autosomes appear to be mostly identical between the two genera based on G-band pattern.

There are some additional slight differences between the chromosomes of the two genera. The longest acrocentric chromosome of *Myotis* has a small but consistently discernable, G and C negative second arm. This is absent in our specimen of *Miniopterus*. The smallest autosome in *Miniopterus* is biarmed and is acrocentric in the two species of *Myotis*. The difference is possibly due to a pericentric inversion. We were unable to demonstrate a positive C-band on either arm but the addition of a heterochromatic arm cannot entirely be ruled out as the region is so small. Bickham (1976) reported this chromosome varies, as either an acrocentric or metacentric, between different species of North American *Myotis*. The variation is explained by the addition of a small piece of heterochromatin in *M. thysanodes* and *M. evotis*.

Bickham (1976) examined the banding patterns of 11 genera of vespertilionid bats and concluded that the karyotype of *Myotis* is most likely the primitive karyotype for the family. This is in agreement with Capanna & Civitelli (1970). *Myotis* is geologically the oldest extant vespertilionid genus and is generally considered to be morphologically primitive (Handley, 1959). *Miniopterus* is also a geologically old genus and the two genera may have evolved from a common ancestor early during the evolution of vespertilionid bats. This early differentiation was accompanied by only a few chromosomal changes and the karyotypes of extant *Miniopterus* and *Myotis* closely reflect what the karyotype must have been in the primitive stock from which they evolved. Other genera of vespertilionids have modified their karyotypes mainly by Robertsonian mechanisms (Baker & Patton, 1967; Bickham, 1976; Capanna & Civitelli, 1970). The early evolutionary divergence of *Miniopterus* and *Myotis*

was accompanied by a rapid morphological divergence on the part of *Miniopterus*. The morphological differences are sufficient to support placing the two genera in different subfamilies. The karyotypes are a more conservative character and reflect the phylogenetic relationship of *Miniopterus* and *Myotis*.

The karyotypes of vespertilionid bats seem to be evolutionarily conservative in general. The genus *Myotis* is a good illustration of this. *Myotis myotis* and *M. oxygnathus* from Yugoslavia were found to be karyotypically identical based on banding pattern. In addition, Bickham (1976) examined the banding patterns of 8 New World species of *Myotis* and found only minor differences in the size of the heterochromatic Y and in the smallest autosome. This conservatism is also reflected in the fact that intraspecific and intrageneric chromosomal variation is uncommon in this family (Baker, 1970; Baker & Patton, 1967; Capanna & Civitelli, 1970). Although our sample size is low we have made every effort to be sure of our presumed interspecific homologies. Several banded karyotypes were prepared for each species and in addition 10-25 banded spreads were examined through the microscope. No intra-individual variation was found, the banding patterns were always consistent from cell to cell. Thus, we feel assured that the data presented are representative of the three species studied.

Appreciation is gratefully extended to Nikola Turtkovic and Patricia G. Dolan for aid in the collection of the specimens. The study was supported from a grant to Drs. Dilford C. Carter and J. Knox Jones, Jr. from the Smithsonian Foreign Currency Program. Dr. John Morrow provided the use of certain laboratory equipment from NIH grant 1-RO1-CA-16207-01. Support was also obtained from NSF grant GB-41105 to Drs. Robert J. Baker and Hugh H. Genoways.

References

Baker, R.J. (1970). Karyotypic trends in bats. In: Biology of Bats Vol. I. W.A. Wimsatt, ed. Academic Press, New York, xii + 406 pp.

Baker, R.J. & J.L. Patton (1967). Karyotypes and karyotypic variation of North American vespertilionid bats. *J. Mammal.* 48: 270-286.

Bickham, J.W. (1976). Chromosomal banding and phylogenetic relationships of vespertilionid bats. Ph.D. thesis, Texas Tech University, Lubbock, Texas, 72 pp.

Bickham, J.W. & R.J. Baker (1976). Chromosome homology and evolution of emydid turtles. *Chromosoma* (Berl.) 54: 201-219.

Capanna, E. & M.V. Civitelli, (1970). Chromosomal mechanisms in the evolution of chiropteran karyotype. Chromosomal tables of Chiroptera. *Caryologia* 23: 79-111.

Caspersson, T., L. Zech, E.J. Modest, G.E. Foley, U. Wagh & E. Simonsson (1969). Chemical differentiation with fluorescent alkylating agents in Vicia faba metaphase chromosomes. *Exp. Cell Res.* 58: 128-140.

Handley, C.O., Jr. (1959). A revision of American bats of the genera Euderma and Plecotus. *Proc. U. S. natn. Mus.* 110: 95-246.

Koopman, K.F. & J.K. Jones, Jr. (1970). Classification of bats. In: About Bats. B.H. Slaughter and D.W. Walton eds. Southern Methodist Univ. Press, Dallas, vii + 339 pp.

Mascarello, J.T., A.D. Stock & S. Pathak (1974). Conservatism in the arrangement of genetic material in rodents. *J. Mammal.* 55: 695-704.

Pathak, S., T.C. Hsu & L. Shirley (1973). Chromosome homology in the climbing rats, genus Tylomys (Rodentia: Cricetidae). *Chromosoma* (Berl.) 42: 215-228.

Seabright, M. (1971). A rapid banding technique for human chromosomes. *Lancet*, 1971, II: 971-972.

Stock, A.D. (1975). Chromosome banding pattern homology and its phylogenetic implications in the bat genera Carollia and Choeroniscus. *Cytogenet. Cell Genet.* 14: 34-41.

Sumner, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* 75: 304-306.