EVOLUTIONARY RELATIONSHIP OF THE BRACHYPHYLLINAE TO THE GLOSSOPHAGINE GENERA GLOSSOPHAGA AND MONOPHYLLUS

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ABSTRACT.—Analysis of the chromosomes of Glossophaga soricina, Monophyllus redmani (Glossophaginae), Phyllonycteris aphylla, Erophylla sezekorni, and Brachypylla nana (Brachyphyllinae) revealed that there are no detectable differences in the G- and C-band patterns of the karyotypes of these species. Data from these species are compatible with the hypothesis that the karyotype of Macrotrus waterhousii is most like that primitive for the family Phyllostomatidae (Patton and Baker, 1979). The karyotype of these five genera can be derived from that of Macrotrus waterhousii by five pericentric inversions, seven fusions, and one fission. If the primitive karyotype for the family Phyllostomatidae is similar to that of M. waterhousii, then these members of the Glossophaginae and the Brachyphyllinae have shared a common ancestor after evolving this highly derived karyotype. These data cast doubt on the validity of the subfamily Brachyphyllinae, which may be more properly classified as a subtaxon of the Glossophaginae. The magnitude of classical morphological variation and the magnitude of chromosomal variation do not appear to be correlated.

This paper concerns the evolutionary relationships of bats of the Brachyphyllinae (Phyllostomatidae), a subfamily currently consisting of three genera whose geographic distributions are restricted to the West Indies (Jones and Carter, 1976). For justification of the use of the name Brachyphyllinae rather than Phyllonycterinae, see Baker (1979).

Bats of the family Phyllostomatidae are so anatomically diverse and complex (Baker et al., 1976, 1977) that there is considerable confusion and disagreement on the evolutionary and systematic affinities of subfamilies and some genera (Smith, 1976). The problem ensues from the fact that several higher taxa show no distinct pattern of morphological similarity to other taxa of equal rank. It is often impossible to determine if the few morphological similarities shared between taxa are the results of a common evolutionary history or of convergence. The genus Brachyphylla, which has been placed in four different subfamilies, is a good example of this problem. The morphological similarity of Brachyphylla to at least three of these subfamilies is probably the result of convergence or shared primitive characters.

Characters that evolve slowly enough to show past evolutionary relationships, yet still exhibit sufficient change to distinguish separate evolutionary lineages, are needed to resolve systematic affinities of questionable taxa. Such criteria should be of a type that are not likely to produce similar character states in independent lines of evolution.

Determination of homology based on G-banded chromosomes promises to provide considerable insight into the evolutionary history of bats within this family. Patton and Baker (1979) found that homologous G-band segments could be identified among the families Phyllostomatidae, Mormoopidae, and Noctilionidae. Furthermore, they found sufficient changes in chromosomal arrangements to show evolutionary affinities of these groups. Baker et al. (in press) also demonstrated that considerable G-band homology was identifiable between chromosomal elements of the phyllostomatid subfamilies Stenodermatinae and Phyllostomatinae. Therefore, G-banding patterns should be valuable in determining if the general karyotypic similarity of the Brachyphyllinae and some species of the Glossophaginae reflects chromosomal homology as proposed by Baker and Lopez (1970) and Gardner (1977). Also, G-band data should
be valuable in determining if Brachyphylla shares an evolutionary history with other brachyphylline genera.

We have examined and compared the G-band and C-band patterns of the chromosomes of members of the genera Glossophaga, Monophyllus (Glossophaginae), Phyllostomus, Erophylla, and Brachyphylla (Brachyphyllinae). Also, we have compared these karyotypes with that of Macrotus waterhousei, which was proposed as primitive for the family Phyllostomatidae (Patton and Baker, 1979).

**MATERIALS AND METHODS**

Specimens examined in this study were taken from natural populations by mist nets. Ear, lung, or embryo biopsies were taken at the collection sites and stored in Ham's F-10 Nutrient Mixture supplemented with 20% fetal calf serum, 1.8% Penicillin-Streptomycin, and 0.9% Mycostatin suspension. Fibroblast tissue cultures were initiated from tissue biopsies. Cultures were maintained in media as described above except without Mycostatin. Five cell-line samples from each individual were frozen at −90°C in 5% dimethyl sulfoxide in growth medium.

Replicated chromosomes were arrested at metaphase with 0.1–0.5 ml 0.0005% Velban in 15 ml of media for 10 to 20 min. Cells were removed with 0.25% trypsin, and karyotypes were prepared as described by Greenbaum et al. (1978) except that 0.22% culture media was used as the hypotonic solution.

Slides to be C-banded were left for 12 to 24 h at room temperature and treated with 0.2 N HCl for 20 to 30 min, 5% BaOH at 46°C for 2 to 15 min, and 1X or 6X SSC at 60°C for 45 min (Sumner, 1972). C-banded slides were stained in 4% Giemsa for 10 min.

All chromosomes in the karyotype of each species were identified according to the proposed standard karyotype for the family (Baker, 1979); numbers in the text and in Figs. 3 and 4 refer to this system. To facilitate comparisons between chromosomes in different stages of contraction, cells were photographed and printed so that all pair 6/7 chromosomes were of equal length (Figs. 3, 4). Several banded karyotypes (at least five) were prepared from each specimen examined, and homologies were determined based on differential longitudinal staining patterns of each chromosome. Only spreads with the diploid number characteristic of the species were used in the comparisons.

*Specimens examined.*—All specimens are deposited in the Division of Mammals, Texas Tech University, Lubbock (TTU), or Carnegie Museum of Natural History, Pittsburgh, Pennsylvania.
(CMNH). All frozen cell lines are deposited in the Division of Living Tissues, Texas Tech University.

_Brachyphyllo lans._—**Haiti:** Dept. de Sud; 1 km E Lebrun, TTU 22762 (♀). _Erophylla sezekorni—Jamaica:_ Westmoreland Parish; Bluefields, CMNH 44519 (♀); St. Ann Parish; Orange Valley, CMNH 44514 (♀), 44516 (♀). _Glossophaga soricina—Jamaica:_ St. Ann Parish; Orange Valley, CMNH 44318 (♂); St. Ann Parish; Green Grotto, CMNH 44308 (♂), 44309 (♂). _Macrotus waterhousii—Jamaica:_ St. Ann Parish; Orange Valley, CMNH 44293 (♂); St. Ann Parish; Green Grotto, CMNH 44274 (♂), 44276 (♂), 44277 (♀). _Monophyllus redmani—Jamaica:_ Westmoreland Parish; Bluefields, CMNH 44370 (♀); St. Ann Parish; Orange Valley, CMNH 44330-44333 (♂ ♀), 44334 (♀). _Phyllonycteris aphylla—Jamaica:_ Westmoreland Parish; Bluefields, CMNH 44536 (♂); St. Ann Parish; Orange Valley, CMNH 44523 (♂).

**RESULTS**

Proposed Homologies between _Glossophaga soricina_ and _Macrotus waterhousii_

Complete G-band karyotypes of _Glossophaga soricina_ and _Erophylla sezekorni_ are shown in Figs. 1 and 2, respectively. Five autosomal pairs (4/5, 6/7, 15/16, 19/20, and 25/26) and the X and Y chromosomes appear identical between _G. soricina_ and _M. waterhousii_ (Fig. 3A). Eight elements (3, 8, 9, 13, 17, 22, 27, and 28) that are acrocentric in _M. waterhousii_ constitute arms of biarmed elements (8/9, 17/3, 28/13, and 27/22) in _G. soricina_ (Fig. 3B). The arms of biarmed chromosome 1/2 of _M. waterhousii_ are found in two different chromosomes in _G. soricina_. Chromosome 18 is fused with chromosomal arm 2 (Fig. 3B). Chromosomal arm 1 also appears to differ in the two karyotypes by a pericentric inversion. Chromosomal arms 12 and 21 of biarmed elements in _G. soricina_ appear homologous to acrocentric elements of _M. waterhousii_, as indicated in Fig. 3C. If the _M. waterhousii_ biarmed chromosomes 10/11 and 23/24 are homologous to individual chromosomal arms of biarmed chromosomes of _G. soricina_ as proposed (Fig. 3C), either pericentric inversions that did not alter the observed banding patterns or centric transpositions occurred prior to fusion. In _G. soricina_, 14 and 29 are biarmed elements, whereas in _M. waterhousii_ these elements are acrocentric. A pericentric inversion in both 14 and 29 best explains these differences (Fig. 3C).
Proposed Homologies among the Representatives of Glossohaga-Monophyllus-Erophylla-Phyllonycteris-Brachyphylla

Proposed homologies for the karyotypes of *Glossohaga soricina*, *Monophyllus redmani*, *Erophylla sezekorni*, *Phyllonycteris aphylla*, and *Brachyphylla nana* are shown in Fig. 4. No differences were observed among these five genera, and all chromosomes appear homologous and unchanged in G- and C-bandering patterns. C-band material was restricted to small amounts at the centromeric region.

**Discussion**

*Primitive Karyotype for the Family Phyllostomatidae*

One of the most important aspects in a study of this nature is the determination of primitive versus derived character states (plesiomorphic versus apomorphic; Hennig,
FIG. 4.—Proposed homologous chromosomes between *Glossophaga, Monophyllus, Erophylla, Phyllonycteris*, and *Brachyphylla*, left to right, respectively. Chromosome 17/3 in *Phyllonycteris* was cut at the centromere and aligned to save space.

1966). Based on outgroup comparisons, Patton and Baker (1979) proposed a primitive karyotype for the family Phyllostomatidae identical to that of *Macrotus waterhousii* (2N = 46; FN = 60). The following discussion provides the most parsimonious explanation for the changes required to derive the chromosomal character states observed
in species of Glossophaga, Monophyllus, Phyllonycteris, Brachyphylla, and Erophylla from the proposed primitive karyotype.

Chromosomal banding data from G. soricina, M. redmani, and genera of the Brachyphyllinae can be related to Patton and Baker’s (1979) hypothesized primitive karyotype for the Phyllostomatidae as follows. The proposed primitive karyotype contains eight pairs of biarmed autosomes (1/2, 4/5, 6/7, 10/11, 15/16, 19/20, 23/24, and 25/26), and 14 pairs of acrocentric autosomes. Sympleiomorphies (shared primitive character states; Hennig, 1966) in the representatives examined from the glossophagine and brachyphylline lineages are five biarmed pairs (4/5, 6/7, 15/16, 19/20, and 25/26). Pairs 10/11 and 23/24 have been maintained as single linkage groups but are altered by pericentric inversions in representatives examined in the two subfamilies.

Because biarmed pairs 10/11 and 23/24 are found in the families Mormoopidae and Noctilionidae as well as in the Phyllostomatidae (Patton and Baker, 1979), the most parsimonious explanation is that pairs 10/11 and 23/24 were primitive as observed in M. waterhoussii and the condition of G. soricina is derived. One hypothesized primitive biarmed pair (chromosome 1/2) is not found as a single linkage group in our sample of glossophagines and brachyphyllines. Chromosome 1/2 is, however, maintained as a single element in the families Mormoopidae and Noctilionidae (Patton and Baker, 1979) and the phyllostomatid subfamilies Phylllostomatinae (Patton and Baker, 1979) and Stenoderminae (Baker et al., in press). Based on these data, the most parsimonious explanation for the independence of chromosome arms 1 and 2 in the Glossophaginae and Brachyphyllinae is a fission of the primitive 1/2 chromosome in the line that gave rise to these two subfamilies.

Fusion of chromosomal arms 8 and 9 has occurred in several phyllostomatines (Micronycteris nicefori, Tonatia minuta, Mimon crenulatum, Phyllostomus discolor, and P. hastatus; Patton and Baker, 1979) as well as in the brachyphyllines and glossophagines examined. However, chromosomal arms 8 and 9 are independent of one another in three other phyllostomatine species, mormoopids, and noctilionids (Patton and Baker, 1979) and stenodermines (Baker et al., in press). Therefore, on the basis of these data we cannot determine if the fusion of chromosomes 8 and 9 is a synapomorphy (the only one) among the phyllostomatines, brachyphyllines, and glossophagines, or is merely the result of a convergent event.

Therefore, we do not find any data from Glossophaga and Monophyllus and the brachyphylline genera that argue against the karyotype of Macrotes waterhoussii as primitive for the family Phyllostomatidae. In fact, there is no other known or hypothesized karyotype that all G-banded chromosomal forms can so easily be derived from as that of M. waterhoussii. If a karyotype like that characteristic of M. waterhoussii evolved into that observed in representatives of Glossophaga, Monophyllus, Phyllonycteris, Erophylla, and Brachyphylla, then the following are synapomorphic (shared derived character states among these genera) for these five genera: four independent fusions of two acrocentrics to form biarmed elements (8/9, 17/3, 28/13, and 27/22); two pericentric inversions of biarmed elements (10/11 and 23/24) to an acrocentric condition with subsequent fusion with another acrocentric (12/10-11 and 21/23-24); fission of chromosome 1/2 with a fusion of one arm to an acrocentric (18/2); and pericentric inversions in 1, 14, and 29 (a total of five inversions, seven fusions, and one fission). The absence of variation in C-band material suggests that this material has played little role in the evolution of these bats.

Evolutionary Implications

The essentially identical and, in our opinion, highly derived karyotypes of the genera Glossophaga, Monophyllus, Phyllonycteris, Erophylla, and Brachyphylla leave little doubt that this karyotype was characteristic of their common ancestor and not
the result of convergence. These data could be interpreted in two ways. First, the karyotype for the brachyphyllines and glossophagines is like that of the progenitor for the entire Glossophaginae and Brachyphyllinae or, second, this karyotype is derived, and the Brachyphyllinae and members of the genera Glossophaga and Monophyllus represent a lineage within the evolution of the nectar-feeding bats, Glossophaginae (as shown in Fig. 8 in Gardner, 1977). Examination of the G-band homology of the various karyotypes found in members of the Glossophaginae will be valuable in determining the probability of the alternative explanations.

Despite the classical morphological distinction between the Glossophaginae and Brachyphyllinae, there will be no evolutionary basis for recognition of the Brachyphyllinae if the karyotype of Glossophaga proves to be derived within the Glossophaginae. Even if the Glossophaga karyotype proves to be the most probable primitive karyotype for the entire Glossophaginae, these data will still show clearly that the Brachyphyllinae and Glossophaginae shared a common ancestor. The problem of recognition of the subfamily then will be one concerned with the anatomical as well as other forms of distinctiveness, as the two lineages could have evolved this primitive karyotype very early. A similar case exists in Sturnira (of the sometimes-recognized Sturnirinae) and Artibeus (Stenoderminae), which have identical karyotypes that are highly derived from the karyotype proposed as primitive for the family (Baker et al., in press). If the Brachyphyllinae and Sturnirinae are recognized, then they are more closely related to the glossophagines and stenodermines, respectively, than either is to any other subfamily of the Phyllostomatidae. Even on the sole basis of classical anatomical studies, Allen (1898) proposed that the brachyphyllines were a subgroup of the Glossophaginae.

The genus Brachyphylla has had a varied taxonomic history, being moved from one subfamily to another at least six times. Chromosomal data clearly show that this genus is closely allied with Erophylla, Phyllonycteris, Glossophaga, and Monophyllus and not with the Stenodermidae (Baker et al., in press). Based on morphology and feeding behavior, Brachyphylla appears to be related to Phyllonycteris and Erophylla (Silva-Taboada and Pine, 1969); these three genera are probably the result of a minor degree of adaptive radiation after their ancestor reached the islands (Baker and Genoways, 1978).

Wilson et al. (1975) hypothesized that, in placental mammals, magnitude of karyotypic evolution is correlated with magnitude of morphological change. Chromosomal and morphological data from the brachyphyllines are contradictory to this hypothesis. Phyllonycteris and Erophylla are sufficiently different morphologically from Glossophaga and Monophyllus that most classical systematists have placed them in separate subfamilies, yet no karyotypic evolution has accompanied this morphological divergence. Furthermore, Brachyphylla is morphologically so unique from other brachyphyllines that it has been classified as a desmodontine (Winge, 1892), stenodermine (Peters, 1861; Dobson, 1878; Miller, 1907), and even placed in its own tribe (Brachyphyllina, Gray, 1866, which is the oldest available name of subfamilial rank for the taxa currently placed in what has been called the Phyllonycterinae). Dobson (1878) also noted the morphological similarity between Brachyphylla and the vampire bats. This morphological divergence of Brachyphylla has not been accompanied by gross structural chromosomal changes detectable by G-band techniques. Another example of extensive anatomical divergence in the absence of chromosomal evolution is the Sturnira-Artibeus variation noted above (Baker et al., in press). These data leave little doubt that, within mammals, substantial morphological change is not always associated with equally large amounts of karyotypic change and may even occur in the absence of karyotypic change. It is also true that large amounts of chromosomal variation are not always associated with large amounts of morphological change. For
instance, a study (Patton and Baker, 1979) of the G-band karyotypes of *Tonatia bidens* (2N = 16) and *T. minuta* (2N = 30) failed to reveal sufficient chromosomal homology between the two taxa for routes of chromosomal change to be hypothesized. However, on morphological grounds, the two are considered congeneric. The implication of the hypothesis of Wilson and his colleagues (1975) is that in mammals chromosomal rearrangements (resulting in regulator gene changes) are involved in a cause-and-effect relationship to accomplish the considerable anatomical variation observed in the living forms of the Mammalia. Even though a general overview of higher mammalian taxa might correlate magnitude of chromosomal rearrangements with magnitude of cranial and exomorphological change, there are several cases where this is not true. Data from the bat family Phylllostomatidae clearly show that the proposed cause-and-effect relationship does not always exist, and that the reason for extensive chromosomal change might be better explained by alternative hypotheses.

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**LITERATURE CITED**


the relationship between the bat genus *Brachyphylla* and the Phyllonycterinae. Biotropica, 1:10–19.


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