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

Henryk LENIEC, Stanislaw FEDYK & Andrzej L. RUPRECHT


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 (Schneider, 1774) were found. The phylogenetic relationships within Plecotini tribe were re-assessed according to these new data.

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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 sub-species. Those that were then described, among others Plecotus auritus wardii Thomas, 1911 and Plecotus auritus meridionalis V. 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 (Topal, 1958; Bauer, 1956; Hanák, 1962, 1966; Ruprecht, 1965, 1969, 1983; Stebbings, 1967).

The karyotype of P. auritus was first described by Mathey & 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 (Fediyk & Fediyk, 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 neartic vesperilionids. Zima (1982) and Fediyk & Ruprecht (1983a) found that such numbering might be used in palaeartic 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, Fediyk & Ruprecht (1983b) proved that G band patterns in P. auritus and T. barbastellus were identical. In light of these results, the differentiating between P. auritus and P. austriacus (suggested by Fediyk & Fediyk (1971)) may be questioned. This study has been undertaken to clarify this uncertainty 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, 1983 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. Fediyk & 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. bar-
bastellus* 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 palearctic 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 *Ple-
cotini*: *P. (Corynorhinus) townsendi*, *P. (Idionycteris) phyllotis* and *Euder-
ma maculatum*. This author, however, has not applied Bickham’s nomen-
clature 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 aban-
doning 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). Ac-
cording to Stock (l.c.) *P. (Corynorhinus) townsendi* and *P. (Idionycteris)
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 sug-
gested 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 I

Comparison of band patterns on chromosomes from palaeartic 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).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Band pattern on chromosomes</th>
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<tr>
<td></td>
<td>Plecotus auritus</td>
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<tr>
<td>Volleth (1985)</td>
<td>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</td>
</tr>
<tr>
<td>Fedyk &amp; Ruprecht (1983b)</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>Plecotus austriacus</td>
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<td></td>
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</tr>
<tr>
<td>This paper</td>
<td>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</td>
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<tr>
<td></td>
<td>Barbastella barbasterllus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fedyk &amp; Ruprecht (1983b)</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td>Plecotus (Corynorhinus) townsendi</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Bickham (1979a)</td>
<td>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</td>
</tr>
<tr>
<td>Stock (1983)</td>
<td>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</td>
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<tr>
<td></td>
<td>Plecotus (Idiomycteris) phyllatis</td>
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<tr>
<td>Bickham (1979a)</td>
<td>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 —</td>
</tr>
<tr>
<td>Stock (1983)</td>
<td>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 —</td>
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<tr>
<td></td>
<td>Euderma maculatum</td>
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<td></td>
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</tr>
<tr>
<td>Stock (1983)</td>
<td>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 —</td>
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<td>inv.</td>
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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 *Euder-
ma 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 *Vesperti-
lionidae* (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 (Idiony-
cteris) 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 *Plec-
ottus (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.

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CHROMOSOMY KILKU GATUNKÓW MROCZKOWATYCH
IV. NOWE DANE DOTYCZĄCE PLECOTINI.

Streszczenie

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