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Chromosomes of some Species of Vespertilionid Bats

III. Banded Chromosomes of *Vespertilio murinus* L., 1758

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With 3 Figures

Introduction

It is a considerable time ago relative that attention was drawn to the fact that karyotypes of *Vespertilionidae* representatives differ in respect of the number of chromosomes, whereas the number of arms of autosomes in the majority of *Vespertilionidae* species is the same ($NFa = 50$) (cf. review in CAPANNA and CIVITELLI 1970). There is thus no doubt that the basis for differentiation of the chromosome formula in *Vespertilionidae* consisted of centric fusions. Conventionally stained chromosomes made it impossible to conclude which of the chromosomes (or arms of chromosomes) are homologous with each other. It was not until the technique of differential staining of chromosomes was applied (chiefly the G-banding technique) that it became possible to identify accurately the different arms of chromosomes. BICKHAM (1979 a, b) introduced model nomenclature for chromosome arms in various North American species of *Vespertilionidae*, and this nomenclature has been successfully applied in studies on European species (ZIMA 1982, FEDYK and RUPRECHT 1983 a, b). It was found that different arms of chromosomes in Palaearctic and Nearctic species have the same banding patterns.

Up to the present time descriptions have been given, on the basis of conventionally stained preparations, of chromosomes in three species of the genus *Vespertilio*. The chromosomes of *V. murinus* have been described by VORONTSOV et al. (1969) and ZIMA (1978). The karyotype of *V. orientalis* was described by OBARA and SAITOH (1977), ANDO et al. (1977) and TSUCHIYA (1979). The chromosomes of *V. superans* are known from the descriptions of VORONTSOV et al. (1969), ANDO et al. (1977) and ANDO et al. (1980). An identical chromosome formula was found in all three species: $2N = 38$; $NFa = 50$.

OBARA and SAITOH (1977) and ANDO et al. (1980) also differentially stained the chromosomes of *V. orientalis* and *V. superans*. They used the C-banding technique, which does not produce sufficiently accurate differentiation of the different chromosome arms. In the present paper the G-banding technique has been used for the first time in differential staining of the chromosomes of *Vespertilio murinus*.

Material and methods

Seven male *V. murinus*, caught during the years 1968–1976 at Białowieża (Białystok Province) — $52^{\circ}42' N$, $23^{\circ}51' E$ — were used for the studies. The chromosomes obtained from these individuals were conventionally stained with lacto-aceto-orceine. One male *V. murinus* was caught on August 10, 1983 at Kowal

(Włocławek Province) — 52°32' N, 19°09' E. The chromosome preparations of this individual were stained by the G-banding method. Thirty minutes before anaesthetizing, the animal was given an intraperitoneal injection of the Colchicéos preparation in amounts of 0.002 mg per 1 gram of body weight. Preparations were made from the spleen and bone marrow, using a 15-minute hypotonic shock in 0.075 M of KCl solution at room temperature. The cells of the spleen and marrow were fixed in Carnoy's fluid (1 : 3 glacial acetic acid and ethanol). The preparations were made by the air-drying method. The chromosomes were conventionally stained with lacto-aceto-orceine, while G-bands were obtained after digesting with trypsin and staining with a buffered Giemsa solution.

Results

38 chromosomes were found in all the males examined, i. e. 6 pairs of large two-armed autosomes with centromeres localized in a median or submedian position, one pair of small autosomes with submedial centromeres and 11 pairs of

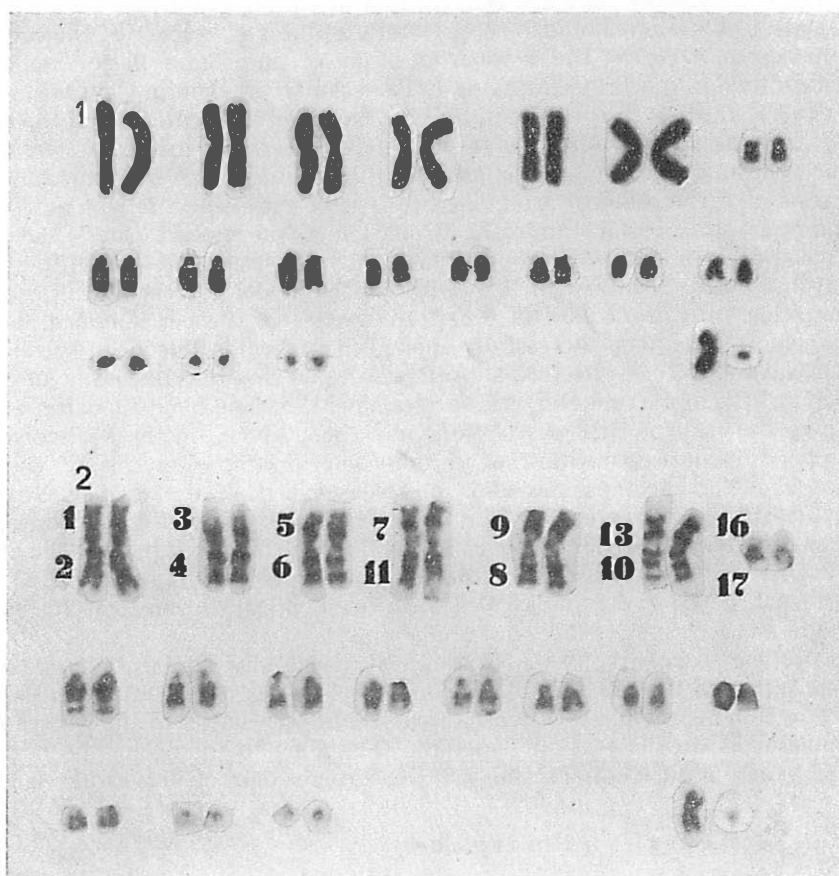


Fig. 1. The karyotype of *Vespertilio murinus* (♂), 2N = 38; NFa = 50

Fig. 2. Banding pattern of *Vespertilio murinus* chromosomes. The chromosomes are numbered after BICKHAM (1979 a)

single-armed autosomes of gradually decreasing dimensions, among which the two smallest pairs of autosomes must be considered as dot-like chromosomes. The second smallest pair of single-armed autosomes has a distinct secondary constriction. Chromosome X is submetacentric, slightly larger than the longest single-armed autosomes, while chromosome Y is a dot-like chromosome (Fig. 1). The number of arms of autosomes in *V. murinus* is thus $NFa = 50$.

The individual arms of the chromosomes were identified by comparing the banding patterns obtained with the patterns given by BICKHAM (1979 a, b) and the model karyotype of *Eptesicus serotinus* and European *Plecotus* and *Barbastella* (FEDYK and RUPRECHT 1983 a, b). The numerical nomenclature of arms (1–25) introduced by BICKHAM (1979 b) was used.

It was found that the composition of arms on 7 two-armed autosomes is as follows: no. 1 connected with no. 2, no. 3 connected with no. 4, no. 5 with no. 6, no. 7 with no. 11, no. 8 with no. 9, no. 13 with no. 10, no. 16 with no. 17. The remaining eleven pairs of single-armed autosomes have the following numeration: 12, 14, 15, 18, 19, 20, 21, 22, 23, 24, 25. The two-armed X chromosome also has the characteristic system of bands identical with that observed in other species of *Vespertilionidae* (Fig. 2).

Discussion

The chromosome formula of *V. murinus* found in the present study ($2N = 38$; $NFa = 50$) is identical with the formula for this species given from the Ussuri region (VORONTSOV et al. 1969). ZIMA (1978), on the other hand, gives $NFa = 52$ for the *V. murinus* population from Czechoslovakia. The difference arises as this author has treated one pair of dot autosomes as two-armed chromosomes. Identical chromosome formulae ($2N = 38$; $NFa = 50$) were found for two Asian species: *Vespertilio orientalis* (OBARA and SAITOH 1977, ANDO et al. 1977, TSUCHIYA 1979) and in *Vespertilio superans* (VORONTSOV et al. 1969, ANDO et al. 1977, 1980). Thus the genus *Vespertilio* is not differentiated in respect of the chromosome formula.

C-banding centromeric heterochromatin examined on the chromosomes of *V. orientalis* (OBARA and SAITOH 1977) and *V. superans* (ANDO et al. 1980) has the same system in both species. The pericentric bands revealed allowed us to draw conclusions as to elimination of centromeric heterochromatin during fusion processes of chromosome arms. This method is, however, not appropriate for distinguishing the different arms of chromosomes, and consequently we have no direct proof that the other two species (*V. superans* and *V. orientalis*) have a combination of arms identical with that of *Vespertilio murinus*. Nevertheless this conclusion would appear correct. It is most probable that processes of specific divergence within the genus *Vespertilio* were not accompanied by divergence of the karyotype.

The karyotype occurring in contemporary species of the genus *Eptesicus* was taken as the ancestral karyotype for *Vespertilionidae*, i. e.: $2N = 50$; $NFa = 48$ (FEDYK and RUPRECHT 1983 a). By means of centric fusion and single pericentric inversion the karyotype characteristic of the majority of species of the genus *Myotis* arose from this karyotype. As a result of these aberrations 4 two-armed chromosomes were formed: 1/2, 3/4, 5/6, 16/17 ($2N = 44$; $NFa = 50$) — BICKHAM (1979 b). The same stage of evolution of the karyotype has been attained by several species of *Pipistrellus* (ZIMA 1982) and *Nyctalus fuscus* (HARADA et al. 1982). These species also have each 44 chromosomes, and among the autosomes four pairs of two-armed chromosomes with arm combinations 1/2, 3/4, 5/6, 16/17.

It has been suggested that all species in which the 1/2, 3/4, 5/6, 16/17 combination of arms was found, have passed through the *Myotis* evolutionary stage and are defined as *Myotini* sensu lato (FEDYK and RUPRECHT 1983 b). The genus *Vespertilio* has also passed through the *Myotini* s. l. stage, during a certain period the ancestors of *Vespertilio* had a karyotype identical with contemporary species of *Myotis* ($2N = 44$; $NFa = 50$). Several radiations appeared at this stage of evolution of *Vespertilionidae* chromosomes. The ancestors of the contemporary species of the genus *Vespertilio* exhibited three further fusions, giving two-armed chromosomes with the arm combinations 7/11, 9/8 and 13/10, which resulted in reduction of $2N$ to 38. Fusion between 13 and 10 also took place in *Nyctalus lasiopterus* ($2N = 42$; $NFa = 50$) – HARADA et al. (1982). It is difficult to decide whether this aberration took place independently in *N. lasiopterus* and *Vespertilio*, or whether the whole genus *Vespertilio* together with *N. lasiopterus* and probably also *N. noctula*, passed through this evolutionary stage of the karyotype. This second possibility suggests a close relationship between *Vespertilio* and *Nyctalus*.

The arm combination 13/10 also occurs in *Rhogeessa tumida* from Honduras ($2N = 34$; $NFa = 50$), on the other hand, in the same species from Trinidad occur the arm combination 11/7 ($2N = 30$; $NFa = 50$) – BICKHAM and BAKER (1977). It must, however, be considered that these two fusions in *Rhogeessa tumida* took

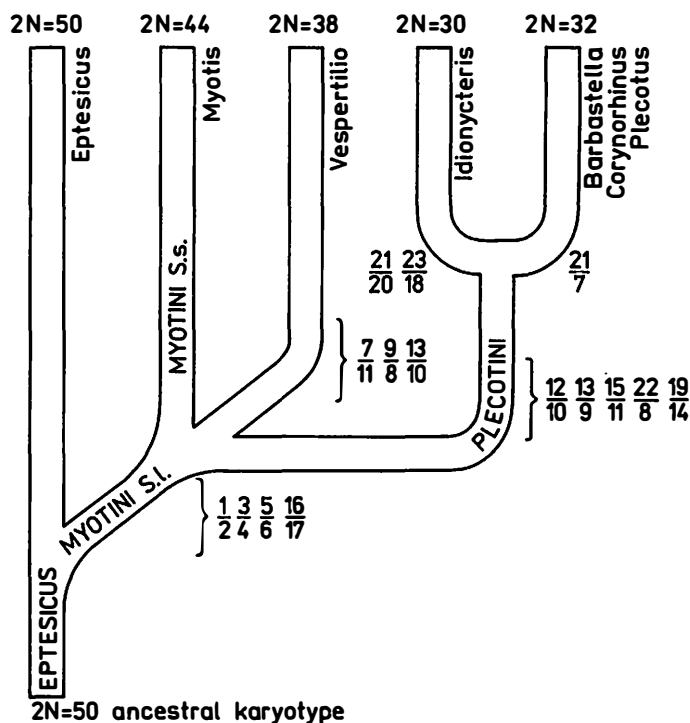


Fig. 3. Hypothetical differentiation of evolutionary lineages of Vespertilionid karyotypes. Capital letters denote 6 stages (evolutionary lines) of the karyotype; fractions represent arm combinations evolving in particular lineages

Table 1. Chromosome arm composition of two-armed chromosomes in some representatives of vespertilionid bats

Species	Arm composition													References
<i>Eptesicus</i> sp.	—	—												BICKHAM (1979 a), FEDYK and RUPRECHT (1983 a)
<i>Miniopterus schreibersi</i>	1/2 5/6													BICKHAM and HAFNER (1978)
<i>Nycticeius humeralis</i>	3/4 (inv)	8/7												BICKHAM (1979 a)
<i>Myotis</i> (11 species)	1/2 3/4 5/6 16/17													BICKHAM (1979 b)
<i>Pipistrellus</i> (3 species)	1/2 3/4 5/6 16/17													ZIMA (1982)
<i>Nyctalus fuscus</i>	1/2 3/4 5/6 16/17													HARADA et al. (1982)
<i>Nyctalus lasiopterus</i>	1/2 3/4 5/6 16/17	13/10												HARADA et al. (1982)
<i>Lasiurus</i> (5 species)	1/2 3/4 5/6 16/17	11/10 14/7 13/8 12/9 19/18 20/15 25/22 21/16-17 acro												BICKHAM (1979 a)
<i>Vespertilio murinus</i>	1/2 3/4 5/6 16/17	13/10 7/11 9/8												this paper
<i>Barbastella Plecotus</i> (<i>Plecotus</i>) <i>P.</i> (<i>Corynorhinus</i>)	1/2 3/4 5/6 16/17	12/10 13/9 15/11 22/8 19/14 21/7												BICKHAM (1979 a), FEDYK and RUPRECHT (1983 b)
<i>Plecotus</i> (<i>Idionycteris</i>)	1/2 3/4 5/6 16/17	12/10 13/9 15/11 22/8 19/14 21/20 23/18												BICKHAM (1979 a)
<i>Rhogeessa tumida</i> (Honduras)	16/17	7/11 15/2 10/4 13/8 5/1 14/19 23/3 22/12 20/8 21/9												BICKHAM and BAKER (1977)
(Trinidad)	16/17	13/10 15/4 11/1 14/19 23/3 22/12 20/8 21/9												BICKHAM and BAKER (1977)

place independently of *Vespertilio*, since the genus *Rhogeessa* forms an independent line in respect of chromosomes, did not pass through the *Myotini* stage and has only one chromosome (16/17) typical of *Myotis*.

The third radiation is formed by *Plecotini* (genera *Barbastella* and *Plecotus* with the subgenera *Idionycteris*, *Corynorhinus* and *Plecotus*). In these forms two-armed chromosomes occur: 1/2, 3/4, 5/6, 16/17 and 5 pairs common to the whole of *Plecotini* (12/10, 23/9, 15/11, 22/8 and 19/14). Further karyological divergence within *Plecotini* consists of the formation of a two-armed chromosome 21/7 in *Barbastella* and the subgenera *Corynorhinus* and *Plecotus*, while in *Idionycteris* two fusions appeared, giving chromosomes 21/20 and 23/18 (BICKHAM 1979 a, Fedyk and Ruprecht 1983 b). As the combination of arms 7/11, 9/8 and 13/10 (typical of *Vespertilio*) does not occur in representatives of *Plecotini* it must be considered that the radiation in *Vespertilio* was completely independent of *Plecotini* (Fig. 3, Table 1).

It must be remembered that certain species represent, from the karyological aspect, a stage intermediate between *Eptesicus* and *Myotis* (cf. Table 1), e.g. *Miniopterus schreibersi* ($2N = 46$; $NFa = 50$) has only two pairs of two-armed autosomes with an arm combination of 1/2 and 5/6 (BICKHAM 1979 a). Also in *Nycticeius humeralis* ($2N = 46$; $NFa = 48$) only two two-armed autosomes occur, but they have an arm combination of 3/4 and 8/7, arm no. 4 being inverted (BICKHAM 1979 a). Species of the genus *Lasiurus* may also be allocated to the intermediate stage, even though they have as many as 11 two-armed autosomes ($2N = 28$; $NFa = 48$), but only chromosomes 1/2, 3/4, and 5/6 occur among them, while the inverted chromosome 16/17 as a single arm has undergone fusion with arm no. 21 (BICKHAM 1979 a). In *Rhogeessa* also, despite the fact that 9–11 fusions of this kind have taken place, only chromosome 16/17, typical of the *Myotini* s. l. stage, occurs (BICKHAM and BAKER 1977).

These intermediate stages should be treated as independent radiations, stemming directly from the ancestral *Eptesicus*-like trunk.

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Summary

Conventional and differential staining of chromosomes revealed that the chromosome formula of *Vespertilio murinus* L., 1758 from the Białowieża and Kujawy populations takes the form of $2N = 38$; $NFa = 50$. Its karyotype consists of 6 pairs of large-size two-armed autosomes with centromeres localized in the medial or submedial position, one pair of small-size autosomes with submedial centromeres and 11 pairs of single-armed autosomes of gradually decreasing size. Chromosome X is submetacentric, slightly larger than the longest single-armed autosomes, while chromosome Y is dot-like. Differential staining (G bands) showed that 7 two-armed pairs of chromosomes in *V. murinus* have the follow-

ing composition of arms: 1/2, 3/4, 5/6, 16/17 (these 4 pairs are typical of a large number of *Vespertilionidae* species) and the remaining 3 pairs the following arm composition: 13/10, 7/11, 9/8 are characteristic of the species examined. The results of differential staining provided a basis for discussing the probable evolution mechanisms of the chromosome formulae in *Vespertilionidae*, taking the karyotype of contemporary species of the genus *Eptesicus* ($2N = 50$; $NFa = 48$) as ancestral.

Zusammenfassung

Herkömmliche und spezifische Chromosomenfärbung (G-banding) zeigten, daß die Chromosomenformel von *Vespertilio murinus* L., 1758 der Populationen von Białowieża und Kujawy $2N = 38$, $NFa = 50$ ist. Der Karyotyp besteht aus 6 Paaren großer, zweischenklicher Autosomen, bei denen die Centromere medial oder submedial lokalisiert sind, einem Paar kleiner Autosomen mit submedialen Centromeren und 11 Paaren einfacher Autosomen von graduell abnehmender Größe. Das x-Chromosom ist submetazentrisch, wenig größer als das längste einfache Autosom, während das y-Chromosom kugelförmig ist. Spezifische Färbungen (G-bands) erwiesen, daß 7 zweischenkliche Chromosomenpaare von *V. murinus* die folgende Zusammensetzung der Schenkel haben: 1/2, 3/4, 5/6, 16/17 (diese 4 Paare sind typisch für eine große Zahl von Vespertilioniden-Arten), die verbleibenden 3 Paare die Zusammensetzung 13/10, 7/11, 9/8, was für die untersuchte Art charakteristisch ist. Die Ergebnisse der spezifischen Färbungen liefern eine Grundlage für die Diskussion der wahrscheinlichen Evolutionsmechanismen der Chromosomenformel der *Vespertilionidae*, wobei der Karyotyp der rezenten Arten der Gattung *Eptesicus* ($2N = 50$; $NFa = 48$) als ursprünglich betrachtet wird.

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