

Examination of Monophyly of Bats: Restriction Map of the Ribosomal DNA Cistron

ROBERT J. BAKER,¹ RODNEY L. HONEYCUTT,² AND
RONALD A. VAN DEN BUSSCHE^{3,4}

ABSTRACT

Two opposing hypotheses concerning the origin of bats, as well as flight in mammals, have been proposed. In one, all bats shared a common ancestor after diverging from the remainder of extant Mammalia, whereas in the other, Megachiroptera, Primates, and Dermoptera shared a common ancestor after diverging from the Microchiroptera. In the latter hypothesis, flight in mammals would have evolved twice. To discriminate between the two competing hypotheses, we mapped 52 restriction sites for the ribosomal cistron (rDNA) for representative taxa using a mole, a shrew, and *Mus* as outgroups. We examined 14 genera representing 13 families of Microchiroptera, 5 genera of Megachiroptera, *Cynocephalus* (order Dermoptera), and *Homo* and *Lemur* (order Primates). Of the 52 mapped restriction sites, 24 were shared among all taxa. Resolution of the two alternative hypotheses was not found within these data. The only

potentially resolving site was a *Pvu* II site in the nontranscribed spacer that united Dermoptera with the five genera of Megachiroptera. No synapomorphic site linked all bats, all Microchiroptera, or Megachiroptera, Dermoptera, and Primates. It is hypothesized that the lack of resolution from these molecular data originates from these taxa sharing a common ancestor for a relatively short time after diverging from the remainder of extant Mammalia. Such a short time in a common ancestor would permit few molecular events in conservatively evolving DNA sequences to become established to document a common origin. Alternatively, events that became established in rapidly evolving molecules would be lost or obscured due to extensive evolution over the long term since the Primates, Megachiroptera, Microchiroptera, and Dermoptera separated from each other.

INTRODUCTION

Systematics is "the study of organismic diversity as that diversity is relevant to some specified kind of relationship thought to exist among populations, species, or higher taxa" (Wiley, 1981). Although this definition may be considered too narrow by some systematists, most would agree that one of the more important exercises in systematics is determining the phylogenetic relationships among taxa prior to any formal taxonomic treatment

of those taxa. One of the most difficult tasks in systematics is the discovery of attributes or taxonomic characters that can be used to diagnose relationships. As Mayr (1982) stated, "the most frequent complaint made by a taxonomist is that the group of animals or plants on which he is working does not supply sufficient characters to allow an unequivocal decision on relationship." Therefore, systematists use a broad array of taxonomic char-

¹ Horn Professor, Department of Biological Sciences and The Museum, Texas Tech University, Lubbock, Texas 79409.

² Associate Professor, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843-2258.

³ Research Associate, Department of Biological Sciences and The Museum, Texas Tech University, Lubbock, Texas 79409.

⁴ Present address: (Sloan Fellow) Department of Biological Sciences, University of Idaho, Moscow, Idaho 83843.

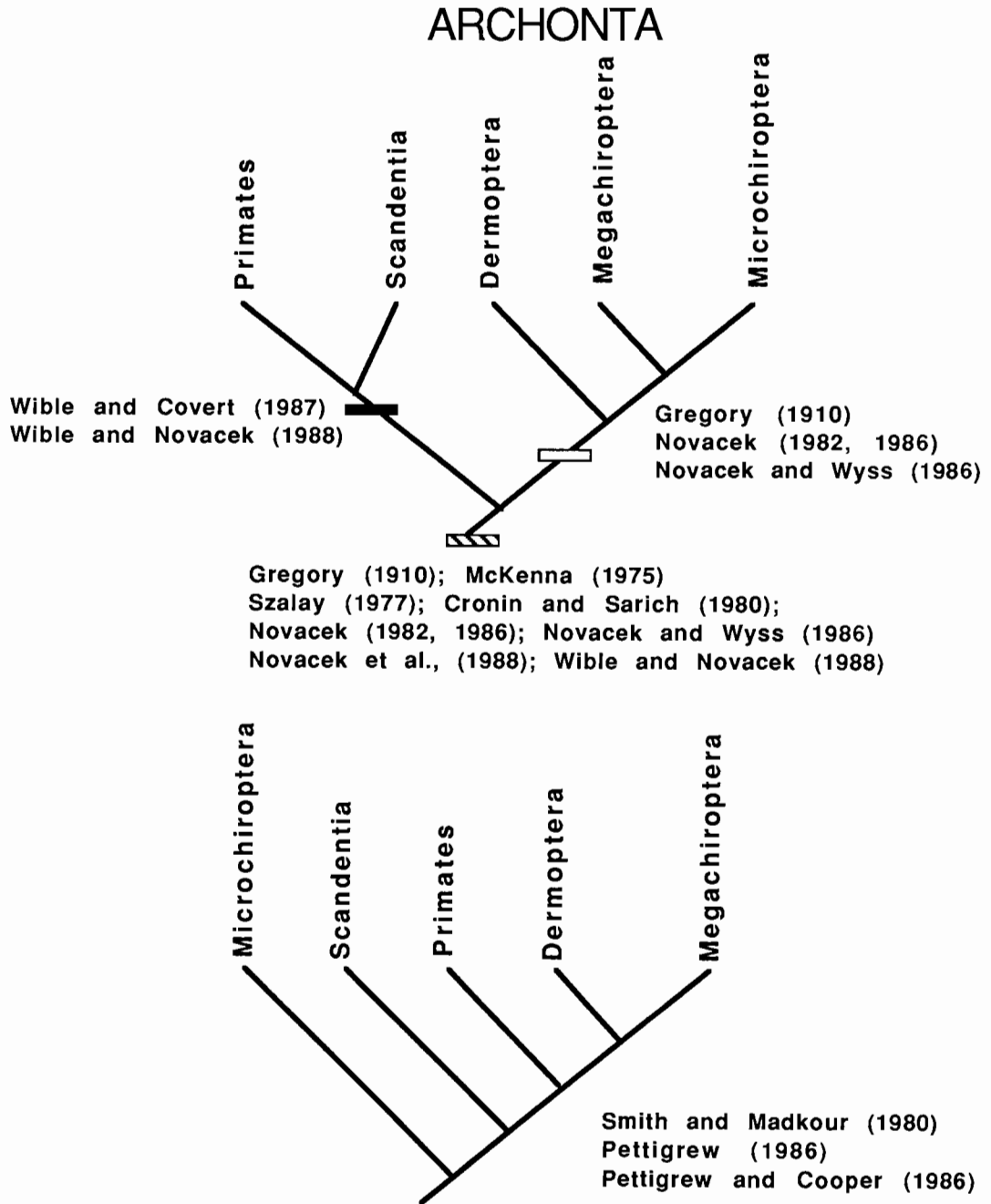


Fig. 1. Phylogenetic hypotheses depicting interordinal relationships in the Archonta. **Above.** Classical hypothesis. **Below.** Pettigrew hypothesis. References for studies supporting particular monophyly within the respective trees of both Archonta and groups within Archonta accompany the cladograms.

acters, including anatomy, histology, behavior, immunology, chromosomes, proteins, and DNA in an effort to resolve phylogenetic relationships and produce a classification.

The order Chiroptera represents a group of mammals that has received extensive attention from systematists for over 100 years, and many problems associated with both the phylogeny and classification of bats still exist. For instance, the New World bat family Phyllostomidae has been intensely studied using many different taxonomic characters, yet a number of questions pertaining to the recognition of monophyletic groups and the classification of phyllostomids persist (Baker et al., 1989). Even more troubling is the question of whether or not the order Chiroptera is a monophyletic group. Classically, the bats have been classified within a single taxon to the exclusion of other mammals (Linnaeus, 1758)—as monophyletic (Linnaeus, 1758)—but several recent authors have suggested that the order is diphyletic (fig. 1; Smith and Madkour, 1980; Pettigrew and Cooper, 1986).

The purposes of this study are twofold. First, a summary of opposing views and existing data that directly pertain to the question of chiropteran monophyly will be addressed. Second, empirical data will be provided for a detailed analysis of ribosomal DNA (rDNA) variation in chiropterans and related orders in an effort to stimulate more molecular systematics research on the chiropteran monophyly question.

CHIROPTERAN MONOPHYLY: Gregory (1910) recognized the superorder Archonta—containing the orders Chiroptera, Dermoptera, Scandentia, and Primates—and this clade was maintained in McKenna's (1975) classification. Although there have been criticisms of this grouping (Cartmill and MacPhee, 1980), several morphological studies and at least one immunological study (Cronin and Sarich, 1980) support the monophyly of this group. Nevertheless, some molecular studies place primates closer to other orders of mammals, including Lagomorpha and Rodentia (Schwale et al., 1984; De Jong, 1985; Miyamoto and Goodman, 1986). The associations among orders within Archonta are less clear (Novacek et al., 1988). Several authors, using erectile tissue of the penis (Smith and Madkour, 1980) and visual pathways of the cen-

tral nervous system (Pettigrew, 1986; Pettigrew and Cooper, 1986; Pettigrew et al., 1989), have suggested that the order Chiroptera is diphyletic (fig. 1), with derived features that are shared between the suborder Megachiroptera and the orders Primates and Dermoptera being absent in Microchiroptera. This arrangement suggests that the mechanism for powered flight in bats, once considered the major feature uniting the two suborders, has been derived independently in the two groups. To the contrary, cranial and postcranial features suggest chiropterans to be monophyletic (fig. 1; Wible and Novacek, 1988). One additional study that disagrees with both the diphyly hypothesis of Pettigrew (1986) and the monophyly of Archonta involved a comparison of mitochondrial DNA nucleotide sequences of the cytochrome III gene (Bennet et al., 1988). The results of this study are unclear, because a true test for chiropteran monophyly was not performed. For instance, one bat genus (*Pteropus*, a megachiropteran) was compared to three mammalian orders: Primates, Artiodactyla, and Rodentia. *Drosophila* was used as an outgroup.

RIBOSOMAL DNA AS A TAXONOMIC CHARACTER: Our study was designed to determine if a cladistic analysis of restriction site data in the ribosomal DNA cistron could provide resolution of these systematic problems. With the recent advances in molecular biology, a plethora of potential systematic probes has been made available to molecular systematists for use in elucidating phylogenetic relationships. However, because the field of molecular systematics is relatively new, it is still unclear which molecules will provide adequate resolution to address a particular systematic question. Previous systematic studies using the ribosomal DNA gene complex have provided valuable information for addressing phylogenetic relationships (Gerbi, 1985; Appels and Honeycutt, 1986; Hillis and Davis, 1986, 1987; Suzuki et al., 1986; Dallas et al., 1988; Seperack et al., 1988; Mindell and Honeycutt, 1989; Van Den Bussche, 1989).

The rDNA gene complex is a tandemly repeated gene family consisting of coding and noncoding sequences that evolve at different rates. Coding regions (18S, 5.8S, 28S rDNA

genes) tend to be conserved across higher taxonomic levels (Elwood et al., 1985; Appels and Honeycutt, 1986). The internally transcribed spacer regions (ITS-1, ITS-2) that separate the coding regions, and the externally transcribed spacer regions (ETS) located at the 5'-end of the 18S rRNA gene, appear to be under fewer selective constraints than the coding regions and, hence, have been found to contain phylogenetically informative characters in many vertebrate groups (Wilson et al., 1984; Hillis and Davis, 1986, 1987; Mindell and Honeycutt, 1989; Van Den Bussche, 1989). Finally, separating the tandemly arranged coding regions is the most variable region, the nontranscribed spacer region (NTS). Subspecific, populational, and interindividual differences have been found for some taxa in this region (Davis, 1986; Suzuki et al., 1986; Suzuki et al., 1987; Dallas et al., 1988). Therefore, results from previous studies suggest that the rDNA complex should contain sufficient restriction site variability, either in the coding regions or in the transcribed spacer regions, to allow assessment of the higher phylogenetic relationships among the Chiroptera.

MATERIALS AND METHODS

High-molecular-weight DNA was isolated from heart, kidney, liver, muscle, and/or placenta from the taxa listed in table 1, essentially following the method of Bingham et al. (1981). Genomic DNA was subjected to single and double digestion involving 18 different restriction endonucleases (*Bam*H I, *Bcl* I, *Bgl* II, *Bst*E II, *Cla* I, *Dra* I, *Eco*R I, *Hinc* II, *Hind* III, *Kpn* I, *Nco* I, *Pst* I, *Pvu* II, *Sal* I, *Sst* I, *Stu* I, *Xba* I, *Xho* I). These 18 restriction endonucleases recognized a total of 108 nucleotides; 55 of these were either cytosine or guanine (50.9%). Therefore, the choice of enzymes was not significantly biased by either A:T- or G:C-rich recognition sequences. Digestion was accomplished with 2–4 units of enzyme per microgram of DNA for 3–12 hours under temperature and buffering conditions specified by the manufacturer. Digested fragments were separated on 0.6–2.0% agarose TAE (0.4 M Tris, 0.1 M Na₂EDTA, 0.05 M sodium acetate) gels with ethidium bromide and run at 30 mAmp for

approximately 14 hours. Higher percentage agarose gels were used to improve resolution of the smaller fragments.

Separated DNA fragments were denatured in the gel by soaking in transfer solution (0.6 M NaCl, 0.4 M NaOH) for 30 minutes, and the denatured DNA was transferred to nylon hybridization membrane (Gene Screen Plus; DuPont) according to the techniques of Southern (1975), with modifications for alkaline transfer (Chomczynski and Qasba, 1984). Membranes were then washed in neutralizing solution (1.0 M Tris, 0.5 M NaCl; pH = 7.0) and allowed to air dry. Prior to hybridization with radioactive probes, the membranes were washed in prehybridization solution (1% sodium dodecyl sulfate [SDS], 50% formamide, 5% Denhardt's solution, 1.5% denatured salmon sperm DNA) at 37°C for 2–4 hours. All gels contained two internal size standards: Lambda DNA digested with *Hind* III and a 1-kilobase (kb) ladder. Additionally, all mapping gels contained a lane with a sample of human DNA. This sample of human DNA served as a control to ensure that digestion had gone to completion, as well as to verify any site changes from the human sequence for this gene complex.

rDNA fragments were detected by hybridization with radioactively labeled rDNA clones of the 18S (p2546) and 28S (pI19) genes of *Mus musculus* (Arnheim, 1979). All rDNA probes were radiolabeled using the random priming method (Feinburg and Vogelstein, 1984). Labeled probes were denatured, combined with prehybridization solution (1×10^6 dpm/ml), and allowed to hybridize with the membranes for at least 12 hours at 37°C. After hybridization, the nylon membranes were washed (three times for 15 minutes at 42°C in $2 \times$ SSC, 0.1% SDS; twice for 30 minutes at 55°C in $0.1 \times$ SSC, 0.1% SDS) and exposed to X-ray film using two intensifying screens at -70°C (Laskey, 1979). Restriction endonuclease site maps for the transcribed portion of the rDNA were constructed using a combination of single and double digestion (Nathans and Smith, 1975). To increase accuracy in constructing restriction site maps, results from the human map were compared to the published sequence of the 18S (Torczynski et al., 1985), 5.8S (Nazar et al., 1976), and 28S (Gonzalez et al., 1985) rDNA genes.

Hypotheses of genealogy were based upon shared-derived characters—synapomorphies—as defined by Hennig (1966). Variable restriction sites, coded as present or absent, were used as phylogenetic characters, and trees were constructed using version 3.0 of PAUP (phylogenetic analysis using parsimony; developed by David Swofford). In addition, potential synapomorphies were also determined by a hands-on cladistic analysis using multiple outgroups, as suggested by Maddison et al. (1984) and Owen (1987). *Mus*, *Scalopus*, and *Crocidura* were used as outgroups in all phylogenetic analyses.

SPECIMENS EXAMINED

Order Rodentia

Mus musculus male (TK 28805): USA: Oklahoma, Cimarron Co., 3 mi E, 1.5 mi S Kenton.

Order Insectivora

Family Talpidae: *Scalopus aquaticus* male (29735): USA: Texas, Montgue Co., 3 mi N, 5 mi E Bowie.

Family Soricidae: *Crocidura* sp. (TK 21587): GABON: Estuaire Prov., Cap Esterias.

Order Primates

Family Hominidae: *Homo sapiens* male (TK 30732): USA: Texas, Lubbock Co., Lubbock, placenta donation from St. Mary's Hospital.

Family Lemuridae: *Lemur catta* male (TK 26899): USA: Texas, specimen from the Fort Worth Zoo; geographic origin unknown.

Order Dermoptera

Family Cynocephalidae: *Cynocephalus volans* male (TK 21407): THAILAND: Surat Thani Prov., Tha Chang Dist., 15 km N, 23 km W Ban Muruan.

Order Chiroptera

SUBORDER MEGACHIROPTERA

Family Pteropodidae: *Pteropus hypomelanus* female (TK 20225): PAPUA NEW GUINEA: East New Britain Prov., Duke of York Island, Rakauda Plantation. *P. macrotus* male (TK 20310): PAPUA NEW GUINEA: Central Prov., Lakoke Quarantine Station, 9 km NE Fort Moresby. *Rousettus* (TK 27199): KENYA: Western Providence, Kakamega District, 6 km S, 6 km W Kakamega. *Megaloglossus* sp. female (TK 21507): GABON:

Estuaire Prov., Cap Esterias. *Megaloglossus* sp. female (TK 21566): GABON: Estuaire Prov., 2.5 km SE Cap Esterias. *Nyctimene* sp. female (TK 20095): NEW GUINEA: East New Britain Prov., Gela Gela Plantations. *Nyctimene* sp. male (TK 20096): NEW GUINEA: East New Britain Prov., Gela Gela Plantations. *Nyctimene* sp. male (TK 20101): NEW GUINEA: East New Britain Prov., Gela Gela Plantations.

Family Macroglossinae: *Macroglossus* sp. female (TK 20239): PAPUA NEW GUINEA: East New Britain Prov., Duke of York Islands, Rakauda Plantations. *Macroglossus* sp. male (TK 20306): PAPUA NEW GUINEA: Central Prov., Lakoke Quarantine Station, 9 km NE Fort Moresby. *M. sobrinus* female (TK 21445): THAILAND: Surat Thani Prov., Tha Chang Dist., 15 km N, 23 km W Ban Muruan.

SUBORDER MICROCHIROPTERA

Family Rhinopomatidae: *Rhinopoma microphyllum* female (TK 25611): JORDAN: Jordan Valley, Tabqat Fahl Ruins. *R. microphyllum* female (TK 25612): JORDAN: Jordan Valley, Tabqat Fahl Ruins. *Rhinopoma* sp. male (TK 25644): PALESTINE: West Bank of Jordan, Jericho, Mt. Quarantania. *Saccolaryx leptura* female (TK 19493): VENEZUELA: Barinas, 38 km SE Varinas on hwy 4.

Family Megadermatidae: *Megaderma lyra* female (TK 21288): THAILAND: Uthi Thani Prov., Lansak Dist., Huai Kha Khang Wildlife Sanctuary, Tam Khe Nok, 3.6 km N, 2.6 km W Sanctuary Headquarters.

Family Nycteridae: *Nycteris graudis* male (TK 21558): GABON: Estuaire Prov., 2 km SE Cap Esterias. *N. graudis* female (TK 21559): GABON: Estuaire Prov., 2 km SE Cap Esterias.

Family Rhinolophidae: *Rhinolophus euryotis* male (TK 20037): NEW GUINEA: East New Britain Prov., 2 km S Gunanur.

Family Hipposideridae: *Hipposideros comersoni* female (TK 33178): KENYA: Coastal Prov., Kwale Dist., Shimba Hills National Reserve, Mwele Forest, 12 km S, 11 km W Kwale.

Family Phyllostomidae: *Macrotus californicus* male (TK 28985): USA: Arizona, Pinal Co., 27.0 mi SW Casa Grande, Old Mammon Mine. *M. waterhousii* female (TK 27727): JAMAICA: St. Ann's Parish, Green Grotto Cave, 23 km SW St. Ann's Bay.

Family Mormoopidae: *Mormoops megalophylla* male (TK 19312): VENEZUELA: Barinas, 7 km NW Barinitas.

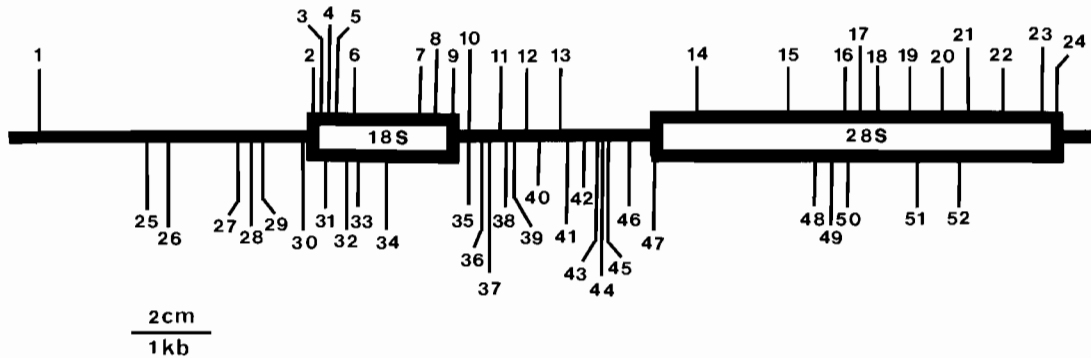


Fig. 2. Restriction endonuclease site map of the transcribed portion of the rDNA gene complex showing conserved sites on top and variable sites below. Sites are numbered as follows: *Bam*H I 15, 19, 25, 32, 33, 42; *Bcl* I 13; *Bgl* III 17; *Bst*E II 4, 7, 40, 46, 50, 52; *Cla* I 1, 33; *Dra* I 5m, 12m, 49; *Eco*R I 8, 23; *Hinc* II 14, 21, 29; *Hind* III 9; *Kpn* I 11; *Nco* I 3, 16, 24, 38; *Pst* I 6, 45, 51; *Pvu* II 20, 26, 41; *Sal* I 27, 36, 47; *Sst* I 18, 22, 31, 34, 43; *Stu* I 30, 37, 48; *Xba* I 2, 10; *Xho* I 28, 39, 44. The numbering of sites is the same as in table 1.

Family Noctilionidae: *Noctilio leporinus* female (TK 19129): VENEZUELA: Bolivar, 8 km S, 5 km E El Manteco. *Noctilio leporinus* male (TK 19077): VENEZUELA: Bolivar, 2.5 km E El Manteco.

Family Molossididae: *Tadarida brasiliensis* male (TK 21816): USA: North Carolina, Cumberland Co., Fayetteville.

Family Natalidae: *Natalus* sp. female (TK 15663): DOMINICA: St. John Co., 0.5 mi N Toucari. *Natalus* sp. male (TK 15665): DOMINICA: St. John Co., 0.5 mi N Toucari.

Family Thyropteridae: *Thyroptera* sp. (TK 19255): VENEZUELA: Bolivar, 8 km W El Manteco.

Family Furipteridae: *Furipterus horrens* female (TK 17149): SURINAME: Saramacca, Voltzbert.

Family Vespertilionidae: *Lasionycteris noctivagans* female (TK 24216): USA: Texas, Lubbock Co., Lubbock.

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RESULTS

The rDNA repeat of the 25 taxa examined was mapped using 18 restriction endonucleases. The overall repeat unit length in these taxa ranged between 38 and 43 kb, with most length variation mapping to the nontranscribed spacer. Few restriction sites in the NTS were conserved among species, and the size of the NTS—in combination with length variation and probes restricted to the transcribed region of the rDNA repeat—limited the value of the NTS for detailed phylogenetic comparisons. Additional length variation (10–70 base pairs) was also found in the 28S gene region, but this variation was restricted to “divergent domains” or “expansion segments” known to vary in length among mammals and other vertebrates (Clark et al., 1984; Hassouna et al., 1984; Hillis and Davis, 1987).

Of the 52 mapped restriction sites, 24 sites were shared among all taxa (fig. 2; table 1). Most of the conserved sites were located within the coding regions (18S and 28S rRNA genes) and the internal transcribed spacer (ITS-1). Two restriction sites, *Hinc* II site 21 (*Homo*, *Megaderma*, and *Nycteris*) and *Sal* I site 47 (*Saccopteryx*), were polymorphic within individuals having two repeat types defined by the presence or absence of these sites (table 1). The 28 variable sites occurred primarily in the internal transcribed spacer,

TABLE 1
Restriction Endonuclease Site Map for the Ribosomal DNA Cistron for 25 Mammalian Genera
 (0 = absent; 1 = present; 2 = polymorphic. Position of each site is identified by character number in figure 1.)

	Enzyme (letter) and character number																								
	C 1	A 2	N 3	Z 4	D 5	T 6	Z 7	E 8	H 9	A 10	K 11	D 12	L 13	R 14	B 15	H 16	G 17	Q 18	B 19	P 20	R 21	Q 22	E 23	N 24	B 25
<i>Mus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1
<i>Scalopus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Crocidura</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Homo</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Lemur</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Cynocephalus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Pteropus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
<i>Rousettus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
<i>Megaloglossus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
<i>Nyctimene</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
<i>Macroglossus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
<i>Rhinopoma</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Saccopteryx</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Megaderma</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
<i>Nycteris</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
<i>Rhinolophus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Hipposideros</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Macrotus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Mormoops</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Noctilio</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Tadarida</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Natalus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Thyroptera</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Furipterus</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Lasionycteris</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

the NTS upstream of the 18S gene, and the 5'-end of the 28S gene. Twelve of these 28 sites have been lost or gained in only one species (autapomorphic for single taxon) and are therefore phylogenetically uninformative.

The remaining 17 variable sites were used to evaluate the alternative phylogenetic hypotheses of chiropteran monophyly versus diphyly. Of these sites, six occur in the NTS and externally transcribed spacer, one in 18S genes, eight in the ITS, and two in the 28S gene. Each of these 17 variable sites was treated as a character and scored as present or absent, and the character matrix (table 1) was used in a parsimony analysis employing version 3.0 of PAUP. Two approaches were used in this analysis, and in each case *Mus* and the insectivores (*Scalopus* and *Crocidura*) were used as outgroups. First, existing phylogenetic hypotheses for relationships among or-

ders in Archonta were tested with the assumption that each chiropteran suborder represented a monophyletic group, with relationships among taxa within suborders based on those proposed by Smith (1976) for Microchiroptera and Haiduk (1983) for Megachiroptera. Second, the data set was analyzed heuristically using global branch-swapping (MULPARS option) in PAUP. As can be seen in figure 3 (a and b), the monophyly and diphyly hypotheses have tree lengths of 46 and 45, respectively. The level of ambiguity in both of these trees can be seen by the low consistency index (CI = 0.348 for fig. 3a and 0.356 for fig. 3b), thus revealing high levels of homoplasy. Several clades are supported by synapomorphies: (1) monophyly of Megachiroptera (3 characters); (2) sister-group relationship of *Mormoops* and *Noctilio* (3 characters); (3) monophyly of Phyllostomatoidea (*Macrotus*, *Noctilio*, and

TABLE 1—(Continued)

Enzyme (letter) and character number																										
P	S	X	R	U	Q	B	C	Q	B	S	U	N	X	Z	P	B	Q	X	T	Z	S	U	D	Z	T	Z
26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	
0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	
0	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	
0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	
1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	
1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	
1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0	
1	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	
1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	
0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1	1	0	1	0	
1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	1
0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	1	1	0	0
0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	
0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	
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0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	
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0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	

Mormoops) (1 character); (4) monophyly of microchiropteran families Hipposideridae, Rhinolophidae, Megadermatidae, Nycteridae, Emballonuridae, and Rhinopomatidae (1 character); and (5) sister-group relationship between order Dermoptera (*Cynocephalus*) and Megachiroptera in figure 3b (1 character). Unfortunately, most synapomorphies characterizing particular clades are homoplastic (have low consistency indices), with the exception of three characters in figure 3a and four characters in figure 3b that have consistency indices of 1.00. One of these characters defines the dermopteran/megachiropteran clade, thus making the order Chiroptera diphyletic. Because this character is not shared by *Homo* or *Cynocephalus*, there is no support for a Primates/Dermoptera/Megachiroptera association.

A heuristic search restricted to the first 100 trees followed by the construction of a strict

consensus tree yielded a tree of length 43. In this analysis, megachiropteran monophyly was supported, as well as a clade uniting *Mormoops* and *Noctilio*. The only other clade was one depicting the associations of *Homo* and *Rhinolophus* (a microchiropteran bat) and *Pteropus/Megaloglossus* (different megachiropteran subfamilies). All other relationships were unresolved.

DISCUSSION

Examination of figure 3 reveals how few synapomorphies document clades in the alternative trees. The only clades supported by synapomorphies that do not involve homoplastic events are the common ancestry of the five genera of Megachiroptera (character 25), the common ancestry of the Megachiroptera and Dermoptera (character 26) to the exclusion of all other taxa examined, and the

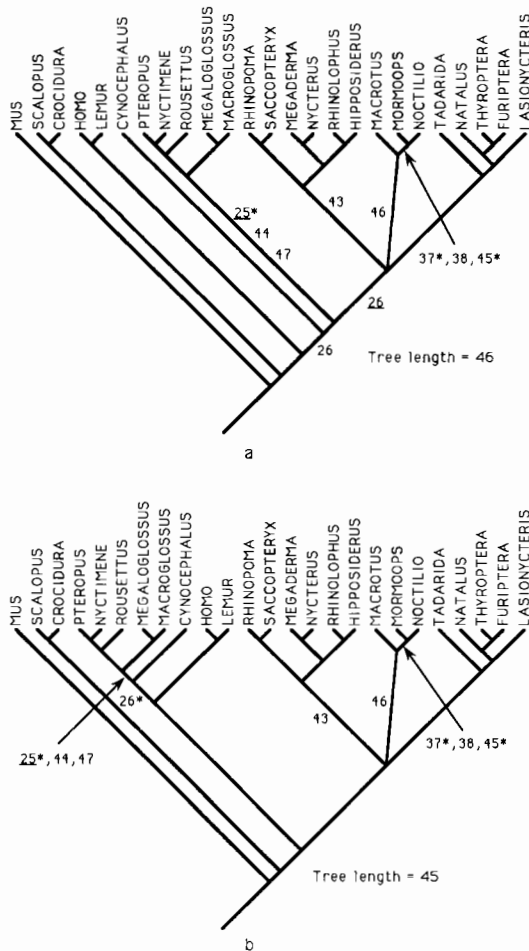


Fig. 3. **a.** Apportionment of characters along branches defining a tree that supports chiropteran monophyly—classical hypothesis. **b.** Characters supporting chiropteran diphyly—Pettigrew hypothesis. The tree lengths and assignment of characters throughout the tree were established by (PAUP) phylogenetic analysis using parsimony. Asterisk (*) identifies characters with a consistency index of 1.00. All other characters have CIs lower than 1.00. Underlined characters are site losses and those with no underline are site gains.

common ancestry of *Mormoops* and *Noctilio* (table 1; fig. 3). We do not conclude that the difference between 46 steps required for the classical hypothesis (fig. 3a) and 45 steps required for the Pettigrew hypothesis (fig. 3b) is significant. The rDNA data do not provide

a synapomorphy that documents either the common ancestry of all bats (classical hypothesis) or a common ancestry between Primates and megachiropterans after divergence from the Microchiroptera (Pettigrew's hypothesis). Therefore, we conclude that this data set provides no clear resolution of the debate over the shared common ancestry of Dermoptera/Primates/Megachiroptera to the exclusion of the Microchiroptera.

The lack of resolution observed among these mammal groups is surprising considering the utility of the rDNA complex in addressing phylogenetic hypotheses associated with higher taxonomic categories of other vertebrate groups (Hillis and Davis, 1986; Mindell and Honeycutt, 1989). Just how serious is this lack of resolution? Is there any level of divergence among these 25 mammalian taxa where the rDNA molecule provides useful phylogenetic information? Unfortunately, the prognosis for the rDNA molecule providing information on mammal relationships is poor. For instance, the following well-established clades based on a variety of morphological studies and standard classifications were not supported by rDNA synapomorphies: (1) monophyly of prosimians (*Lemur*) and simians (*Homo*); (2) monophyly of insectivores (*Crocidura/Scalopus*); and (3) monophyly of the Rhinolophidae (*Rhinolophus/Hipposideros*). Collectively these results suggest that the "evolutionary window" provided by the rDNA restriction site data is narrow relative to resolving particular relationships among mammals and that even when synapomorphies are provided, the number of these characters supporting nodes will be small and the consistency of individual synapomorphies at nodes will be low. We therefore conclude that the rDNA molecule provides little insight into the mammalian origins and cladogenetic events leading to these 25 taxa.

What then do our data indicate? The only synapomorphy that may provide some information pertinent to the question of chiropteran monophyly is character 26, which unites *Cynocephalus* with the five megachiropteran genera. This character has a consistency of 1.00 and does depict a site gain as opposed to a site loss. If we accept character

26 as supporting a common ancestry for the Dermoptera and the suborder Megachiroptera, then the order Chiroptera (including Megachiroptera and Microchiroptera) is diphyletic and flight would by necessity have arisen twice or alternatively would have been lost in the Dermoptera. The question then becomes, "How much credibility can we give to character 26?" In light of the extreme inconsistency associated with other characters, we suggest that one should be cautious of overinterpreting single synapomorphies. The only other clade concordant with other character sets is the Phyllostomidae (*Macrotus*, *Noctilio*, and *Mormoops*), which was supported by one synapomorphy in this study. However, this character is homoplastic. Some examples (table 1) of the inconsistency in our data include character 27 (uniting *Homo*, *Pteropus*, *Megaloglossus*, and *Rhinolophus*), character 38 (uniting *Megaderma*, *Noctilio*, and *Mormoops*), character 40 (uniting *Megaderma*, *Natalus*, and *Thyroptera*), character 42 (uniting *Rousettus* and *Lasionycteris*), character 43 (uniting *Cynocephalus*, *Saccopteryx*, *Nycteris*, *Rhinolophus*, *Pteropus*, *Rousettus*, *Megaloglossus*, *Macroglossus*, *Nyctimene*, and *Macrotus*), and character 45 (uniting *Macrotus* and *Noctilio*).

Are there any other explanations for the lack of resolution provided by restriction site variation of the rDNA cistron relative to the relationships among bat families and other mammalian taxa? One possibility is that these taxa underwent a rapid radiation, having a short time frame of shared common ancestry during which synapomorphies could become established. If this is true, then other molecular studies of early bat evolution will likely encounter problems similar to those observed in this data set. Synapomorphies from any data set that document most ordinal-level relationships in mammals as well as familial-level relationships in bats are few. Perhaps systematic studies should be concerned not only with topologies, contents of clades, and levels of incongruence, but also with the actual internode lengths defining particular monophyletic groups. It could be that a combination of relatively short periods of shared common ancestry between diverging lineages and long periods of time subsequent to par-

ticular cladogenic events makes resolving branching patterns rather difficult regardless of the characters used to diagnose relationships. It is interesting that a study of 45 genera of phyllostomid bats by Van Den Bussche (1989) found a greater amount of resolution and fewer obvious homoplastic events than the present study. However, his study also encountered extreme conservatism in the evolution of this gene complex. If the internode length explanation of the absence of synapomorphies is correct, then most well-established clades documented by rDNA synapomorphies (Van Den Bussche, 1989) can be expected to be supported by unrelated sets of characters.

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