ACTA THERIOLOGICA
Vol. 32, 18: 307—314, 1987

Chromosomes of Some Species of Vespertilionid Bats IV. New Data on the Plecotine Bats

Henryk LENIEC, Stanisław FEDYK & Andrzej L. RUPRECHT

Leniec H., Fedyk S. & Ruprecht A. L., 1987: Chromosomes of some species of verspertilionid bats IV. New data on the plecotine bats. Acta theriol., 32, 18: 307—314 [With 1 Table & Plate X].

The chromosomes from three individuals of *Plecotus austriacus* (Fischer, 1829) collected in Central Poland were studied. Set 2N=32, and the pattern of G-bands was identical as that in *Plecotus auritus* (Linnaeus, 1758) and *Barbastella barbastellus* (Schreber, 1774) were found. The phylogenetic relationships within *Plecotini* tribe were re-assessed according to thesew ne data.

[Laboratory of Genetics and Evolutionary Biology, University of Warsaw, Branch in Białystok, Sosnowa 64, 15-887 Białystok (H. L. & S. F.) and Mammals Research Institute, Polish Academy of Sciences, 17-230 Białowieża (A.L.R.), Poland]

1. INTRODUCTION

The taxonomic status of the European representatives of genus *Plecotus* was established by Bauer (1956). As early as at the beginning of the 19th century it was noted, however, that the European populations of *Plecotus auritus* were not of uniform character (Jenyns, 1828). It was not before 20th century when some of them were given status of subspecies. Those that were then described, among others *Plecotus auritus wardii* Thomas, 1911 and *Plecotus auritus meridionalis* V. et E. Martino, 1940 were regarded by Bauer (1956) as analogous with *Vespertilio auritus austriacus* Fischer, 1829 from Austria. As the differences between *Plecotus auritus* and the above subspecies were of full specific rank, Bauer (1956) suggested re-naming the second one *Plecotus austriacus* (Fischer, 1829). There are differences between the two species suggesting their full isolation (Topál, 1958; Bauer, 1956; Hanák, 1962, 1966; Ruprecht, 1965, 1969, 1983; Stebbings, 1967).

The karyotype of *P. auritus* was first described by Matthey & Bovey (1948) and Bovey (1949), who determined 2N=32, NFa=50, NF=54. The data have been confirmed on the material from Poland (Fedyk & Fedyk, 1970) and Czechoslovakia (Zima, 1978). The identical chromosomal formula has been found in *P. austriacus* (Fedyk & Fedyk, 1970, 1971) and in *B. barbastellus* (Capanna *et al.*, 1968; Fedyk & Ruprecht, 1983b).

The karyotype analysis carried out on conventionally stained material

suggested differentiation in three autosomal pairs and X-chromosome. The measurements revealed differences in centromere positioning (Fedyk & Fedyk, 1971). Such differences may result from imperfection of the method used or may materially exist as, for example, the result of a pericentric inversion. Using a differential staining method may provide much more detailed information.

Bickham (1979a, b) introduced numbering of autosome arms, from 1 to 25 on the basis of G band pattern in the nearctic vespertilionids. Zima (1982) and Fedyk & Ruprecht (1983a) found that such numbering might be used in palaearctic species as well because of full homology in band patterns of autosome arms in the representatives of *Vespertilionidae* from both zoogeographic regions. Using Bickham's nomenclature of arms, Fedyk & Ruprecht (1983b) proved that G band patterns in *P. auritus* and *P. barbastellus* were identical. In light of these results, the differentiating between *P. auritus* and *P. austriacus* (suggested by Fedyk & Fedyk (1971)) may be questioned. This study has been undertaken to clarify this uncertainity by analysing material stained by a differential method.

2. MATERIAL AND METHOD

The material used in this study consisted of three young males of *P. austriacus* collected on August 10, 1083 at Kowal locality (52°32'N, 19°09'E, Włocławek Province). Chromosome preparations were made from the bone marrow and spleen. Prior to anesthetics, the animals were administered colcemide, peritoneally, in 0.002 mg dose per 1 g of body weight per 0.5 h. Hypotonization was carried out in 0.075 M KCl for 0.5 h in room temperature. The preparations were then air dried, and, after a week, digested in trypsin and next stained by Giemsa stain according to Seabright's (1971) method. The description of karyotype follows nomenclature applied by Bickham (1979a, b).

3. RESULTS

The karyotype of *P. austriacus* was compared with that of previously described *P. auritus* (Fig. 1, Plate X). The number of chromosomes is equal for both species: 2N=32, the number of arms NF=54. Differential staining revealed the following combination of arms in metacentric autosomes: 1/2, 3/4, 5/6, 16/17, 15/11, 12/10, 13/9, 22/8, 19/14, 21/7. It was identical to that found in *P. auritus* (*cf.* Fedyk & Ruprecht, 1983b). Among ten pairs of two-arm autosomes the sequence of arms could be firmly established for the following six pairs: 1/2, 3/4, 5/6, 16/17, 15/11, 12/10. More problems were posed by attempted determination of arm

sequence in the remaining pairs. These covered two groups of arms that are difficult to discriminate: 7, 8 and 19, 21, 22. They form metacentrics: 13/9, 21/7, 22/8, 19/14 whose band patterns were fuzzy on plates of lower quality.

The X-chromosome is undoubtedly identical in respect to G band pattern in both European *Plecotus* species. It has a characteristic large dark block within the longer arm. The shorter arm has a distinct band in the centre.

The one-arm autosomes and Y-chromosome have less discernible bands nevertheless they are comparable in the two species. They have been labelled as follows: 18, 20, 23, 24, 25.

4. DISCUSSION

Studying conventionally stained material, Fedyk & Fedyk (1971) found that *P. auritus* and *P. austriacus* differed in the centromere position in the following chromosomes: m1, m3, Sm2 and X (their nomenclature). In Bickham's nomenclature these are: m1=1/2, m3=12/10, Sm2=22/8, X=X. Identification of Sm2 chromosome was the most difficult task. Fedyk & Fedyk (1971) distinguished in karyotypes only two submetacentric autosomes hence, according to Bickham's classification, these could be only 22/8 and 21/7. Since arm 22 is smaller than 21, and arm 8 is smaller than 7 thus the whole chromosome 22/8 is smaller and must correspond to Sm2 according to nomenclature given by Fedyk & Fedyk (1971). The measurements of acrocentric autosomes and of Y-chromosome did not revealed any discernible differences between the species. This was confirmed by band patterns obtained in this study.

The results reported by Fedyk & Fedyk (1971) point out similarities in chromosomes of *P. auritus* and *P. austriacus* except for four questioned pairs. The results of this study did not provide any data suggesting lack of homology between chromosomes in these two species. The agreement of band patterns in all the chromosomes allows conclusion that the karyotypes in *P. auritus* and *P. austriacus* and hence *B. barbastellus* are identical. The differences found by Fedyk & Fedyk (1971) should be explained rather by imperfection of the method applied. Various degree of spiralization may render chromosome length measurement unreliable. Yet the presence of pericentric inversions of very short chromosome sections cannot be excluded. Stock (1983) pointed out the high proportion of perimetacentric inversions in the evolution of *Vespertilionidae*. Such inversions might actually be more frequent than suggested by recent data because they could be detected only in pre-

parations of quite elongated chromosomes, and because some of the near--centromere inversions could be virtually undetectable. In light of these recent findings the idea of possible exchange of genetic material between nonhomologous chromosomes suggested by Fedyk & Fedyk (1971) must be discarded.

Volleth (1985) presented Q-banded karyotypes of *P. auritus* and *B. barbastellus* from Bavaria. Arm combination reported by Volleth (1985) differs largely from that described by Fedyk & Ruprecht (1983b) (Table 1). It is generally accepted that bands G and Q are comparable although bands Q are always less distinct than G. Hence it does not seem probable that the material from Bavaria may have different arm sequence from those collected in Poland.

It may be thus assumed that the palaearctic forms of *P. auritus*, *P. austriacus*, and *B. barbastellus* have identical band patterns and 2N while nearctic forms of *Plecotini* are more diversified (*cf.* also Iyad & Hoffmeister, 1983). Bickham (1979a) found, on the basis of G bands, that the difference between *Plecotus (Corynorhinus) townsendi* and *Plecotus (Idionycteris) phyllotis* appeared only in respect to two chromosomes. Stock (1983) showed banded karyotypes of three nearctic species of *Plecotini: P. (Corynorhinus) townsendi, P. (Idionycteris) phyllotis* and *Euderma maculatum*. This author, however, has not applied Bickham's nomenclature thus making difficult any comparisons with other studies. It may be suspected that Stock (1983) considered the high number of pericentric inversions occurring in *Vespertilionidae* as a sufficient reason for abandoning uniform numbering of autosome arms. As the band patterns in *P. townsendi* seems to be identical in both Bickham's (1979a) and Stock's (1983) accounts we attempted to apply Bickham's nomenclature (1979a) to the results reported by Stock (1983) (Table 1).

The results obtained by Stock (1983) indicate even more diversity among nearctic vespertilionid bats than found by Bickham (1979a). According to Stock (*l.c.*) *P. (Corynorhinus) townsendi* and *P. (Idinycteris) phyllotis* differ from each other in as many as six pairs of metacentric autosomes. He also found differences between *Euderma maculatum* and *P. (Idionycteris) phyllotis* pertaining to two pericentric inversions (Table 1). Owing to exceptionally high quality of G bands shown in Stock's (*l.c.*) study, his results should be judged more accurate.

Fedyk & Ruprecht (1983b) and Stock (1983) have independently suggested similar models of karyotype evolution within *Vespertilionidae*, assuming that the ancestral form of this group of bats had 50 acrocentric chromosomes (as in recent *Eptesicus*). This possibility was also discussed by Bickham (1979a) but it was abandoned (on account of far advancement of morphological evolution in *Eptesicus*) in favour of

Table 1

Comparison of band patterns on chromosomes from palaearctic and nearctic representatives of *Plecotini*. Data from Stock (1983) are arranged according to the nomenclature of chromosome arms proposed by Bickham (1979b) (see text for explanation).

Reference	Band pattern on chromosomes														
							Plecotus auritus								
Volleth (1985)	**1/2**	3/4	5/6	16/17	12/9	14/11	15/10	19/7	21/8	18/13	**20**	**22**	23	24	25
Fedyk & Ruprecht (1983b)	1/2	3/4	5/6	16/17	15/11	12/10	13/9	22/8	19/14	21/7	18	20	23	24	25
							Plecotus austriacus								
This paper	1/2	3/4	5/6	16/17	15/11	12/10	13/9	22/8	19/14	21/7	18	20	23	24	25
							Barbastella barbastellus								
Fedyk & Ruprecht (1983b)	1/2	3/4	5/6	16/17	15/11	12/10	13/9	22/8	19/14	21/7	18	20	23	24	25
							Plecotus (Corynorhinus) townsendi								
Bickham (1979a)	1/2	3/4	5/6	16/17	15/11	12/10	13/9	22/8	19/14	21/7	18	20	23	24	25
Stock (1983)	1/2	3/4	5/6	16/17	15/11	12/10	13/9	22/8	19/14	21/7	18	20	23	24	25
							Plecotus (Idionycteris) phyllotis								
Bickham (1979a)	1/2	3/4	5/6	16/17	15/11	12/10	13/9	22/8	19/14	23/18	21/10	7	24	25	—
Stock (1983)	1/2	3/4	5/6	16/17	15/11	13/10	21/9	19/8	22/12	18/14	23/20	7	24	25	—
							Euderma maculatum								
Stock (1983)	1/2	3/4 inv.	5/6	16/17	15/11	13/10	21/9	19/8	22/12	18/14	23/20	7 inv.	24	25	—

choosing the karyotype of recent *Myotis* as the ancestral one. More discussion about the correlation between the evolution of karyotype and that of morphological traits can be found in an earlier work (Fedyk & Ruprecht, 1983b).

Recently available data on G band patterns in chromosomes of *Euderma maculatum* (Stock, 1983), as well as the revision of G band pattern in *P. (Idionycteris) phyllotis* (Stock, 1983) made us to review critically our earlier model of karyotype evolution within *Plecotini* tribe (Fedyk & Ruprecht, 1983b). In light of these new findings we accept the model of evolution proposed by Stock (1983) for this group of bats broadening its scope to encompass the palaearctic species as well.

Hence 2N=50 karyotype should be regarded as ancestral for *Vespertilionidae* (*cf.* Fedyk & Ruprecht, 1983b, 1985), *Myotini* group (*sensu lato*) separated very early. It had three centric fusions: 1/2, 3/4, 5/6 and the inversion that created pair 16/17. Meanwhile the initial line evolved morphologically without concurrent chromosomal alternations resulting in emergence of recent *Eptesicus* species. On the other hand, part of *Myotini* (*sensu lato*) remained morphologically conservative (recent species of genus *Myotis*).

From a *Myotini* (*sensu lato*) stem, separated the *Plecotini* group with a metacentric 15/11 to divide later into two branches. One branch includes recent species of *Barbastella*, *Plecotus*, and nearctic representatives of *Plecotus* (*Corynorhinus*) which acquired fusions: 12/10, 13/9, 22/8, 19/14, 21/7. The other branch embraces two nearctic species: *Plecotus* (*Idionycteris*) *phyllotis* and *Euderma maculatum* sharing 15/11 metacentric with all *Plecotine*, and having fusions: 13/10, 21/9, 19/8, 22/12, 18/14, 23/20. Differentiation between them consists of two pericentric inversions (Table 1).

It should be inferred that *Euderma* and *Plecotus* (*Idionycteris*) took over Nearctic region having already acquired fusion 15/11 while *Plecotus* (*Corynorhinus*) did so not earlier than after getting fusions 15/11, 13/9, 22/8, 19/14 and 21/7. The pericentric inversion in X-chromosome in subgenus *Idionycteris* must have occurred within the Nearctic region.

Acknowledgements: We wish to thank Mr. J. M. Wójcik for his help in laboratory work, and Mr. L. K. Ruprecht for help in collecting bats. Separate thanks are due Miss H. Bałachowicz for comprehensive assistance with field work.

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Received 23 March 1986, Accepted 21 June 1986.

Henryk LENIEC, Stanisław FEDYK & Andrzej L. RUPRECHT

CHROMOSOMY KILKU GATUNKÓW MROCZKOWATYCH IV. NOWE DANE DOTYCZĄCE *PLECOTINI*.

Streszczenie

Zbadano chromosomy trzech samców gacka szarego, *Plecotus austriacus* (Fischer, 1829) odłowionych w Kowalu (woj. włocławskie). Potwierdzono wcześniej ustaloną liczbę chromosomów u tego gatunku (2N=32). Zastosowanie barwienia różnicującego (prążki G) potwierdziło całkowitą homologię ramion autosomów i chromosomów płci u europejskich *Plecotini* (rodzaje *Plecotus* i *Barbastella*). Przedyskutowano filogenetyczne zależności między reprezentantami trybu *Plecotini*. Nowe dane kariologiczne dotyczące prążków na chromosomach *P.* (*Idionycteris*) *phyllotis* i *Euderma maculatum* podane przez Stocka (1983), pozwoliły zrewidować wcześniejsze (Fedyk & Ruprecht, 1983b) poglądy na ewolucję *Plecotini*. Dywergencja *Euderma* i *Idionycteris* nastąpiła we wczesnym stadium ewolucyjnym *Plecotini*, po uzyskaniu fuzji ramion autosomów 15/11.

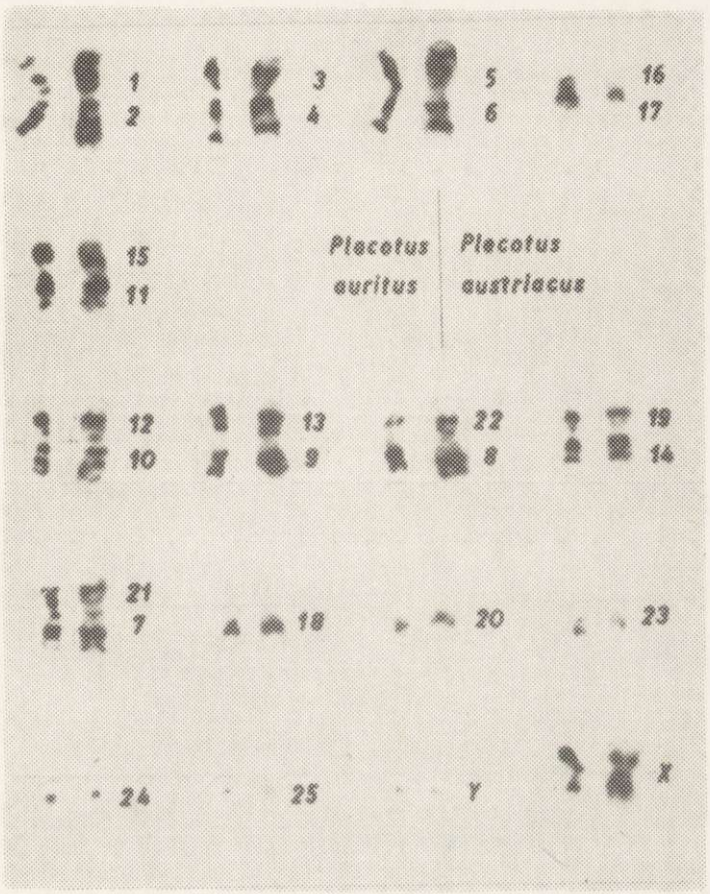

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Fig. 1. Band patterns on chromosomes of *P. auritus* (left) and *P. austriacus* (right).