CHROMOSOMAL HOMOLOGY AND EVOLUTION OF PHYLLOSTOMATOIDS

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Abstract

Patton, J. C., and R. J. Baker (Department of Biological Sciences and The Museum, Texas Tech University, Lubbock, Texas 79409) 1978. Chromosomal homology and evolution of phyllostomatoid bats. Syst. Zool. 27:449-462.-G- and C-banding analyses were used to determine chromosome homology from representatives of bat families Mormoopidae, Noctilionidae and Phyllostomatidae, subfamily Phyllostomatiniae. The karyotype of Macrotus waterhousii 2n = 46, FN = 60 is proposed as primitive for the Phyllostomatoidea. The major strategy involved in karyotypic evolution of taxa analyzed appears to have been reduction of the number of autosomal linkage groups by Robertsonian fusion from the 22 of Macrotus waterhousii to near 15 for the majority of taxa. Chromosome arms were found to be highly conserved. The X chromosomes of all taxa did not vary and were similar to the pattern thought to be characteristic of the primitive X of mammals. Two systematic groups appear recognizable within the Phyllostomatoidea above the family level (Phyllostomatiniae in one group and the Noctilionidae and Mormoopidae in the other), whereas three systematic groups are discernible within the Phyllostomatiniae. The three groups within the Phyllostomatiniae include Macrotus waterhousii in the Macrotus-group, Tonatia minuta, Mimon crenulatum, Phyllostomus discolor and P. hastatus in the Tonatia-Mimon-Phyllostomus-group, and Micronycteris nicefori, M. brachypotis, and M. minuta in the Micronycteris-group. Tonatia bidens and Micronycteris megalotis were karyotypically so divergent that based solely on chromosomal data they could not be placed with any grouping. Examples are shown where extensive chromosomal evolution has been accompanied by little morphological evolution and where extensive morphological evolution has been accompanied by little chromosomal evolution. Such examples are the basis for questioning the hypothesis that chromosomal changes altering the position of regulator genes are a primary mechanism in the evolution of the large magnitude of morphological differentiation in the class Mammalia. [Phyllostomatoidea; Phyllostomatidae; Phyllostomatiniae; Mormoopidae; Noctilionidae; G-bands; chromosome; phylogenetic relationships.]

Evolutionary events and strategies involving the karyotypes of vertebrates have been difficult to determine because chromosome homology could only be inferred. Data based on longitudinal differential staining patterns for each chromosome can be used as a test to reject or accept proposed homologies, and thereby add to our understanding of mammalian evolutionary processes and phylogenies. G-banding and C-banding patterns provide some of the same advantages to cytogeneticists working with mammalian chromosomes that the differentially banded chromosomes of Drosophila have provided to evolutionists studying fruit flies.

This report is based on an examination of the G-banding and C-banding patterns of bats of the genera Macrotus, Micronycteris, Tonatia, Mimon, Phyllostomus (subfamily Phyllostomatiniae, family Phyllostomatidae), Pteronotus (family Mormoopidae), and Noctilio (family Noctilionidae). This particular combination of taxa offers the opportunity to examine 1) considerable chromosomal variation as diploid number varies from 16 to 46 and fundamental number ranges from 20 to 68, which allows a determination of the extent to which such chromosomal variation has altered the G-banding patterns, 2) representatives of phyllostomatids that are believed most like the ancestral stock based on classical studies (see, Smith, 1972, 1976), and 3) representatives of all three families that are presently considered to have been involved in early phyllostomatoid evolution (Smith, 1972, 1976). If

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banding pattern homologies can be demonstrated among these three families, then chromosomes having banding patterns common to all three families can be proposed as being primitive. Reconstruction of a primitive karyotype and the most parsimonious patterns of change would allow the proposal of direction of chromosomal evolution. Such data offer an opportunity to test published hypotheses relative to the evolutionary origin, evolutionary strategies, and systematic relationships of phyllostomatoid bats (Baker, 1973; Gardner, 1977).

The cytogenetic basis of C- and G-bandings has been discussed at both the organismal and molecular level by numerous authors (for example, Caspersson and Zech, 1973). Analysis of the C-band patterns will determine the amount of karyotypic variation attributable to variation in the amount and distribution of constitutive heterochromatin. Additions or deletions of constitutive heterochromatin have been shown to be a principal component of karyotypic variation in Peromyscus, a cricetid rodent, and in the highly variable mammalian Y chromosome (Duffey, 1972; Pathak et al., 1973; Greenbaum et al., 1978). Preliminary data suggest bats may have karyotypes characterized by reduced amounts of C-band material.

G-band patterns have been used to demonstrate types of chromosomal rearrangement and overall conservatism of gene sequences at the interspecific level for rodents (Pathak et al., 1973; Mascarello and Hsu, 1976; Greenbaum et al., 1978), the intergeneric level for rodents (Mascarello et al., 1974), the interfamilial level for primates (de Grouchy et al., 1973; Turleau et al., 1972) and turtles (Bickham and Baker, 1976), and the interordinal level for birds (Stock et al., 1974).

An intrinsic portion of this study relative to determining evolutionary events and their systematic implications is an elucidation of the primitive versus the derived karyotypes. Because there is no fossil record for karyotypes, this cytogenetic aspect has been difficult to ascertain. Baker (1973) has proposed that the $2n = 30$ or $32$, $FN = 56$ or $60$ karyotype may be primitive for the family Phyllostomatidae. Baker explained the karyotypic similarity among most subfamilies as conservatism with many or most chromosomes being essentially homologous among the taxa within subfamilies. If this is true the G-banding analyses should reflect this similarity and add credulity to the $2n = 30$ or $32$, $FN = 56$ or $60$ primitive karyotype theory. On the other hand, a lack of similarity in G-band patterns between many chromosomes of those similar karyotypes (based on overall chromosome morphology, diploid value, and fundamental number) would suggest that in spite of many successful chromosomal rearrangements there has been independent selection for a $2n = 30$ or $32$ karyotype composed primarily of biarmed autosomes from an ancestral form which possessed a higher diploid number. This would support an alternative theory that a specific number of linkage groups (in this case 15–16) and a complement rich in biarmed chromosomes are critical to the evolutionary strategy of these bats.

One important aspect of this study involves testing the role of centric fission in the evolution of the Phyllostomatinae. The importance of centric fission has been debated at length (for instance, see Lawlor, 1974; Baker et al., 1975). If G-bands are as conservative from an evolutionary standpoint as preliminary data indicate (Mascarello et al., 1974) and Baker’s (1973) hypothesis regarding the primitive phyllostomatid karyotype is correct, there should be considerable homology between banding patterns of chromosomes from various taxa having $2n = 30$ or $32$, $FN = 56$ or $60$. If this similarity exists then the karyotypes of bats such as Macrotus waterhousii ($2n = 40$ and $46$; $FN = 60$) most likely evolved via fission. If, on the other hand, the mechanism involved proved to be independent fusions then a karyotype rich in acrocentric chromosomes such as M. waterhousii ($2n = 46$; $FN = 60$) might be
found to be the karyotypically primitive rather than the karyotypically derived form (Baker, 1967). In this case, fission would not be as important a mechanism in the karyotypic evolution of phyllostomatine bats as previously hypothesized.

**MATERIALS AND METHODS**

Bats analyzed in this study were taken from natural populations with mist nets. Lung and ear biopsies served as the source for primary cultures used to obtain mitotic spreads. Biopsies taken in the field were transported in 10–15 ml Nutrient Mixture F-10 (Ham’s) supplemented with 20 percent fetal calf serum and antibacterial and fungicidal agents. Cultures were grown in media identical to that used for transport media without the fungicidal agents.

Actively dividing cells were arrested at metaphase from 15 minutes to 3 hours with either Colcemid or Velban (time of mitotic inhibitor was adjusted as a function of growth rate of a particular cell line). Cells were removed from flasks with trypsin and karyotyped as described by Baker (1970) except that preparations were air dried by dropping the cell suspension onto slides containing a layer of 40 percent aqueous acetic acid. Slides for G-banding were then placed on a slide warmer for 2–24 hours at 60°C. G-bands were induced by trypsin following essentially the technique of Seabright (1971). Slides to be C-banded were left overnight at room temperature. C-bands were induced by slight modifications of the technique described by Sumner (1972). Staining was with 2 percent Giemsa Blood Stain in phosphate buffer pH 6.8–7.0. Banding results were photographed us-

**TABLE 1. BIARMED Macrotus waterhousii ELEMENTS PROPOSED HOMOLOGOUS TO ELEMENTS OF CHROMOSOME COMPLEMENTS OF THE TAXA ANALYSED.**

<table>
<thead>
<tr>
<th>Macrotus waterhousii</th>
<th>1/2</th>
<th>4/5</th>
<th>6/7</th>
<th>10/11</th>
<th>15/16</th>
<th>19/20</th>
<th>23/24</th>
<th>25/26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronycteris nicefori</td>
<td>1/2, 3/2</td>
<td>4/5</td>
<td>6/7</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>24/23-27</td>
<td></td>
</tr>
<tr>
<td>M. brachyotis</td>
<td>1/2</td>
<td>4/5</td>
<td>6a/7</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>26/25-13</td>
</tr>
<tr>
<td>M. minuta</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>26/25-13</td>
<td></td>
</tr>
<tr>
<td>M. megalotis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Tonatia minuta</td>
<td>1/2</td>
<td>4/5 inv.</td>
<td>6/7</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>25/26</td>
</tr>
<tr>
<td>T. bidens</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Mimon crenulatum</td>
<td>1/2</td>
<td>4/5 inv.</td>
<td>6/7</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>25/26</td>
</tr>
<tr>
<td>Phyllostomus discolor</td>
<td>1/2</td>
<td>4/5 inv.</td>
<td>6/7</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>25/26</td>
</tr>
<tr>
<td>P. hastatus</td>
<td>1/2</td>
<td>4/5 inv.</td>
<td>6/7</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>25/26</td>
</tr>
<tr>
<td>Pteronotus parnellii</td>
<td>1/2</td>
<td>4/5</td>
<td>6,7 inv.</td>
<td>10/11</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>25/26</td>
</tr>
<tr>
<td>Noctilio albiventris</td>
<td>1/2</td>
<td>4/5</td>
<td>6/7</td>
<td>10/11 inv.</td>
<td>15/16</td>
<td>19/20</td>
<td>23/24</td>
<td>25/26</td>
</tr>
</tbody>
</table>

1 Dashes indicate chromosomes which are not identifiable with respect to the biarmed autosomes of Macrotus.

2 Chromosomes separated by commas represent elements which are now found as separate or segments of separate chromosomes.
Fig. 2.—G-banded karyotype of *Micronycteris* examined. Chromosome arms proposed homologous to chromosome arms of *M. waterhousii* labeled as in Fig. 1. Karyotype A) *Micronycteris nicefori*, B) *M. brachyotis*, C) *M. minuta*, and D) *M. megalotis*. Asterisks indicate segments in the *M. brachyotis* karyotype not identified.

Individual spreads were analyzed on a side by side basis to determine the karyotype of a specific individual as well as homologies shared by the various chiropteran taxa.

The karyotype of *Macrotus waterhousii* is used as a standard reference to identify homologous elements in all Phyllostomatoids (Baker, 1978, and Fig. 1). The figure published by Baker (1978) should be used as final reference in determining any questionable identity.

**RESULTS**

Biarmed chromosomes of each species studied that are identifiable with respect to the biarmed chromosomes of *Macrotus waterhousii* are listed in Table 1. Robertsonian fusion products are listed in Table 2.

**Table 2. Proposed fusion patterns of the acrocentric elements of *Macrotus waterhousii* within the taxa analyzed.**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Fusion Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micronycteris nicefori</em></td>
<td>22/14, 8/9, 12/3, 18/17, 1/21, 30/2</td>
</tr>
<tr>
<td><em>M. brachyotis</em></td>
<td>22/14, 9/3, 8/12, 29/27</td>
</tr>
<tr>
<td><em>M. megalotis</em></td>
<td>22/14, 12/9</td>
</tr>
<tr>
<td><em>M. minuta</em></td>
<td>biarmed chromosomes not identified</td>
</tr>
<tr>
<td><em>Tonatia minuta</em></td>
<td>8/9–22, 18/3, 17/12, 30/13–14a, 28/21, 29/27</td>
</tr>
<tr>
<td><em>T. bidens</em></td>
<td>biarmed chromosomes not identified</td>
</tr>
<tr>
<td><em>Mimon crenulatum</em></td>
<td>8/9, 18/3, 17/12, 22/13, 14a/21, 29/27, 30/28</td>
</tr>
<tr>
<td><em>Phyllostomus discolor</em></td>
<td>8/9, 18/3, 17/12, 22/13, 14a/21, 29/27, 30/28</td>
</tr>
<tr>
<td><em>P. hastatus</em></td>
<td>8/9, 18/3, 17/12, 22/13, 14a/21, 29/27</td>
</tr>
<tr>
<td><em>Pteronotus parnelli</em></td>
<td>21/14, 17/9, 13/8, 22/3, 18/12</td>
</tr>
<tr>
<td><em>Noctilio albiventris</em></td>
<td>21/14, 17/9, 13/8, 22/3, 18/12, 30/28</td>
</tr>
</tbody>
</table>
Description of Karyotypes

Macrotus.—G-banded autosomes of Macrotrus waterhousii (2n = 46; FN = 60) have been arranged in a graded series from largest to smallest (Fig. 1) and all autosomal arms have been characterized and consecutively numbered (1–30). The X chromosome is a medium sized submetacentric element and the Y chromosome is a small acrocentric element. No variation in G-banding was detected between specimens from Jamaica and Haiti, and no variation could be detected between standard karyotypes of island Macrotrus waterhousii and mainland forms (see Greenbaum and Baker, 1976). C-banding analysis revealed only small amounts of centric heterochromatin.

Micronycteris.—Four species of Micronycteris representing three subgenera were analyzed: M. (Trinyceris) nicefori (2n = 28; FN = 52); M. (Lampronycteris) brachyotis (2n = 32; FN = 60); M. (Micronycteris) megalotis (2n = 40; FN = 68), and M. (Micronycteris) minuta (2n = 28; FN = 50). Their G-banded karyotypes are shown in Fig. 2. Chromosomes 26/25–13 and 22/14 are unique to Micronycteris and are shared by members of all three subgenera (Fig. 3).

All chromosomes of Macrotrus waterhousii were found to be referable to the G-banded Micronycteris (Trinyceris) nicefori karyotype (Figs. 2A and 4). The five biarmed autosomes which appear homologous to biarmed chromosomes of waterhousii are placed to the right of their proposed homolog in waterhousii (Fig. 4A). Rearrangements of biarmed waterhousii chromosomes 1/2, 23/24, 25/25 in nicefori are proposed in Fig. 4B. The fusion patterns of the remaining nicefori chromosomes with respect to acrocentric elements of waterhousii are shown in Fig. 4C. The X chromosome of nicefori appears identical to the X chromosome of waterhousii. The morphology of the Y chromosome could not be determined as no male was analyzed.

Results of the G-banding analysis for Micronycteris (Lampronycteris) brachyotis.
FIG. 6.—Composite karyotype of Mimon crenulatum, Phyllostomus discolor, P. hastatus, and Tonatia minuta, respectively. Asterisks indicate where the T. minuta karyotype differs from that of the other three. Sex chromosomes of M. crenulatum (C), P. discolor (D), P. hastatus (H), and T. minuta (M) placed at lower right.

otis are shown in Fig. 2B. Chromosome arms proposed to be homologous to chromosome arms of Macrotus waterhousii are appropriately labeled. All eight biarmed autosomes of waterhousii appear to be present in brachyotis although two (6a/7 and 26/25) and possibly three of the chromosomes (a small inversion is suspected in arm 11 of 10/11) have been rearranged. The X chromosome of brachyotis appears identical to the X chromosome of waterhousii. The morphology of the Y chromosome is unknown as no male was studied. C-band analysis revealed only centromeric heterochromatin (Fig. 5).

G-band analysis of the karyotype of Micronycteris (Micronycteris) minuta revealed no autosomes homologous to the biarmed autosomes of Macrotus waterhousii except the rearranged chromosome 26/25–13 (Fig. 2C). The X chromosome of minuta appears indistinguishable from the X chromosome of waterhousii. The nature of the Y chromosome is unknown.

Analyses of the G-banded autosomes of Micronycteris (Micronycteris) megalotis were not referable to the autosomes of Macrotus waterhousii (Fig. 2D). The X chromosome of megalotis appears homologous to the X chromosome of waterhousii. The Y chromosome is a small acrocentric element.

Mimon.—All chromosome arms of Macrotus waterhousii, with the possible exception of chromosome arm 24b, could be identified in the Mimon crenulatum (2n = 32; FN = 60) karyotype. All eight biarmed autosomes of waterhousii can be identified in the Mimon karyotype, and seven of the eight are identical to those of waterhousii. Chromosome 4/5 of waterhousii is inverted in Mimon, as well as in Tonatia minuta and Phyllostomus. Robertsonian fusion products common to Mimon and Phyllostomus discolor (see following account) are 18/3, 8/9, 17/12, 14a/21, 22/13, 29/27, and 30/28 (Table 2; Fig. 6). The X chromosome of Mimon appears like that described for waterhousii. The Y chromosome is a small biarmed element. C-band analysis revealed only centromeric heterochromatin.

Phyllostomus.—All chromosomes of Macrotus waterhousii could be identified in the Phyllostomus discolor (2n = 32; FN = 60) karyotype (Fig. 9). The presumed homologs of seven of the eight biarmed autosomal linkage groups of waterhousii are compared to chromosomes of P. discolor in Fig. 9A. In Fig. 9B the inversion of chromosome 4/5 of waterhousii is shown. The proposed fusion patterns of the biarmed elements of discolor with respect to the waterhousii karyotype are shown in Fig. 9C. The X chromosome of discolor is indistinguisher-
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 Able from the X of waterhousii. The Y chromosome is the smallest chromosome of the complement. C-band analysis revealed only centromeric heterochromatin.

*Phyllostomus hastatus* (2n = 32; FN = 58) was analyzed by G-banding and found to differ from *discolor* only by an apparent inversion in chromosome 30/28 (Fig. 6) which converts this biarmed element of *discolor* to an acrocentric in *hastatus*.

*Tonatia.*—G-band analysis of *Tonatia minuta* (2n = 30; FN = 56) revealed that all chromosome arms of *Macrotus waterhousii* can be identified. All eight biarmed autosomes of *waterhousii* are identifiable in the *T. minuta* karyotype with seven of the eight showing no evidence of rearrangement (4/5 is the exception). Robertsonian fusion products 18/3, 8/9, 17/12 and 29/27 are also found in species examined of *Mimon* and *Phyllostomus*, as is the inversion 4/5 (Table 1; Fig. 6). The X chromosome of *minuta* appears to be indistinguishable from the X chromosome of *waterhousii*. No males were examined, so the morphology of the Y chromosome could not be determined. C-band analysis revealed only the presence of centromeric heterochromatin (Fig. 7).

Although the positions of many of the chromosome arms of *waterhousii* can be speculated, the extent of chromosomal rearrangement in *Tonatia bidens* (2n = 16; FN = 20; Fig. 8) obscures any definite conclusion concerning chromosome homology between these two taxa. The X chromosome of *bidens* is a submetacentric element which appears homologous to the X chromosome of *waterhousii*. The morphology of the Y was not determined as no males were examined.

*Pteronotus.*—All chromosomes of *Macrotus waterhousii* were found to be referable to elements of the *Pteronotus parrnelli* (2n = 38; FN = 60) karyotype (Fig. 10). A comparison of seven of the eight biarmed chromosomes of *waterhousii* and their proposed homologs in *parrnelli* is shown in Fig. 10A. Chromosome 6/7 of *waterhousii* which is represented by two acrocentric elements in *parrnelli* and four small chromosomes which remain unfused in both *parrnelli* and *waterhousii* are shown in Fig. 10B. Figure 10C illustrates the proposed fusion pattern of *parrnelli* chromosomes relative to the elements of *waterhousii*. The proposed fusion patterns of *parrnelli* are unique to *parrnelli* and *Noctilio albiventris* (see following account). The X chromosome of *parrnelli* appears homologous to the X chromosome of *waterhousii*. The Y chromosome is a subteloacentric element roughly two-thirds the size of the X chromosome.

*Noctilio.*—All chromosomes of *Noctilio albiventris* (2n = 34; FN = 62) were found referable to chromosomes of *Macrotus waterhousii*. All eight biarmed chromosomes of *waterhousii* are found in *albiventris*, although chromosome 10/
**DISCUSSION**

**Primitive Karyotype**

The major contribution from this study concerns the determination of the primitive (plesiomorphic) karyotype for the phyllostomatoid bats. Such data are extremely valuable in understanding the direction of evolutionary events. Baker (1973), using standard karyotypes, hypothesized a primitive karyotype of \(2n = 32; \) \(FN = 60,\) as primitive for the Phyllostomatoidea. Gardner (1977), using standard karyotypes, proposed a primitive karyotype with a diploid number of 36 to 40 and a fundamental number approaching the minimum possible (presumably \(FN = 40\)). Data from this study support the proposed \(FN = 60\) as primitive not only for phyllostomatine, but mormoopid and noctilionid bats as well. In a comparison of the G-banded karyotypes of representatives of *Macrotus, Pteronotus,* and *Noctilio,* homologous segments have a readily distinguishable counterpart in the karyotypes of the other two genera (Figs. 10 and 11). Such data suggest that the karyotypes of these three genera evolved from an ancestor which possessed a karyotype with these common 30 pairs of homologous autosomal arms. We therefore interpret the homology of banding patterns for the autosomal arms of these three genera representing the three families of the Phyllostomatoidea to be a symplesiomorphic (shared primitive) character state. The alternative explanation, that the G-banding similarity between these three taxa is the result of the evolution of convergent G-banding patterns in the same number of pairs of autosomal arms (30), seems less probable. Data from G-banding patterns of the other taxa studied support, and in no way refute, the \(FN = 60\) hypothesis as primitive (plesiomorphic) for the Phyllostomatoidea. Derivation of the various karyotypes of the taxa studied from any of the karyotypes with the more aberrant fundamental numbers (such as *Tonatia bidens* \(FN = 20\) or *Micronycteris mega-
lotis (FN = 68) requires numerous additional steps to keep from concluding that Macrotus waterhousii, Noctilio and Pteronotus are more closely related to each other than Macrotus is to the other members of the Phyllostomatinae.

Concerning diploid number, it is concluded that a 2n = 46 (with 16 biarmed autosomes, 28 acrocentric autosomes, plus sex elements) is the most likely candidate for the primitive condition. Essentially, this is the karyotype of Macrotus waterhousii. The data which support this conclusion are that seven of the eight pairs of biarmed elements of Macrotus waterhousii are shared by Noctilio albiventris, Pteronotus parnellii, Tonatia minuta, Mimon crenulatum and Phyllostomus discolor and P. hastatus. However, one of the seven shared by P. parnellii and M. waterhousii (415) is not the same as one shared by M. waterhousii and the other three phyllostomatine genera (617; see Tables 1 and 2; Figs. 4, 6, 9, 10 and 11). The majority of these eight are present in most other karyotypes studied. Therefore, it is probable that these eight biarmed pairs were plesiomorphic for the phyllostomatoid karyotype. Although the karyotypes of most species studied are composed of biarmed elements in addition to the eight described as common for Noctilio albiventris and Macrotus waterhousii, the banding patterns in these biarmed elements suggest that they are the product of independent fusions of acrocentric elements in the respective lineages.

An alternative hypothesis would be to propose a noctilionid–mormoopid-like karyotype as primitive. This would, however, require additional events in that fissions would have to precede several independent fusions. As demonstrated by Mascarello et al. (1974) for rodents, and as suggested in this study, evolutionary selection for fission products is quite uncommon, whereas Robertsonian fusion products may be the most common type of euchromatic variation. Therefore, fissions preceding fusions appear less probable. The following discussion and proposed common ancestry for respective subtaxa of the Phyllostomatoidea are based on the hypothesis that the plesiomorphic condition for phyllostomatoids was a 2n = 46, FN = 60 similar to Macrotus waterhousii with 16 biarmed autosomes and 28 acrocentric autosomes plus the sex elements.

**Karyotypic Relationships**

From a Macrotus waterhousii karyotype three distinct karyotypic clades are evident in the phyllostomatoids analyzed (Fig. 12). They are the Micronycteris clade, Tonatia–Mimon–Phyllostomus clade, and the Noctilio–Pteronotus clade.

**Micronycteris clade.**—This karyotypic grouping has two synapomorphic characters (Hennig, 1966)—one terminal translocation (26/25–13) and one Robertsonian fusion (22/114)—which are shared by all three Micronycteris subgenera (see Figs. 2, 3 and 4). All subsequent rearrangements within Micronycteris appear to have been achieved through independent events unique to a subgenus. The proposed plesiomorphic karyotype for the subgenera Trincycteris, Lampycteris, and Micronycteris would therefore be 2n = 42; FN = 58.

**Tonatia–Mimon–Phyllostomus clade.**—These taxa are characterized by four synapomorphic Robertsonian fusions (18/3, 8/9, 17/12 and 29/27) and one synapomorphic inversion (4/5 inv.). These chromosomes are common to Tonatia minuta, Phyllostomus discolor, P. hastatus and Mimon crenulatum (Fig. 6). Three additional fusion products (22/13, 14a/21 and 30/28) are synapomorphic to Phyllostomus discolor, P. hastatus, and Mimon crenulatum (Fig. 6).

These data are best explained by the divergence of Tonatia minuta from the Mimon and Phyllostomus ancestor at a 2n = 38, FN = 60 level, whereas the divergence of Phyllostomus and Mimon (Anthorhina) crenulatum lineages occurred at a 2n = 32 or 34, FN = 60 level. A 2n = 34 divergence cannot be discounted because Robertsonian fusion products occurring independently in
forms containing only two acrocentric linkage groups could only lead to a similar fusion product. The possibility of a $2n = 34$ divergence is strengthened by *Mimon (Mimon) cozumelae* possessing a $2n = 34$, $FN = 60$ karyotype (S. L. Williams, pers. comm.).

The $2n = 16$, $FN = 20$ karyotype of *Tonatia bidens* is so unlike that of any other species examined, based on its banded karyotypes, that no relationships are proposed. Intermediate karyotypes which would allow an accurate assessment of these rearrangements may never be found as knowledge of the karyotypic variation in this genus is available for five of the six known species (Baker, 1973, 1978; Gardner, 1977).

**Pteronotus—Noctilio clade.**—This karyotypic grouping is characterized by five synapomorphic Robertsonian fusions (21/14, 17/9, 13/8, 22/3, and 18/12). Data indicate these taxa diverged at a $2n = 36$, $FN = 60$ level. The G-banding karyotypic similarity of representatives of *Noctilio* and *Pteronotus* indicates that these bats are more closely related to each other than either is to the phyllostomatine forms.

**Phylogenetic Relationships**

With the exception of two species (*Tonatia bidens* and *Micronycteris megalo- tis*) placement of all taxa in Fig. 12 is based on chromosomal data. Using representatives of the Mormoopidae and Noctilionidae as outgroup comparisons, the most parsimonious plesiomorphic chromosomal condition for the family Phyllostomatidae is $2n = 46$, $FN = 60$. The most plausible alternate interpretation is a $2n = 48$, $FN = 60$ as primitive, as it is possible that chromosome 6/7 of *Noctilio* and the phyllostomatids represents a convergent character with the plesiomorphic state for the chromosome being like that in *Pteronotus* (Figs. 10 and 11). However, the chance that chromosome arms 6 and 7 fused in *Noctilio* and in each of the phyllostomatid clades independently is less likely than the acrocentrics of *Pteronotus* simply representing a fission of chromosome 6/7. A major point that should be considered is that chromosomal evolution is in no way a time dependent phenomenon. This should be evident solely from the data presented herein, although numerous other examples can be cited. For this reason, a cladogram constructed on chromosomal grounds cannot be construed as representing a time divergence model.

The relationships within the families (and genera) of the Phyllostomatoidea have been studied by numerous authors (see Smith, 1976, for review). Based on standard morphology, there are no data to suggest that all phyllostomatine bats (including *Macrotus waterhousii*) do not share a common ancestor and, to the contrary, there are numerous characters which suggest that they are monophyletic. Finally, there is no reason for us to consider the karyotype of *Macrotus waterhousii* to be anything more than a conserved primitive character. As such, this is a case where cladistic interpretation based solely on chromosomes is inadequate and the synapomorphic character states revealed by classical morphological studies are required if one is to get a realistic interpretation of the relationships of these bats. As no marker chromosomes were found to exist between any two of the three phyllostomatine groups, it is impossible to determine the sequence of divergence of the *Tonatia—Mimon—Phyllostomus* group and the *Micronycteris* group. The dichotomy between *Macrotus* and the *Phyllostomus*-like forms has been discussed by several authors (Walton and Walton, 1968; Slaughter, 1970; Smith, 1972, 1976).

**Rates of Karyotypic Change**

Considerable data generated by this study can be used to determine the degree of karyotypic dissimilarity characteristic of various taxonomic levels. Data suggest that investigators who attempt to elevate or sink taxa on the basis of the degree of correlation or lack of correlation between either standard or banded karyotypes must use extreme caution.
Proposed Primitive Karyotype for Phyllostomatoidea

2N=46; FN=60

Biarmed Chromosomes 1/2, 4/5, 6/7, 10/11, 15/16, 19/20, 23/24, 25/26
Acrocentric Chromosomes 3, 8, 9, 12, 13, 14, 17, 18, 21, 22, 27, 28, 29, 30

Fig. 12.—Cladogram of proposed chromosomal changes in the karyotypic evolution of taxa studied assuming that the primitive condition was like that characteristic of *Macrotus waterhousii*. F = fusion of acrocentrics, T = translocation, Fi = fission of biarm to two acrocentrics, H+ = addition of heterochromatic short arm, * = unidentified segment present, ND = homology of chromosomal arms not determined with placement in cladogram based on currently accepted systematic position.

Specific cases of why restraint must be exercised are exemplified in the genera *Micronycteris*, *Tonatia* (family Phyllostomatidae) *Pteronotus* (family Mormopidae), and *Noctilio* (family Noctilionidae).

We examined four species of *Micronycteris* from three subgenera. The karyo-
types of two species *M. nicefori* (subgenus *Trinycteris*) and *M. brachyotis* (subgenus *Lampronycteris*) are easily related and distinguished by few chromosomal events. The karyotypes of *M. minuta* and *M. megalotis* in the subgenus *Micronycteris* are so chromosomally unique that apparently most or all of the elements of their karyotypes cannot be parsimoniously related to the karyotypes of each other or any other phyllostomatoid. In *Tonatia* we find a similar situation where the two species examined show essentially no chromosomal similarity. When these intragenic differences are contrasted with the degree of similarity of karyotypes between *Pteronotus parnellii* (Mormopidae) and *Noctilio albiventris* (Noctilionidae) the discordant nature of agreement between variation in skeletal and exomorphology on the one hand and chromosomal morphology on the other is obvious.

Comparison of the karyotypes of *Pteronotus* and *Noctilio* reveals the karyotypes differ in only three euchromatic changes. This is the least amount of karyotypic change thus far documented between two mammalian families. In light of such data it is always proper to question the validity of the families, but in this case there is little question that the magnitude of morphological distinctness used to justify the recognition of the two families is as great or greater than that which distinguishes most mammalian families. Prior to this study it is doubtful that any agreement could be reached as to which family *Noctilio* (the family Noctilionidae contains a single genus) should be placed in if it were not awarded familial distinction.

Therefore, these data further document that karyotypic change is not a prerequisite for the evolution of a higher taxon. That so few changes in primitive linkage groups are often characteristic of rather divergent taxa (see also, Mascarello et al., 1974; Stock and Hsu, 1973) leads one to the hypothesis that, through time, numerous mammalian taxa have evolved primarily via point mutations and in many cases the primitive gene arrangements have been conserved. This hypothesis is also supported by chromosome banding analyses of turtles (Bickham and Baker, 1975) and birds (Stock et al., 1974).

Recently, it has been proposed that the rapid evolution of extensive morphological variation in the class Mammalia (relative to other vertebrate subgroups, such as frogs) is a result of chromosomal evolution which has altered the gene positions (Wilson et al., 1974). If this hypothesis is true, then one might predict that when extensive chromosomal evolution has occurred it would be accompanied by a similar magnitude of morphological evolution. This is certainly not the case in the genera *Tonatia* and *Micronycteris*. Conversely, when there has been a large amount of morphological evolution one might predict that this would be accompanied by extensive chromosomal rearrangement. This also is not true (at least with our techniques) of the degree of karyotype distinction that separates *Macrotus* (Phyllostomatidae), *Noctilio* (Noctilionidae) and *Mormoops* (Mormoopidae). If the hypothesis of extensive morphological evolution via chromosomally altered regulator genes is to have credulity, it needs to be able to account for such contradictory data as presented above.

**Karyotypic Patterns**

Euchromatic rearrangements observed within the genera analyzed included Robertsonian changes, translocations and inversions. By far the most common rearrangement within the taxa analyzed was Robertsonian fusion. Both partial arm and terminal (centromere-telomere) translocations were found to occur in several of the taxa analyzed. Within the taxa studied, rearrangements by terminal translocations appeared to be more common than by partial arm translocations. Inversions were found in the *Tonatia-Mimon-Phyllostomus* clade, chromosome 4/5, *Noctilio albiventris*, 10/11, and *Phyllostomus hastatus*, 30/28. Within
Micronycteris inversions probably occurred within the subgenus Micronycteris and possibly within the subgenus Lampronycteris (Fig. 2). Evidence of fission was obtained only from Pteronotus (Figs. 10 and 11); however, fission was likely involved in the rearrangements of the primitively biarmed chromosome 1/2 of the Micronycteris nicefori karyotype (Fig. 4).

The karyotypic patterns of these bats appear to be basically a reduction of linkage groups and retention of the number and banding patterns of the primitive chromosome arms. The tightening of linkage groups has been achieved primarily by Robertsonian fusions. The data suggest that Baker’s (1973) hypothesized FN of near 60 is correct; however, the frequent occurrence of 2n = 30 and 32 chromosome forms noted by Baker is best explained by Robertsonian fusions from a Macrotus waterhousii-like karyotype which resulted in a convergent diploid number near 30 or 32. Gardner’s (1977) proposal of a primitive 2n near 40 is more accurate than Baker’s 2n = 32, although Baker (1967) did point out that the possibility of evolving the lower diploid numbers from fusions in a Macrotus-like karyotype. We find no data to support Gardner’s contention that the primitive FN was near 40.

The amount and position of constitutive heterochromatin was not found to contribute substantially to chromosomal rearrangements observed between taxa (Figs. 5 and 7).

**Evolutionary Origin**

Our interpretation of data from this study suggests an initial dichotomy from an ancestral taxon to the Phyllostomidae and Noctilionidae-Mormoopidae. Smith (1976) opted for evolution of the Phyllostomatoidea directly from the Paleochiroptera rather than from an emballonurid-like form. Although no chromosome banding data are yet available for emballonurid forms, the emballonurids which have been found to possess 32 or more chromosomes (4 genera) have chromosome morphology strikingly similar to the chromosome morphology of phyllostomatoid bats which possess primitive chromosome arms for the Phyllostomatoidea. Fundamental number for the four emballonurid genera with a 2n = 32 ranges from 58–60. Chromosome banding analyses of these taxa could prove enlightening.

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**REFERENCES**


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APPENDIX

Specimens examined
All specimens and suspended cell lines are deposited in The Museum, Texas Tech University.

Macrotus waterhousii, 1 male, Haiti: Dept. du Sud; 2 km N, 2 km E Lebrun, TK 8548. 1 male, Jamaica: St. Ann Parish; 2 mi E Discovery Bay, Green Grotto Cave, TK 8121.

Micronycteris nicefori, 1 female, Trinidad: Nariva Co.; Guayaguayare, TK 8383.

M. brachyotis, 1 female, Trinidad: Nariva Co.; Guayaguayare, TK 8351.

M. minuta, 1 female, Trinidad: St. George Co.; 2 mi N St. Joseph, TK 8376.

M. megalotis, 1 male, Trinidad: St. George Co.; Maracas, TK 8463.

Tonatia minuta, 1 female, Trinidad: St. Andrew Co.; 2 mi N, 2 mi W Valencia, TK 8409.

T. bidens, 2 females, Trinidad: St. George Co.; Las Cuevas, TK 8299 and 8306.

Mimon crenulatum, 1 male, Trinidad: St. George Co.; 2 mi W San Rafael, TK 8450.

Phyllostomus discolor, 1 male, El Salvador: La Paz; 3 mi NW La Herradura, TK 5195.

P. hastatus, 2 males, Trinidad: St. George Co.; Las Cuevas, TK 8319-20.

Pteronotus parnellii, 1 male, Trinidad: St. Andrew Co.; 2 mi N, 2 mi W Valencia, TK 8415.

Noctilio albiventris, 1 male, Honduras: Nacaome; 10 mi SSW Nacaome, TK 9000.