PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Phylogenetic Relations Between Microbats, Megabats and Primates (Mammalia: Chiroptera and Primates)

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PHYLOGENETIC RELATIONS BETWEEN MICROBATS, MEGABATS AND PRIMATES (MAMMALIA: CHIROPTERA AND PRIMATES)

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We examine the paraphyletic hypothesis of bat origins, both in the light of previous discussions, and in the light of new evidence from our analyses of neurological traits and wing morphology. Megabats share with primates a variety of complex details in the organization of neural pathways that have not been found in any other mammalian group, particularly not in microbats. The features previously used to link microbats and megabats have been examined and found to be questionable bases for support of a monophyletic origin. In particular, morphological analyses of the musculoskeletal adaptations associated with the flight apparatus are consistent with two separate origins of the mammalian wing. Taken together, these analyses suggest that megabats evolved from an early branch of the primate lineage. This branch was comprised of moderate-sized, phytophagous gliders, of which the other living descendants are the dermopterans. Microbats, in contrast, probably evolved much earlier from small, agile insectivores whose forelimbs had long metacarpals in relation to their phalanges.

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1. Introduction

Two distinct assemblages of flying mammals have been recognized since well before Dobson (1875) formally divided the bats into two suborders; the Megachiroptera (or megabats, with a single family, Pteropodidae) and the Microchiroptera (or microbats, with many families) (see Smith (1980) for a history of bat classification). The list of differences between these two groups of bats is long. The majority of investigations so far done on both suborders has revealed that they have contrasting attributes (table 1). Despite these differences, bats have traditionally been placed in one order, with the underlying presumption that mammalian flight has evolved only once. In this account, we consider the alternative hypothesis that mammalian flight has evolved twice. This paraphyletic hypothesis of bat origins has been advanced repeatedly before (for example, Jones & Genoways 1970; Smith 1976; Hill & Smith 1984; Pettigrew 1986), but has never been widely accepted, for reasons that are examined and rejected in this paper.

What then, is the basis for the firm conviction of so many scholars that bats are monophyletic (Winge 1941; Van Valen 1979; Koopman 1982; Novacek 1982), when there seems to be so little to link the megabats with the microbats across the gulf seen separating them in table 1? First, it should be made clear that a list of differences does not constitute an argument for paraphyly. The two groups of bats may, for example, have diverged sufficiently long ago that they have acquired a large number of differences. A more compelling argument would require that one of the chiropteran suborders share its differences with another mammalian group and that these shared differences be derived rather than representing some common mammalian inheritance. Although more work is required to determine which shared features are derived, in this respect, it is of interest that megabats agree with primates in 29 of the 33 differences listed in table 1, whereas microbats and primates agree in only one.

There appear to be two main obstacles to the acceptance of a paraphylectic origin for bats. The first is the strong similarity between the complex of musculoskeletal modifications that has given rise to the wing in each kind of bat. This similarity, combined with the obvious dissimilarity of the pterosaurian and avian forelimb modifications (Padian 1983), both from each other and from that of the bats, has had a powerful effect on the perception of taxonomists. For example, Koopman (1982) admitted that this similarity in wing structure 'overrides all other considerations'. Others (see, for example, Smith 1976, 1977), have stressed the need for caution here, in view of the strong possibility of parallelism, which has proven to be the rule rather than the exception in many phylogenetic systems (see Gosliner & Ghiselin 1984). Extensive homoplasy (or parallelism) is found to be an empirical feature of most phylogenetic reconstructions using parsimony analysis; rarely, if ever, does a phylogenetic tree have a consistency index of 1.0, indicating no homoplasy (see, for example, Jamieson *et al.* 1987). It has been claimed that 'little more than a glance' makes it obvious that megabat and microbat wings have a common derivation (Van Valen 1979), but perhaps some careful analysis would be advisable instead. Morphometric analysis has certainly revealed several points of difference between megabat and microbat wings (Smith & Starrett 1979).

The second obstacle to the acceptance of chiropteran paraphyly come from the absence of a recognizable sister group to which either group of bats can be clearly linked. The origin of bats seems to have been close to the stem of the eutherian tree within the 'Insectivora' (Hill & Smith 1984). The diversity and taxonomic difficulties within the latter group, which is

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TABLE 1. MICROBAT–MEGABAT[a] CONTRASTS

Note that of the 34 differences listed, one (terrestrial locomotion) agrees in microbats and primates, whereas 29 agree in primates and megabats (marked with an asterisk). The sources of the data are identified in the following list: 1, Hill & Smith (1984); 2, Andersen (1912); 3, Walker (1964); 4, Marshall (1985); 5, Koopman(1970); 6, Koopman & MacIntyre (1980); 7, Novick (1977); 8, Neuweiler *et al.* (1980); 9, Fenton (1984); 10, Novick (1958); 11, Sales & Pye (1984); 12, Neuweiler (1962); 13, Fenton & Crerar (1984); 14, Vaughn (1959); 15, Pettigrew (unpublished); 16, Wise *et al.* (1986); 17, M. Tuttle, personal communication; 18, Leen & Novick (1969); 19, Bauchot & Stephan (1970); 20, Stephan & Pirlot (1970); 21, Henson (1970); 22, Pettigrew (1986); 23, Cooper & Pettigrew (1986); 24, Theodor (1967); 25, Marshall (1981); 26, Dwyer (1971); 27, Bartholomew *et al.* (1964); 28, Henshaw (1970); 29, Rouse & Robson (1986); 30, Smith & Madkour (1980); 31, Haiduk (1983); 32, Haiduk *et al.* (1980); 33, Haiduk *et al.* (1981); 34, Baker & Bickham (1980); 35, Leen & Novick (1969); 36, Norberg (1976*a*); 37, Norberg (1976*b*); 38, Griffin *et al.* (1960); 39, Griffin (1958); 40, Norberg (1972); 35, Kennedy *et al.* (1987); 36, Nudo & Masterton (1985); 37, Pye & Hinchcliffe (1969); 38, Quay (1962); 39, Pettigrew *et al.* (1989); 40, Graydon *et al.* (1988); 41, Pettigrew *et al.* (1988); 42, Calford *et al.* (1985); 43, Wise *et al.* (1985); 44, Suga (1982); 45, Calford & McAnally (1987); 46, Novacek (1985); 47 Vogt & Vogt (1907); 48, R. Straney, quoted in Litos (1988).

	microbats	megabats	references
distribution	worldwide	*palaeotropical	1–5
diet and dentition	primarily insectivorous with secondary adaptations for fruit, flesh, blood and nectar; teeth with, or derivable from, w-shaped ectolophs	phytophagous with secondary adaptations for nectar; teeth simplified and not readily derivable from insectivorous w-ectoloph pattern	1,6
brain and behaviour			
laryngeal sonar	universally present in all species examined	*universally absent; non-laryngeal sonar in one or two genera	7–11
orientation	predominantly acoustic	*predominantly visual	8–12
roost posture	neck extended	*neck flexed	13
terrestrial locomotion	can move limbs independently; many species can run on ground	awkward, symmetrical forelimb movements while weight bearing	14,15
agonistic display	usually acoustic; wing spreading not recorded	often visual, involving wing spreading	17
pollex and hallux	limited independent use of these digits	*dexterous use of these digits; pollex long, hallux opposable	18
midbrain	inferior colliculus larger than superior colliculus	*superior colliculus larger than inferior colliculus	19,20
retinotectal pathway	plesiomorphous pattern	*apomorphous primate-like pattern	22,23
accessory optic system	medial terminal nucleus prominent	*medial terminal nucleus reduced	23
lateral geniculate nuclues	unlaminated	*laminated	45
spinal cord	cervical grey enlarged: large myelinated dorsal roots with medial entry; marginal nucleus of Hofmann-Koelliker	*unremarkable cervical spinal cord; marginal nucleus absent	21
forebrain	generally less well developed than hindbrain except in some advanced phyllostomids	*complex expansion of forebrain (e.g. cerebral cortex) in all species	1
somatosensory cortex	small hindlimb representation; two somatotopic maps (SI & SII)	*large hindlimb representation; three somatotopic maps (1, 3*b* & SII)	16
motor cortex	primitive arrangement of cortico-spinal areas	*advanced primate-like arrangement of cortico-spinal motoneuron fields	35,36
frontal eye fields	absent	*present	47
auditory cortex	low frequencies represented caudally	*low frequencies represented rostrally	43–45
visual cortex	no middle temporal area with direct input from area 17	*middle temporal area (MT) with direct input from area 17	39,42

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TABLE 1. (*cont.*)

retina			
nutrition	simple retina; choroidal circulation	choroidal papillations with retinal capillary loops as well as choroidal circulation	40
ganglion cells	streak below optic disc	*streak above optic disc	41
tapetum lucidum	never present	*often present	41
visual development	altricial; eyes open postnatally	*precocial; eyes open prenatally	15
parasites	nyceteribiid ectoparasites in subfamily nycteribiinae; thaumopsylline fleas	nycteribiid parasites in subfamilies archinycteribiinae and cyclopodiinae; ischnopsylline fleas	24
torpor	highly developed in 'main sequence' of insectivorous microbats	*generally absent except for possible partial heterothermia in macroglossines	25,28
sperm morphology	diverse; usually small acrosome; small subacrosomal space	large acrosome and subacrosomal space; unique origin for axoneme central singlets	29
penis	corpus spongiosum not enlarged to form glans penis	*corpus spongiosum enlarged to form glans penis	30
karyotype	high degree of chromosomal change; fusions most common type of rearrangement	*low degree of chromosomal change; heterochromatic additions most common type of rearrangement	31,34
pinna	interrupted anteriorly; tragus present	*complete; no tragus	1
middle ear	Paaw's cartilage present	*Paaw's cartilage absent	21,37
cochlea	non-allometric; acoustically isolated	*allometric	21,46
forelimb	long metacarpals in relation to phalanges	*metacarpals and phalanges similar in length	39
hindlimb	long metatarsals in relation to phalanges	*metatarsals and phalanges similar in length	39
	flexor tendons separated from gastrocnemius	flexor tendons with gastrocnemius in tunnel independent of calcaneus	48
cranium	post-orbital process absent	*post-orbital process present	1
skin	striated pilo-erector muscles	*smooth pilo-erector muscles	38

We recognize that some scholars are unhappy about the use of the neologisms 'megabat' and 'microbat' to refer to megachiropteran and microchiropteran bats, respectively. In spite of the mixed etymology, these terms can be justified, we believe, on the grounds that the commonly used alternatives are either awkward or ambiguous. For example, the terms 'microchiropteran' and 'megachiropteran' are, strictly speaking, adjectives that tend to stilt the text when they are used repeatedly as nouns, quite apart from their large number of syllables. The use of the term 'fruit bats' to describe the megabats is ambiguous because of the large radiation of frugivorous, phyllostomid microbats.

undoubtedly polyphyletic itself (see, for example, Kingdon 1974), have meant that it has been easier to link the two bat groups together, via the wing, than to find a sister group for either.

The present account attempts to overcome these obstacles. First, we show that primates can be justifiably regarded as a sister group for the megabats; the two groups share a large number of derived characters, which *in toto* cannot parsimoniously be considered parallelisms, and they are not found in microbats or in other mammals. The placement of bats within the primates has a long history that goes back to Linnaeus (1758). Rather than removing all bats from the order Primates, as scholars after Linnaeus have done (see Winge 1892), we would retain the megabats but remove the microbats. A specific line of investigation on the primate–megabat link was begun by Smith & Madkour (1980), using the morphology of the penis. The corpus spongiosum of the penis is enlarged into a glans in primates, megabats, and dermopterans, but

not in microbats. We have extended this approach by looking for derived features in the brain, especially in the visual pathway, the specific elaborations of which are among the most important characteristic of primates (Allman 1977; Martin 1986). In the past, neural pathways have been used successfully to illuminate primate relationships. For example, neural characters played a very important role in the argument about the relationship of tree-shrews to primates (Campbell 1974; Martin 1986*a*, *b*). Neural characters in the visual system should therefore be an appropriate basis for comparing primates with other putative 'sister groups'. We have done such a comparison by using formal cladistic analysis in the tradition of Hennig (1966).

Secondly, we present evidence that points to the independent evolution of megabat and microbat wings, from the morphometrics of the hindlimb and forelimb digits. We have tried to find a measure of the forelimb that shows the least amount of functional constraint and which therefore should be least affected by convergent similarity in the wings of the two groups of bats. One such measure, the metacarpophalangeal index, supports the idea that the megabat wing evolved from a primate or dermopteran, gliding precursor, while at the same time indicating that the microbat wing may have had separate evolutionary origins. The origins of bats are discussed in the light of the neural, skeletal and molecular evidence.

2. Material and methods

2.1. *Visual pathways analysis*

Both anterograde (eye-to-brain) and retrograde (brain-to-eye) labelling of neural pathways was done, as previously described (see Cooper & Pettigrew 1979; Pettigrew 1986; Pettigrew & Cooper 1986; Pettigrew *et al.* 1988) on the visual pathways of the chiropteran and non-chiropteran taxa shown in table 2. The non-chiropteran taxa in the orders Macroscelidea, Primates, Hyracoidea and Edentata were studied to supplement the information already available from the literature on these orders. In addition, we were able to obtain information on the visual pathways of one specimen of the dermopteran colugo, *Cynocephalus variegatus*, whose possible affinities with the bats are of great interest. Although there is extensive information available about the visual pathways of myomorph, cavimorph and sciuromorph rodents, we studied an additional gliding sciuromorph, *Petaurista petaurista*. This nocturnal, highly visual, phytophagous, aerial rodent provides a valuable comparison with dermopterans and megabats, both of which are also nocturnal, highly visual, phytophagus and aerial. We were therefore given the opportunity to examine the possibility of functional convergence within the nervous system of a rodent and a dermopteran with similar lifestyle.

2.2. *Choice of taxa for formal cladistic analysis*

There is a limit to the size of the data matrix we could run easily on a small laboratory computer. Also, we have found empirically that the topology of a computer-generated tree can be altered, and indeed biased, by the disproportionate representation that results from the inclusion of an excessive number of taxa in a particular group. For these reasons, the formal analysis below is based on a sample of two microbats and two megabats from the larger sample in table 2. Each of the microbat taxa would have been equivalent and interchangeable, as each had the same states for the neural characters of the matrix. This also holds true for the megabats (see below).

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Table 2. Taxa studied for visual pathways

Order Edentata				
Family Bradypodidae				
Bradypus tridactylus[a]	F	French Guiana	ARG	MNHN
Order Dermoptera				
Cynocephalus variegatus[a]	F	Thailand	HRP/ARG	UQ;MNHN
Suborder Megachiroptera				
Rousettus aegyptiacus[a]	F	Africa	ARG	MNHN
Pteropus alecto	L	Australia	HRP	UQ
Pretopus poliocephalus[a]	L	Australia	HRP	UQ
Pteropus scapulatus	L	Australia	HRP	UQ
Dobsonia moluccensis	L	Australia	HRP	UQ
Penthetor lucasi	F	Borneo	HRP	UQ
Eonycteris spelaea	F	Borneo	ARG	UQ
Syconycteris australis	L	Australia	HRP	UQ
Macroglossus minimus	F	Bornea	ARG	UQ
Suborder Microchiroptera				
Family Emballonuridae				
Emballonura alecto	F	Borneo	ARG	UQ
Taphozous georgianus	L	Australia	HRP	UQ
Taphozous melanopogon	F	Borneo	ARG	UQ
Family Megadermatidae				
Macroderma gigas[a]	L	Australia	HRP	UQ
Family Phyllostomidae				
Artibeus cinereus	L	Peru	HRP	UQ
Family Vespertilionidae				
Myotis adversus	L	Australia	HRP	UQ
Chalinolobus morio	L	Australia	HRP	UQ
Miniopteris australis	L	Australia	HRP	UQ
Nyctophilus gouldi	L	Australia	HRP	UQ
Family Molossidae				
Tadarida plicata	F	Borneo	ARG	UQ
Mormopterus beccarii[a]	L	Australia	HRP	UQ
Order Primates				
Family Cheirogaleidae				
Microcebus murinus	L	MNHN	HRP;ARG	MNHN
Family Lorisidae				
Nycticebus coucang	F	Thailand	HRP	UQ;MNHN
Family Tarsiidae				
Tarsius bancanus[a]	F	Borneo	ARG	SM;MNHN
Family Hylobatidae				
Hylobates lar[a]	L	INSERM	ARG	INSERM
Order Hyracoidea				
Procavia capensis	L	Africa	ARG	UQ;MRI
Order Rodentia				
Family Sciuridae				
Petaurista petaurista[a]	F	Thailand	HRP;ANG	UQ;MNHN
Order Macroscelidea				
Macroscelides proboscideus	L	Africa	HRP	UQ;MRI
Elephantulus brachyrhynchus	L	Africa	HRP	UQ;MRI
Elephantulus myurus[a]	L	Africa	HRP	UQ;MRI
Petrodromus tetradactylus	L	Africa	ARG;HRP	UQ;MRI

[a] Taxon was used to construct character matrix of table 3 and the cladogram; F, initial preparation carried out in the field; L, complete investigation carried out in the Laboratory; HRP, horse radish peroxidase injections and histochemistry; ARG, tritium-labelled amino acid injections and autoradiography; MRI, Mammal Research Institute (Pretoria); SM, Sarawak Museum, (Kuching); MNHN, Museum National D'Histoire Naturelle (Paris); INSERM, Lab.de Neuropsychologie Exp. U 94(Bron), UQ, University of Queensland.

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2.3. *Cladistic analysis*

Twenty-four quantitative and qualitative characters were derived from the central nervous system of 14 eutherian species representing the Microchiroptera ('microbats'), Megachiroptera ('megabats'), Edentata, Macroscelidea, Rodentia, Scandentia, Dermoptera and Primates. Plesiomorphic states were deduced from a survey of out-groups (marsupials, monotremes) and of putatively plesiomorphic eutherians including in-group taxa such as edentates. A hypothetical taxon with the deduced plesiomorphic states for all characters was used as the ancestor to provide the root for the otherwise unrooted Wagner tree(s). The attributes employed in the present study, and decisions as to plesiomorph–apomorph polarity for each character, are given in table 3 and are justified in more detail below. Plesiomorph states are coded as zero, while more apomorph states are coded as 1, if a two-state character, or of numerals $(1, 2 \ldots n)$ if they form a multistate transformation series.

Cladistic relationships were computed with the Phylogenetic Analysis Using Parsimony program (PAUP, version 2.1) of Swofford (1984). PAUP is a computer program for inferring phylogenies based on the principle of maximum parsimony. It uses a 'Wagner algorithm' (see Farris 1970; Swofford 1984) to find the shortest tree, the length of which is defined as the total number of evolutionary 'steps' (transformations from one character state to another) needed to produce the tree. PAUP is an unrestricted parsimony program, that is, it does not prohibit reversals from an apomorph state back to a more plesiomorph one, and it does not limit the number of origins of a character state. In addition, PAUP is able to treat unordered (disordered) characters, i.e. states the relationships between which are not considered deducible before the analysis (see Platnick (1987) for a comparison with other algorithms).

By using the hypothetical ancestor (see above), or using the Lundberg rooting option (see Swofford 1984), the shortest uprooted tree obtained for the in-group taxa is rooted at the position in which the hypothetical ancestor (containing only plesiomorphic states for all characters) would join the tree. This is a reasonable approach but does not, of course, obviate the difficulty that the choice of plesiomorphies, i.e. direction of change deduced, may be incorrect for one or all characters and can never be deduced with certainty. However, if most characters have a consistency index (CI) of 1 (i.e. no homoplasies), the plesiomorphic states for a subset of characters of more questionable polarity (particularly those with low CIs) can be deduced (at least for the set of characters used) by observing any transformation, from the supposed plesiomorph state, between the hypothetical ancestor and the root of the remainder of the tree for each character. The state observed may experimentally be given plesiomorphic status and the effect of this on the CI of the character can then be computed (see Jamieson *et al.* 1987). If the index increases, particularly if it alters to a value of 1 (indicating no homoplasies), the character may be rescored with the new polarity if it appears to conform to a rational series of evolutionary events for that character. Recourse to this procedure was made for characters 1 and 2 in this study.

2.4. *Brain characters used for cladistic analysis*

Character 1*: position of horizontal streak in iso-density contour map of retinal ganglion cells*

In most mammals, retinal wholemounts reveal a topographical organization of the retinal ganglion cell layer with an elongation of the iso-density contours parallel to the horizon, the

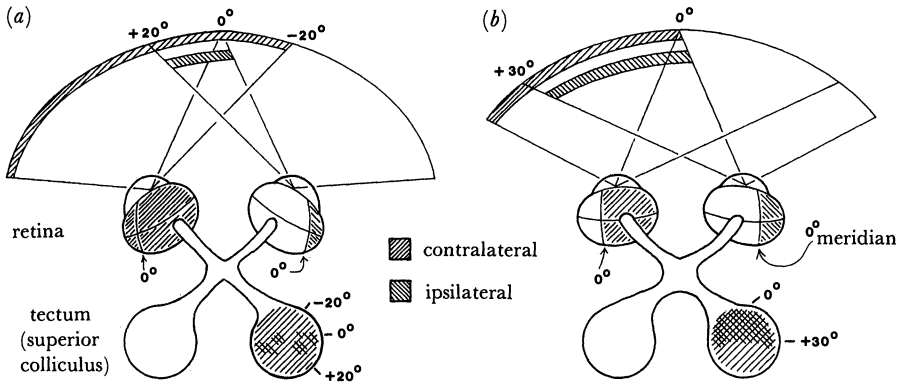

FIGURE 1. Retinotectal organization in primates and non-primates. Differences between the retinotectal pathways of primates (*b*) and all other vertebrates so far studied (*a*).

(*a*) plesiomorphic state (non-primate vertebrates)	(*b*) apomorphic state (primates)
(i) Large fraction of the total retinofugal axons; not much lower in density than the retinothalamic pathway.	Reduced in comparison to the retinothalamic pathway, which may have ten times the density of retinal neurons.
Retinotectal input is largely, if not exclusively, restricted to the contralateral eye.	Balanced as to the size of the contribution from each eye.
(iii) Retinotectal input comes from whole contralateral retina; not restricted to one hemifield.	Decussated so that only the contralateral hemifield of visual space is represented.
(iv) The front of the superior colliculus (or optic tectum) represents the far temporal edge of the retina.	Arranged in a particular pattern on the surface of the superior colliculus, so that its anterior margin represents the zero retinal meridian.

horizontal streak. The streak may not be very pronounced (e.g. in microchiropteran bats it is only just discernible, Pettigrew *et al.* (1988)), but only in rare cases (for example, the teleost *Amblyglyphidodon*, Collin & Pettigrew (1988)) can it not be discerned.

This character refers to the relation of the horizontal streak relative to the optic-nerve head. In the vast majority of vertebrates, the horizontal streak passes superior to the optic nerve head. This arrangement is seen in anurans (see Graydon & Giorgi 1984), Chondricthyes (Collin 1988), birds (Bravo & Pettigrew 1981, Moroney & Pettigrew 1987), Metatheria (Hokoc & Oswaldo-Cruz 1979) and many eutherians (see Hughes (1977) for an overview). The exceptions to this rule include the following eutherian taxa all of which have a horizontal streak that passes inferior to the optic-nerve head: elephant (Halasz & Stone 1986), elephant shrews (J. D. Pettigrew, unpublished data on *Petrodromus tetradactylus* and *Elephantulus myurus*), hyrax (unpublished data on *Procavia capensis*), rabbit (Hughes 1971), edentates (M. L. Cooper & J. D. Pettigrew, in preparation), two sciuromorph rodent species compared with many myomorph and cavimorph rodents that have been surveyed (see Stone 1981) and microchiropteran bats (Pettigrew *et al.* 1988). Because of the widespread occurrence across all major vertebrate out-groups of the condition with the streak superior to the optic disc, we considered at first that this condition was likely to be plesiomorphic. A more parsimonious tree was generated, however (i.e. homoplasy was minimized) when this assignment was reversed. We have also found some teleost retinae with the streak inferior to the disc (Collin & Pettigrew

1988). Accordingly, in table 3, the presence of a streak below the optic nerve head has been given the value of zero (indicating plesiomorphy), even though we recognize that most other non-mammalian vertebrate taxa investigated would consequently have a character state of 1, indicating that they were apomorphic in possessing a superior streak. Although this method may go against accepted cladistic practice, it must be stressed that the decision as to polarity in this case was largely immaterial as it affected only the number of steps in the tree. The topology of the tree generated by the computer (figure 8) was not altered by reversing the polarity of this character. We think it likely that some characters have reversed their polarity more than once during vertebrate or mammalian evolution. Perhaps a better documented case is character 19, discussed below. The latter character, in all likelihood, has reversed twice in mammalian phylogeny; once when the early mammals acquired a large auditory midbrain, in contrast with reptilian outgroups, and again when visual specialists such as carnivores and primates appeared.

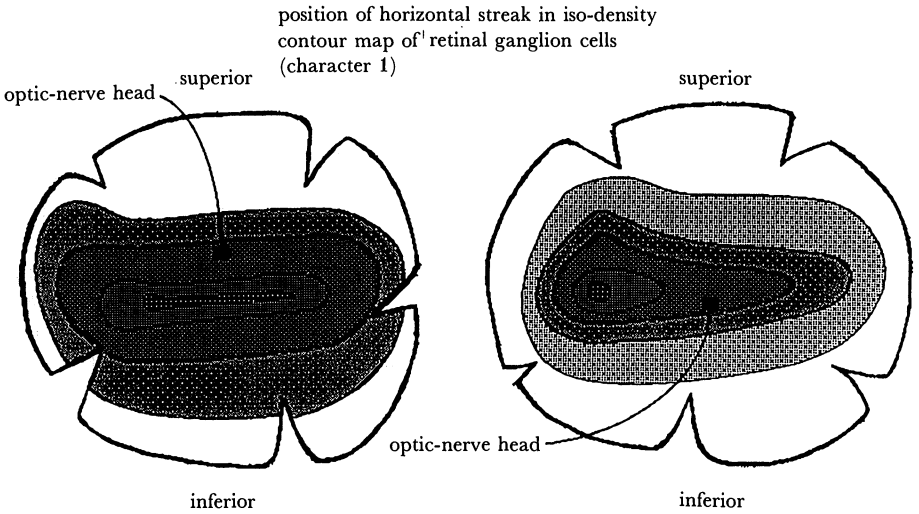

FIGURE 2. Two possible dispositions of the mammalian retinal horizontal streak with respect to the optic nerve head (character 1 in table 3). There is a horizontally oriented region of increased ganglion cell density – the horizontal 'streak'. Note that the streak may lie below (*a*) the optic-nerve head, as in edentates, microbats, lagomorphs, elephants and elephant shrews, or above (*b*) the optic-nerve head, as in carnivores, ungulates, primates, dermopterans and megabats.

Character 2: lacunar demarcation between ipsilateral and contralateral inputs

This feature is evident only after anterograde transport labelling from one eye with a radioactive amino acid or horseradish peroxidase (Pettigrew & Cooper 1986; Cooper *et al.* 1979; Cooper & Pettigrew 1979). Islands of label surrounded by an area clear of label in one lateral geniculate nucleus (LGN) have exactly matching lacunae of no label, surrounded by an area of label in the opposite LGN. The rounded border of sharp demarcation between the regions of ipsilateral and contralateral eye representation give the suggestion of having been punched out with a 'cookie-cutter'. This extremely sharp cut-off contrasts markedly with the binocular overlap that is often observed between the domains of each eye in the LGN of metatherians (Dasyurids are a good example; see Sanderson (1986), whereas the rounded boundaries with a sharper curvature than the LGN borders themselves contrast with the laminar boundaries between the domains for each eye as shown by most eutherians (see character 3). It is difficult to see this feature of lacunar demarcation of LGN organization as transitional to

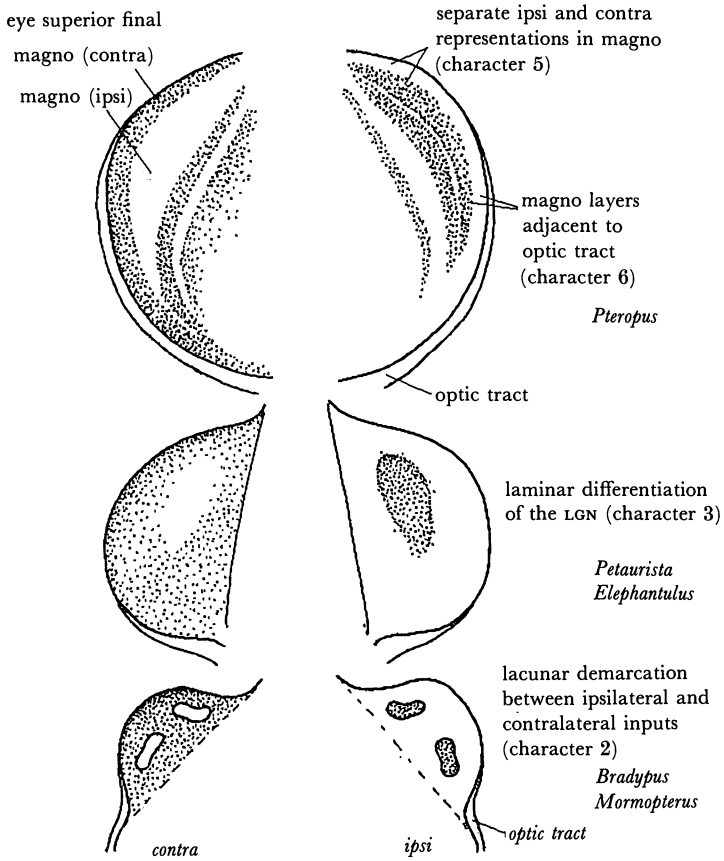

FIGURE 3. Mammalian lateral geniculate nucleus I. Characters 2, 3, 5 and 6 in table 3 and §2.4. Shading represents label transported anterogradely from an eye injection. Contra = contralateral to the injected eye, ipsi = ipsilateral to the injected eye. Note that *Pteropus* (upper) has six layers that are separately labelled (three well-separated layers on the contralateral side, one well separated and two separated but adjacent layers on the ipsilateral side). For further description see text.

the laminar organization or to the diffuse organization with binocular overlap, and we initially assigned a 1 (the apomorphic state of a binary character) to this state, which we have observed so far only in the dentate tree sloth, *Bradypus* (M. L. Cooper, unpublished data) and in all of the microchiropteran bats that we have examined with the anterograde technique (*Chalinolobus morio*, *Nyctophilus gouldi*, *Miniopteris australis* and *Myotis adversus* in the family Vespertilionidae; *Taphozous georgianus* and *Emballonura alecto* in the family Emballonuridae; *Macroderma gigas* in the family Megadermatidae; *Mormopterus beccariae*, *Tadarida plicata* and *Mormopterus loriae* in the family Molossidae) (Pettigrew *et al.* 1988; Pettigrew 1989 in preparation). This distribution resulted in one homoplasy (consistency index 0.5) for the state, whereas, if regarded as a plesiomorphy, its consistency index was 1. We therefore scored it as zero in table 2, while recognizing that it, and laminar differentiation, may be independent apomorphies from a plesiomorphic condition not represented in the eutherians studied here (see character 3).

Character 3: laminar differentiation of the LGN

The usual pattern of mammalian LGN organization shows laminar organization, wherein inputs from each eye are segregated into laminae, whose borders run roughly parallel to the

external boundary of the LGN itself. It is seen in table 3, as the apomorphic state 1, in all taxa except the microbats *Macroderma*, *Mormopterus* and the edentate *Bradypus*, which show the plesiomorph state in which it is lacking. Although the distribution of this character is exactly complementary to character 2 among the present group of taxa, note that the two characters are not logical correlations as the pangolin, *Manis*, lacks both the lacunar demarcation (character 2) and laminar differentiation (character 3) and the rock hyrax, *Procavia capensis*, has laminar differentiation but has broadly overlapping regions of ipsilateral and contralateral labelling (data not shown).

Character 4*: presence of differentiated magnocellular layer or layers*

The presence of a separate, clearly differentiated layer of large cells (state 1) is a prominent feature of many mammalian LGNs, particularly among the carnivores and primates (Sanderson 1986). This feature is not seen in rodents, microchiropterans, edentates or elephant shrews (Pettigrew & Cooper, unpublished data).

Character 5*: separate ipsilateral and contralateral eye representations within the magnocellular layers*

As revealed by anterograde transport labelling, there are two separate magnocellular laminae, one for each eye (state 1). In the taxa chosen for this study, the distribution of this character is the same as for character 4, but note that this would not be the case if we had included some marsupials (e.g. *Sminthopsis* or *Dasyurus* from the family Dasyuridae), which have a separate magnocellular layer (character 4), but have ipsilateral and contralateral eye inputs overlapping within that lamina.

Character 6*: magnocellular layers immediately adjacent to the optic tract*

Although the tree shrew, *Tupaia*, has differentiated magnocellular layers, these are located deep in the LGN rather than on the external surface subjacent to the optic tract (Conley *et al.* 1987). This location of the magnocellular layers adjacent to the optic tract (state 1), is known only for the primates, megachiropterans and dermopterans. Even where, in some higher primates, a sparse, interrupted lamina of irregularly sized cells intervenes between the magnocellular layers and the optic tract (S or O laminae), we have scored this character in the same way because of the irregular and incomplete nature of the interposed layer.

Character 7*: ipsilateral magnocellular layer external to the contralateral layer*

When both layers are present, there are two possible orientations of the paired magnocellular layers (one for each eye) with respect to each other. We have arbitrarily assigned 1 to the state found only in *Tarsius* and *Cynocephalus*, where the ipsilateral eye's lamina lies external to the contralateral eye's lamina. We have no way of knowing whether this state is apomorphic or not and think that it is equally likely that *Tarsius* and *Cynocephalus* share a feature that was more widespread in early primate phylogeny. The zero state has been assigned to the more common (but not necessarily plesiomorphic)state found in most primates and megachiropterans where the contralateral eye's lamina lies external to that of the ipsilateral eye.

Character 8*: proportions of ipsilateral and contralateral eye input in* LGN

The relative proportions of the LGN taken up by inputs from the two eyes can be estimated from anterograde transport studies. The proportion of the LGN taken up by the ipsilateral eye's

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input varies from less than 10 % in microchiropterans to 50 % in the higher primates. We have scored this character on a scale from 0 (less than 10 % ipsilateral input) to 6 (50 % ipsilateral input).

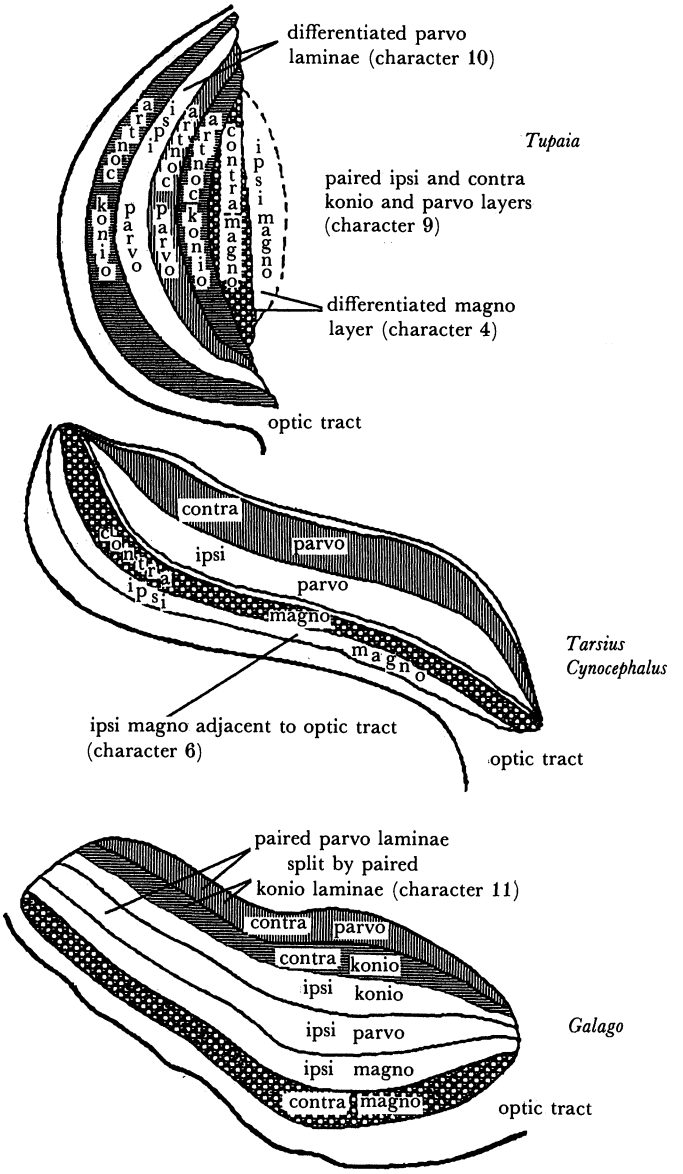

FIGURE 4. Mammalian lateral geniculate nucleus II. Characters 4, 6, 10 and 11 in table 3. Contralateral layers are all shown with label (ipsilateral layers are shown blank). In addition, contralateral layers are each shown with stippling to indicate the type of cells in the layer. Koniocellular layer (konio), horizontal stippling; parvocellular layer (parvo), vertical stippling; magnocellular layer (magno), round stippling. The small konio layers in *Tarsius* and *Cynocephalus* are not separately identified.

Character 9*: paired ipsilateral and contralateral koniocellular and parvocellular layers*

While the tree shrew has six layers, two of these (probably corresponding to the konio layers of primates) are unpaired, each being driven by the contralateral eye (Conley *et al.* 1987). By contrast, primates and megachiropterans have matched pairs of layers, one for each eye. In other words, in addition to the right- and left-eye magnocellular pair, there are a further

two right-eye, left-eye pairs for the parvo- and koniocellular layers. Distinction between konio and parvo layers is not a routine matter and requires further investigation in many cases such as that recently completed for the tree shrew (Conley *et al.* 1987). We do not yet know the exact disposition of parvo and konio layers in *Pteropus*, although it is clear that in this species there are two pairs of layers in addition to the pair of magnocellular layers.

Character 10*: differentiated parvocellular laminae with segregated inputs from each eye*

This feature has been described so far only in primates, tree shrews, *Cynocephalus* and megachiropterans. The present data show that the distribution of this character is identical to that of character 4 (differentiated magnocellular layer), but note once again that the lateral geniculate nuclei of some marsupials have character 4 but not character 10 (Sanderson 1986).

Character 11*: paired parvocellular laminae split by paired koniocellular laminae*

Two koniocellular layers (one for each eye) are immediately adjacent to each other but are interposed between the ipsilateral eye's parvocellular lamina (on the side closest to the optic tract) and the contralateral eye's lamina (on the side furthest from the optic tract). Although it is theoretically possible to have the arrangement where the ipsi- and contra-parvocellular layers have reversed their positions about the paired konio layers, this has not so far been observed in any mammal (marsupials included). We have not therefore allowed for this possibility in our character set.

This unusual arrangement where parvo layers are split by a pair of konio layers has been described for the following five prosimian primates, *Lemur catta*, *Microcebus murinus*, *Galago senegalensis*, *Nycticebus coucouang* and *Periodicticus potto* (Cooper & Pettigrew (in preparation); Conley *et al.* 1987; Kaas *et al.* 1978), but is not known for any investigated simian, nor in the 'prosimian', *Tarsius* (Cooper & Pettigrew, in preparation). In the present data set it is unique to *Galago* and therefore does not contribute to the configuration of the phylogram. It is included because we have found it useful in ongoing studies that include the wider range of prosimians mentioned above.

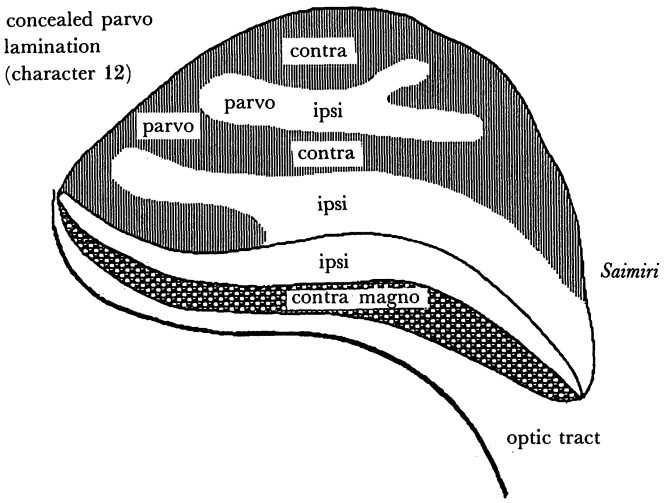

FIGURE 5. Mammalian lateral geniculate nucleus III. Character 12. Conventions as for figure 4. Description in text.

Character 12*: concealed parvocellular lamination*

While in all primates the parvocellular cell mass is clearly differentiated from the magno- and koniocellular layers (see character 10), in all Old World primates whose LGN is known, the parvocellular region is divided by cell-sparse interlaminar zones into separate layers for each eye that are recognizable in conventional Nissl-stained sections; the separate layers for each eye can thus be recognized without the benefit of labelling, although labelling is necessary to assign a layer to a particular eye. In contrast, in the New World Monkeys, *Callithrix* and *Saimiri*, the separation of the parvocellular mass into separate layers for each eye may not be apparent unless anterograde label from the eye is used to define the separate regions within the mass. The condition is termed concealed lamination (Kaas *et al.* 1978). In most non-primate mammalian out-groups, lamination is concealed, but there is no parvo–magno laminar differentiation. This we consider to constitute the plesiomorphic condition of parvo–magno differentiation (state 0). We have recognized a transformation series where the apomorph state (1) is the presence of visible lamination in the parvocellular laminae of Nissl-stained material. It has proved parsimonious to regard concealed parvo lamination of the type seen in the New World monkeys (*Saimiri*) as a further apomorphic state (state 2). This multi-state arrangement was found to have a better consistency index than the alternative binary arrangement with two separate characters (one for the presence or absence of concealed parvocellular lamination, one for the presence or absence of visible lamination).

Character 13*: parvocellular leaflets*

Reduplication of the parvocellular laminae is a prominent feature of the LGN of the New World saki and squirrel monkeys, the Old World monkeys *Cercopithecus*, *Macaca* and *Papio*, as well as chimpanzee and humans, but it is not observed in the gibbon, New World marmosets, prosimians or tarsier (Kaas *et al.* 1978; Cooper & Pettigrew 1989, in preparation). This condition is almost certainly an apomorphic feature (state 1) associated with the huge increase in numbers of parvocellular elements found in the visual pathways of diurnal primates (Kaas *et al.* 1972).

Character 14*: ratio of ipsilateral to contralateral eye input to superior colliculus*

This ratio varies from zero in microchiropterans, where there is no discernible input to the superior colliculus from the ipsilateral eye, to 1 in the gibbon where the inputs from each eye are exactly balanced. We have assigned four apomorph states to the character and have assumed that extreme contralateral bias is the plesiomorphic condition on the grounds that balanced binocularity in the optic tectum is very rarely encountered in out-groups such as metatherians, birds, anurans and fish. Absence of reversals, whether this character is treated as ordered or unordered, endorses this decision. On the other hand, there are cases of balanced binocularity in one of the small-fibre diameter pathways of some fish, so it is possible that this character has reversed polarity at least once (see, for example, Reperant *et al.* 1976; see Ebbeson (1984) for review).

Character 15*: surface representation of superior colliculus taken by ipsilateral eye's input*

There is considerable variation in the position, shape and extent of the ipsilateral eye's representation on the superior colliculus (figure 6). In the gibbon, the ipsilateral eye

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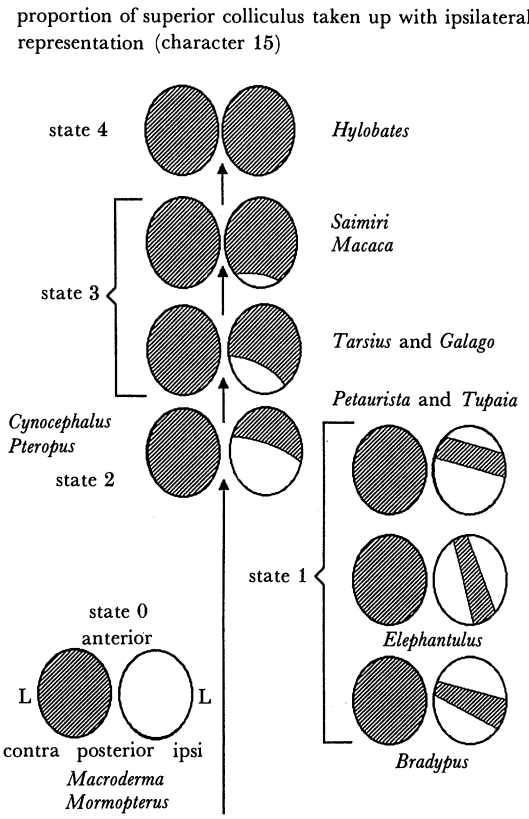

FIGURE 6. Ipsilateral representation in the mammalian superior colliculus: character 15 of table 3. The stippling represents the proportion of the dorsal surface of the superior colliculus taken up with label transported from one eye. Anterior is to the top and the ipsilateral colliculus is on the right in all cases (as shown at bottom left). Note that the size of the representation may vary from zero (the microbats, *Macroderma* and *Mormopterus*), through increasing fractions in the rodents (*Petaurista*) and elephant shrews (*Elephantulus*), until it becomes a significant proportion in the primates, megabats and dermopterans. Note that the latter group are all characterized by ipsilateral label reaching the anterior pole of the superior colliculus, and that the gibbon, *Hylobates*, has involvement of the complete surface of the ipsilateral colliculus.

representation occupies the complete rostrocaudal extent of the colliculus (state 4). In the simians and prosimians there is a crescent of absent label at the caudal pole of the colliculus (state 3). In megachiropterans and in *Cynocephalus* the label from the ipsilateral eye extends right to the rostral-most pole of the colliculus, as in primates, but there is a fairly large region of absent label from the caudal half to one third of the colliculus (state 2). In the flying squirrel, *Petaurista*, as in other rodents, there are two regions devoid of label, one at the rostral pole and one at the caudal pole, with the result that label is confined to a belt that runs obliquely across the middle of the collicular surface (state 1). A similar pattern is seen in the tree shrew, the tree sloth and the elephant shrew, although the extreme obliquity of the 'belt' in the elephant shrew means that its anterior edge reaches the rostral pole of the colliculus, just as it does in the rabbit (Pettigrew 1989, in preparation). We have not tried to take this variation in the obliquity of the belt into account in the present study, but feel that it would be important to do this in any study that included both the rabbit and elephant shrew. These share several unusual visual pathway features to a degree which supports McKenna's (1975) Ernotheria. In microchiropterans the ipsilateral representation is so small as to be indiscernible (0).

Character 16*: ratio of retinogeniculate to retinotectal ganglion cells*

In all vertebrates, most retinal ganglion cells project to the midbrain optic tectum (superior colliculus in mammals) with only a small majority sending axons to the thalamic lateral geniculate nucleus. The only known exceptions to this rule are the primates, megachiropterans and *Cynocephalus*, which have a minority of retinotectal ganglion cells and a majority of retinothalamic ganglion cells. We have ordered this character from 0 in tree shrew, flying squirrel, microchiropterans, tree sloth and elephant shrew, all of which have retinal ganglion cell populations dominated by the retinotectal group (Pettigrew 1986; Pettigrew & Cooper, in preparation) to 3 in the gibbon, simians and prosimians. Intermediate values of 2 are given to the tarsier and megachiropterans because of the strong tectal projection in the tarsier despite its massive retinothalamic contribution and the relatively small total size of the retinothalamic population in the megachiropterans whose retinotectal population is tiny, as it is in most primates (Pettigrew 1986; Pettigrew & Cooper 1989, in preparation). *Cynocephalus* has a relatively small LGN compared with primates and megachiropterans, so we have scored it 1, even though it has a reduced retinotectal input.

Character 17*: decussation in the retinotectal ganglion cell population*

A sharp decussation line of retinotectal ganglion cells is found at the vertical meridian only in primates, megachiropterans and *Cynocephalus* (Pettigrew 1986; Allman 1977; Kaas 1986; Pettigrew & Cooper 1989. (In preparation.)

The possession of derived states for characters 14–17 has been attributed to the functional acquisition of stereopsis by animals occupying the fine branch niche (Martin 1986*b*). According to this viewpoint, homoplasy of the derived states of these midbrain characters would be expected in taxa that have independently acquired the ability to carry out stereoscopic processing in the fine branch niche. There are problems with this viewpoint which include,

1. Stereoscopic processing takes place in the retino-geniculostriate pathway, not in the retinotectal pathway (see review in Pettigrew (1986*b*)).

2. Excluding primates, stereopsis has evolved independently in several other taxa whose visual pathways are well described. In none of these non-primate cases has there been any modification of the retinotectal pathway. For example, cats (Barlow *et al.* 1967), owls (Pettigrew 1979) and ungulates (Clarke *et al.* 1976) all have stereoscopic processing, but with a retinotectal pathway lacking in any of the derived features under consideration.

3. There is a good correlation between elaborations of the geniculostriate visual pathway and specializations for the fine branch niche. We therefore find multi-laminate lateral geniculate nuclei in arboreal squirrels and phalangers as well as primates (Sanderson 1986). Squirrels and phalangers nevertheless have a plesiomorphic retinotectal visual pathway and their own distinctive patterns of lamination in the LGN.

In summary, there is no strong reason to argue that the retinotectal characters are subject to more functional constraint than any other neural characters. There is no obvious functional explanation for the sharply defined distribution of derived retinotectal characters within mammals. It is possible that the primate reduction in the direct retinal input to the tectum has led to an increased influence of the indirect tectal input from the retinogeniculostriate pathway whose derived decussation pattern and balanced binocularity is then imposed upon

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the tectum. Such an explanation can account for some of the characters, but fails to account for the primary reduction of the retinotectal pathway, such a rare feature of visual systems.

Character 18*: reduced medial terminal nucleus*

There are three main accessory optic nuclei in mammals, the medial, dorsal and lateral terminal nuclei. The medial terminal nucleus is usually very prominent, but is reduced in primates (except, curiously, for the tarsier), megachiropterans, *Cynocephalus* and *Bradypus*. As a large medial terminal nucleus is a feature of major mammalian out-groups, such as Metatheria (see, for example, Sanderson & Pearson 1981), we have assigned 0 to this condition and 1 to the reduction observed in most primates.

Character 19*: ratio of inferior colliculus to superior colliculus*

The inferior colliculus (IC) is an auditary midbrain structure that lies immediately behind the visual midbrain superior colliculus, against which it can readily be measured. In some mammals, such as the microchiropteran bats, the inferior colliculus dominates the midbrain in such a way that the superior colliculus (SC) is difficult to discern on macroscopic inspection of the brain. In contrast, more visual mammals such as the tree shrew, primates, *Petaurista* and *Elephantulus* have enlarged superior colliculi. We have assigned 1 to the condition where the IC > SC, but recognize that this is tentative. No change in the consistency index is obtained when the polarity is changed. The visual midbrain dominates in most non-mammalian taxa, but it is quite likely that the earliest mammals had an auditory midbrain which was more prominent than the visual midbrain (Jerison 1974).

Character 20*: auditory pathway specializations for sonar*

Microbats have several features in the auditory pathway that set them apart from other mammals, and which are probably related in some way to echolocation and sonar. Some examples include the specialization within the nuclei of the lateral lemniscus thought to be associated with the processing of time information (Covey & Casseday 1986), the elaboration of auditory cortical regions (Suga 1986) and large cochlea, which has enabled microchiropterans to be distinguished from megachiropterans, even in fossil material (Novacek 1985). These characters of microchiropterans have been grouped together (as state 1), for convenience, to avoid undue duplication of characters confined to microchiropterans.

Character 21*: laryngeal sonar*

Although the neurological substrate for it is still in the process of elucidation (see, for example, Sculler 1979), high-frequency sonar cells emitted from the larynx are a universal feature of microchiropterans (Sales & Pye 1974; Fenton 1984). This ability is absent in megachiropterans, despite the well-recognized ability of *Rousettus* to echolocate using clicks generated by striking the tongue against the roof of the mouth (Kulzer 1960) and recent suggestions that *Eonycteris* may echolocate using wing claps (Gould 1988).

Character 22*: middle temporal visual cortical area*

A cortical visual area devoted to the processing of movement has been described in the temporal lobe of primates (Allman 1977; Kaas 1986). A middle temporal visual cortical area (MT) can be recognized by several different criteria and appears to be confined to primates,

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megachiropterans and *Cynocephalus*. The criteria include: direct input from visual cortical area 17; circumscribed boundaries in myelin-stained material, and location anterior to and not adjoining area 18. Based on these criteria, as well as physiological studies, MT is present in the higher primates and the prosimian *Galago* (Allman 1977). By cytoarchitectonic criteria it is almost certainly present in *Tarsius* (Pettigrew & Cooper, in preparation) and there is similar evidence for its presence in *Cynocephalus*. *Pteropus* clearly has an MT based on direct projections from area 17 to a temporal cortical area, combined with mapping studies of cortex (Calford *et al.* 1985). We have assigned 1 to this character because of its limited distribution within these mammals and its apparent absence from out-group taxa.

Character 23*: spinal cord with greatly enlarged dorsal roots and dorsal horn*

Microchiropteran bats have an unusual specialization in the spinal cord (figure 7) (state 1 of this neural character), where entering dorsal roots and their associated ganglia are enlarged and the dorsal columns are surrounded by a greatly expanded dorsal horn (Henson 1970).

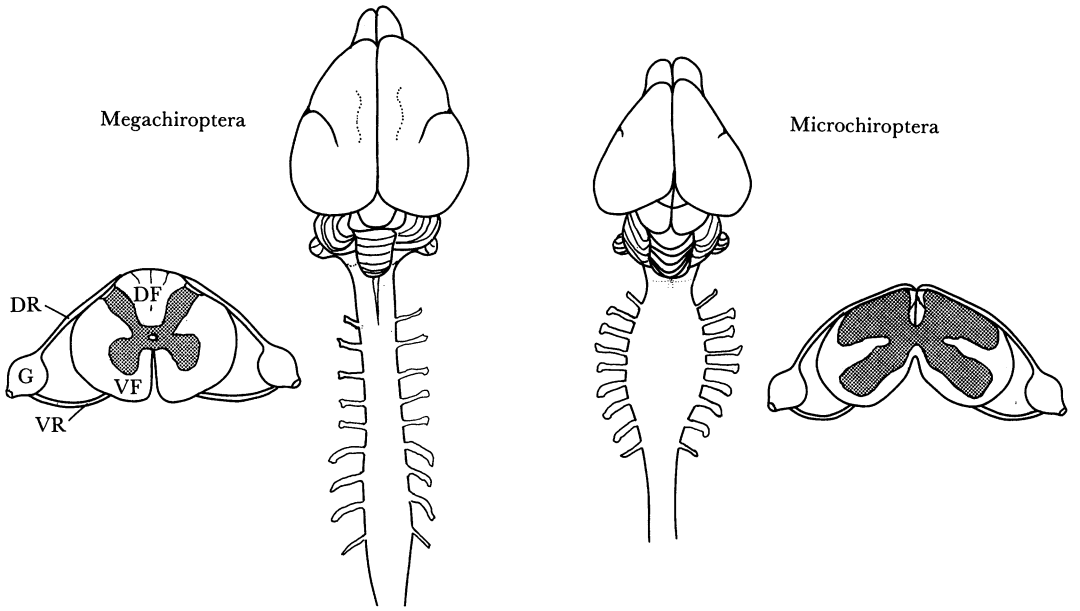

FIGURE 7. Derived spinal cord arrangement in microbats: megachiroptera have a spinal cord (left) like most other mammals, with dorsal roots entering the dorsal horn of the cord just lateral to the dorsal funiculi (DF). In contrast, the cervical cord of microbats is enlarged and afferents entering in the dorsal roots traverse to the midline and enter the expanded dorsal column from the medial site. The dorsal root afferents of microbats are also very large in diameter, heavily myelinated and have very large cell bodies in the dorsal root ganglion (G), when compared with other mammals. VR, ventral root; VF, ventral funiculus.

Character 24*: premotor area C*

Nudo (1985) has described a third field of cortico-spinal neurons (area C) in the premotor cortex of primates (*Macaca*, *Galago*, *Callithrix* and *Saimiri*), which is absent from seven other mammalian orders that he studied. We have extended this work to metatherians (*Trichosurus* and *Sminthopsis*), macroscelidids (*Elephantulus* and *Macroscelides*) and the microbats, *Macroderma*, *Artibeus* and *Myotis*, all of which also lack area C. *Pteropus* has an area C very similar in size, shape, location and cell composition to that found in *Galago* (see, for example, Kennedy *et al.* 1987).

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2.5. *Limb measurements*

The lengths of the forelimb digital bones were either measured with dial calipers, or obtained from the literature (Andersen 1905, 1912; Dobson 1878; Freeman 1981; Habersetzer & Storch 1987; Hill 1983) for 597 different chiropteran taxa. Measurements were obtained for all 175 known megachiropteran species (including the fossil megabat, *Archaeopteropus*) and 423 representative species from all the living families of the Microchiroptera, in addition to the oldest fossil microbat genera, *Icaronycteris* and *Palaeochiropteryx*. An effort was made to obtain information from all genera (such as those with very large and very small body size within each family) that might contain taxa† representing the extremes of variation in wing morphology. Where possible, more than one specimen of each taxon were measured to obtain a mean value, but we found that intra-specific variation was very small for the ratios taken. Digital measurements were also taken from the forelimbs and hindlimbs of 68 non-chiropteran mammal species from 11 orders and 22 families (see table 4).

X-radiography was used to obtain the lengths of the digital bones in the forefeet of small mammals and the hindfeet of bats, both cases where we were unable to take the measurements directly through the skin. We tried to ensure that the X-radiographs did not give a foreshortened view of any bones, particularly of the proximal phalanx which is often bent at an angle to the metatarsal in skin specimens. This constraint limited our sample of hindfeet considerably, as we had to eliminate many cases where the metatarsophalangeal ratio may have been artificially elevated because of foreshortening of the proximal phalanx. In the larger mammals, digital measurements could be made directly, particularly if the specimen had been prepared as a skeleton.

Measurements were done at the Queensland Museum, the Sarawak Museum, the British Museum (Natural History), the Transvaal Museum, the Mammal Research Institute, the Museum of Vertebrate Zoology at Berkeley and the Department of Zoology Museum at University of Queensland.

(*a*) *Metacarpophalangeal index*

To facilitate comparison between taxa of greatly different sizes, the digital measurements are presented as a ratio of the length of the metacarpal to the length of the proximal phalanx of the same digit. Data were not easily obtainable from some digits, particularly the first (because of its small size and variability) and the second (which lacks phalanges in some bats). Initial results indicated that data from the fifth digit had a strong functional component, which led to similar values for unrelated, but functionally similar, chiropteran taxa. For these reasons, particular attention has been directed in the present analysis to the values obtained from the third and fourth digits. To simplify the data presentation, the metacarpophalangeal ratios for the third and fourth digits have been added together to provide a single index that is used through this paper (M/P). In few taxa, X-radiography enabled a metatarsophalangeal index to be determined in a similar way for the hindlimb.

(*b*) *Developmental studies of M/P*

Developmental series of digital forelimb measurements were available from juveniles of the microchiropterans *Tadarida brasiliensis* (forearm measurements [FA], 18–46 mm) and *Myotis*

† The full list of taxa and measurements is available to anyone interested who writes to the principal author.

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velifer (FA = 12–45 mm) and both foetuses and juveniles of the microchiropterans *Macroderma gigas* (FA = 15–100 mm) and the megachiropteran *Pteropus scapulatus* (FA = 5–70 mm).

2.6. *Haemoglobin sequence analysis*

We used the similarity coefficient of Russell & Rao (1940) to compute resemblances of amino acid sequences in the β-globin chain from published sequences for haemoglobin primary structure. This coefficient gives equal weight to matched and unmatched pairs, excludes negative matches in the numerator and is expressed as: $S = n_{JK}/n$, where S is the similarity between taxa J and K, n_{JK} is the number of positive matches, and n is the total number of characters.

A total of 10 chiropteran sequences are available from the work of Dr G. Braunitzer and his collaborators in Martinsreid, four from the megabats *Pteropus poliocephalus*, *Pteropus alecto*, *Cynopterus sphinx* and *Rousettus aegyptiacus* and six from the microbats *Myotis velifer*, *Antrozous pallidus* (family Vespertilionidae), *Tadarida brasiliensis* (family Molossidae), *Megaderma lyra* (family Megadermatidae), *Rhinopoma hardwickei* (family Phinopomatidae) and *Macrotus californicus* (family Phyllostomidae) (see Kleinschmidt *et al.* (1988) for the most recent data on megabat haemoglobin sequences and for the references to earlier bat sequences which have been determined in Dr Braunitzer's laboratory). The chiropteran data were compared with β-globin sequence data from 24 other eutherian mammals, including two rodents (*Mus* and *Citellus*); five ungulates (llama, pig (order Artiodactyla); zebra, horse and lowland tapid (order Perissodactlya, *Tapir terrestris*); two carnivores (cat and dog (order Carnivora)); 10 primates (human (family Hominidae); gibbon (family Hylobatidae, *Hylobates* spp.); rhesus macaque (family Cercopithecidae, *Macaca mulatta*); cotton-headed tamarin (family Callithricidae, *Saguinas oedipus*); squirrel monkey (family Cebidae, *Saimiri sciureus*); slow loris (family Lorisidae, *Nycticebus coucang*); greater galago (family Lorisidae, *Galago crassicaudatus*); Western tarsier (family Tarsiidae, *Tarsius bancanus*); brown lemur (family Lemuridae, *Lemur fulvus*) and ring-tailed lemur (family Lemuridae, *Lemur catta*); rock hyrax (order Hyracoidea, *Procavia habessinica*); tree shrew (order Scandentia, *Tupaia glis*); rabbit (order Lagomorpha); nine-banded armadillo (order Edentata, *Dasypus novemcinctus*) and grey kangaroo (order Marsupialia, *Macropus giganteus*).

As PAUP analyses did not reach a resolution in less than 100 trees, resemblances between amino acid substitution sites were computed using the coefficient of Russell & Rao (1940), followed by UPGMA (group average) sorting. Data input for computation were prepared in three distinct ways, two of which are cladistic modifications of this similarity coefficient, the third being purely phenetic.

(*a*) *Cladistic resemblance*

In this procedure an out-group relative to the included eutherian mammals was selected, namely the grey kangaroo, *Macropus giganteus*. This was (*a*) attributed plesiomorphy (a zero state) for all 140 amino acids or (*b*), more appropriately, was used as the basis for determination of a hypothetical ancestor for the eutherian in-group. In alternative (*c*), all considered taxa were input into a PAUP analysis that, for this data set, terminated at the 100-tree limit. The apomorphic changes from the kangaroo sequence to the first node of the in-group tree (26 of the 146 sites) revealed in this analysis were incorporated, with the 120 unchanged sites, into the sequence recognized as the hypothetical ancestral molecule (*hypanc*)

for the eutherians. The *hypanc* agreed closely with that deduced 'intuitively' by inspection involving comparisons of kangaroo sequences with those in disparate in-group taxa. When plesiomorph states have been deduced by out-group comparison and are represented by zeros, the Russell & Rao (1940) coefficient provides a measure of the cladistic resemblance between taxa because shared zero (plesiomorphic) states do not contribute to the coefficient. In the absence of a subsequent subprogram for branch swapping, this method does not guarantee determination of the shortest tree(s) but it at least establishes relationship purely on the basis of shared advanced states (synapomorphies) and avoids establishment of relationship on a mixture of these and shared primitive characters (symplesiomorphies) inherent in phenetic methods.

In the program prepared for the first method, integers were substituted for the letters representing the amino acids of all taxa, 0 for the plesiomorph acid and 1 to $(n_a{-}1)$ for the apomorphic amino acids at each of the 146 sites, where n_a is the number of alternative acids at a site for all taxa. This, in practice, reduced the number of alternative states at a given site to well below the limit of 16 symbols permitted in PAUP analyses for the computer used. Retension of lettered states for more than 20 amino acids potentially present, would have exceeded this limit.

(*b*) *Cladistic resemblance based on DNA triplets*

In this method, as in method 1, either the kangaroo or the *hypanc* was included and treated as a wholly plesiomorphic taxon. As the 16-symbol limit of PAUP was not involved, states were retained as the letters denoting their amino acids. The Russell & Rao similarity measure was used and shared plesiomorphies again did not contribute to the similarity score in the numerator, whereas identical states at a given site between two taxa gave a score of 1. However, where the states differed, and would have been computed as a mismatch adding 0 to the numerator in method 1, a score (maximally 1) was given here that reflected the DNA base separation of the acids. In this method the denominator was reduced by the number of negative (0−0) matches (symplesiomorphies) at each site. On the other hand, in methods 1 and 3, although the program allowed for this, the alternative of including negative matches in the denominator was chosen.

(*c*) *Phenetic resemblance*

If, alternatively, resemblance between all amino acid sites is computed using the Russell & Rao coefficient, without recourse to considerations of plesiomorphy or apomorphy, there are no negative matches and the coefficient effectively corresponds to that of Jaccard (1908): $S = n_{JK}/(n_{JK}+u)$, where u is the number of mismatches. The method is a phenetic rather than a cladistic or phylogenetic method. It has the advantage of objectivity in that determination of character polarity is not involved, but some spurious relationships based on symplesiomorphies (albeit difficult to determine) are inevitable. For discussion of clustering and the various coefficients we have used, the reader is referred to Sokal & Sneath (1963).

We used Swofford's PAUP program on several smaller haemoglobin data sets than were examined with the similarity coefficient method. The greater computing requirements of PAUP when large data sets are used on a laboratory computer have limited the number of taxa that we could examine at one time. A comparison of the effectiveness of PAUP, and other programs for parsimony analysis, for revealing mammalian relationships when the data are derived from

protein sequences, neural characters and morphological features, respectively, will be the subject of a later report (Pettigrew & Jamieson 1989). (In preparation.)

3. Brain characters shared by megabats and primates

3.1. *Megabat visual pathways*

Previous results on *Pteropus* spp. that showed the presence of all of the primate features of the retinotectal pathway (Pettigrew 1986) have been confirmed on the wider set of taxa from the Megachiroptera. In addition, the lateral geniculate nucleus (LGN) of megabats, as revealed by anterograde labelling techniques, has now been delineated in greater detail so that it can be compared with the LGN organization of other mammalian taxa. The LGN of all megabats so far studied (table 2) has a '2×3' (two eyes by three cell types) lamination pattern, with three layers (parvocellular, koniocellular and magnocellular) for each eye and with magnocellular layers lying externally, just underneath the optic tract. This is an unusual pattern in mammals, particularly when one considers the large number of possible combinations of ordering six layers, but it is the same as that found in primates and the dermopteran, *Cynocephalus*, and quite distinct from that found in the tree shrew, *Tupaia*, whose LGN has six layers but a totally different (4+2) arrangement with four layers for the contralateral eye and two layers for the ipsilateral eye. This complex of characters has enabled us to distinguish the visual pathway of primates from the visual pathways of all other vertebrates so far studied, including the tree shrews, which are often considered to be the sister-group of primates.

The four characters related to the retinotectal pathway from the eye to the midbrain superior colliculus are detailed in figures 1 and 6. The tectum on one side of the primate brain, by these rules of connectivity, represents the ipsilateral hemifield of both retinas (Allman 1977). This has been demonstrated in prosimians (Lane *et al.* 1973), New World monkeys (Kadoya *et al.* 1972), Old World monkeys (Lane *et al.* 1973; Cynader & Berman 1972) and gibbons (Cooper *et al.* 1986). In contrast, the tectum of non-primates represents the whole of the contralateral retina and is dominated by the synaptic inputs from that retina. This has been demonstrated in anurans (Gaze 1958); teleosts (Schwassman & Kruger 1965); birds (Bravo & Pettigrew 1981); reptiles (Stein & Gaither 1981); marsupials (Ramoa *et al.* 1985); various rodents (Lashley 1934, Woolsey *et al.* 1971); lagomorphs (Hughes 1971); elephant shrews (Pettigrew, unpublished data); edentates and pholidotes (Cooper & Pettigrew 1989) (In preparation.); hyraxes (J. D. Pettigrew, unpublished data); ungulates (Pettigrew *et al.* 1981); carnivores (Straschill & Hoffman 1972) and tree shrews (Kaas *et al.* 1974).

While microbats conform to the plesiomorphic pattern for these characters shown by most vertebrates, megabats show the primate pattern, both quantitatively and qualitatively, as all four characters in the retinotectal pathway (Pettigrew 1986), and for other characters in the accessory optic system, lateral geniculate nucleus and retino-hypothalamic pathway (Cooper & Pettigrew 1986). This has been shown for nine species of megabats in seven genera (*Pteropus*, *Rousettus*, *Penthetor*, *Eonycteris*, *Dobsonia*, *Cynopterus* and *Syconycteris*), covering the full spectrum of size and feeding adaptations of this suborder.

3.2. *Microbat visual pathways*

In microbats, there was a little more interspecific variation in the organization of the visual pathways than found in megabats. For example, there were marked variations in the depth and

TABLE 3. TAXA AND THEIR CHARACTERS

	taxon 1 *Hylobates*	2 *Macaca*	3 *Saimiri*	4 *Galago*	5 *Tarsius*	6 *Cynocephalus*	7 *Rousettus*	8 *Pteropus*	9 *Tupaia*	10 *Petaurista*	11 *Macroderma*	12 *Mormopterus*	13 *Bradypus*	14 *Elephantulus*	
1. position of horizontal streak relative to optic-nerve head; inferior (P); superior (A)	1	1	1	1	1	1	1	1	1	1	0	0	0	0	
2. lacunar demarcation between ipsi and contra inputs; not lacunar (P); lacunar (A)	1	1	1	1	1	1	1	1	1	1	0	0	0	1	
3. laminar differentiation of the LGN; not laminar (P); laminar (A)	1	1	1	1	1	1	1	1	1	1	0	0	0	1	
4. presence of differentiated magno layer; not differentiated (P); differentiated (A)	1	1	1	1	1	1	1	1	1	0	0	0	0	0	
5. separate ipsi- and contra-lateral magnocellular layers; not separate (P); separate (A)	1	1	1	1	1	1	1	1	1	0	0	0	0	0	
6. magnocellular layers adjacent to the optic tract; not adjacent (P); adjacent (A)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	
7. ipsi magnocellular layer external to the contra layer; contra external to ipsi (P); reverse (A)	0	0	0	0	1	1	0	0	0	0	0	0	0	0	
8. [a]ratio of ipsilateral to contralateral eye input in LGN; < 10% (P); to 50% (A1–6)	6	6	6	5	5	3	4	4	3	2	0	0	1	1	
9. [a]paired ipsi and contra konio- and parvocellular layers; no differentiation or pairing (P); 1 pair (A1); 2 pairs (A2)	2	2	2	2	2	2	2	2	1	0	0	0	0	0	
10. parvo laminae with each eye input segregated; not segregated (P); segregated (A)	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1
11. paired parvo laminae split by paired konio laminae; not split (P); split (A)	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1.
12. [a]concealed parvocellular lamination; parvo-magno differentiation absent (P); visible (1); concealed (2)	1	1	2	1	1	1	1	1	1	0	0	0	0	0	1.(
13. parvocellular leaflets; not reduplicated (P); reduplicated (A)	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1.
14. [a]ratio of ipsi to contra eye input to SC; 0 (P); ratio increasing to 1 (A1–4)	4	3	3	3	3	2	2	2	1	1	0	0	2	1	0
15. [a]proportion of SC; taken by ipsi representation 1; negligible (P); to entire rostro-caudal SC (A1–4)	4	3	3	3	3	2	2	2	1	1	0	0	1	1	1
16. [a]ratio retinogeniculate: retinotectal (RT) ganglion cells; ganglion cells mostly RT (P); increased retinothalamic (A1–3)	3	3	3	3	2	1	2	2	0	0	0	0	0	0	
17. decussation of retinotectal ganglion cell population; not sharp (P); sharp (A)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	
18. reduced medial terminal nucleus; MTN prominent (P); MTN reduced (A)	1	1	1	1	0	1	1	1	0	0	0	0	1	0	(
19. ratio of inferior colliculus to superior colliculus; IC > SC (P); SC > IC (A)	1	1	1	1	1	1	1	1	1	1	0	0	0	1	;
20. auditory pathway specializations for sonar; sonar specializations absent (P); present (A)	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
21. laryngeal sonar; HF sonar neuronal substrate absent (P); present (A)	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
22. middle temporal visual cortical area (MT; MT absent (P); MT present (A)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1
23. spinal dorsal roots & dorsal horn greatly enlarged; dorsal roots and ganglia not enlarged (P); enlarged; (A)	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
24. premotor area C; PMAC absent (P); present (A)	1	1	1	1	?	?	?	1	0	0	0	0	0	0	1

[a] Multistate characters. Other characters are binary. Abbreviations: P, plesiomorphic; A or A1-n, apomorphic state; CI, Consistency Index (a measure of homoplasy; CI = 1.0 when there are no homoplasies).

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degree of branching of the medial terminal nucleus (MTN). The MTN is enormously expanded in microbats compared with megabats, where the nucleus can be discerned only with the greatest difficulty. In the emballonurid genera *Taphozous* and *Emballonura* the MTN had several branches extending deep into the tegmentum, and in the molossids *Tadarida* and *Mormopterus*, the MTN also ramified deeply. The LGN of molossids was also distinct from that found in other microbat families, with a central patch of retinal terminals with a densely clustered arrangement, surrounded by the more usual uniformly spaced terminals. Despite the interfamilial variation in the exact morphology of the retinal terminations and the retino-recipient nuclei, none of the microbats examined showed any evidence of the primate-like specializations. We found no microbat that showed any evidence of lamination in the lateral geniculate projections, nor any with the retinotectal features found in all megabats. The retinogeniculate projection of all microbats studied showed a primitive, 'lacunar' organization. This pattern, with a small island or two of ipsilateral input and a matching hole or holes in the contralateral input, is unlike that of any other mammalian group with which we are familiar, with the possible exception of the edentate tree sloth *Bradypus*.

3.3. *Representative sample of bat visual systems?*

A question has arisen as to how representative our sample of chiropteran visual systems is, given the extreme diversity of bats and the limitations imposed by experimental labelling of the visual pathway (e.g. Wible & Novacek 1988). In the case of megabats, there can be little doubt that our sample is representative, as we have included some of the atypical cavernicolous megabats (*Eonycteris*, *Rousettus* and *Penthetor*), the nectar-feeding specialist *Syconycteris*, (which is also one of the smallest megabats), as well as a range of different sizes and lifestyles from the widespread genera *Pteropus* and *Cynopterus*. In all of these cases we found the same set of visual pathway characters, with virtually no inter-specific variation. Compared with other megabats, the tectal label was slightly weaker and more patchy in *Rousettus* and *Eonycteris*, perhaps related to the cavernicolous habits of these two genera, but the overall distribution and pattern of labelling was identical in all megabats.

In the case of microbat representation there is a little more room for doubt, as the technical difficulties of working with the tiny microchiropteran eye have conspired with their enormous diversity (129 Recent genera) to limit the completeness of the sample. The present sample of visual pathways comes from 11 species in 10 genera from five families (table 2). The shape of some of the target nuclei (particularly the MTN) varies from family to family, yet the overall pattern of retinal termination is invariant. For this reason any of the microchiropteran taxa that we have studied could be substituted for *Mormopterus* or *Macroderma* in the cladistic analysis of figure 8 without changing the result.

What are the chances that a microbat will subsequently be found with a visual pathway that breaks the general rules which are unbroken by our sample so far? On a purely statistical basis, it might seem likely that there will prove to be exceptions somewhere among the remaining 95% of microbats yet to be studied. Nevertheless, on logical grounds, we consider it most unlikely that future investigation will reveal a visual pathway with the primate-like pattern in some as-yet-unstudied microbat. The reason relates to the nature of the primate-like visual specializations themselves, all an indication of a visually advanced lifestyle. While the significance of such specializations as a multi-laminated LGN and a hemi-decussated retinotectal

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pathway are presently unclear, we know enough to be reasonably confident that they are unlikely to be found in taxa for which vision is not the primary sensory modality. Put another way, of all the microbats, those most likely to show the primate-like visual specializations are the highly visual microchiropteran taxa, such as the megadermatids and emballonurids, for example. In this sense, the initial choice of the megadermatid *Macroderma gigas*, was a strong control. *Macroderma gigas* has the largest eye, the best visual acuity, the largest number of retinal ganglion cells and therefore the most highly developed vision of any microbat known (Pettigrew *et al.* 1988). This generalization is likely to hold, because of the dominant role played by eye size in limiting chiropteran visual ability (Pettigrew *et al.* 1988), if we take into account the fact that neither of the other microbats in *Macroderma*'s size range (neither *Hipposideros commersoni* nor *Vampyrum*) has a larger eye. The fact that *Macroderma* and visually well-endowed emballonurids, molossids or phyllostomids did not show any of the primate visual features, makes it rather unlikely that less visual microbats such as noctilionids, mormoopids, rhinolophoids and natalids will prove to be exceptions in the future.

This should not be taken to indicate that we consider it is a good general strategy to choose a highly specialized taxon for a phylogenetic analysis. The specializations might prove to be unrepresentative of the group, like some of the complicated reproductive strategies of phyllostomid microbats, which are misleading when used to compare microbat reproduction as a whole with other groups. A plesiomorphic representative, nearest the ground plan of a given group, *ipso facto* gives a more accurate picture of relationships outside the group. In the present case, if a more-visually specialized microbat such as *Macroderma*, had shown the primate-like features, one would still have to set about establishing whether this was typical for microbats, or whether it might be a homoplasy, as has already been suggested for megabats (Martin 1986*b*). That hypothetical situation has not arisen, and we have yet another example where the megabats appear to share a derived feature with primates that they do not share with microbats.

4. Cladistic analysis

4.1. *Cladogram generated with neural characters*

There are clearly several brain characters shared in common between primates and megabats. We have formally and objectively analysed such characters to see what they reveal about the relationships between the taxa under consideration. For reasons already explained, we have restricted the data matrix to the 14 taxa and 24 characters in table 3.

Using this data matrix, the PAUP computation, with the ordered branch and bound option and the option Mulpars, provided three most parsimonious trees, each of 43 steps and each with a consistency index of 0.907. They differed only in the configuration of the two megabats. One (figure 8), grouped the two megabats as a monophyletic group; the other two showed the megabats as paraphyletic, with either *Pteropus* or *Rousettus* linking first with the primates as its sister-group. On *a priori* grounds, paraphyly of pteropids seems unacceptable, but the arrangement does serve to highlight their close relationships to primates. Furthermore, if the three trees are 'tested' against one or more of three non-neural characters (presence of chloroidal papillae, type of retinal circulation and presence of a patagium), in conjunction with the 24 neural characters, only monophyly of the two pteropids is supported. The trees that include the three non-neural characters are equivocal as to whether the colugo, *Cynocephalus*, is paraphyletic or monophyletic with the megabats and it may well be that association in a

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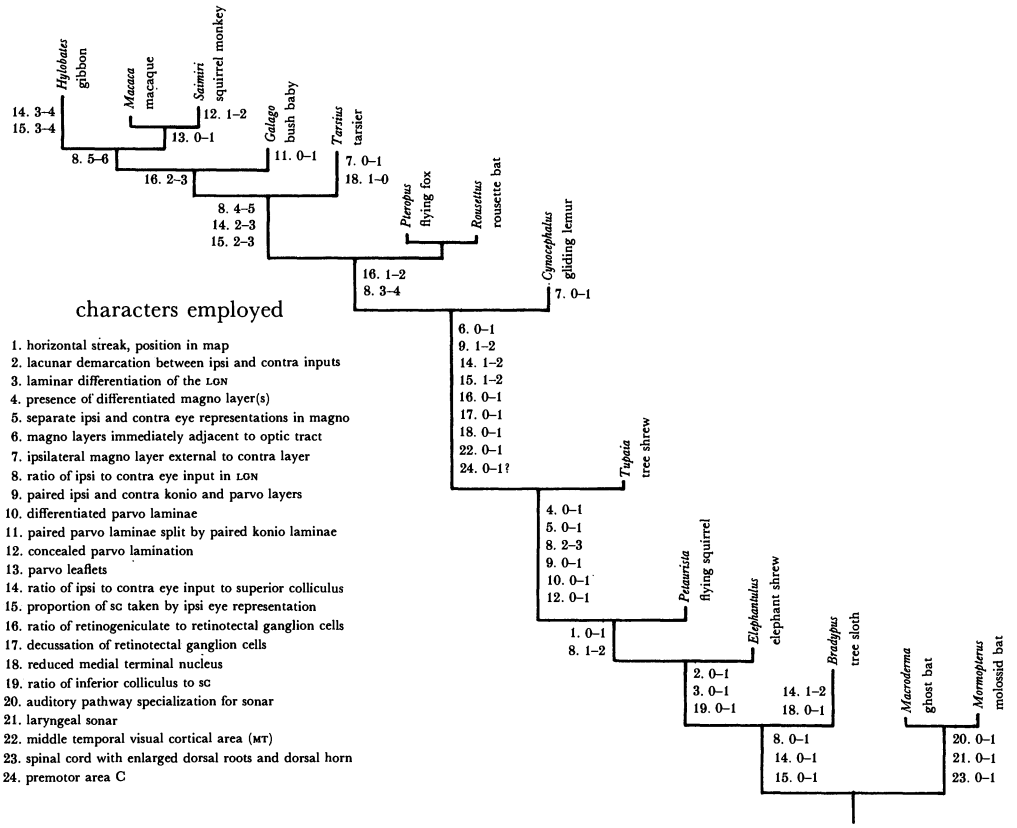

FIGURE 8. The most parsimonious cladogram generated from the data matrix of 24 neural characters and 14 taxa in table 3, using the PAUP program and the ordered, branch and bound option; there were 43 steps and the consistency index was 0.907. Note that the neural characters have successfully identified the primates and their relations. The two megabats, *Rousettus* and *Pteropus*, form a sister group to the primates, as does the dermopteran, *Cynocephalus*. The two microbats, *Mormopterus* and *Macroderma*, are widely separated from the megabats, by other mammalian orders, including tree shrews (*Tupaia*) rodents (*Petaurista*), elephant shrews (*Elephantulus*) and edentates (*Bradypus*). The neural characters, as well as failing to recognize any affinity between megabats and microbats, also fail to reveal any special affinity between the two gliding mammals, *Cynocephalus* (which the cladogram places with the primates) and *Petaurista* (which was a perfectly typical rodent as far as its nervous system was concerned).

monophyletic group with the pteropids is phylogenetically 'correct' (see §4.4). It will be noted that the two pteropids show no neural synapomorphies (of the characters used) beyond those which they share with the hypothetical ancestor of the primates. They do, however, have a unique synapomorphy (autapomorphy), the possession of choroid papillae. Inclusion of choroid papillae in conjunction with the neural characters gave a single parsimonious tree of 44 steps with a consistency index (CI) of 0.909, agreeing in topology totally with figure 8.

If trees were computed for only brain characters, with all characters unordered, the same CI (0.907), and a number of steps (43), were obtained, but the number of equally parsimonious trees rose from three to ten. In these trees, the megabats, *Tarsius* and *Cynocephalus* showed varying relationships with a group formed by the remainder of the primates. The remainder of the tree, from the ancestor to *Tupaia*, was identical with that in figure 8.

The chief features of the 'ordered' tree (figure 8), on which all apomorphies are indicated, are:

1. the Megachiroptera (*Pteropus*, *Rousettus*) from the plesiomorphic sister-group of the

primates (*Tarsius*, *Galago*, *Saimiri*, *Macaca* and *Hylobates*) with the colugo, *Cynocephalus* as the plesiomorphic sister-taxon of this assemblage;

2. the tree shrew, *Tupaia* is the plesiomorphic sister-taxon of the *Cynocephalus*–megabat–primate asemblage and shows no apomorphies relative to the common hypothetical ancestor (HTU) of these combined taxa, therefore representing their ground plan for these characters;

3. successive (chained) plesiomorphic sister-taxa towards the base of the tree are the flying squirrel, *Petaurista*; the elephant shrew, *Elephantulus*; the tree sloth, *Bradypus*, and, basally, a unified Microchiroptera, represented by the Australian ghost bat, *Macroderma*, and, as its sister taxon, the mastiff bat, *Mormopterus*.

4.2. *Relationship to accepted mammalian phylogenies*

In many respects the tree (figure 7) conforms to accepted views of mammalian phylogeny. For example, anthropoids, such as the macaque, squirrel monkey and gibbon, form a monophyletic group. The tarsier, whose status as a primate has never been questioned, has nevertheless been the subject of constant debate as to its phylogenetic position within the primates. It has been placed in the haplorhine primates (anthropoids) or alternatively in the prosimians (represented in our study by *Galago*). Our cladogram shows that both of these affiliations are invalid as they result in paraphyletic assemblages. The tarsier shares no apomorphies (for the characters used) with other primates which are not common to all primates. This applies to the prosimian *Galago* used for our analysis, to all other prosimians such as lemurs and cheirogaleids (see §2.4*b*), and to the anthropoids. This conclusion is further strengthened by the apparent retention in the tarsier of a large medial terminal nucleus (character 18), a feature lost in all other living primates studied. Although supporting the primate status of the tarsier, the cladogram at the same time highlights the difficulty of placing it within one of the named assemblages of primates.

4.3. *The dermopteran–primate–megachiropteran association*

The position of *Cynocephalus* in the cladogram is consonant with placement by early taxonomists, either in the primates (Linnaeus 1758), or close to them (hence 'flying lemur' (Gregory 1910), and interestingly, 'flying maucauco', the maucauco being the loris (Pennant 1781). A close link to primates is also indicated by recent serological evidence (Cronin & Sarich 1978). The question therefore arises, once again, whether this single genus of two species warrants separate ordinal status as the sole living representative of the Dermoptera. If megabats can be accepted as primates, perhaps *Cynocephalus* is better regarded as an aberrant primate, highly specialized as a primary folivore, whose true status has eluded us in the same way that the primate aye-aye, *Daubentonia*, was placed with the rodents for some time because of unusual specializations such as its large diastema and chisel-shaped incisors (Oxnard 1981). A further element in the 'disguise' of *Cynocephalus* (in having an uncharacteristically small brain and enlargement of the cerebral ventricles) may be the phenotypic impact of the folivorous niche on its brain development. A similar syndrome of small brain and ventricular enlargement appears to have been independently acquired by folivorous genera of three different orders: in Dermoptera by *Cynocephalus*; in the Marsupialia by the koala, *Phascolarctos*; and in the Edentata by the tree sloth, *Bradypus*. The only plausible link between these three taxa is the fact that they are primary folivores exposed during brain development to the deleterious effects of high

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circulating levels of phytogenous toxins (J. D. Pettigrew, M. L. Cooper & J. D. Haight, unpublished data).

In addition to the primate–dermopteran link, early scholars of mammals, such as Lêché (1886), were sufficiently impressed by the number of shared characters between Dermoptera and Megachiroptera to propose a phylogenetic link between them. The proposal never gained much ground, largely because the rapidly increasing corpus of knowledge about the Microchiroptera revealed so many points of difference between these bats and dermopterans. Winge (1892), in particular, took Lêché to task and raised thirteen points which were supposed to demolish the thesis that bats may have evolved via a dermopteran-type ancestor. Close examination of these points reveals, however, that the valid points of difference all involve characters found in Microchiroptera which are not concordant in Megachiroptera, or are at least controversial or difficult to discern. An example of the latter concerns the derivation of the various components of the tympanic cavity, which Winge (1892) confidently asserts are different in bats and Dermoptera, yet which may well be homologous in Dermoptera and Megachiroptera (Novacek 1980). The basic problem here lies with the assumption that bats are monophyletic, as Winge's (1892) contention that Dermoptera and bats are far apart is reasonable only if one is forced by this assumption to include for comparison the diverse Microchiroptera, whose numerous sources of difference with the Megachiroptera have already been emphasized (table 1). The subject is therefore worthy of re-examination in the light of the present thesis that the Megachiroptera represent a line of flying mammals totally separate from the Microchiroptera. Novacek (1980) has allied dermopterans with both microbats and megabats on the basis of basicranial features and patagium. A microbat–dermopteran link is not supported by the following features in visual pathways, forelimb digits, diet, size, distribution and locomotion, which are nevertheless support a megabat–dermopteran association.

4.4. *Features linking dermopterans and megabats*

Both groups have large frontally placed eyes that aid foraging at night. The lateral geniculate nucleus of *Cynocephalus* has a number of characters so far confined to primates and megabats (Kaas *et al.* 1978; see also §2.4). Recent work has shown that *Cynocephalus variegatus* has the primate pattern of organization of the retinotectal pathway, as in megabats (Pettigrew & Cooper 1986).

There is a clearly homologous relationship between the patagium of bats and that of the colugo, whose patagium is unique among gliding mammals (Lêché 1886; Gregory 1910; Szalay 1969). In contrast to the other five families of gliding mammals, all of which have patagia attaching to the forelimb at some point proximal to the digits (Rayner 1981), *Cynocephalus* has a flight membrane that extends to the phalanges of the digits, as in chiropteran patagia, with which it shares the same pattern of innervation and musculature (Lêché 1886).

Both *Cynocephalus* and megabats share with primates the derived, low value for the metacarpophalangeal index (see below and figure 8).

Dermoptera, Pteropodidae and Primates have a glans penis that is formed by an extension of the corpus spongiosum (Smith & Madkour 1980). These three groups of mammals are the only ones known to have a glans penis formed in this way.

Both pteropodids and dermopterans are phytophagous, the dermopteran diet and dentition having become somewhat specialized for the shredding of leaves. Although the details of the

diets are different in this way, the specialization of Dermoptera for leaf-eating conforms to some expectations of evolutionary theory if our proposal of a gliding, frugivorous, dermopteran-type precursor to the Pteropodidae is correct. This is because dermopterans would have had to find a new 'adaptive zone' once true flapping flight appeared in a successor; that is, they would have been forced to find a more specialized niche to avoid competition with their now more mobile, frugivorous flying successors. This line of reasoning is supported by the present-day scarcity of dermopteran species (two in the single genus of this order) compared with the more diverse dermopterans of the past (Rose & Simons 1977; Szalay 1976) and the diverse pteropodids of the present (around 174 species in 43 genera).

Both living and fossil dermopterans have moderate sizes (estimated forearm length around 100 mm), like that of the postulated plesiomorph state for megabats (see below). This contrasts with the microbats, which have also been linked to dermopterans (Novacek 1982, 1986), but whose plesiomorphic size was probably small (estimated forearm length less than 40 mm).

Both dermopterans and megabats are limited to the Palaeotropical zone, in contrast to the microbats, which have a diverse radiation in the Neotropics. Dermopterans and pteropodids are extremely awkward on the ground, in contrast to microbats, which show considerable quadrupedal agility, including running (see, for example, molossids, G. C. Richards in Strahan (1983)) and leaping (desmodontines, Yalden & Morris (1975)). Apparently lacking the normal mammalian (and microbat) anti-gravity reflexes necessary to support their body mass on three limbs while they extend a fourth limb, both megabats and *Cynocephalus* are forced to move along the ground by symmetrical 'rowing' movements of the forelimbs. Symmetrical movements of this kind are also used for vertical climbing by *Cynocephalus*. In contrast to this 'symmetrical' behaviour, when the animals are suspended in the trees and the flexors are acting as the anti-gravity muscles rather than the extensors, both have a comparable pattern of locomotion involving alternate, independent movements of both forelimbs and hindlimbs (J. D. P. Walker 1964; personal observations by J D. Pettigrew).

The large number of derived features in common to the brains of *Cynocephalus* and megabats have already been described (table 3, figure 8 and §2.4).

4.5. *Consistency of neural characters*

The characters we have used all have a high degree of consistency within the generated cladogram, as can be seen in table 3 and figure 7. There are several possible explanations for this consistency, which contrasts with the generally low values obtained with molecular sequence data on the mammals (see Wyss *et al.* 1986 for a comparison of several studies in mammalian phylogeny). The first possible explanation might be that we have selected the characters, unwittingly or not, in a way that was influenced by preconceptions about the relationships of the taxa of interest. In this connection, it could be pointed out that there is one group of neural characters shared between both kinds of bats that we have failed to include for consideration. These relate to the representation of the wing found in the somatosensory cortex (SI) of bats, whether these are of the microchiropteran or megachiropteran variety. First, there is the presence of receptive fields which can be plotted for SI cortical neurons on the wing surface (Zook & Fowler 1982; Calford *et al.* 1985). A feature so consequential upon the presence of the wing itself can hardly be included as a neural character. Second, there is the topological reversal of the representation of the forelimb digits reflecting the position of the digits in flight

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(Calford *et al.* 1985; Wise *et al.* 1986). We did not include consideration of such characters in the present study because of their obvious relation to the musculoskeletal adaptations of the forelimb. In addition, such characters cannot be demonstrated anatomically and their distribution across different taxa is therefore incompletely known. Although the microbat, *Macroderma gigas* shares the topological reversal with the megabat, *Pteropus poliocephalus*, this is not true of all microbats. The reversal is not found in the vespertilionid microbat, *Antrozous pallidus*, which spends time on the ground where it uses its forelimbs in a posture unlike that used for flight (Zook & Fowler 1982; Zook, personal communication).

We have therefore avoided the use of such cortical traits involving physiological determinations of topology within subsections of a map that may also be subject to developmental and experimental variations. In answer to the charge that we may have biased the data set by eliminating a somatosensory character that appears to unite bats at the exclusion of other mammals (cf. Wible & Novacek 1988), we reply that there are other topological characters of cortex that we have not used and whose inclusion has the opposite effect; to unite the megabats with primates and to split off the microbats. Examples include the multiple representations of the body surface found within SI of both primates and megabats but not microbats, the polarity of the tonotopic map within auditory cortical area AI, which has the low frequencies represented rostrally in primates and megabats but has low frequencies represented caudally in microbats, the presence of frontal eye fields in the cortex of both primates and megabats but not microbats and the enlarged hindlimb representation in the somatosensory cortex of megabats and primates but not microbats (see table 1 for references). The distribution of all these characters is incompletely known. For this reason, and for the reasons already given above, they were not used in the matrix we analysed in this paper. Their inclusion in a smaller data matrix strengthens the major conclusion of this study that megabats are more closely associated to the primate lineage than to microbats.

We think that a possible reason for the high consistency of the neural characters may lie with properties of the nervous system itself. The hierarchical organization and conservative evolution of the nervous system may lend itself to phylogenetic analysis. Although homoplasy of nervous system structures occurs, such cases can usually be readily recognized as such from the details of the wiring diagram in each case. We can take two well-studied examples:

1. the independent evolution of the Doppler-shift strategy for sonar in acoustically cluttered environments by two separate groups of microbats, the New World pteronotids and the Old World rhinolophoids (see Neuweiler 1984; Neuweiler *et al.* 1980);

2. the independent evolution of pathways for binocular vision by owls and cats (Pettigrew & Konishi 1976).

Despite the great similarities between the overall outcome, the neuroscientist has no difficulty in detecting the detailed structural differences between the pteronotid and rhinolophoid solutions that reflect their separate origins (Neuweiler 1984). Similarly, the owl's solution to binocular vision is so different from the mammalian one that there is no real difficulty in recognizing the homoplasy (Pettigrew 1979). In other words, detailed structural analysis of the nervous system has the power to recognize homoplasies because of the great variety of wiring diagrams that will produce the same result. Perhaps the nervous system is constrained by development and by inter-relationships between its subsystems in a way that ensures the phylogeny of a given brain is always recognizable, despite the functional

adaptations. To take an example from the present data set, brain structure and visual pathways of the gliding squirrel, *Petaurista*, were those of a typical sciuromorph rodent and bore no resemblance to those of *Cynocephalus*, despite the close similarities of these two comparable-sized, nocturnal, phytophagous, palaeotropical gliders (see §§2.4 and 4). We know of another striking case in birds where it proved much easier to establish phylogenetic relationships from the avian nervous system than from external morphology (Pettigrew & Frost 1985).

5. Parallel evolution of flight in megabats and microbats?

There is a close identity between the megabat pattern of brain organization and the primate pattern. Are these parallel evolutionary events in these two highly visual and dextrous groups, as Martin (1986*b*) has suggested? Or did megabats and primates share a common ancestor, as the cladogram in figure 8 suggests? The first possibility is one that would immediately be put forward in the context of a monophyletic Chiroptera. It is made less likely by the fact that other mammals with even more highly developed visual systems than megabats, such as carnivores, squirrels and phalangers have not acquired the primate-like visual projection pattern despite ecological pressures in aboreal, visually directed predatory niches that can be considered comparable to those thought to have given rise to the primate visual organization (Cartmill 1972). That this pattern is also not found in the highly visual microbats (such as *Macroderma gigas*, *Taphozous* spp., *Artibeus* and *Mormopterus*) further supports the rarity of the arrangement, even in animals occupying similar flying niches to those that are proposed, in this scenario, to have brought about the arrangement in megabats. A further difficulty with the proposal of parallelism is the presence of other synapomorphies which link primates and megabats, such as those in the penis (Smith & Madkour 1980), in the motor pathways (Nudo 1985; Kennedy *et al.* 1987) in the haemoglobin molecules (Kleinschmidt *et al.* 1988), and in the skeleton (see below). As there is no reason to link details of the visual pathway to the arrangement of the corpus spongiosum in forming the glans penis, or either of these to the triple motor arrangement, one is forced in this scenario to propose not one, but an implausible number of parallel evolutionary events in the megabats and primates. The lack of parsimony of this arrangement is evident, even with the neural data itself, as can be assessed objectively by the dramatic increase in the number of steps needed to change the tree in figure 8 to one with microbats and megabats united.

Compared with the unusual primate-like neural characters, flying is not rare among vertebrates. Powered, flapping flight has evolved independently in teleosts, pterosaurs, birds and mammals (Rayner 1981). Gliding flight has evolved numerous times in different vertebrate orders (see, for example, Rayner 1981), with three separate inventions in the marsupials alone (Archer 1984). One may therefore be on safer ground in proposing that flight has evolved in parallel in a branch of the primates relative to microbats, rather than proposing that flight in megabats and microbats is monophyletic and that megabats have independently evolved all the functionally obscure details of neural connections found in primates. We say 'functionally obscure' to emphasize the fact that there is currently little understanding of the functional significance of most of the neural features used in our analysis, such as the particular order of layers in the lateral geniculate nucleus or the primate-like retinotectal wiring diagram. This stands in contrast to the well-recognized functional constraints operating on a wing. The

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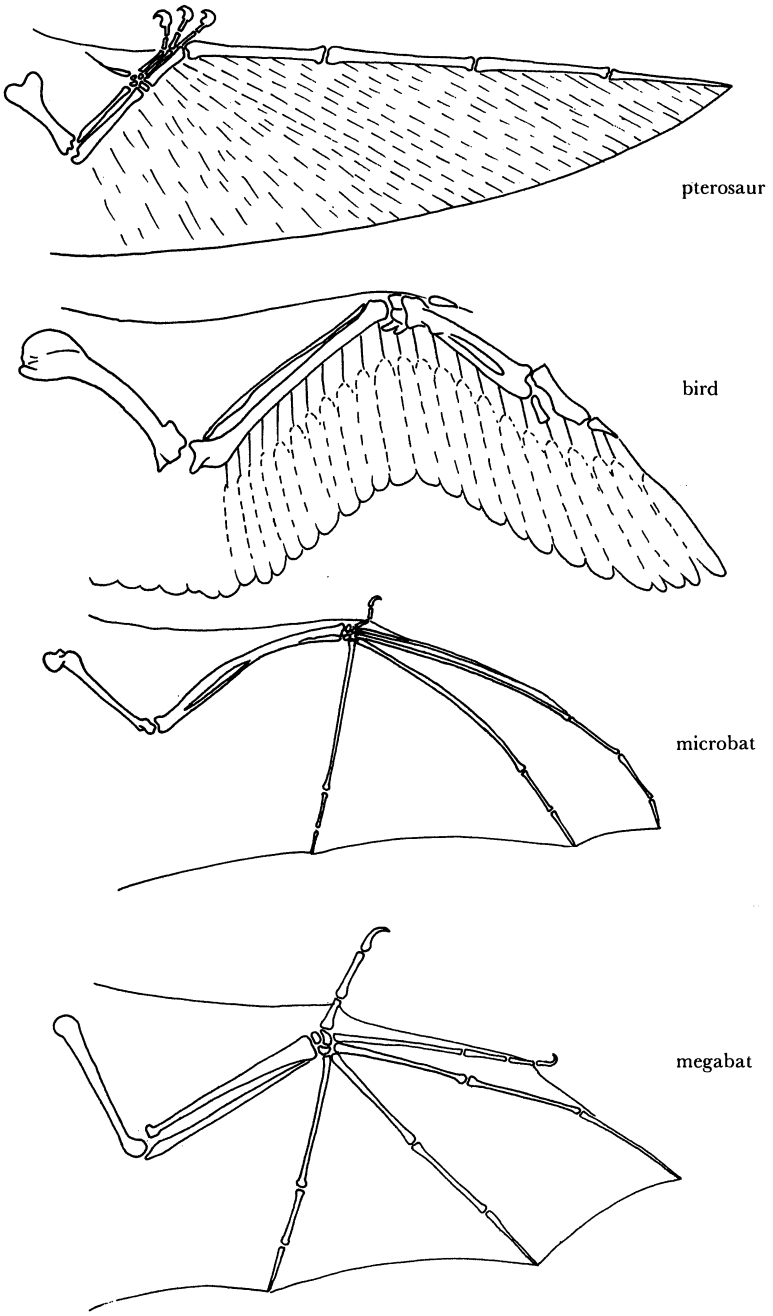

FIGURE 9. Four kinds of vertebrate wing. The pterosaur wing is supported anteriorly by a spar formed from an enlarged fourth digit; the wing membrane is supported by close-packed, parallel fibres or actinofibrillae. The avian wing is supported by feathers and their shafts; both kinds of bat wing have a thin membrane supported by digits that are locked into place to stretch the membrane into an aerofoil; the close resemblance of the two kinds of bat wing to each other need not imply a common origin, as mammals did not have options like feathers or actinofibrillae which permitted the contrasting designs found in pterosaurian and avian wings.

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high degree of similarity between the arrangement of digits within the membrane of both the megabat wing and microbat wing might equally well reflect such constraints as it might reflect a common origin. It must also be borne in mind that early flying mammals had neither the feathers, which were available to birds as wing-supporting structures, nor the actinofibrillae that appeared to provide structural support to the wing of pterosaurs (figure 9). Actinofibrillae were long, straight, close spaced fibres that radiated through the pterosaur wing in a pattern

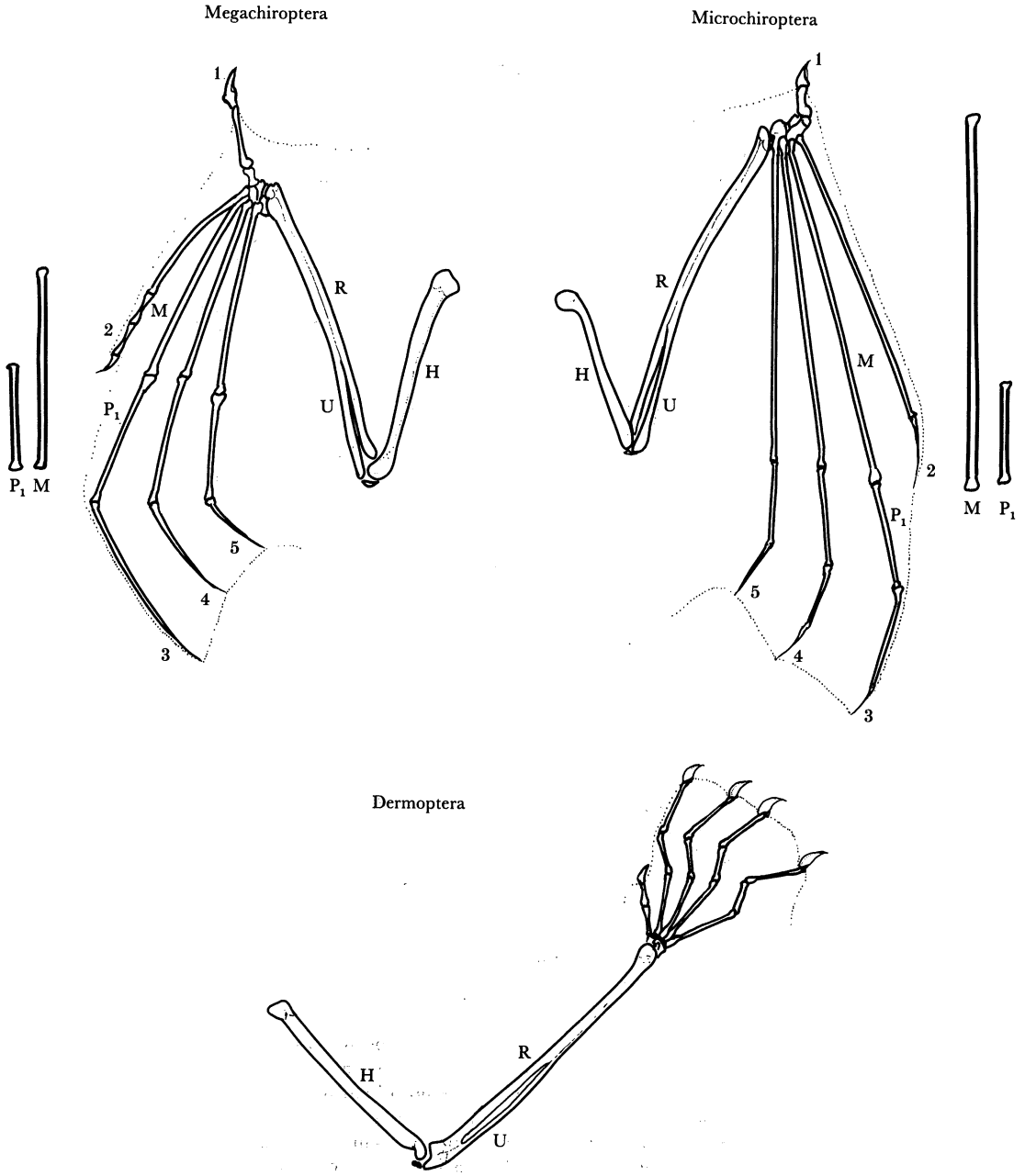

FIGURE 10. Skeletal structure of megabat, microbat and dermopteran forelimb to show digital bones supporting the patagium. Note the more similar length of metacarpal (M) and proximal phalanx (P1) in Megachiroptera compared with Microchiroptera. The dermopteran forelimb, although having shorter digits than both kinds of bats, clearly resembles the megachiropteran forelimb with respect to the relative length of metacarpal and first phalanx. Abbreviations: H, humerus; U, ulna; R, radius.

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reminiscent of feather shafts in birds or supporting digits in bats (Welnhofer 1975). The finding of these actinofibrillar, supporting structures within pterosaur wing membranes emphasizes that similar constraints may be operating to maintain wing form in different kinds of vertebrate wing, with actinofibrillae performing the same function in pterosaur wings as feathers perform in bird wings and bony digits perform in both megabat and microbat wings. In place of the wing-stiffening elements used in the wings of birds and pterosaurs (Padian 1985), mammals have used the digital bones to stiffen the wing membrane. The fact that both megabats and microbats have extended all five digits to form the wing, in contrast to the extension of a single fourth digit used by pterosaurs, has been presented as evidence for a common origin of flight for bats (Novacek & Wyss 1986). The assumption here is that such similarities are unlikely to have evolved by chance, given the different possibilities for vertebrate flight that are apparently open if one surveys the four different vertebrate orders that have powered flapping flight. We agree that the similarity has not occurred by chance, but is nevertheless two independent responses to the need to strengthen the wing in the absence of feathers or actinofibrillae. As already reasoned, the single-digit extension used by pterosaurs may not have been available as an option to mammals because this option also required the presence of actinofibrillae that mammals lack. Also, the well-developed, pentadactyl clawed limb of the megabat and microbat precursor, as well as their quadrupedal gait, stands in contrast to the bipedal pterosaurs and bird precursors and may have exercised other constraints on the final pattern of the mammalian flight apparatus to make it distinct from others. We believe that the perceptual difficulties in viewing the megabat flight apparatus as independently evolved from the microbat flight apparatus were largely responsible for the original placement of the two groups together despite the numerous, long-known differences between them.

It is therefore important to look beyond the superficial similarities in megachiropteran and microchiropteran wing structure (which may exist because they are necessary for flight), in the hope of finding some features that better reflect the genetic origins of the bearer. One such feature appears to be the relative length of the metacarpals and phalanges, which show clear differences between the megachiropteran wing and the microchiropteran wing (figure 10) and which indicate different patterns of evolution in these two kinds of wing, as argued below. Other differences supporting independent evolution are found in the shoulder joints (Strickler 1978) and in the likely pattern of evolution of wing sizes in the two kinds of bat (see §7.0).

6. Metacarpophalangeal index (M/P)

6.1. *Summary of findings*

The M/P index was determined (as detailed in §2.5) by combining, for the third and fourth digits, the ratio of the metacarpal length to the length of the first plalanx. In bats the M/P index covers a wide range, from 2.9 in some of the smaller *Pteropus* spp., to 12, in *Amorphochilus schnablii*, an unusual South American microbat in the family Furipteridae (figure 14). In mammals other than bats, the range is not quite so wide, from highest values in the Edentata (6.1 in the giant anteater, *Myrmecophaga tridactyla*) and the Pholidota (5.5 in the Malayan pangolin, *Manis javanica*) to lowest values in primates (for examples 1.6 in the tarsiers, 2.4 in the Cercopithecidae) (figure 13). Megabats all have low values clustered around 3.1, despite the wide variation in size in this group (figure 11). Microbats have M/P values that do not overlap with those of megabats (figure 11). The lowest M/P index we found in 423 microbat

taxa from all 17 families was 3.9 in *Cheiromeles torquatus*, the largest, most-derived species in the Molossidae (Freeman 1981). There is much overlap between different microbat families (figures 12–14). Phyllostomidae, for example, covers almost the total range found in microbats, from low values in the carnivorous species, which can take prey from the ground (*Trachops cirrhosus*, 4.9; *Vampyrum spectrum*, 4.3), to high values in the vampires (*Desmodus rotundus*, 10; *Diphylla ecaudata*, 9.7). Thus there is some overlap between Phyllostomidae and the

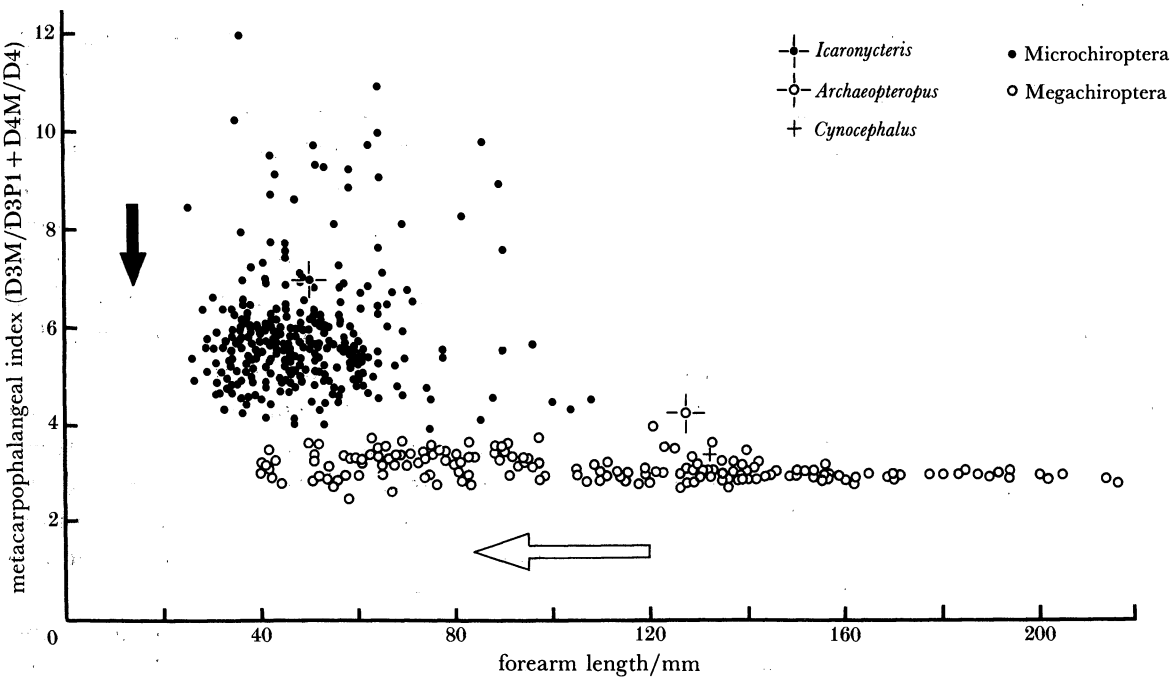

FIGURE 11. Separate distributions of two wing characters (M/P index and size) in microbats and megabats. Note that there is virtually no overlap when these two wing measures are considered together in the two groups of bats. Note also that the direction of evolutionary change (arrows indicate polarity of the character) has been opposite for these wing characters in the two groups of bats. Points represent forearm length versus the sum of the ratios of metacarpal/proximal phalangeal length for digits D3 and D4 (M/P index). Arrows indicate the direction of evolution or polarity, from plesiomorphic (primitive) to apomorphic (derived) for M/P index in microbats (black arrow) and for the evolution of size in megabats (open arrow). (A complete species-by-species printout of raw vaues is available on request from the principal author). Megabats: values of 174 species were used. Microbats: breakdown by family is shown in table 4. The values for the fossil microbat *Icaronycteris* were taken from Jepsen (1970), for the fossil megabat *Archaeopteropus*, from Andersen (1912) and Habersetzer & Storch (1987).

Mormoopidae (mean $M/P = 8.8$, $N = 5$) and the Noctilionidae (mean $M/P = 9.9$, $N = 2$), two microbat families with the highest M/P (excepting the two species making up the family Furipteridae, mean $M/P = 11$).

6.2. *Separation of megabat and microbat wings by M/P index*

The M/P index is a forelimb character which provides a useful new insight into chiropteran phylogeny. The consistent difference between microbats and megabats, which we have demonstrated for this index (see figure 11), however it be interpreted, objectively denies the claimed high degree of similarity between the two kinds of wing. Despite unity of function, the metacarpophalangeal character has maintained two distinct statistical assemblages when

compared with other overall measures of the wing. As they have not been related to differences of function, these differences in the M/P index may reasonably be attributed to differences in phylogenetic origin of the groups which display them. We now detail a number of reasons that support the proposition that the M/P index reflects phylogenetic relationships more than functional relationships:

1. M/P index enables a clear separation of all megabats from all microbats, but does not enable different microbat families to be clearly separated (figures 11–14).

2. Bats which have separate phylogenetic origins yet similar lifestyles, may have wings that cannot be separated, by conventional means, on the basis of their extremely similar wing morphometrics (see, for example, Struhsaker 1961; Norberg 1981). Such bats may nevertheless be separated on the basis of M/P index. For example, the megachiropteran macroglossines (family Pteropodidae) and the microchiropteran glossophagines (family Phyllostomidae) each have extremely similar wing characteristics, which reflect their similar nectarivorous lifestyles (Norberg, 1981*a*; Norberg & Rayner, 1987). Despite the similar wing morphometry, they are widely separated by M/P index indicating their separate origins, with glossophagines having M/P indices around 6, twice the values for macroglossines ($M/P = 3$).

3. As a corollary of 2, there are numerous examples of microbat wings with identical M/P indices but with radically different functional properties and morphometrics. For example: (*a*) the molossid microbat genus, *Cheiromeles*, has wings with high loading and high aspect ratio compared with the nycterid genus, *Nycteris*, whose wings have low loading and low aspect ratio (Norberg & Rayner, 1987), yet both of these contrasting wings have the same M/P index, about 4; (*b*) at the high end of the M/P spectrum, both *Natalus* and *Noctilio* have large M/P indices (around 9), yet the manoeuvrable insectivore *Natalus* has very large wing area, average aspect ratio and extremely low wing loading compared with the strikingly high aspect ratio and moderate wing loading of the piscivore, *Noctilio*; (*c*) in Norberg & Rayner's (1987) figure 8*e*, molossid and vespertilionid wings tend to be at morphometrically opposite extremes, with the molossids having both high wing loading and high aspect ratio, whereas the vespertilionids show the reverse tendency toward low values on both of these variables. Nevertheless, M/P indices are similar in these two families. A specific comparison between the extremes of the range in each group reveals that *Tadarida fulminans*, with the greatest combined values of wing loading and aspect ratio, has the same M/P index (5) as *Kerivoula argentata*, which is among those vespertilionids with the lowest combined values for loading and aspect ratio.

4. Despite the enormously increased length of the digits of the modified forelimb compared with the hindlimb, there is a correlation between the M/P index taken from the forelimb and the metatarsophalangeal ratio taken from the hindlimb of the same bat ($r = 0.65$, $n = 29$, table 5). This suggests that there are genetic factors responsible for the relative lengths of the phalanges to both metacarpals and metatarsals in the same taxon and that the influence of these factors can be detected despite the effects of the specialized developmental programmes for the wing. In other words, the clear separation between microbat wings and megabat wings that the M/P index provides (figure 11) has its counterpart in the metatarsophalangeal index, which also separates the two suborders of bats.

5. The developmental sequence of M/P index is different in the two suborders of bats, with a gradual decrease in M/P index throughout embryonic and post-natal development of microbats compared with no change, or a very slight increase, in M/P index during development in megabats.

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6.3. *Polarity of the metacarpophalangeal index*

Because of the large amount of interspecific variation of the M/P index and also in view of the separation, which this character appears to provide between different phylogenetic groups of mammals (table 4, figures 11–15), it seemed worthwhile to attempt to use it in a cladistic analysis. The first step in such an analysis is to determine which states of the character are

TABLE 4. MAMMALIAN M/P RATIOS

	M/P	N
order Edentata	6.0	5
order Pholidota	5.5	1
order Insectivora	—	—
family Solenodontidae	4.0	2
family Tenrecidae	3.6	9
family Erinaceidae	5.0	2
family Soricidae	3.6	4
order Scandentia, family Tupaidae	3.6	4
order Dermoptera, family Cynocephalidae	3.2	1
order Chiroptera	—	—
suborder Megachiroptera	—	—
fossil Genus *Archaeopteropus*	4.1	1
family Pteropodidae	3.1	174
suborder Microchiroptera	—	—
fossil Genera, *Palaeochiropteryx* and *Icaronycteris*	6.6	2
family Rhinopomatidae	7.5	3
family Emballonuridae	6.6	33
family Craseonycteridae	8.5	1
family Nycteridae	4.3	6
family Megadermatidae	5.1	5
family Rhinolophidae	6.0	41
family Hipposideridae	5.6	14
family Noctilionidae	9.9	2
family Mormoopidae	8.8	5
family Phyllostomidae	7.2	139
family Natalidae	8.0	2
family Furipteridae	11.1	2
family Thyropteridae	5.7	2
family Myzopodidae	5.0	1
family Vespertilionidae	6.0	73
family Mystacinidae	7.4	2
family Molossidae	5.3	90
order Primates	—	—
family Cheirogaleidae	2.7	2
family Lemuridae	2.6	1
family Lorisidae	2.0	2
family Tarsiidae	1.6	2
family Callithricidae	2.6	2
family Cercopithecidae	2.4	5
family Hylobatidae	2.3	2
family Pongidae	2.2	2
order Carnivora	4.2	4
order Hyracoidea	4.4	2
order Tubulidentata	2.6	1
order Pholidota	5.5	1
order Rodentia	4.5	7
order Lagomorpha	3.7	3
order Macroscelidea	5.3	5

Abbreviation: N, number of different species sampled to give the mean value for the group.

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derived and which are primitive. In trying to take this first step, we have made the simplifying assumption that the rank-ordering of M/P index within the microbats represents a single direction of evolutionary change in this character (while realizing that the reality may have been more complicated, with for example, dispersion in different directions away from the original condition, or reversals).

There are two fairly well accepted approaches to the determination of polarity: ontogenetic and out-group comparisons (Hennig 1966; Wiley 1976). As described below, both of these approaches indicate that a small metacarpophalangeal M/P index is derived within mammals. The large values which characterize the forelimbs of microbats, but not megabats, therefore represent the primitive condition. While the consideration of each single example of these approaches does not always give a compelling case for the polarity of this character, we are impressed by the fact that every test we can envisage indicates the same polarity.

(*a*) *Out-group analysis*

Among mammals, the smallest M/P index is found in primates ($M/P = 2.6$), particularly the Tarsiidae ($M/P = 1.6$); megachiropterans also have low values ($M/P = 3.1$). All other mammalian orders have higher values (see figure 15 and table 4). On this basis, one would be justified in concluding that a low value for the M/P index is the derived condition. Within the microbats there is some variation in M/P index, both from family to family, but also within some of the large, diverse families such as the Phyllostomidae. It is therefore possible to extend the out-group analysis to families within the Microchiroptera. For example, the families Mormoopidae and Noctilionidae are both recognized as lineages which branched off the early phyllostomoid lineage, and which therefore represent appropriate out-groups to the phyllostomidae (Patton & Baker 1978). Both of these families have very large M/P ratios when compared with other microbats, including phyllostomids, thereby supporting the conclusion that lower values indicate a more derived condition. Within the Phyllostomidae itself, those

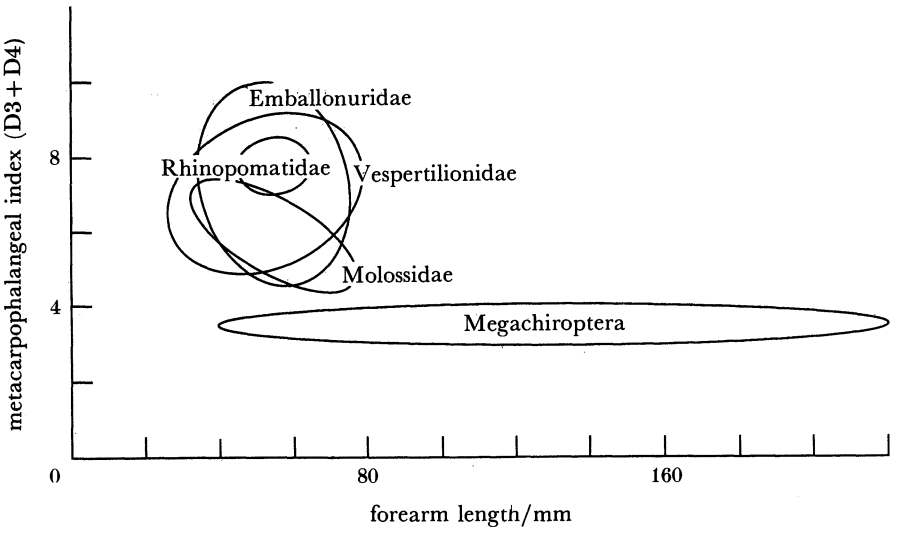

FIGURE 12. Distributions of M/P index and forearm lengths in wings from different families of microbats and megabats: ellipses enclose data points shown in figure 11 on a family by family basis. Note the extensive overlap of the microchiropteran families, Emballonuridae, Rhinopomatidae, Vespertilionidae and Molossidae, compared with the well-separated megachiropteran family Pteropodidae.

derived subfamilies, such as the glossophagines, which have become highly specialized for nectar feeding, also have lower M/P indices than those subfamilies (e.g. phyllostomines, represented by the type genus *Phyllostomus*) that could be regarded as out-groups.

(*c*) *M/P index of fossil microbats*

The present lack of agreement about the phylogenetic relationships of the different microbat families limits the degree to which our assignment of the polarity of the M/P character can be tested. On the other hand, every test that we do on the basis of tentative out-group assignment has led to the same conclusions about polarity. One such test worth mentioning involves the use of fossil microbat values for M/P index. One must stress the limitations of the fossil record, as only a small number of taxa are known. If one confines attention to the oldest fossil microbats, namely, 50 Ma old *Icaronycteris* and 45 Ma old *Palaeochiropteryx* and *Hassianycteris*, M/P indices are greater (around 7 in these taxa) than both the mean (5.9) and the mode (5.4) for living microbats. This is in accord with our assignment of polarity, although there are several extant microbats with M/P indices larger than the fossil values, such as those found in the furipterids, natalids, vampires, mormoopids and noctilionids.

(*d*) *Ontogenetic method*

The M/P index shows differing developmental trends in microbats and megabats. The data from the molossid microbat, *Tadarida brasiliensis*, (84 different juveniles) and the vespertilionid, *Myotis velifer* (24 juveniles) indicate a clear, gradual decrease in the M/P index during post-natal development in each case. A similar trend has been observed in more limited embryonic material from the microbats, *Macroderma gigas*, and *Myotis adversus* (J. D. Pettigrew 1989, unpublished observations). In contrast, the megabat *Pteropus scapulatus* shows a barely perceptible change in M/P index during embryonic and post-natal development. There are problems with the use of ontogenetic material to determine the polarity of characters used in phylogenetic analysis that have been pointed out by De Quieroz (1986). Nevertheless, our ontogenetic data support the many out-group comparisons we have done, which all indicate that where smaller values for the M/P index occur in microbats, they are derived. The different developmental trends in microbats and megabats also support our opinion that the wings are independent in their derivations. More data on the development of M/P, particularly in the various mammalian out-groups, would be valuable.

(*e*) *Cladistics of M/P index*

We tested our assignment of the polarity of M/P against the neural data in §4. When we added M/P values for each of the taxa in table 3 and ran the new matrix on the PAUP program, the general form of the tree was unchanged, so long as the polarity adopted for the M/P character was the same as we have argued here; that is, with large values representing the plesiomorphic condition. If, on the other hand, we set a large value instead of a small value of M/P in the hypothetical ancestor, there was an increase in the number of steps in the most parsimonious tree, the overall consistency index of the tree fell (to 0.88), and there was an obligatory change of M/P at the base of the tree to a value corresponding to the highest M/P found in the data set. This empirical test provided no support whatever for the contrary view that large values of M/P might be derived.

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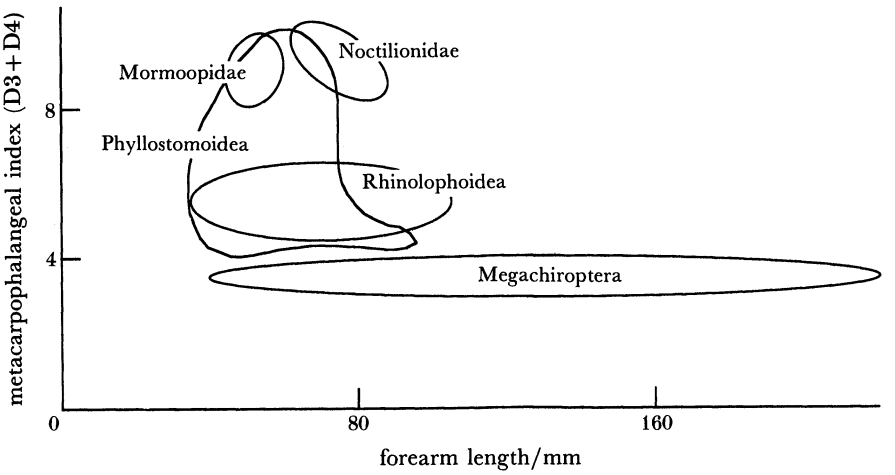

FIGURE 13. Distributions of M/P index and forearm length in wings of different families of microbats and megabats. Conventions as for figure 11, but for the microchiropteran families, Mormoopidae and Noctilionidae, and the Superfamilies, Phyllostomoideae (including Phyllostomidae, Desmodontidae) and Rhinolophoideae (including Rhinolophidae, Hipposideridae, Megadermatidae and Nycteridae).

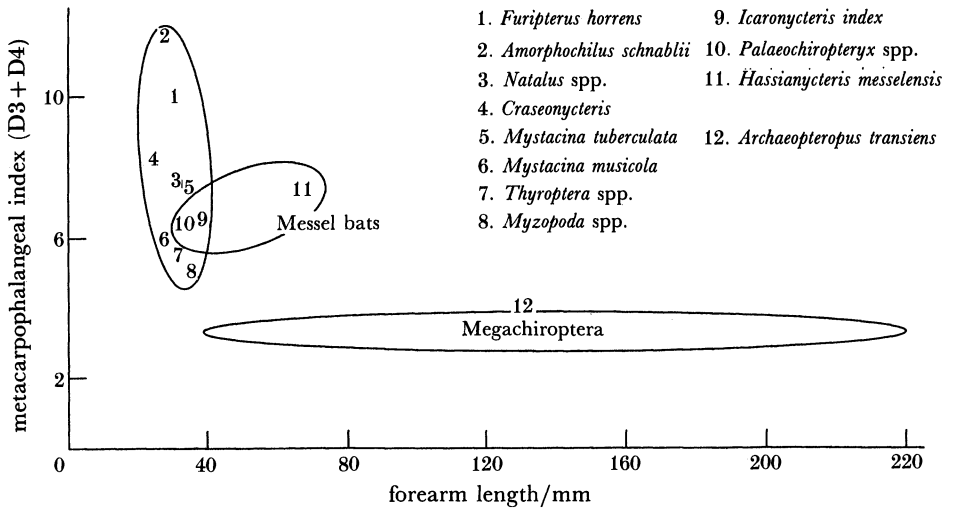

FIGURE 14. Distribution of M/P index and forearm length in different families of bats. Vertical ellipse encloses values from assorted microbats, either fossil (*Icaronycteris* and *Palaeochiropteryx*) or living, small or monotypic families, such as Furipteridae (*Furipterus* and *Amorphochilus*), Natalidae (*Natalus*), Craseonycteridae (*Craseonycteris*), Mystacinidae (*Mystacina*), Thyropteridae (*Thyroptera*) and Myzopodidae (*Myzopoda*).

6.4. *Comparisons of M/P index within Phyllostomidae*

Taking microbat families one at a time, we find that the most highly derived specis within each family have smaller M/P indices. An appropriate family to begin with is the new World Phyllostomidae (Koopman 1984), which has 46 genera (compared with 43 genera of the Pteropodidae) (Corbet & Hill 1980), and where there is wide variation of M/P index, from 4 to 10 (mean = 7.2, 2.1 s.d.). In this family, small values of M/P are invariably found among the taxa with highly derived flight characteristics, such as the glossophagines, which can hover

in front of flowers to feed (mean value for M/P in this subfamily is 5.9), and species so highly specialized for flight that they can successfully capture vertevrate prey while on the wing (e.g. *Trachops cirrhosus* $M/P = 4.9$; Tuttle & Ryan 1981; *Vampyrum spectrum*, $M/P = 4.4$; Vehrencamp *et al.* 1977). At the other end of the spectrum of M/P values in the Phyllostomoidae, we find species with less sophisticated, more direct flight such as the noctilionids ($M/P = 9.5$), the vampires ($M/P = 10.4$) and *Phyllostomus hastatus* ($M/P = 9.0$). Although some of these species are highly specialized in their lifestyles (*Noctilio* using a sophisticated echolocation system to fish over water (see, for example, Suthers & Fattu 1973), they are generally considered to be primitive when compared with the other more derived phyllostomoids (see, for example, Baker & Bickham 1980).

6.5. *Other examples of M/P comparisons in microbats*

Within other families, the proposed assignment for the polarity of the M/P ratio is consistent with generally accepted views about specialization for flight. For example, we may take the microbat species with the smallest M/P ratio of the whole suborder, *Cheiromeles torquatus*, with $M/P = 3.9$. According to our analysis, this taxon should come from a family which is the most derived with respect to flight, a prediction confirmed by Norberg & Rayner's (1987) statement that the family Molossidae (mean M/P ratio = 5.0) is the most specialized for flight, and Freeman's (1981) statement that *Cheiromeles* is the most highly derived taxon within Molossidae.

6.6. *Zoogeography and M/P index*

Chorological analysis (Hennig 1966) is difficult for microbats because of their diversity and the wide geographic distribution of many genera, but may support our determination of the polarity of M/P if we consider some geographically restricted taxa, forming small, often monotypic, families, such as Mystacinidae (*Mystacina*, $M/P = 7.5$), Furipteridae (*Furipterus*, $M/P = 10.0$, *Amorphochilus*, $M/P = 12.0$), and Natalidae (*Natalus*, $M/P = 8.0$). These families may be considered as relictual Gondwanan groups with only distant relationships to other microbat families (§9.2). The Mystacinidae have a distant relationship with the Phyllostomoids (Pierson *et al.* 1986), from which they have been separated presumably since the time New

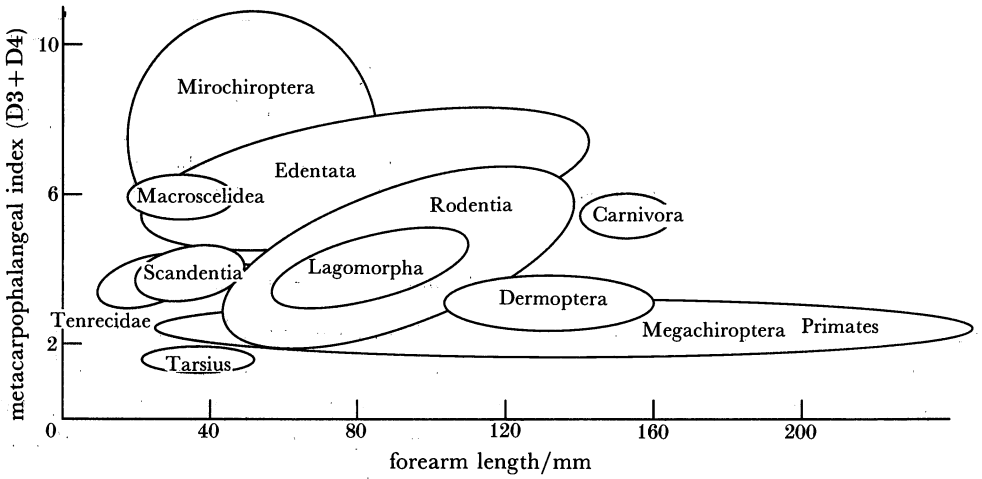

FIGURE 15. Distributions of M/P ratio and forearm length in different orders of mammals. Mean values for each group are given in table 4. Note that smallest values of M/P are found in primates and that all other mammalian groups have larger values, particularly edentates and microbats.

Zealand and South America were close enough for an exchange of bats to occur. On this basis, the large M/P index is in keeping with our postulate of the polarity, if one assumes that the relictual taxon does not undergo much evolutionary change. Similar arguments can be made for the other two families, although present knowledge of relationships in their cases is more vague (see Van Valen 1979).

The Craseonycteridae ($M/P = 8.5$) can also be included in this group because of its extremely narrow geographic distribution (less than 100 km in diameter), on the Gondwanan fragment in western Thailand (see §9.2). The affinities of this Family seem to lie with the Emballonuridae or Rhinopomatidae (Hill 1974; Hill & Smith 1981; Van Valen 1979), so the large M/P index conforms to our predictions, on two grounds, chorological and based on the comparison with Emballonuridae (mean M/P index = 6.6) or with Rhinopomatidae ($M/P = 6.6$).

7. Size as a character in megabats and microbats

7.1. *Moderate size is plesiomorphic in Megachiroptera*

The mean forearm length in the Megachiroptera is 111 mm (figure 11). There are few megabat species with a mean forearm length shorter than 50 mm, and excluding individuals, no megachiropteran species has a mean forearm length shorter than 38 mm, which sets a lower limit, or barrier, for this group. The failure of any megabats to cross this 'barrier', in contrast to the presence of many microbats with forearms less than 30 mm, immediately poses a difficulty for the counter-hypothesis that small size is plesiomorphic in megabats. If small size is the ancestral condition in megabats, why should they have so much difficulty in achieving sizes at the low end of the size spectrum where many microbats are found? Part of this difference is only apparent as there is different scaling between body mass and forearm in the two kinds of bats, such that megabats tend to have shorter forearms than microbats of the same body mass (Norberg & Rayner 1987). This discrepancy in forearm length becomes quite evident when one compares the macroglossine megabats of the Old World with the glossophagine microbats, which occupy a similar nectar-feeding niche in the New World. There is a difference in the size ranges of these two groups of bats that is reminiscent of the difference between the small New World hummingbirds, which tend to hover while feeding, and the somewhat larger, Australian honeyeaters, which also feed on nectar, but which prefer to perch rather than hover as they feed (Pyke 1981). The absence of macroglossine megabats in the small size categories occupied by glossophagine microbats, could perhaps be related to the preference of macroglossines for feeding while perched (Start 1973) compared with the combined feeding-hovering mode that is known to be adopted by many of the glossophagines (Leen & Novick 1969). Alternatively, as hovering is not unknown in megabats (see, for example, Start (1973), J. M. V. Rayner & J. D. Pettigrew, unpublished data), torpor may play a role in the size difference because it is highly developed in microbats, but not megabats (table 1).

Torpor may enable the glossophagine microbats to attain small sizes which would make macroglossines too vulnerable to fluctuations in climate and food supply. A third possibility relates to the difficulty of obtaining a high visual acuity in a small eye because of the limits placed by diffraction and cell packing. The visual megabats may be 'barred' from the small end of the size range because of these optical factors and their dependence on vision for foraging

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TABLE 5. FORELIMB–HINDLIMB COMPARISON

	FA	MPI	MTI
Family Pteropodidae			
Epomorophus crypturus	82	3.8	2.0
Epomorophus wahlbergi	65	3.8	1.8
Pteropus poliocephalus	198	2.9	1.9
Syconycteris australis	41	3.1	2.2
Family Emballonuridae			
Coleura afra	48	5.6	3.2
Rhynconycteris naso	36	6.5	2.6
Family Megadermatidae			
Cardioderma cor	55	5.4	3.2
Lavia frons	58	4.9	3.1
Family Hipposideridae			
Hipposideros commersoni	99	4.8	3.0
Triaenops persicus	51	6.5	3.4
Family Noctilionidae			
Noctilio albiventris[a]	57	11	2.0
Noctilio leporinus[a]	84	9.4	2.0
Family Mormoopidae			
Pteronotus davyi	44	8.3	6.3
Pteronotus personatus	43	9.9	4.9
Pteronotus parnellii	56	8.9	5.0
Mormoops megalophylla	55	6.7	4.8
Family Phyllostomidae			
Macrotus californicus	50	4.9	3.0
Phyllostomus discolor	59	8.7	3.1
Phyllostomus hastatus	90	9.2	2.8
Leptonycteris sanborni	53	7.3	5.4
Leptonycteris nivalis	57	7.1	4.4
Lonchophylla concava	35	6.3	3.7
Lonchophylla mordax	36	4.7	3.2
Carollia perspicillata	42	5.3	4.5
Carollia castanea	40	4.7	3.0
Sturnira lilium	44	5.9	3.0
Uroderma bilobatum	42	5.4	2.8
Vampyrops helleri	39	6.5	2.4
Vampyrodes caraccioli	53	5.9	2.4
Chiroderma salvini	51	5.3	2.8
Artibeus literatus	69	6.1	2.8
Artibeus phaeotis	37	6.2	3.8
Desmodus rotundus	58	10	3.8
Family Vespertilionidae			
Myotis albescens	36	6.5	2.6
Myotis evotis	38	7.4	2.8
Myotis nigricans	36	7.0	3.1
Myotis yumanensis	34	7.5	3.9
Pizonyx vivesi[a]	57	6.4	2.1
Eptesicus fuscus	45	6.0	3.0
Scotophilus leucogaster	45	6.4	3.8
Scotophilus nigrita	53	6.1	2.8
Lasiurus cinereus	51	8.0	2.9
Miniopterus schreibersi	48	9.0	5.1
Antrozous pallidus	56	6.7	3.0
Family Molossidae			
Tadarida brasiliensis	44	6.4	3.8
Tadarida aegyptiaca	48	5.0	3.4
Molossus rufus	52	4.6	3.8

[a] M/T tends to be greater in the microbats than in megabats; these three piscivores are exceptions to the rule.

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and orientation. Whatever its ultimate explanation, the absence of megabats in the size range occupied by ecologically equivalent microbats argues rather strongly against the megabats having originated at the small end of their present size spectrum. Looking at the argument from another direction, we can see that moderate size is plesiomorphic within the megabat group. This conclusion is supported if we apply some of Hennig's (1966) 'rules' for indicating polarities and therefore look for the pteropodid genus which has the least specialization, the largest numbers of species and the widest geographical distribution. By these criteria, the living genera which provide the best guide to the plesiomorphic size condition are *Rousettus* and *Pteropus*. Both are unspecialized genera in other respects. *Pteropus* has more than 50 species with a geographical range extending from the eastern seaboard of Africa in the west, to the Phillipines and Pacific islands in the east (Andersen 1912; Koopman 1984). *Rousettus*, with nine species, has the largest range of any pteropodid, as it also includes the African mainland from which *Pteropus* may have been excluded by a variety of more highly specialized megabats found there (Kingdon 1974). *Pteropus* has a mean forearm length of 142 mm, ($N = 79$ species, s.d. = 27) and no species with a forearm smaller than 90 mm. *Rousettus* has a mean forearm length of 84 mm (s.d. = 9) with no species with a mean smaller than 70 mm (data from Andersen (1912)). *Rousettus* has been proposed as an ancestral pteropodid form on a variety of grounds (Andersen 1912) including karyotype and serum proteins (Haiduk 1983), but is specialized for cave roosting, having evolved a mechanism for echolocation with tongue-clicks that is independent of the laryngeal mechanisms used by the microbats (Novick 1977; Kulzer 1960). For these reasons, our own preference is for a *Pteropus*-like ancestral form rather than one modelled on the more specialized *Rousettus*. In either case, the conclusion about moderate size being plesiomorphic in the Pteropodidae is unaltered, because *Rousettus* is also of moderate size, albeit slightly smaller than the average *Pteropus* species.

Finally, the palaeontological evidence, although scanty and controversial (Smith 1976), is at least consistent with the hypothesis that moderate, as opposed to small, size was the ancestral condition in megabats. The Oligocene fossil megabat, *Archaeopteropus*, had a forearm length of about 120 mm (Andersen 1912). The polarity of the size character is therefore from moderate-sized plesiomorphy to small-sized apomorphy in megabats. Another, perhaps more accurate way to express the size polarity in megabats is to say that there has been an increase in dispersion across the size range from an ancestral, moderately sized form.

7.2. *Size is derived in microbats*

We know of only six genera of Microchiroptera in which the wing has a mean forearm length greater than 80 mm. These come from five families, each of which has a large number of smaller, less specialized species. *Hipposideros commersoni* (*gigas*) (forearm mean length = 108 mm) and *Hipposideros lankadiva* (FA = 97 mm) are specialized for large prey within the Hipposideridae (Hill & Smith 1984). *Macroderma gigas* (mean forearm length = 100 mm) is likewise specialized within the Megadermatidae (Douglas 1967). In the Vespertilonidae, with around 350 species (Corbet & Hill 1980), only a single taxon exceeds the forearm limit of 80 mm, *Scotophilus gigas* (*nigrita*) (FA = 89 mm). Within the Phyllostomidae, (mean forearm length = 49 mm for the family), *Vampyrum spectrum* (mean forearm length = 104 mm) and to a lesser extent, *Phyllostomus hastatus* (mean forearm length = 82 mm) are similarly specialized and *Noctilio leporinus* (family Noctilionidae) (86 mm) takes fish. Taken together with the mean and modal values for the microchiropteran suborder (48 mm and 45 mm, respectively), as well as the

value (45 mm) for the oldest fossil microchiropteran known (*Icaronycteris*) (Jepsen 1970), it seems that the character of size has the opposite polarity in Microchiroptera to that indicated for Megachiroptera. This can be summarized by the generalization that, within any given family of microbats, the largest members will be found to be highly derived taxa (e.g. *Cheiromeles* in the Molossidae, *Macroderma gigas* in the Megadermatidae and *Hipposideros commersoni* in the Hipposideridae, and *Vampyrum spectrum* in the Phyllostomidae), whereas in the Megachiroptera, the smallest species will be found to be highly derived (e.g. the nectar feeders *Syconycteris*, *Macroglossus* and *Megaloglossus* and the 'peculiarly specialized' *Balionycteris* (Anderson 1912).

7.3. *A common origin for megabats and microbats?*

The assignment of polarities argued above, if correct, poses a number of problems for any phylogenetic scenario that attempts to derive both lines of bats from a common flying ancestor. The first problem is the absence of any overlap in figure 11. The second problem is the implausibility of any evolutionary path linking the two data sets.

Suppose, for the sake of argument, that there was a protobat which gave rise to both megabats and microbats. Using the characters of size and M/P index, we can see that such a protobat could have had four possible combinations of these characters, namely: (*a*) moderate size, large M/P index; (*b*) moderate size, small M/P index; (*c*) small size, large M/P index, and (*d*) small size, small M/P index. These extreme possibilities would be represented by regions in the four corners of figure 11.

Possibility (*a*), where both size and M/P index are large (upper right corner of figure 11) can be eliminated immediately because it does not occur in any living bat or in any fossil.

Possibiity (*b*), large size but small M/P index, (lower right corner of figure 11) would require an evolutionary path from moderate-sized megachiropterans to small-sized species, a direction which we have already argued to be the one which has, in fact, occurred. The problem with this scenario involves the transition from a small megabat with a low M/P index to the microbat line in which we have already argued that the primitive condition was a high M/P index. If these arguments are correct, then a transition from a small protobat (with megabat affinities) to an early microbat would require an implausibly sudden leap to the top values for M/P. A transition across the narrow gap between small megabats with low M/P indices and microbats with small M/P indices appears unlikely, because the latter are all highly derived taxa and because subsequent evolution of microbats would then have to have been in the opposite direction to that already argued. This scenario is also inconsistent with the derived characters (such as those involving the visual and motor systems), which the megabats do not share with the microbats, and with the fossil record (see §12.2).

Possibility (*c*), small size but large M/P, (beginning in the upper left) also seems implausible. Constructing an evolutionary path between the two data sets according to this scenario, first requires *ad hoc* bridging assumptions, as well as a circuitous route from a small protobat with high M/P index 'down' the left-hand side of the diagram to a microbat with a more derived wing design (and lower M/P index) then a move right along the line formed by the larger, derived microbats (*Macroderma*, *Vampyrum* and *Hipposideros commersoni*) before a final reduction of M/P to the low megabat value once absolute size had reached a moderate value (say around 100–120 mm forearm length). Apart from the implausibly large number of twists and turns, this evolutionary path has the grave difficulty that intermediate steps have to involve the large microbats, which are highly derived and which bear separate, unmistakable signs of the family of origin (such as the type of noseleaf, tragus, sonar, etc.).

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Possibility (*d*) involves a common ancestor for both the microbat and megabat lines which had small values for both characters. The difficulties with this possibility are as great as those with the preceding three, because both lines would then have to have evolved in the opposite direction to that indicated by the evidence. Also, there is no overlap between these characters in living megabats and microbats, so one is again forced to postulate an intermediate form for which there is no evidence. The difficulties are magnified when it is recalled that living megabats and microbats with small values for both these characters are highly derived taxa of a different kind. One is therefore hoping to find common ground among a megabat group of non-echolocating flower-feeding specialists and a microbat group of sophisticated insectivores with elaborate pinnae and ultrasonic sonar. All of these possibilities are implausible compared with the evolutionary route for megabats through the Dermoptera already proposed.

8. Two claws

The case for parallelism in megachiropteran and microchiropteran wings made previously, appears weakened by the finding that the fossil *Icaronycteris*, an undeniable microchiropteran (Novacek 1985) had claws on both D1 and D2, like the modern pteropodids (see figure 7 in Hand (1984)). This finding has been interpreted to indicate the retention of a primitive character in megabats (Van Valen 1979) with the corollary that modern microchiropterans have lost the second claw along with the evolution of more sophisticated flight. The latter corollary is eminently reasonable, because *Icaronycteris* was clearly microchiropteran and there are no double-clawed microchiropterans in existence today. The inference that megabats can be linked to the Microchiroptera via the dual claw is more suspect.

Because the mammalian wing evolved from a clawed forelimb, we can presume that all such wings, whether ancestral megachiropteran or microchiropteran, have passed through a many-clawed stage. An alternative conclusion to be drawn from the pteropodid/*Icaronycteris* comparison may therefore be that the megabats have not progressed so far along their own evolutionary path; an interpretation that could be related to their more recent origin. Alternatively, the phytophagous habits of megabats, with their attendant needs to clamber about trees may have favoured the retention of the second claw to a greater degree than in the more aerially biased microchiropterans. In this respect it is interesting to note that five megachiropteran genera, *Eonycteris*, *Nesonycteris*, *Neopteryx*, *Notopteris* and *Dobsonia*, have lost the second claw (Andersen 1912; K. F. Koopman, personal communication); a proof of parallelism in this loss, unless it be argued that the two-clawed megabats are the plesiomorphic sister group of an assemblage of one-clawed microbats and megabats! Given this variability of the character and the considerations above, it may be facile, but wrong, to link the two groups of bats because many megabats have two claws like the earliest known microbat. To use the language of Hennig (1966) the retention of a second claw is a plesiomorphy that has no validity for linking the megabats to microbats.

9. Microbat origins

9.1. *A sister group for the microbats?*

Perhaps the greatest weakness with the current thesis is the absence of a recognizable sister-group for the microbats. There can be little argument about the monophyly of the microbats, based on the complex of derived features which they share such as a greatly enlarged cochlea

(Novacek 1984), laryngeal sonar (Neuweiler *et al.* 1980) and the particular forelimb modifications for flight. Except for the forelimb, a problematical link to the megabats that has already been discussed, none of the derived features characterizing the microbats has been found in another group of mammals. The enlarged cochlea is not found among any of the 'insectivores', such as the tenrecs or soricid shrews, which might have been thought perhaps to have provided a basic stock for the microbats (M. Novacek, personal communication), and it is likewise difficult to relate the laryngeal sonar used for echolocation by microbats to the primitive forms of echolocation found in some other mammalian groups (Gould 1976). If one accepts our arguments that the primitive microbat forelimb had a very long metacarpus in relation to the phalanges (high M/P), it is again very difficult to find a living mammalian group to which the microbat hand can be related by virtue of a comparable metacarpo-phalangeal ratio. The closest groups using this comparison would be the Edentates, and the Pholidote, *Manis*, both of which have a high M/P index (table 4 and figure 15). The edentate tree sloth, *Bradypus*, also shares an unusual visual pathway feature with microbats (lacunar demarcation in the LGN; §2.4). Because of the near-reptilian features of its spermatozoa (L. Leung, personal communication), *Manis* may be regarded as the most primitive eutherian mammal known. The high M/P index shown by some microbats, edentates and the pangolin, may therefore represent a retained primitive feature from the base of the early eutherian radiation. If this is so, then microbats may have branched off at a very early point and have no easily recognizable, living sister group, if indeed this has survived into the recent fauna.

It is therefore easy to see why there has been a general reluctance to abandon the megabats as the nearest sister group for the microbats, based on the derived characters which both groups appear to share in the forelimb. To retain this relation in the face of the conflicting data from brain and genitalia requires one of two postulates: (*a*) the convergent evolution of an implausibily large number of the same characters in the primates and megabats (i.e. motor and visual pathway features, genital features, shared substitutions in haemoglobin, similar M/P) or (*b*) the loss in microbats of the same large number of characters. Neither of these possibilities is compelling (§12.2).

9.2. *Ancient origin for microbats*

There seems to be little choice, but to accept that microbats have no living close sister group. This might be accounted for in terms of the ancient origins we postulate for the microbats. Noctuid moths, adapted specifically to evade the sonar of microbats (Roeder & Treat 1962), may have been present 75 Ma ago (Gall & Tiffney 1983). This circumstantial evidence, but no positive fossil evidence so far, places the origin of the microbats in the late Cretaceous when the great angiosperm and insect radiation was taking place (Marshall 1983; Muller 1981; Takhtadzhian 1958). One might expect that an aerial, mammalian insectivore would not be too long in evolving after the great insect radiation, and this expectation is partially fulfilled by the finding of *Icaronycteris index*, a microbat with advanced flight capabilities and sonar only 15 Ma from the end of the Cretaceous (Jepsen 1970; Novacek 1985). It is difficult to imagine both of these extremely advanced abilities evolving overnight, particularly as *Icaronycteris index* appeared to have had a cochlea and wings that were even more derived than many living microbats (see Novacek 1985; Habersetzer & Storch 1987 and §6.3). Their presence in a microbat close to the dawn of the Tertiary implies that sonar and flight must first have appeared earlier, probably in the Cretaceous.

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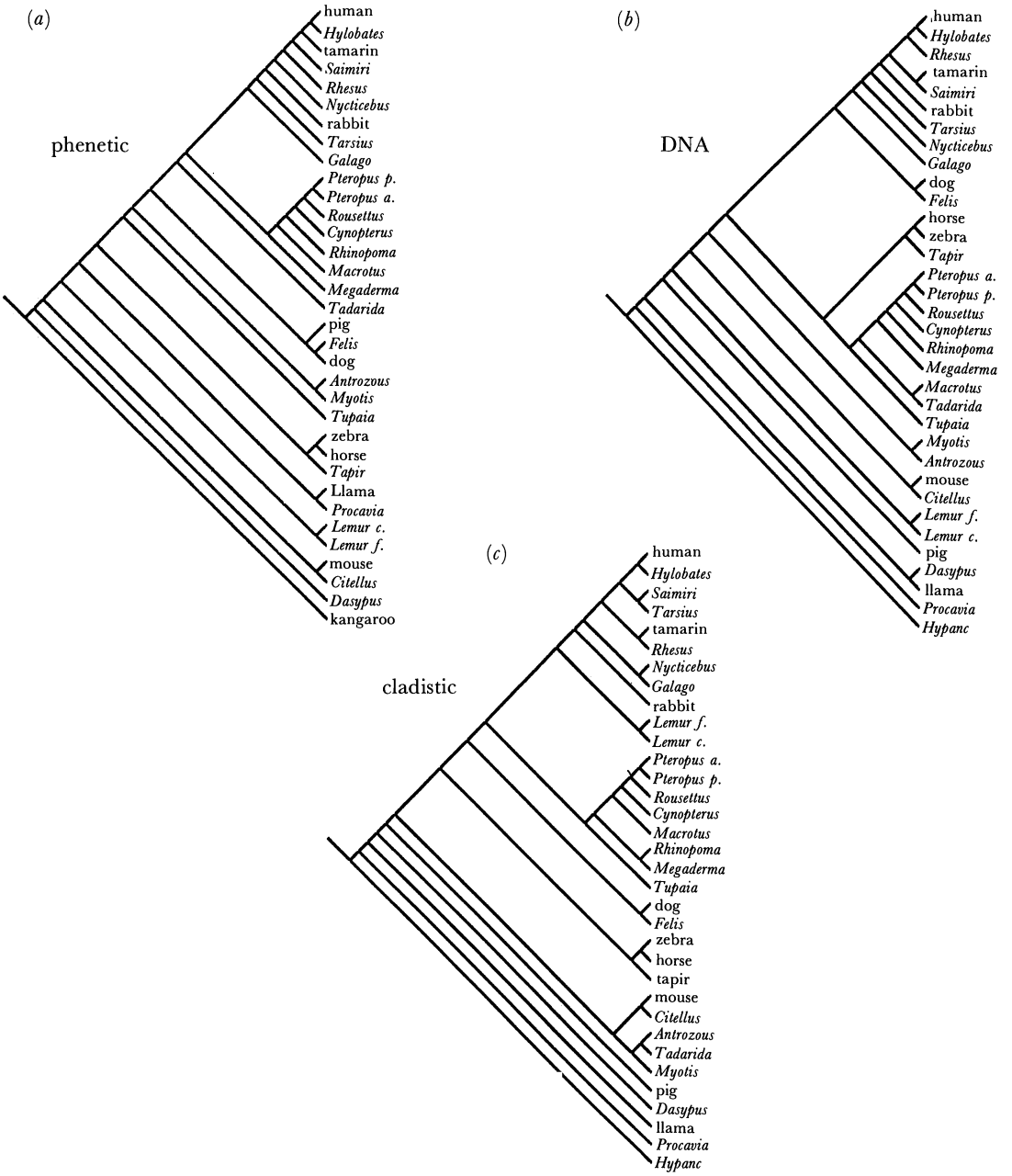

FIGURE 16. Phylogenetic relations of 34 mammals in 12 Orders, as revealed by three different measures of similarity in their β-globin chains. (*a*) Tree based on purely phenetic similarity (Russell & Rao). Note that the primates cluster together as a cohesive group, except for the separation of the two lemurs and the presence of the rabbit, the sole member of the lagomorphs represented; megabats (*Pteropus*, *Rousettus* and *Cynopterus*) form a sister group to the primates; microbats have a variable relation to the megabats, with four species from different families (*Rhinopoma*, *Macrotus*, *Megaderma* and *Tadarida*) linked closely, but two species from the Vespertilionidae (*Antrozous* and *Myotis*) in an outlying relation beyond the carnivores. (*b*) Tree derived from cladistic resemblance (where shared plesiomorphies did not contribute to the similarity score) based on DNA triplets; features of the tree, such the outlying position of the edentate (*Dasupus*) and the tight cluster of non-lemurine primates and the split grouping of the microbats, are like those in (*a*); differences include a change in the position of the pig (from an association with the two carnivores to an isolated position near the edentate), a change in the position of *Tupaia* (to join some of the bats) and a change in the position of the vespertilionid pair of microbats (*Antrozous* and *Myotis*. (*c*) Tree based on cladistic resemblance (see §2.6*a*). Note that the vespertilionid pair of microbats is now associated with the molossid microbat to form a vespertilionoid assemblage in an even more outlying position, separated from other bats and primates by tree shrew, carnivores and perissodactyls.

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Table 6. Russell & Rao nearest neighbour similarities of 34 mammalian β globins

species	phenetic (positive matches/*N*)		cladistic DNA (distance computed)		cladistic (synapomorphies/*N*)	
Pteropus a.	*Pteropus p.*	98.8	*Pteropus p.*	98.1	*Pteropus p./Rousettus*	19.3
Pteropus p.	*Pteropus a.*	98.8	*Pteropus a.*	98.1	*Pteropus a.*	19.3
human	*Hylobates*	97.7	*Hylobates*	92.9	*Hylobates*	19.3
Hylobates	human	97.7	human	92.9	human	19.3
horse	zebra	97.7	zebra	95.0	zebra	28.4
zebra	horse	97.7	horse	95.0	horse	28.4
Rousettus	*Pteropus a.*	95.4	*Pteropus a.*	93.6	*Pteropus a.*	19.3
tamarin	*Hylobates*	94.3	*Saimiri*	92.4	*Saimiri*	19.3
Saimiri	tamarin	94.3	tamarin	92.4	*Tarsius*/tamarin	19.3
Cynopterus	*Pteropus a.*	93.1	*Pteropus a.*	87.8	*Pteropus a./Rousettus*	18.1
Rhesus	*Hylobates*	93.1	*Hylobates*	87.3	tamarin/*Hylobates*	17.0
Rhinopoma	*Pteropus a.*	90.9	*Pteropus a.*	87.8	*Megaderma/Galago*	17.0
Nycticebus	*Hylobates*	88.6	rabbit	79.4	*Galago*	17.0
Tarsius	*Saimiri*	87.5	*Saimiri*	82.1	*Saimiri*	19.3
rabbit	tamarin	87.5	tamarin	81.9	tamarin	14.7
Megadema	*Rhinopoma*	86.3	*Rhinopoma*	82.7	*Rhinopoma*	17.0
tapir	zebra	84.0	zebra	85.8	zebra	21.5
dog	*Hylobates*	84.0	*Felis*	76.6	*Felis/ Tarsius*	14.7
Macrotus	*Cynopterus*	82.9	*Cynopterus*	78.8	*Cynopterus*	17.0
Galago	*Nycticebus*	82.9	*Rhinopoma*	77.4	*Nycticebus/ Tarsius* *Rhinopoma*	17.0
Lemur fulvus	*Lemur catta*	80.6	*Lemur catta*	75.6	*Lemur catta*	22.7
Lemur catta	*Lemur fulvus*	80.6	*Lemur fulvus*	75.6	*Lemur fulvus*	22.7
Felis	dog	80.6	dog	76.6	dog	14.7
Myotis	*Antrozous*	79.5	*Antrozous*	72.4	*Antrozous/ Tarsius*	12.5
Tadarida	*Pteropus a./Rousettus* *Cynopterus/Rhinopoma*	79.5	*Cynopterus*	75.5	*Rhinopoma/Macrotus*	14.7
Antrozous	*Myotis*	79.5	*Myotis*	72.4	*Tadarida*	13.6
Tupaia	*Pteropus a./Rousettus*	78.4	*Tadarida*	72.3	*Rousettus*	14.7
pig	*Pteropus a./Pteropus p.*	77.2	zebra	70.7	*Felis*/zebra/horse	9.0
llama	*Pteropus a./Pteropus p.*	76.1	rabbit	65.5	*Saimiri*	9.0
Procavia	*Pteropus a./Pteropus p.* *Hylobates*/pig	73.8	mouse	54.9	mouse	5.6
mouse	*Citellus*	72.7	*Citellus*	74.6	*Citellus*	20.4
Citellus	mouse	72.7	mouse	74.6	mouse	20.4
Dasypus	*Tarsius*	68.1	*Tapir*	60.1	*Tarsius*	11.3
kangaroo	dog	60.2	(replaced with *hypanc*)		(replaced with *hypanc*)	

A final piece of circumstantial evidence for the Cretaceous origins of microbats comes from zoogeography. There are a number of cases where close microbat relatives live on separate Gondwanan fragments to which they are unlikely to have dispersed after Gondwanaland broke up towards the end of the Cretaceous. For example, the closest relatives of many New Zealand fauna are found in South America (see Briggs (1987) for a review). This is true of the New Zealand microbat genus *Mystacina* (Daniel 1979), whose closest living relative is the South American genus *Noctilio* (Pierson *et al.* 1986). Their flight capabilities notwithstanding, these two genera of microbats are unlikely to have separated much later than 70–80 Ma ago when New Zealand and South America were close enough to the Antarctic and Australasian land masses to provide interchange (Briggs 1987). Similar cases may be made for the African vespertilionid genus *Glauconycteris* and the Australasian vespertilionid genus *Chalinolobus* (Ryan 1966; Koopman 1971), the sucker-footed families Myzopoda (in Madagascar) and Thyroptera (in South America) (Yalden & Morris 1975), and possibly for the leaf-nosed Rhinolophoids in

the Old World and the leaf-nosed Phyllostomids in the New World (Van Valen 1979). If one accepts our arguments indicating that a large M/P index is primitive, it is of some interest that all of the most primitive microbat families by this criterion ($M/P > 8$) are restricted to Gondwanan locations; furipterids ($M/P = 11$), noctilionids ($M/P = 9.9$), desmodontines ($M/P = 9$), mormoopids ($M/P = 8.8$) and natalids ($M/P = 8$) in South America. An apparent exception appears to be the monotypic genus and family comprising *Craseonycteris thonglongyae* ($M/P = 8.5$) from Thailand. This smallest of all microbats is restricted to a zone less than 100 km in diameter near Sai Yok in Western Thailand, lat. 14° 26′ N, long. 98° 51′ E (Hill 1974; Hill & Smith 1981). Closer examination reveals that this location is a belt of Permian limestone which is part of the Indo-Australian plate derived from Gonwanaland (Audley-Charles 1983; Archbold *et al.* 1982; Buffetaut & Rucha 1985). *Craseonycteris* can therefore be regarded as occupying a Gondwanan segment like the other microbats with large M/P indices.

The likely forest habitat of the early microbats would not favour preservation of whole bats as fossils, but perhaps some of the presently unassignable mammalian teeth from the Cretaceous should be re-examined with the new surface microscopical techniques (Lester *et al.* 1988). It is possible that some features in the dental enamel of microbats might be sufficiently characteristic to enable them to be recognized, if present, in ancient teeth currently assigned to the Insectivora.

10. Haemoglobin sequence data from megabats and microbats

10.1. *General features of the mammalian tree*

The complete amino acid sequences are available for both the α- and β-chains from haemoglobins in ten different species of bat, six microbats, *Antrozous pallidus* and *Myotis velifer* in the Vespertilionidae, *Tadarida brasiliensis* in the Molossidae, *Macrotus californicus* in the Phyllostomidae, *Megaderma lyra* in the Megadermatidae and *Rhinopoma hardwickei* in the Rhinopomatidae, and four megabats, *Rousettus aegyptiacus*, *Cynopterus sphinx*, *Pteropus poliocephalus* and *Pteropus alecto* (Kleinschmidt *et al.* 1988). We compared the ten sequences from the β-chains of bats with the sequences from the β-chains of 24 other mammals in 11 different mammalian orders, Marsupialia, Edentata, Scandentia, Perissodactyla, Artiodactyla, Carnivora, Lagomorpha, Primates, Hyracoidea, Rodentia and Primates (see figure 16 and §2.2 for the full list of taxa). We used a variety of different algorithms, both cladistic and phenetic, for classification of the sequence data. Figure 14 shows the results of one study using three variants of the Russell & Rao similarity coefficient (see §2.5*a* for details).

The placement of many mammals within the phenetic tree (figure 16*a*) generated by the haemoglobin sequence data is in accord with accepted views of mammalian phylogeny. For example, the marsupial kangaroo is placed outside all the other eutherian taxa studied, and the edentate armadillo is, in turn, placed outside of all of the other eutherian mammals. Some pairings occur as might be expected, such as horse with zebra, the two kinds of lemur with each other, the two rodents and the two species of *Pteropus*. Some clusterings also conform to expectations, such as the primate grouping of *Galago*–tarsier–*Nycticebus*–*Rhesus*–squirrel monkey–tamarin–gibbon–human. One problem with this last grouping, however, is the placement of the rabbit between tarsier and *Nycticebus*. The 'anomalous' placement of a taxon from the order Lagomorpha in the midst of the order Primates is not a peculiarity of our

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particular method in this case, as we observed the same feature with all three of our techniques (see figures 16*a–c*). We also saw the same placement of the rabbit when we ran a smaller data set with branch-swapping on PAUP (data not shown), and note that it is a feature of other phylogenetic reconstructions using haemoglobin sequence data (Goodman *et al.* 1985).

The general form of the trees is similar to the phenetic tree already described, when they are constructed from the more cladistic similarity measurements we have made (figures 16*b* and *c*; §2.6). One notable change, compared with the phenetic tree (figure 16*a*), is that the lemurs are found closer to the rest of the primates, separated from them only by the rabbit in the cladistic tree (figure 16*c*) rather than in an outlying position with the edentate, although they remain separated from the other primates by many taxa in the tree generated when we tried to take account of DNA base changes (figure 16*b*). There is also some change in the relative positions of some other primate taxa and the rabbit within their clade. There is a variable relationship of the three microbats *Rhinopoma*, *Megaderma* and *Macrotus* relative to each other, but their position with respect to the haplorhine primates, strepsirhine primates and megabats appears constant.

10.2. *Position of the bats in the* β*-globin tree*

All of the four megabats tested, *Pteropus poliocephalus*, *P. alecto*, *Cynopterus* and *Rousettus* cluster tightly together in the same part of the tree, in a sister-group relationship to the primates (ignoring association with some microbats, and in one tree, *Tupaia*) in all three trees (figures 16*a–c*). The six microbats, on the other hand, taken as a whole, have no clear relationship to each other, or to the other mammals. *Rhinopoma*, *Macrotus* and *Megaderma*, each from the separate familes Rhinopomatidae, Phyllostomidae and Megadermatidae, respectively, associate with the megabats in the sister-group-relation the primates. In contrast, *Myotis* and *Antrozous*, both microbats from the family Vespertilionidae, appear together, further out on the tree, beyond the branches containing the carnivores (figure 16*a*) or separated by the scandentian tree shrew in the DNA tree (figure 16*b*), and even further out, beyond the rodents, in the cladistic tree in which *Myotis* and *Antrozous* have been joined by *Tadarida* (figure 16*c*). *Tadarida*, from the family Molossidae, which has been aligned with the Vespertilionidae to form the Vespertilionoidea (Koopman & Jones 1970), appears in a variable position in the present trees. In studies with other algorithms, we have again found that the position of *Tadarida* is not constant, but varies according to the other sequences being considered at the same time. Some appreciation of this can be gained from an examination of table 6, which gives the values computed for β-globin similarities between nearest neighbours (as distinct from the averages used to construct the trees). Note, for example, that although the nearest neighbour of the microbat, *Antrozous*, is consistently *Myotis*, it has an equal relationship with *Tadarida* in cladistic data. In runs using PAUP, *Tadarida* often clustered with *Myotis* and *Antrozous*, as in the cladistic tree (figure 16*c*), with all three vespertilionoid taxa consistently split from the other three microbats by members of several intervening mammalian orders, including scandentians, carnivores, ungulates and rodents.

10.3. *Interpretation of* β*-globin tree in light of paraphyly hypothesis*

The results from the haemoglobin sequence analysis cannot be regarded as definitive. The present study can be regarded only as a very preliminary one, both because of our inability to carry out branch-swapping analysis on a large data set and because of the variable placement of some of the key taxa in the trees generated from the haemoglobin sequence data by different methods. Some tentative conclusions can be formulated, however.

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1. There is a clear and strong association between megabats and primates. The only non-primate taxon that associates more strongly with primates than megabats is the rabbit. The position of the rabbit can be qualified by the fact that it is the sole representative of the lagomorphs. For this reason, the possibility of a chance similarity to the primate pattern of sequences cannot be ruled out until we have β-globin sequences from other members of the Lagomorpha. This qualification, based on inadequate representation of taxa, does not apply to the close placement of megabats next to primates. All four megachiropteran taxa are consistently linked both with each other and with the primates.

2. The situation with respect to the microbats, on the other hand, is difficult to resolve. Proponents of the monophyly of bats would no doubt place emphasis on the grouping of *Rhinopoma*, *Megaderma* and *Macrotus*, and sometimes *Tadarida*, the microbats which associate both with primates and with megabats. From this viewpoint the other three microbats, *Myotis* and *Antrozous* and sometimes *Tadarida*, might be seen as anomalies appearing for various reasons in the 'wrong' part of the tree, such as the rabbit whose sequence associates with primates. On the other hand, there are reasons for placing some weight on the three microbats in the more outlying position, particularly the vespertilionids, *Antrozous* and *Myotis*. As closely related taxa, the vespertilionoids are less likely to be the subject of chance similarity in sequence data than an isolated taxon from a single family, like each of the three remaining microbats in the Phyllostomidae, Rhinopomatidae and Megadermatidae. On this point, it is worth emphasizing the very small number of common substitutions that can be used to define the microbats from the β-globin sequences. Of the 23 sites which show substitutions restricted to the microbats in our sample, only one is found in the β-chain of *Macrotus* (site 112), one site is changed similarly in *Rhinopoma* (site 77) and two sites (69, 76) are changed in *Megaderma*. The corresponding numbers of sites for *Antrozous*, *Myotis* and *Tadarida*, respectively, are: eight (sites 5, 6, 51, 69, 76, 77, 130, 135), nine (sites 5, 6, 51, 69, 76, 77, 130, 134, 135) and seven (sites 5, 69, 76, 77, 112, 130, 134). The data set would obviously be improved if there were sequences from other microbat taxa whose closer relationship with *Macrotus*, *Rhinopoma* or *Megaderma* might provide an increased number of common substitutions over the present limited set.

The variable and split relationships of the microbats, some associating with megabats, others far distant from them, suggest that the similarity of some of the microbat haemoglobin sequences to the megabat sequences may have arisen from functional convergence in two groups of flying animals with similar thermal and metabolic demands on the oxygen carrying system. Functional convergence of amino acid sequence structure is known for haemoglobin (Perutz 1983), but a particularly striking example concerns the adaptive evolution of stomach lysozymes in two distantly related, but phytophagous, mammals, the cow and the hanuman langur. Amino acid sequence similarity shared between the stomach lysozyme of cow and of langur is sufficiently high that parsimony analysis wrongly places the monkey on the cow's branch of the mammalian tree (Stewart *et al.* 1987). Although it may sound like special pleading to try to account for the 'awkward' positions of *Rhinopoma*, *Macrotus*, *Megaderma* and sometimes *Tadarida*, in this way, it has to be admitted that such 'upward' convergence of haemoglobin structure toward that of other taxa of similar lifestyle is more plausible than the 'downward' convergence of haemoglobin structure from *Antrozous* and *Myotis*, with our without *Tadarida*, toward the haemoglobins of grossly dissimilar taxa with which they associate, such as rodents, edentates and other non-primates. The only way to settle this question will be to examine more microbat haemoglobins until a reliable clustering is achieved.

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11. Evidence against the paraphyletic hypothesis

Two studies have specifically rejected the paraphyletic hypothesis of mammalian flight on grounds apart from those already considered in this paper. Novacek (1980) found a suite of 15 characters (his characters 48–63) that he considered were synapomorphic in megabats and microbats. Cronin & Sarich (1980) also rejected the paraphyletic hypothesis on serological grounds based on an examination of immunological distances of albumins and transferrins from different mammals. These findings will be discussed in detail because they appear, upon first examination at least, to argue strongly in the opposite direction to that taken in this paper.

Table 7. Some differences between megabat and microbat flight muscles

	microbats	megabats
occipitopollicalis muscle	single muscular origin, tendinous attachments to ventral flight muscles	multiple muscular origins, distal muscle belly, no tendinous attachments to ventral flight muscles
trapezius muscle	multiple divisions, extensive vertebral origins, extensive scapular and clavicular insertions	fused into one mass, thoracic origin, limited insertion onto scapula, no clavicular insertion
deltoid muscle	extensive origin involving both scapula and transverse ligament	origin limited to acromion process of scapula
triceps muscle	caput laterale has very proximal origin, involving trochiter on humerus	caput lateral occupies two thirds of length of humerus
subscapularis and teres minor muscles	moderate to large	very small
infraspinous fossa of scapula	usually three facets (two in *Nycteris*)	one facet
acromio-clavicular joint	weak or absent: acromion delicate: scapula usually bound to clavicle by ligaments or by coracoid process	strongly reinforced joint with a robust acromion and clavicle

11.1. *Skeletal characters*

Ten of Novacek's (1980) characters (51–60) can be legitimately subsumed under the musculoskeletal adaptations making up the wing. For this reason, they should be used with caution because of the possibility that they may be homoplasies between microbats and megabats rather than synapomorphies. A specific case of this kind can be made for one of Novacek's characters (60 – the occipitopollicalis muscle). This is really a group of muscles of uncertain origin with a number of clear and consistent differences between microbats and megabats (table 7 and see also Strickler (1978)). The presence of the muscle is essential to maintain a taut leading edge of the wing, despite changes in the separation of the thumb from the body. It may therefore be an obligatory functional convergence in the two kinds of bats. The presence of clear differences between this essential flight muscle in the two groups of bats makes the case for homoplasy easier to argue in this case, but the possibility of homoplasy should be carefully considered and eliminated in less obvious cases before any character associated with the flight apparatus is accepted as synapomorphic. Because characters 50–59 have not been examined in this way and because character 60, the occipitopollicalis, falls far short of strong synapomorphy when examined, the use of these ten characters to link megabats and microbats cannot be regarded as a strong case. The following are a few examples. The elongation of the digits to support a flight membrane (56) provides a weak link alongside the

quantitative details of the digital elongation shown in figure 11, which supports a schism, rather than a link, between megabats and microbats. Similarly, the rotation of the manus (57), the hindlimb rotation (58), and the various forearm features (51–55) are all more or less to be expected, if not obligatory, in a hand wing. The similarities in the scapula, which has a large infraspinous fossa (51) in both suborders of bats, are considerably weakened by the fact that this fossa has more facets in microbats than in megabats (Strickler 1978). Of the non-skeletal characters used, those related to foetal membranes (61–63) also appear to be arguable. Homoplasic characters associated with placentation are well known (e.g. in the phyllostomid bats, which have many features found also in higher, but not lower, primates (Luckett 1980). Homoplasy seems likely in the case of one of these characters (62) because the prominent layer of extracellular material, which has often been referred to as either the intrasyncytial lamina or the interstitial membrane of bats, has been acquired independently by Carnivora (e.g. Odvor-Okelo & Neaves 1982) and, therefore, cannot be used with any great confidence as a synapomorphy to link the two groups of bats. The use of foetal membranes to sort out relationships within the Microchiroptera can not be regarded as successful because of the common occurrence of homoplasies at higher taxonomic levels and because none of the features of placentation seem to be capable of grouping microbats at lower levels (Luckett 1980; Novacek 1980). In view of the limitations which foetal membrane characters exhibit in defining microchiropteran relations, it is perhaps expecting too much for these characters to be helpful in sorting out relationships between the Microchiroptera and Megachiroptera. Indeed, some authors have used foetal membranes to argue for the paraphyletic hypothesis of bat origins rather than against it (Jones & Genoways 1970).

The remaining cranioskeletal characters (48, 49) do not appear strong because the use of characters from the skull leads to an alignment of megabats with primates, which is as plausible as any alignment with microbats (Hill & Smith 1984; see §11.3).

11.2. *Immunological studies*

The serological evidence is more puzzling, but cannot be used to reject the paraphyletic hypothesis because key data do not yet appear to have been collected. The only serological study to investigate the paraphyletic hypothesis is that of Cronin & Sarich (1980). As part of a broader study into the evolutionary relationships of the Tupaiids using albumin immunological distances, they claimed that 80 'units of albumin change' had occurred along the bat lineage since the separation of bats and primates. The two chiropteran suborders are considered to differ in only 55 units of change, and hence the common ancestry of both groups is assumed. The basis for the primate/bat distance of 80 units, according to the caption of their figure 1, is the assumption that 'bats and primates are at least as closely related to each other as either is to the edentates'. This assumption would be violated if the paraphyletic hypothesis is correct, so we do not consider this to be a valid investigation of such an hypothesis. Given the possible association of microbats and edentates (forelimb, brain and haemoglobin data each raise this as a distant possibility), combined with the possible very ancient origins of microbats (§9.2), and the enormous divergence of albumin immunological distances in some microbat families such as the phyllostomid, noctilionid and mormoopid families, we feel that a serological investigation of the paraphyletic hypothesis should use a more appropriate outgroup than the edentates, such as a monotreme or a marsupial.

There is direct evidence suggesting unequal rates of change in the albumin of different

microchiropteran families, with the phyllostomid, mormoopid and noctilionid clade showing a divergence greater than the 'bats' as a whole. The dendrogram for the familial relationships within the microchiroptera calculated purely on albumin immunological evidence would therefore not be congruent with the morphological data if one assumes a constant rate of change. This result cautions against the use of albumin immunological distances as a determinant of megachiropteran and microchiropteran relationships. Given the lack of congruence between the different mammalian interordinal phylogenies, as a function of the methodology and data sets used (Wyss *et al.* 1987), and the discrepancies between the phylogenies of Cronin & Sarich (1980) and those based on other molecular data sets (for example, haemoglobin, Goodman *et al.* (1985); α-crystallin A, de Jong (1982)), we see no reason to accept the immunological data of Cronin & Sarich (1980) as representing the definitive answer to the question of paraphyly.

TABLE 8. CRANIAL CHARACTERS USED BY WIBLE & NOVACEK (1988) TO LINK MEGABATS AND MICROBATS

character	difficulties
15 (premaxilla greatly reduced)	also present in out-groups such as edentates and primates
16 (jugal greatly reduced)	megachiropteran jugal is expanded to form post-orbital process
17 (tegmen tympani tapers to slender process)	*Pteropus*, *Cynopterus* and *Syconycteris* have a stout tegmen with multiple expansions at tip: similarity between microbats and megabats is limited and confined to the acoustically specialized *Rousettus*
18–20 (basicranial features involving the relationships of vasculature and cranial nerves)	present in out-groups; distribution incompletely known

11.3. *Cranial synapomorphies of megabats and microbats?*

In a recent paper, Wible & Novacek (1988) provide further argument, from a consideration of cranial features, for the opposing view that bats are monophyletic. They provide some new cranial characters, which they argue are derived and confined to the bats. There are problems with respect to most of these cranial features that we detail below and summarize in table 8. Consider character 15, ('premaxilla greatly reduced'), one of the six new characters, 15–20, put forward as synapomorphies for bats. A small or absent premaxilla is claimed to be synapomorphic for megabats and microbats, yet the crania of other major groups, such as edentates and primates, exhibit the same feature. It is perhaps justifiable to eliminate from consideration an edentate–bat connection, although there are reasons, which we have already raised, for thinking that microbats might be linked to an ancient lineage such as the edentates. Wible & Novacek (1988) do not deny that this character, which is supposed to characterize bats, is present in the primate out-group. But given the intense interest focused on the primates as a sister group for megabats it is difficult for us to see justification for the way in which the small premaxilla of primates is discounted as a 'secondary reduction'. In an attempt to justify their dismissal of the presence of this character in primates as a reduction, Wible & Novacek (1988) note no reduction of the premaxilla in *Plesiadapis*, a fossil taxon that many primatologists (see, for example, Martin 1986*a*) would not admit as a primate in the first place. There seems no need to resort to such a dubious out-group taxon when there are so many undisputed primates available, both living and fossil. Our own observation, based on the examination of

skulls of several megabat and prosimian taxa, is that there is a great similarity between both the nature and the degree of premaxillary reduction in prosimians and megabats. As a test of this alternative view point, we asked colleagues (and we invite the reader) to pick one skull rostrum, from the series of six illustrated in figure 1 of Wible & Novacek (1988), most closely resembling a megabat skull (*Pteropus poliocephalus* was provided for comparison). These colleagues were unaware of the identity of any of the illustrations or of the comparison skull. All chose *Notharctos* the fossil primate (figure 1*a* of Wible & Novacek (1988)) as having the closest similarity to the megabat's premaxilla.

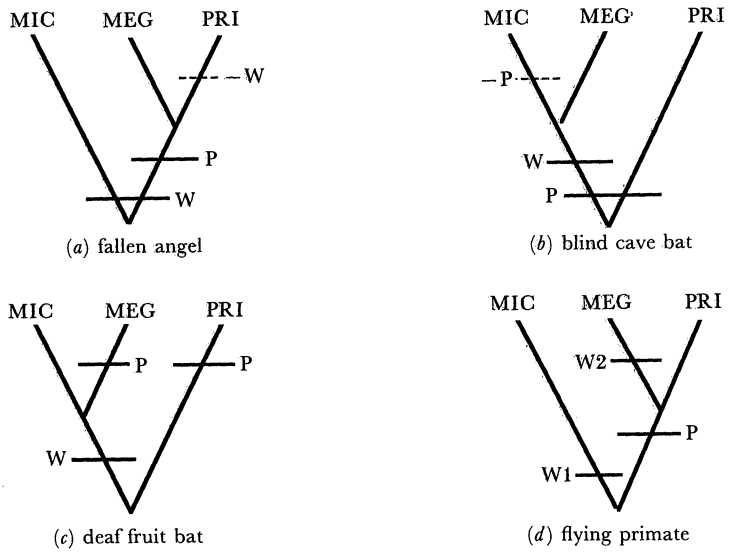

FIGURE 17. Four possible topologies for the phylogenetic relationships of microbats, megabats and primates: colloquial titles refer to the differing evolutionary scenarios which could have given rise to these differing topologies. (*a*) 'Fallen angel' scenario has wings (W) as a shared feature in the ancestry of all three lineages. Wings are subsequently lost (−W) in the primate line, but retained as a shared feature of both kinds of bats; this unlikely scenario is not compatible with the rich fossil record of primates. (*b*) 'Blind cave bat' scenario has wings as a shared feature (W) in the ancestry of megabats and microbats, with the loss of the primate brain, *M*/*P* and genital features in microbats (−P) as a shared feature defining this group; this scenario is unparsimonious in many respects and conflicts both with the fossil record concerning the relative age of microbats and megabats and with the presence of the primate features in *Cynocephalus*; its redeeming feature is its ability to reconcile some conflicting data. (*c*) 'Deaf fruit bat' scenario, like (*b*), has wings in the ancestry of both megabats and microbats, but with megabats being derived from microbats by the acquisition of the primate features and loss of the microbat features of sonar and auditory specialisation; this scenario is consistent with evidence indicating that megabats are a more recent group, but is unparsimonious, lacks any rationale for the independent appearance and disappearance of so many features in the megabats and, like (*b*), conflicts with the evidence from *Cynocephalus*. (*d*) 'Flying primate' scenario has independent evolution of wings, once in an early pre-microbat (W1) and again later in the branch of the primate lineage leading to megabats (W2); this scenario is the most parsimonious way to account for all the data from brain, skeleton, genital anatomy, haemoglobin sequences, the transitional form *Cynocephalus*, fossil record and zoogeography, notwithstanding the apparent homoplasy in the wings. See §12 for more details. Abbreviations: MIC, microbats; MEG, megabats; PRI, primates.

There are several other problems with this character, such as the choice of *Dobsonia moluccensis* as a representative megabat taxon when it has an atypical rostrum, as well as with the arbitrary nature of the out-group comparison.

We are at a loss to know what Wible & Novacek (1988) can have in mind as regards character 16, 'jugal greatly reduced', because megabats have a jugal with a process which extends toward a similar, larger process on the frontal, to form a primordial post-orbital bar.

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We admit that the megabat post-orbital processes do not form a complete bar, like that found in primates and some other mammalian orders, but the megabat post-orbital process is clearly present, in contrast to microbats. We believe that an appropriately objective, cladistic analysis of bat skeletal characters would have to include the post-orbital processes of megabats as a possible synapomorphy with the similar process in primates, although we realise it may be a weak synapomorphy because scandentians, carnivores, ungulates all show similar processes (McKenna 1975). Weak or not as a character, the extension of the post-orbital process in megabats hardly implies a jugal reduction, particularly if megabats, microbats and primates are being compared. We therefore reject the proposition that this character is a satisfactory basis for linking the two kinds of bats.

The choice by Wible & Novacek (1988) of what might prove to be atypical taxa also applies to character 17, ('Tegmen tympani tapers to a slender process projecting ventrally into the middle ear cavity medial to the epi-tympanic recess.') The choice of *Rousettus* as the megabat to represent this character may be problematic, as *Rousettus* is a cavernicolous species, with some auditory specializations that are atypical for megabats as a whole. Our own investigations suggest that the tegmen tympani of *Rousettus* may not be typical of megabats, as *Pteropus poliocephalus*, *P. scapulatus*, *P. alecto* and *Cynopterus brachyotis* each have a tegmen, which is stocky rather than slender, with multiple expansions at the tip contrasting with the typical narrow tip on the microbat tegmen. As with character 15, it is not clear to us that the megabat tegmen (exemplified by the generalized genus *Pteropus*, as opposed to the specialized *Rousettus*) is closer in its morphology to the microbat tegmen than it is to the tegmen of some prosimians.

Rousettus is often regarded as an appropriate megachiropteran genus for comparison with microbats because it has primitive echolocation, is of small size for a megabat and is cavernicolous. The hidden assumption here may be that a taxon such as *Rousettus* could represent a transitional form between megabats and microbats. On the other hand, it could be misleading to choose *Rousettus* as a typical megachiropteran if all the features that it appears to share with Microchiroptera are derived within the Megachiroptera. In fact, as already argued above, small size, tongue-clicking echolocation, cavernicolous roosting behaviour and increased reliance on acoustic orientation may all be derived within the Megachiroptera. It is therefore essential, as Wible & Novacek (1988) themselves point out, to sample wisely from the groups under consideration. To us this would mean a consideration of the least derived genus within the Megachiroptera, which we consider to be *Pteropus*. Wible & Novacek (1988) may err in placing so much emphasis upon atypical megabats such as *Rousettus* (whose tegmen could link it to microbats by a parallel acoustic dependence rather than phylogenetically). Although no certainty can be attached to our conclusion that *Pteropus* is less derived within the Megachiroptera than *Rousettus* or *Dobsonia*, the contrary hypothesis should be explicitly tested rather than assumed as a basis for choosing *Rousettus* or *Dobsonia* as the reference megachiropteran taxon in microchiropteran–megachiropteran comparisons.

Three other characters (18–20), involving complex relationships in the basicranium, are also put forward to bring the total of 'new' cranial bat synapomorphies to six. We are not competent to judge these characters, but note that there are difficulties with the out-group comparison in each case (in other words, none of these characters is unique to the bats).

There seem to be difficulties with all of these characters, particularly with respect to the assignment of polarity based on out-group analysis, which in many cases has not dealt fairly

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with the possibility that primates might be the in-group. It would be of great interest to see how such skeletal characters perform on a more objective, cladistic exercise such as we have performed on the brain characters of figure 8.

12. Four alternative interpretations of primate–megabat–microbat relationships

Haemoglobin sequence data, neural data from a variety of different systems, including the visual pathways, skeletal data such as the derived features in the forelimbs shared by primates and megabats and data on the genital system, all point to a megabat–primate association. On the other hand, musculoskeletal modifications of the forelimb for flight, cranial morphology and immunology of serum proteins have been interpreted by some workers to argue for a monophyletic assemblage of megabats and microbats. In the absence of an obvious sister group for the microbats, the focus is therefore on the relationship of three mammalian groups; the megabats, the primates and the microbats. There are three possible basic topologies for the phylogenetic relations of these three monophyletic taxa: (*a*) megabats and primates are monophyletic, with microbats as a sister group; (*b*) megabats and microbats are monophyletic, with primates as a sister group, and (*c*) microbats and primates are monophyletic with megabats as a sister group. In the absence of any evidence to support the third possibility, this will not be considered further, but two different versions each of topology (*a*) and topology (*b*) will be considered, according to the point(s) in the tree at which flight is supposed to have evolved and according to the relative order of appearance of megabats and microbats. These four phylogenetic possibilities are shown in figure 17. As a mnemonic aid we have given each of these alternative phylogenies a caricatured title: (*a*) 'fallen angel' scenario (where wings evolve first, as a shared feature in the ancestry of all three groups, but have been lost subsequently by the primate lineage); (*b*) 'blind cave bat' scenario (where primate brain and genital features evolve first, wings evolve after the divergence of the primate line, and microbats later lose all of the primate brain and genital features); (*c*) 'deaf fruit bat' scenario (where megabats arise from the microbat lineage by the loss of sonar and acoustic specializations and the simultaneous acquisition of the primate brain and genital features); (*d*) 'flying primate' scenario (microbats evolve first, followed by a monophyletic lineage characterized by primate brain and genital features; megabats come off an early branch of this primate lineage).

12.1. '*Fallen angel*' *scenario*

This alternative is mentioned for logical completeness only, as there is little positive evidence to support it over the other three possible scenarios. The long and extensive fossil record of primates (see, for example, Szalay & Delson 1979) gives no indication that this lineage may have arisen from winged ancestors. According to this scenario, megabats would have preceded primates, when the fossil record indicates the opposite order of appearance. The oldest known fossil megabat is 30 Ma (Dal Piaz 1937), whereas there are possible fossil primates frm the late Cretaceous 70 Ma ago and undisputed primates from the early Eocene, 55 Ma ago (see Archer & Aplin (1984) for a review). This alternative is also contradicted by the neural pathway data (§4.4), penial morphology (Smith & Madkour 1980) and immunological studies (Cronin & Sarich 1978) from *Cynocephalus*, all of which place the origin of this important 'transitional'

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form after the microbats and before the primate–megabat assemblage, not before all three groups, as this alternative predicts. The idea that primates should have arisen from a lineage of flying mammals by loss of the flight apparatus, while having some poetic appeal, we feel to be a somewhat preposterous attempt to reconcile the data, even when one considers the debates about primate origins (Szalay & Delson 1979; Martin 1986*a*). The possible loss by microbats of a similarly complicated set of characters (neural pathway characters, as opposed to flight characters) is the basis of the 'blind cave bat' scenario (see §12.3) discussed below. There is the added problem of accounting for the reversal to a prehensile forelimb from one which has been extensively modified for flight. The possibility that early primates lost flight we rate as only marginally less likely than the next scenario, that early microbats lost the complex set of neural characters defining primates.

12.2. '*Blind cave bat*' *scenario*

It is possible to reconcile the conflicting immunological data and to incorporate those aspects of the haemoglobin and cranial data that might be used to link both kinds of bats to the primates, in the following way. Suppose that all early bats had the primate features we have decribed in the living megabats, but that the microbats lost these features subsequently, perhaps as they passed through an evolutionary bottleneck involving the cavernicolous niche. We have called this the 'blind cave bat' scenario in reference to the ease with which some cavernicolous vertebrates appear to be able to 'lose' visual capabilities (Voneida & Sligar 1976). According to this scenario, the immunological and skeletal features apparently shared by microbats and megabats reflect a common ancestry between the two groups of bats, but the links to primates (particularly the neural, haemoglobin and genital characters) have been retained to a greater extent by the megabats. Apart from its lack of parsimony in several losses necessary in microbats, there are several problems with this scenario that appear to us to outweigh its advantages in reconciling immunological data, which are, in any case, subject to qualification (see §11.2).

The major problem concerns the relative timing of the microbat and megabat lineages. The 'blind cave bat' scenario requires that microbats represent the younger lineage, a necessary corollary of their derivation from the prior megabat line. This is consonant with the view that megabats are more primitive in their flight apparatus, most species having two claws on the wing (see §8) and less derived features than microbats in the attachment of flight muscles to the humerus (Van Valen 1979). The view that megabats are an older lineage, because their flight apparatus is not as advanced as microbats, is a view that presupposes monophyly. If monophyly is rejected, a recent megabat lineage having less-advanced wings is perfectly compatible with an older microbat lineage characterized by diverse, advanced flight.

In conflict with the inference from monophyly that microbats evolved later, there are two concrete pieces of evidence which argue that megabats are the younger lineage, not the microbats. The first is from the fossils. Flight and auditory apparatus more advanced than many modern microbats have been found in an unmistakeable microchiropteran, *Icaronycteris index* from the early Eocene, 50 Ma old (Jepsen 1970; Novacek 1985). There are some grounds for believing that the antecedents of *Icaronycteris* were another 30 Ma older (see §9.2). In contrast, the oldest known megabat fossil, *Archaeopteropus transiens*, is from the middle Oligocene, 30 Ma old (Dal Piaz 1937). There is a clear transition to increasingly derived dentition as one moves, in turn, from the cuspidate *Archaeopteropus transiens* to the less-cuspidate *Propotto leakeyi*, a megabat fossil from the Miocene, 10–25 Ma ago (Walker 1969), to modern

megabats that have lost their cusps almost completely (Van Valen 1979). From this, it can reasonably be concluded that *Archaeopteropus* represents an early stage in the megachiropteran lineage, in contrast to the relatively advanced position of *Icaronycteris* in the microbat lineage. In other words, the origin of microbats was earlier than that of megabats, by at least 20 Ma and perhaps by as much as 50 Ma.

The second piece of concrete evidence supporting a more recent origin for megabats comes from the haemoglobin sequence data. The megabat sequences from four species in three representative genera are tightly clustered with a range of similarity values which is comparable with the range found among the anthropoid primates (see figure 16 and table 6). Assuming that mutation rates have ben comparable in megabats and anthropoids, we can estimate from other values calculated for the anthropoids (see Britten 1986; Goodman *et al.* 1962), that the megabat divergence is between 30 and 40 Ma old. As the higher primates appear to have a slower DNA-sequence mutation rate (Britten 1986), this estimate of the megabat divergence may have to be revised to an even smaller figure. In contrast, microbat haemoglobin sequences from five different families show much lower similarities (see §10, figure 16 and table 7), comparable to values shown between primates and non-primates, and with a wide scatter. Using the same scales for molecular evolution of mammals that were used to estimate the megabat divergence, we can estimate a divergence time amongst different microbat families of greater than 60 Ma.

In summary, the fossil record and molecular phylogeny provide independent evidence that the microchiropteran lineage is 20–30 Ma older than the megabat lineage, in direct conflict with the predictions of the 'blind cave bat' scenario of microbat origins. If megabats are a younger group but are less advanced than microbats, the monophyletic hypothesis has an extra problem in the form of the apparent stasis exhibited by megabats in the face of the diversity evident in microbat evolution. This problem is not present in the 'flying primate' scenario where the separate invention of flight by the megabats is a more recent event and there has been less time for the diversity of flight solutions evolved by microbats over a much longer period.

12.3. '*Deaf fruit bat*' *scenario*

This scenario was suggested as a likely interpretation of the presence of primate brain features in megabats when these data were first published (Martin 1986*b*). While the 'deaf fruit bat' scenario is consistent with the more recent origin of the megabats (§12.2), and with the interpretation of the immunological and musculoskeletal data that bats are monophyletic (§11.1, 11.2), it has many problems that have been dealt with already and which will be summarized here:

1. Mosaic of primitive and derived wing characters in megabats: if megabats have been derived from a microbat line, there are difficulties in accounting for the combination of a highly derived M/P (§6) with primitive features of the wing such as two claws (§8) and the non-derived insertions of muscles into the humerus (Strickler 1978). If megabats originated from the microbat lineage before the microchiropteran wing had lost the second claw, as this scenario requires, it is difficult to explain the apparent stasis of most features of the megabat flight apparatus alongside of the advancement of M/P. On the other hand, if megabats came from a primate lineage which already had a high, derived M/P (scenario 4), this problem does not arise.

2. Size and M/P: as already argued (§7.3), one cannot make a plausible case for a transition

from the microbat to the megabat lineage on the basis of size and M/P, unless one postulates forms for which there is no evidence, either living or in the fossil record.

3. Lack of parsimony: the details of the neural data set alone do not allow a parsimonious solution if megabats and microbats are monophyletic (§4). This problem would be compounded by consideration of the other characters; for example, all the characters in table 1, incompletely determined as they are with respect to phylogenetic polarity, would have to be switched at the microbat–megabat transition.

4. Lack of plausible selective advantage: it is difficult to see why the megabat precursor from the microbat lineage should lose the key features of sonar, enlarged cochlea and specialized auditory processing that are found in all microbats. Recent evidence (Novacek 1980) rules out the possibility that megabats could have diverged before the acquisition of these features by the microbats, so one is forced to postulate an extensive loss without a rationale to account for it. A similar objection can be raised to the independent acquisition by megabats of all the primate neural features, which have no presently known selective advantage (§2.4*q*).

5. *Cynocephalus*. If one accepts the dermopteran as a representative form, transitional to one or both of the suborders of bats, as all authors so far seem to agree (§4.4), then the primate characters of this taxon are in conflict with the 'deaf fruit bat' scenario. *Cynocephalus* has most of the primate neural features, as well as the primate genital features. Incorporating these findings into the present scenario requires: (*a*) either that the microbat lineage lost the primate brain and genital features they inherited from the dermopteran lineage, with their subsequent re-emergence in the megabats or (*b*) three independent inventions of both neural and genital character sets, in dermopterans, primates and megabats. As already pointed out, further work on the molecular phylogeny, ontogeny and neural pathways of *Cynocephalus* will play a key role in distinguishing these scenarios. In the meantime, present data point to the next, 'flying primate' scenario as the most likely interpretation.

12.4. '*Flying primate*' *scenario*

The thesis of this paper is that an early branch of the primate lineage evolved the power of flight independent of the earlier evolution of flight in microbats. Despite the apparent lack of parsimony involved in the postulate of two independent flight mechanisms with such a high degree of similarity, this scenario has the greatest consistency with the large number of derived characters shared by megabats and primates but not by microbats; in the brain (over 15 characters in separate systems); in the forelimbs and hindlimbs (M/P and M/T index); in the genital system (a number of penial characters); in the haemoglobin molecules. This proposal successfully accounts for the earlier fossil appearance of the more advanced wings of microbats, as well as the different zoogeography of the two groups of bats. A key element of this proposal is the role played by the dermopterans in providing an intermediate gliding link between the early primate lineage and megabats. While a role for dermopterans in the evolution of both kinds of bats has been proposed (see, for example, Novacek 1982), we specifically exclude the microbats. We predict that further work on the molecular phylogeny, ontogeny and neurology of *Cynocephalus* will confirm that it occupies a position above the microbats and below the megabats, as shown in our cladogram (figure 8), rather than below both kinds of bat, as predicted by other scenarios.

The title 'flying primate' could give the wrong impression that we are claiming that megabats are at a level comparable with primates in general. In fact there are a number of

reasons, including the cladogram of neural characters, the fossil record and the haemoglobin tree, for considering them to be a very early branch of the primate lineage. Other features that support an earlier divergence of the megachiropteran line compared with the lineages that gave rise to the living strepsirhine and haplorhine primates are the following, found in all living primates but not in megabats: (*a*) a retinal arterial circulation, present in all primates, even in what is arguably the most plesomorphic living primate species, *Microcebus murinus* (Cooper *et al.* 1979, figure 1) but absent in megabats in which choroidal capillary loops supply the retina instead of a retinal artery (Graydon & Giorgi 1987); (*b*) the dimeric *Alu* repeat DNA sequence found in interspersed regions of the genome in all primates (Deininger & Daniels 1986), but not in megabats (P. L. Deininger & V. Slagel, unpublished data); (*c*) the expanded, osseous, petrosal bulla, which characterizes primates (Martin 1986*a*), is absent from megabats that may, in consequence, have reduced sensitivity to low frequencies (Calford & McAnally 1987). As has already been discussed (Pettigrew & Jamieson 1987), the question whether megabats should be called primates is quite separate from the question whether primates and megabats are monophyletic. If these two groups do prove to be monophyletic, as we propose, it will have to be recognized that the megabat–primate assemblage would be ranked ordinally on the same basis as other mammalian Orders such as the Rodentia or the Carnivora.

Speculative implications of the paraphyletic hypothesis

Acceptance of the paraphyletic hypothesis, we believe, opens the way to more fruitful discussion and investigation of several difficult problems in the phylogeny of bats. In particular, if flight has evolved twice in bats it may be much more enlightening to consider separate evolutionary scenarios for microbats and megabats rather than to try to force both into a single picture for the appearance of flight. Some of the competing hypotheses for the evolution of mammalian flight may then turn out to be compatible after all. To take an example, the gliding-to-flying transition (Smith 1976) is much more plausible for the phytophagous megabats than for aerial insectivores like the microbats, because gliders do not have sufficient manoeuvrability to catch insects in the air. If one takes the view that the earliest microbat was an aerial insectivore, an alternative scenario for the emergence of flight in the much smaller microbat line might involve the transition from a climbing insectivore capable of acrobatic, controlled leaps after aerial prey (Pirlot 1977; Caple *et al.* 1983). In one rejects this idea in favour of a commuting, gliding, insectivorous lifestyle in the pre-microbats, with the evolution of aerial insect-catching at a later stage (Rayner 1986), there are still implications of the smaller size we propose for the microbat lineage. Dramatic changes in aerodynamics can occur with slight changes in the body plan of small organisms (see Kingsolver & Koehl 1985), and there are improved mechanical aspects of performance as well as a broader energy margin at small size (Norberg & Rayner 1987; Rayner 1985). Evolution of flight in microbats may therefore have followed a different initial path from the much larger megabats, with perhaps some resulting differences in flight mechanics despite the overall similarities in wing design. Fruitful lines of future research could involve the further examination of differences in flight mechanics between the two groups of bats, as well as the enlarged cervical spinal cord of the microbats (table 1). The latter may represent neural machinery that is placed as close as possible to the flight musculature and sense organs so as to reduce reaction times in an acrobatic insect-catcher. Similarly, the origins of bats are presently obscure and may be illuminated if

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'primate flight' and microbat flight are considered separately. This may be particularly true for palaeozoogeographic considerations, because the megabats, like other primates, may have radiated from the Palaeotropics; whereas our data suggest a Gondwanan origin for microbats, as discussed already in §9.2. The phylogenetic implications of the dermopteran–megabat–primate link are also testable, particularly with molecular techniques in megabats and *Cynocephalus*. There is an increasing amount of molecular data available for primates (see, for example, Goodman *et al.* 1985; Deininger & Daniels 1986), but little is available for the bats or *Cynocephalus*. Acceptance of a separate, primate origin of megabats also has implications for the understanding of the brain evolution. Primate visual organization is complex and widely separated from that of other mammals (Allman 1977), so further understanding of the complex, and similar, visual organization we have revealed in the megabats could be invaluable in the further understanding of primate brain evolution.

Conclusion

Megabats and primates share several derived features in the visual pathways, in the motor pathways, in penial morphology, in the skeleton and in the structure of the globin chains which they do not share with microbats nor with other mammalian groups. A monophyletic origin of megabats and primates is the most parsimonious hypothesis that accounts for these findings. Such an hypothesis is also consistent with the presence of many of these features in the dermopteran *Cynocephalus*, as well as with the fossil record and with present zoogeography. The opposing hypothesis, that microbats and megabats are monophyletic, is in conflict with the evidence for a primate–dermopteran–megabat link, but is supported by data from immunology and from morphology. These data, particularly the morphological data from the wing, can be interpreted differently, however, in a way which is consistent with separate origins of mammalian flight in microbats and megabats. Discrimination between these two opposing hypotheses, megabat–primate monophyly versus megabat–microbat monophyly, will require the collection of more data from the key taxa, particularly from *Cynocephalus*. We predict that further studies on *Cynocephalus* will confirm the present conclusion (based on brain, forelimb and penial morphology) that the colugo occupies a phylogenetic position *between* microbats and megabats, and not *before* both microbats and megabats, as predicted by the monophyletic hypothesis.

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