Flying primates: crashed, or crashed through?

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Synopsis

The flying primate hypothesis originated from the finding that megabats shared a number of advanced visual pathway characters with primates that were not found in any other mammalian order, nor in microbats. This hypothesis indicates that primates, colugos and megabats share a common ancestor with each other more recent than any shared with microbats. The hypothesis has found increasing support from other sources of evidence. Examples reviewed here include further derived brain features, both visual and non-visual, immunological studies of serum proteins with monoclonal antibodies and analysis of restriction sites and protein sequences (globins α and β-crystallin). DNA sequence data, while supporting the colugo-primate association, have been used to reject a primate-megabat connection, even though the total evidence for a colugo-megabat link is better than the generally accepted evidence for a colugo-primate link, and even though DNA sequence data and protein sequence data on the same genes give conflicting phylogenies. A resolution to this conflict is suggested by a bias in all the published DNA sequence data on bats. The shared substitutions claimed in support of bat monophyly are mostly of adenine (A) or thymine (T), in the same direction as the bias that exists in the overall base composition of DNA from metabolically-active, volant organisms. If the AT content of DNA is taken into account by using the NZ algorithm, the much-vaunted claims for bat monophyly based on DNA sequences are not supported. It is more parsimonious to assume that the AT bias responsible for the claimed association arose independently in the two lines of flying mammals.

Introduction

The ‘flying primate’ hypothesis arose from the unexpected finding that megabats shared a number of derived brain features with primates that were not shared with other mammals, particularly not with microbats (Pettigrew 1986). Of the many controversial aspects of this hypothesis, perhaps the most contentious is its corollary: that powered mammalian flight has evolved more than once (see the four-part debate on this topic; Pettigrew 1991a, b;
Baker, Novacek & Simmons 1991; Simmons, Novacek & Falk 1991). Alternative explanations, such as the convergent evolution of primate-like brain organization in one branch of the bats, have become increasingly unlikely as more primate-like details of neural organization are revealed in different brain systems (Pettigrew, Jamieson, Robson, Hall, McAnally & Cooper 1989; Dann & Buhl 1990; Buhl & Dann 1991; Rosa, Schmid, Krubitzer & Pettigrew 1993; Rosa, Schmid & Pettigrew 1994; Rosa & Schmid 1994).

The hypothesis accounts readily for each of a long list of puzzling differences between the two kinds of flying mammals (Table 1). One example, from the 54 listed, is the absence of laryngeal sonar in megabats despite the favourable energetics and the useful role that would be played by this ability in a nocturnal flier (Speakman 1993). Proponents of the rival hypothesis have provided no satisfactory explanation to account for these wide-ranging differences, which are readily encompassed by the flying primate hypothesis. Supporting molecular evidence for the flying primate hypothesis has also accumulated from protein sequence data on α-crystallin (De Jong, Leunissen & Wistow 1993), from globins (Kleinschmidt, Sgouras, Pettigrew & Brainitzer 1988) and from immunological studies of serum proteins (Schreib, Bauer & Bauer 1994).

The above evidence, together with support for the flying primate hypothesis from brain research (Calford, Graydon, Huerta, Kaas & Pettigrew 1985; Kennedy 1991; Krubitzer & Calford 1992; Krubitzer, Calford & Schmid 1993) has been completely overshadowed by the results of DNA sequencing studies of bats. Six separate studies were unanimous in rejecting the flying primate hypothesis after sequencing DNA from a variety of mammals. Each study found greater similarity between the DNA sequences of microbats and megabats than between the megabat DNA and primate DNA (Bennett, Alexander, Crozier & Mackinlay 1988; Adkins & Honeycutt 1991; Midell, Dick & Baker 1991; Ammerman & Hillis 1992; Bailey, Slightom & Godman 1992; Stanhope, Czelusniak, Si, Nickerson & Goodman 1992). These data have been given wide coverage in the literature, together with reports of the "crash" of the flying primate hypothesis that provoked the present title. The difficulties of reconciling these new data with the large body of evidence from brain, behaviour and proteins are largely ignored in the reverence that currently attends DNA scripture. Also ignored, by each of these six studies, is a hefty bias towards adenine (A) and thymine (T) in the base composition of the DNA substitutions claimed as evidence of affinity between microbats and megabats.

The increased number of shared AT substitutions in the DNA of microbats and megabats has a more plausible and more parsimonious explanation than the shared flying ancestor proposed by the proponents of monophyly. High AT content of DNA is well-described for bats (Table 2). A new model of DNA evolution, allowing for AT bias, must be incorporated into the analysis before there is justification for ignoring all the morphological and molecular evidence that conflicts with the DNA sequence data.

In the present account I will summarize the evidence in favour of the flying primate hypothesis and give some examples of its heuristic power. I will then attempt to deal with the problem of conflicting evidence, using both the 'total evidence' approach of Kluge (1989) and the consensus approach (Lanyon 1993). Both approaches provide support for the hypothesis, with the extra step required for a second invention of flight outweighed by the many extra steps required by monophyly.

Primate brain features

Since the hypothesis involves a branch from the early evolution of primates, the choice of an appropriately plesiomorphic (i.e. phylogenetically primitive) primate for comparison is paramount. If they arose from the stem of the primate tree as proposed, megabats are not likely to bear comparison with an advanced primate having features not shared with primates in general. For example, the three cone pigments found in anthropoid primates, but not in non-primates, might be thought to provide a clear-cut defining feature of primates, until one discovers that this feature is absent from lemurs and lorises (Jacobs 1993). Megabats have cone photoreceptors, but it would be unreasonable to "fail" them as primates because they do not measure up to the sophistication of the anthropoid cone photopigments. In fact, megabats, carnivores and prosimians appear to share similar cone types.

Tarsier as a test case

The two most plesiomorphic living primate taxa are Microcebus and Tarsius. There are three reasons for bringing Tarsius into the present discussion of the flying primate hypothesis. (1) Although it is an undisputed primate, Tarsius does not occupy an undisputed rank within the primates. The sources of the dispute are instructive and relevant to the debate concerning bats. (2) Tarsius appears to represent a basal stem of the primate tree and is therefore a more appropriate taxon for comparison with putative sister taxa than the derived primate taxa commonly used in this context. (3) Tarsius shares some characters with the colugo, Cynocephalus, that are not found in any other taxa, primate or non-primate.

Lack of consensus over the position of the tarsier

As shown in Fig. 1, there are three possible tree topologies for the relationships of the three primate groups, tarsiers, anthropoids and lemurs + lorises (loosely referred to as prosimians). Each of these three alternatives has received some support from molecular studies. While a recent influential review has endorsed the anthropoid affinity of Tarsius (Martin 1993), many workers in the field, particularly palaeontologists, are increasingly uncomfortable with this assignment (Fig. 1b). Accepting Tarsius into the anthropoids has the corollary
Table 1. Fifty-four microbat/megabat contrasts. Asterisks mark features shared with primates.

<table>
<thead>
<tr>
<th>Microbats</th>
<th>Megabats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Distribution Worldwide; Neotropical focus</td>
<td>Palaeotropical</td>
</tr>
<tr>
<td>2. Diet and dentition Insectivorous with diverse adaptations</td>
<td>Phytogahous</td>
</tr>
<tr>
<td>3. Perceptive sound Present in all species</td>
<td>Land before taking food</td>
</tr>
<tr>
<td>4. Orientation and navigation Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>5. Delection/micturition at roost Predominantly acoustic</td>
<td>Present in 2 genera</td>
</tr>
<tr>
<td>8. Carrying young Agile, brisk; running, jumping</td>
<td>Predominantly visual</td>
</tr>
<tr>
<td>9. Hindlimb Acoustic</td>
<td>Upright posture: hanging from pollines</td>
</tr>
<tr>
<td>10. Terrestrial locomotion Agile, brisk; running, jumping</td>
<td>* Neonates carried during foraging</td>
</tr>
<tr>
<td>11. Agnostic display Acoustic</td>
<td>* Reaching, carrying, grooming</td>
</tr>
<tr>
<td>12. Pollex</td>
<td>Awkward, poor alternation</td>
</tr>
<tr>
<td>13. Spinal cord Expanding; marginal nucleus</td>
<td>Visual</td>
</tr>
<tr>
<td>14. Midbrain Superior colliculus &gt; inferior colliculus</td>
<td>Dextorous, manipulative use in flexion</td>
</tr>
<tr>
<td>15. Accessory optic system Primitive pattern</td>
<td>Not expanded; no marginal nucleus</td>
</tr>
<tr>
<td>16. LGN: laminar segregation by eye Prominent medial terminal nucleus</td>
<td>* Superior colliculus &gt; inferior colliculus</td>
</tr>
<tr>
<td>17. LGN: magnocellular layers Absent</td>
<td>* Advanced, primate-like pattern</td>
</tr>
<tr>
<td>19. Rhinal fissure No magnocellular differentiation</td>
<td>* Medial terminal nucleus reduced</td>
</tr>
<tr>
<td>20. Hippocampus Visible on lateral surface of forebrain</td>
<td>* Present</td>
</tr>
<tr>
<td>22. Primary visual cortex Primitive pattern</td>
<td>* Paired externally next to optic tract</td>
</tr>
<tr>
<td>23. V2: extrastriate cortex</td>
<td>Hidden ventrally by neocortical expansion</td>
</tr>
<tr>
<td>24. V3 and MT: extrastriate cortex</td>
<td>Anthropic pattern in five of seven features</td>
</tr>
<tr>
<td>25. Frontal eye fields</td>
<td>* High</td>
</tr>
<tr>
<td>27. Somatosensory cortex</td>
<td>* Highest fraction of neocortex in mammals</td>
</tr>
<tr>
<td>28. Spinal cord</td>
<td>Large</td>
</tr>
<tr>
<td>29. Spleen</td>
<td>Present</td>
</tr>
<tr>
<td>30. Karyotype</td>
<td>Present</td>
</tr>
<tr>
<td>31. Middle ear: Paaw's cartilage</td>
<td>6 somatotopic representations</td>
</tr>
<tr>
<td>32. Cochlea</td>
<td>4 separate corticospinal areas</td>
</tr>
<tr>
<td>33. Metacarpals</td>
<td>Low frequencies rostrally</td>
</tr>
<tr>
<td>34. Hindlimb metatarsals</td>
<td>Absent; A1 absent</td>
</tr>
<tr>
<td>35. Ankle joint</td>
<td>Present</td>
</tr>
<tr>
<td>36. Cranial: postorbital process</td>
<td>Retinal capillary loops + choroidal</td>
</tr>
<tr>
<td>37. Skin: pilo-erector muscle</td>
<td>Above optic nerve head</td>
</tr>
<tr>
<td>38. DNA: AT content</td>
<td>Present in many taxa</td>
</tr>
<tr>
<td>39. Serum protein epitopes Heterogeneous; slight overall elevation</td>
<td>Precocial; eyes open postnataally</td>
</tr>
<tr>
<td>40. Lens α-crystallin Few shared with primates</td>
<td>Cyclopodini; Archycterini; Ichnopitulinae</td>
</tr>
<tr>
<td>41. Globin</td>
<td>General; pleiomorphic</td>
</tr>
<tr>
<td>42. Liver lobulation Flexor tendons separated from gastrocnemius</td>
<td>Small acrosomal and subacrosomal space</td>
</tr>
<tr>
<td>43. Palaeotropical</td>
<td>Coarse fibres originate with fine fibres</td>
</tr>
<tr>
<td>44. Phytogahous</td>
<td>Fibrous or bony glans</td>
</tr>
<tr>
<td>45. * Land before taking food</td>
<td>Diverse</td>
</tr>
<tr>
<td>46. Present in 2 genera</td>
<td>Tragus present; incomplete</td>
</tr>
<tr>
<td>47. Predominantly visual</td>
<td>Absent</td>
</tr>
<tr>
<td>48. Upright posture: hanging from pollines</td>
<td>Enoseal; completely anteriorly</td>
</tr>
<tr>
<td>49. * Neonates carried during foraging</td>
<td>Present</td>
</tr>
<tr>
<td>50. * Reaching, carrying, grooming</td>
<td>Allometric</td>
</tr>
<tr>
<td>51. Awkward, poor alternation</td>
<td>Short</td>
</tr>
<tr>
<td>52. Visual</td>
<td>Short</td>
</tr>
<tr>
<td>53. Dextorous, manipulative use in flexion</td>
<td>Tunnel with flexors and gastrocnemius</td>
</tr>
<tr>
<td>54. Not expanded; no marginal nucleus</td>
<td>Present</td>
</tr>
<tr>
<td>55. * Superior colliculus &gt; inferior colliculus</td>
<td>Smooth</td>
</tr>
<tr>
<td>56. * Advanced, primate-like pattern</td>
<td>Highest known for mammals</td>
</tr>
<tr>
<td>57. * Medial terminal nucleus reduced</td>
<td>Primate-like pattern</td>
</tr>
<tr>
<td>58. * Paired externally next to optic tract</td>
<td>Carnivore-ungulate pattern</td>
</tr>
<tr>
<td>59. * Hidden ventrally by neocortical expansion</td>
<td>Primate pattern</td>
</tr>
<tr>
<td>60. Anthropic pattern in five of seven features</td>
<td>No Spigelian lobe</td>
</tr>
<tr>
<td>(Cont.)</td>
<td></td>
</tr>
</tbody>
</table>

The phylogenetic significance of a Table of Differences has justifiably been questioned, on the grounds that polarities are unknown and cladistic analysis is therefore impossible. Note, however, that this problem also attends all molecular sequence data, where outgroup comparison is the only method available to provide character polarity in analysis. Present uncertainties about the mammalian phylogenetic tree make it hazardous, and circular, to choose a eutherian outgroup. If monontomises are used as an outgroup in the above Table, megabats and primates share the derived state for 29 of 54 characters, while microbats share no derived states with primates. If cednates are used as the outgroup, the derived state is shared between megabats and primates for 25 of 54 characters, and for microbats and primates one of the 49 characters is shared derived. The polarity of many character states in the Table can be soundly established by using criteria other than outgroup comparison, such as ontogenetic criteria and character state transformations within rigorously established phylogenies (e.g. the brain data), but the polarity of others flips back and forth as different eutherian outgroups are chosen, like the states of bases and amino acids in molecular sequence data as outgroups are changed.
that living tarsioids are somehow divorced from the well-described, obviously
tarsier-like, omomyids, with their extensive ancient fossil record.

The tree topology with *Tarsius* paraphyletic to the other two groups of living
primates (Fig. 1a) is the one that is regaining acceptance. The brain data are
quite unequivocal in their support of this phylogeny. *Tarsius* has by far the most
plesiomorphic brain of any living primate. Its well-developed medial terminal
nucleus is unlike any other primate's and quite similar to that found in carnivores.
The small cerebellum and corpus callosum also have no parallel in any other living
primate. The lateral geniculate nucleus (LGN) is remarkable because on the one
hand it strongly affirms that *Tarsius* is a primate (with three pairs of laminae
and a prominent pair of external magnocellular laminae), while on the other it
reveals a feature that is found in no other primate (reversal of the order of the
magnocellular laminae, with the ipsilateral lamina lying externally). This unusual
arrangement would be of no use in phylogenetic analysis except for the fact that
it is present only in the tarsier and in the colugo, *Cynocephalus*, another putative
primate sister taxon. Along with the series of plesiomorphic brain characters, this
feature shared with the colugo can be explained only by a very early divergence
of *Tarsius* before the diversification of the living primates. There is also growing
corroborating evidence that the colugo is a close relative of primates. The unusual

Fig. 1. Three possible topologies for the relationships of tarsiers to anthropoid and lorisiform
("prosimian") primates. Arrangement (b) is widely accepted, but note that there is growing
support for arrangement (a) where tarsiers are paraphyletic to all other primates. The present
lack of agreement about the position of tarsiers within the primates underscores the importance
of the new phylogenetic investigations of primates and their putative sister taxa. The brain data
place the tarsier firmly in the position shown in (a).
arrangement shared in the LGN between *Tarsius* and *Cynocephalus* tends to confirm the primate status of the colugo while emphasizing the basal position of *Tarsius* within primates (Rosa, Pettigrew & Cooper in press).

**Functional convergence in *Tarsius***

What of the derived morphological characters apparently shared between anthropoids and *Tarsius*? A bony post-orbital septum is found in both *Tarsius* and anthropoids, as has been eloquently emphasized (Cartmill 1972), but this structure is almost certainly independently derived in the two taxa. In *Tarsius* it is a necessary functional acquisition that provides bony protection for the lateral part of the eyeball as it extends far beyond the lateral margin of the skull. This interpretation of the post-orbital septum is supported by developmental work that failed to find homology between the growth patterns of the post-orbital septum in *Tarsius* and in anthropoids (Simons & Rasmussen 1989).

The other characters used to link *Tarsius* and anthropoids, such as a retinal fovea and discoid placentation, have a scattered phylogenetic distribution that reduces their value in making such links.

**Colugo: a primate in disguise?***

Brain characters throw new light on the living dermopterans or colugos. A mammalian order with a single family, a single genus *Cynocephalus*, and two species, *C. volans* (Philippines) and *C. variegatus* (South-East Asia), the Dermoptera were originally placed with primates by Linnaeus and have hovered nearly in classifications ever since. The recent resurgence of interest in bat origins has provided new evidence from DNA sequence data to link colugos to primates (Ammerman & Hillis 1992; Bailey *et al.* 1992), in support of earlier molecular work using albumin immunology (Cronin & Sarich 1980). The difficulties of recognizing the primate affinities of the colugo by using morphological characters may be revealing in the present context of controversy over bats. Colugo physiology is dominated by the peculiar requirements of its folivorous niche. Like the koala, the tree sloth and the hoatzin, the colugo is a primary folivore with unusual digestive requirements, including the need to handle a high load of dietary phytotoxins. In consequence, all of these vertebrates have a degree of behavioural sluggishness as well as retarded brain development that tend to disguise their true relationships with more active, bigger-brained sister taxa. The fact that the colugo has a brain–body weight relation that is below the norm for primates and megabats (Martin 1993) may not be adequate grounds for its exclusion from a close relationship with primates. The low brain weight is probably a phenotypic feature, acquired in utero along with hydrocephalus, as a result of high circulating levels of phytotoxins. Folivorous koalas and tree sloths also have enlarged cerebral ventricles and tiny brains (Haight & Nelson 1987).

**Colugo–megabat link***

The reservations about the colugo's phylogenetic status notwithstanding, a close relationship between colugo and primates is increasingly accepted. The new molecular data hence provide verification for one important prediction of the flying primate hypothesis, which proposed that dermopterans were the early primate branch that gave rise to the megabats. The reasons for proposing a megabat–colugo link are many, but include similarities in the patagium and striking similarities in the brain and behaviour of the two taxa. The parallels between the megabat and colugo patagium are particularly evident when each is transilluminated against a bright sky and the intrinsic humero-patagialis muscle and vasculature are visible. Some patagial characters linking megabat and colugo are also present in micromabts, but there are a number of behavioural features, not obviously related to flight, that tie colugo and megabat to the exclusion of micromabts and all other mammals. These include the following:

1. Defecation and micturition: accomplished in megabats and the colugo from an erect posture supported by the pollex, the inverse of the normal hanging posture. This contrasts with the micrombat posture for excretion, involving some dorsiflexion but no inversion from the normal, inverted hanging posture.

2. Terrestrial locomotion: awkward, with much use of symmetrical limb movements, in contrast to the brisk, well co-ordinated quadrupedal locomotion of micromabts.

3. Arboreal locomotion: versatile, with frequent use of an inverted hanging gait where flexor muscles of fore- and hind-limbs are loaded. This inverted, alternating, quadrupedal, arboreal gait is not observed in micromabts.

4. Young: carried on forays, to a considerable age, in contrast to the micrombat behaviour of carrying the young only for short distances or 'parking' them during foraging.

5. Neck posture: in the inverted, hanging posture, both colugo and megabat usually flex the neck ventrally to change the view in elevation; micromabts place the neck in extension and have modified neck vertebrae to suit.

6. Hindlimb: both colugos and megabats make extensive use of the hindlimbs for grooming and, in the case of megabats, for manipulation and for carrying food in flight. The importance of the hindlimb for megabat behaviour is reflected in the greatly increased representation of the hindlimb in the somatosensory cortex. Micromabts use the hindlimb only for locomotion and hanging and have a tiny somatosensory representation of the hindlimb.

An ethogram based on these behavioural features strongly links the colugo and megabats. Moreover, the ethogram further supports a primate–colugo–megabat link. Given the stability of Lorenz's ethologically-generated phylogeny of ducks, as well as its abundant support from other studies such as DNA–DNA hybridization (Sibley & Ahlquist 1986), it is difficult to dismiss this link between megabats and colugos, yet this is exactly what has been done as a result of the DNA
sequence data. The problem can be put in the following way: the molecular and neural grounds for supporting a colugo-primate link are persuasive, but this case is weak compared with the case for a megabat-colugo link. If colugos are related to primates and megabats are related to colugos, then megabats should be related to primates. Why is it that the DNA data support only the weakest arm of the triad?

This conflict is resolvable if the DNA of bats has evolved along different lines from the DNA of other mammals as a consequence of flight. Convergence of microbat and megabat DNA towards high AT content, as a metabolic consequence of flight, could contribute to a coincidental similarity between them on the one hand, and disguise their true affinities on the other. In support of this idea, when they are not subject to the complicating effects of the high AT content, sequence data link colugo, megabat and primate to the exclusion of microbats. For example, in all of the six DNA data sets claiming monophyly, guanine (G) and cytosine (C) substitutions in isolation from AT substitutions support the rival, flying primate hypothesis (Table 2). Similarly, protein sequence data do not provide support for monophyly.

Increasing support from other brain characters

There is not space to recount all the new information that has accumulated on the flying fox brain (see Calford et al. 1985; Krubitzer et al. 1993; Rosa, Schmid, Krubitzer & Pettigrew 1993; Rosa, Schmid & Pettigrew 1994; Rosa & Schmid 1994). It is fair to say that the accumulating data provide increasing support for the flying primate hypothesis. Much of the work has involved exploration of the neocortex. This part of the brain has considerable developmental and phylogenetic plasticity and therefore requires caution interpretation in the present context. Nevertheless, flying fox neocortical specializations have by far the closest similarity to those of primates amongst all mammals studied, including the intensively-investigated microbat cortex. It is also worth noting that primates are remarkable for their degree of neocortical specialization, so it is appropriate to make some comparisons at this level.

Somatosensory cortex

Flying foxes have six separate representations of the body surface in this cortex, similar to the macaque monkey, the most advanced primate so far studied in this regard. Non-primate mammals have many fewer representations than six (three in microbats, four in cats, three or four in rodents), despite intensive investigation. Moreover, the shape and arrangement of the areas are similar in both primate and megabat.

Extrastriate visual areas

The flying fox has a large number of separate representations of the visual field in the extrastriate visual cortex lying between V1 and somatosensory cortex. These bear a remarkable similarity to extrastriate areas that have been described in primates, and sometimes also in carnivores, but are much less easy to relate to those in other mammals. For example, V2 is very large in area and has a split in the visual field representation, in primates and carnivores (Rosa, Schmid & Pettigrew 1994). Anterior to V2 there are at least four separate visual areas whose shape and arrangement are like V3, V3A, DM and MT, which have been described only in primates. Because there is still disagreement between laboratories about the exact nature and relationship of primate extrastriate areas (Rosa, Schmid & Pettigrew 1994), it is not possible to be dogmatic about homology between primate and megabat extrastriate cortices. No other mammal apart from the megabat, not even the cat with its well-developed multiple visual cortical areas, comes so close to the primate level of organization in the extrastriate cortex.

Retinal target nuclei

Attention was first drawn to the possible primate affinities of megabats by the finding of the unusual derived pattern of projections from the retina to the visual target nuclei, particularly the reduced, hemidescussate pattern of connections to the visual midbrain and the unusual pattern of lamination in the lateral geniculate nucleus (Pettigrew 1991a, b). Authors critical of these findings have drawn attention to the fact that megabats do not exactly match the primate states for these visual pathways in a number of respects (Thiele, Vogelsang & Hoffmann 1991; Kaas & Preuss 1993). In this regard it is important to bear in mind my remarks in the first section concerning the choice of an appropriate, plesiomorphic primate for comparison. In concluding that their data from the megabat, Rousettus, do not conform to the primate pattern because there is some degree of invasion of the ipsilateral hemifield by the retinotectal inputs, Thiele et al. (1991) are adopting a criterion from anthropoids that is inappropriate for a basal primate. In all mammals except primates, the ipsilateral invasion is complete. The fact that Rousettus has an incomplete invasion immediately places it with the primates rather than with other mammals. Kaas & Preuss (1993) have put forward a criticism that the external, magnocellular layers of the megabat LGN are not homologous with those of primates because they have not been characterized with respect to all of the many techniques that have been applied to the primate LGN over the years. This is an unduly restrictive view, since the megabats share with primates a number of LGN features (large cell layers for each eye lying externally) that are not found in other mammals. In future, if there prove to be differences between these large cell layers in megabats and the corresponding layers in primates, it would be important to explore them fully.
primates, such differences will help to illuminate the phylogeny, particularly with respect to LGN evolution in very early primates and sister taxa. In the meantime, the tools used so far to check LGN characters in megabats have revealed no examples of character states which are in conflict with the hypothesis. For example, CAT 301 antibodies, specific for the magnocellular pathways, reveal a pattern of labelling in the LGN of *Pteropus* that is primate-like, with only the external pair of laminae labelled, in accord with their assignment as magnocellular layers (S. Hockfield & G. Kelly unpubl.).

**Motor pathways**

In the debate about whether tree shrews were primates, Campbell's (1974) evidence on the pyramidal tract and motor pathways played a crucial role in removing the tree shrews to a more distant relationship with primates. By Campbell's criteria, megabats have a primate-like pyramidal tract. Moreover, they also have a pattern of corticospinal neurons that is found in primates but not in other primate orders of mammals (Nudo 1985; Kennedy 1991).

**Pattern of parvalbumin labelling and neuronal density in the neocortex**

Primates are distinct from other mammals in a number of ways relating to the density of cortical neurons that stain the various calcium-binding proteins (Glezer, Hof, Leranth & Morgan 1993). Microbats have a pattern that is typical of basal eutherians and quite unlike the primate pattern, whereas megabats have the primate pattern (P. R. Hof pers. comm.).

**Hippocampus**

Megabats share with primates five of seven hippocampal features that distinguish them from other mammalian orders (Buhl & Dann 1991). None of these features is found in the hippocampus of the two microbats studied, *Macroderma gigas* (Megadermatidae) and *Mormopterus planiceps* (Molossidae) (E.H. Buhl & J.D. Pettigrew unpubl.).

**Support from proteins**

**Monoclonal antibodies to serum proteins**

Using 86 different monoclonal antibodies, Schreiber et al. (1994) have looked for epitopes on 26 serum proteins in a variety of mammals, including two microbats and two megabats. The pattern of epitopes lends itself readily to parsimony analysis, with the result shown in Fig. 2. It can be seen that all trees separate microbats from megabats and all trees have the megabats in a close sister-group relation to the single primate representative, the human. Another interesting feature of the trees is the close relation of the carnivores to the primate-megabat assemblage, a feature that is also seen in the brain data and the data from α-crystallin.

**α-Crystallin**

Following a protracted study of mammalian relationships using amino acid sequences from α-crystallin, De Jong et al. (1993) recently sequenced this protein from a microbat (*Artibeus*) and a megabat (*Pteropus*). As shown in Fig. 3, these two taxa are separated on the most parsimonious trees, with the megabat grouping with carnivores.

**Globins**

There are considerable amino acid sequence data available from globins (Figs 4, 5). Phylogenetic analysis of these data does not support monophyly of
bats, although there is not unequivocal support for the flying primate hypothesis either. While megabats are close to primates in all analyses, the problem is that some microbats tend to cluster in the analysis with megabats while most microbats clearly do not (Pettigrew et al. 1989). In view of the AT bias in the DNA of bats and the fact that the globin genes are located in the parts of the genome (L isochores) where this bias is greatest, future analyses could focus on the very small number of amino acid substitutions that are responsible for the splitting of microbats when globin data are used.
Conflict between protein and DNA data: AT base compositional bias

In view of the molecular support for flying primates from both amino acid sequence data and serum proteins, the unanimous rejection of the hypothesis by six independent DNA sequence studies of bats is notable. The conflict within the molecular data is all the more notable when taken together with the other conflicting data from brain, behaviour, skeleton, genitalia and all the other systems where primates and megabats share derived features to the exclusion of microbats (Table 1). The DNA data make up a unanimous voice in opposition, but they also are unanimous in a bias towards AT. This bias could explain the conflict in the same way that it has explained other conflicts between phylogenies that are DNA-based and those that are based on amino acid sequences.

AT base compositional bias

All DNA sequence data so far collected on the bat problem have a pronounced AT bias. All six sets of sequences show a 4:1 AT:GC bias in the substitutions that are claimed in support of monophyly. While the total number of substitutions supporting the flying primate hypothesis in the same data set is smaller, they show no AT bias. The number of GC substitutions supporting flying primates is greater, in the same data set, than the number of GC substitutions supporting bat monophyly (Table 2). This bias in the data claimed in support of bat monophyly assumes greater significance alongside the fact that the DNA of bats, particularly megabats, has higher than normal levels of AT. Megabat DNA has the highest proportion of AT known for any vertebrate, in excess of 70% (Arrhigi, Lidicker, Mandel & Bergendahl 1972; Bernardi 1993).

Biases in base composition can lead to preposterous phylogenies when DNA sequence data are used blindly, without allowing explicitly for the bias in the underlying model of DNA evolution assumed in the analysis. A well-documented recent example is the case of the eukaryote, Dictyostelium, whose AT-rich DNA causes it to be placed firmly within the prokaryotes by the commonly-used methods of phylogenetic analysis! In contrast, protein sequence data place Dictyostelium in a more appropriate place on the tree of life, amongst the eukaryotes (Loomis & Smith 1990). The base compositional bias in the DNA data from bats suggests that a similar distortion may be taking place in these phylogenetic analyses as well (Pettigrew 1994).

None of the six published papers drew attention to the bias, let alone took steps to tackle it. The computer packages used to analyse the DNA data presumably used a model of evolution, such as the Jukes–Cantor model, that made no allowance for the kind of mutational bias responsible for the extreme AT content in megabat DNA and the heterogeneous AT content of microbat DNA. Recent work has provided a method for dealing with data that have base compositional biases, but this currently allows a test on only four taxa at a time (Steel, Lockhart & Penny 1993).

AT homoplasy? Or shared derived high AT?

In the light of the ever-growing list of differences between megabats and microbats (Table 1), it would not be surprising if proponents of monophyly claimed the high AT content of bat DNA as a shared derived feature linking megabats and microbats. As I have pointed out already (Pettigrew 1991b), the convergent pressures operating on organisms with powered flight may have exerted on other systems besides the wings. My suggestion that the evolution of flight might have consequences for DNA has been derided (Gibbons 1992), but appears to be the most parsimonious way to account for the AT biases found so far in all DNA sequence data from bats. The alternative proposal, that high
AT was inherited from a common ancestor of megabats and microbats, can be ruled out on the following grounds:

1. All megabats have an AT content that is higher than is found in any microbat. In view of the normal AT content found in many microbats, compared with the uniformly high AT content of all species of megabats, is it really plausible that high AT was present in a common bat ancestor? One would have to do violence to accepted views about the monophyly of microbats to make a phylogeny unifying all bats that is at the same time compatible with the present distribution of AT content. Such a phylogeny would split the microbats in an attempt to place the megabats on the clade of microbats with moderate AT. This is unacceptable, despite the fact that recent molecular phylogenies of bats have sometimes had this feature. For similar reasons it is not possible to use the reduced cellular content of DNA (C-value) as a shared derived feature of all bats, as shown in Fig. 6.

2. High AT levels are found in the DNA from a variety of unrelated organisms, all of which have unusual metabolism. As can be seen in Fig. 7, high AT is found in the DNA of Dicyostelium slime moulds, bees, birds, shrews, microbats and megabats. All of these cases can be explained in terms of the increased adenine nucleotide levels that would confront DNA replication and repair machinery when compared to control organisms. In most cases the increased adenine nucleotide levels (e.g. ATP) would be the result of increased metabolism, as in the bees, shrews and powered flying vertebrates. Note, however, that while Dicyostelium does not have a highly active metabolism, it does have very high levels of the adenine nucleotide, cAMP, used as a secreted signal. DNA from slime moulds using other signals not based on adenine nucleotides, such as Acasis and Polysphondylium, do not have high AT levels (Dutta & Mandell 1972). This comparison in the slime moulds supports the interpretation that the increased AT content is related to levels of adenine nucleotide precursor pools. Another instructive comparison, with the same conclusion, is the lower AT content found in ant DNA compared with bee DNA (Bennett et al. 1988). These two groups of hymenopterans are distinguished mainly by the reduction in flight abilities of the ants. The high temperatures and high metabolism of bees in flight have been well described.

![Diagram of cellular DNA content in homeotherms](image)

**Fig. 6.** C-value (diploid cellular DNA content) in homeotherms. The C-value is smallest in volant birds and in megabats. Note wide range of C-value in microbats but uniformly low C-value in megabats. Reduction in C-value reduces nuclear and cellular size and may therefore help to explain the changes in these metabolically-active organisms that would benefit from increased efficiency of transmembrane exchanges. The common occurrence of C-value reductions in unrelated taxa, along with the absence of C-value reduction in some basal microbats such as Noctilio and emballonurids, argue that C-value reduction has been acquired independently in the two bat lineages.

![Diagram of AT content in DNA](image)

**Fig. 7.** Mutational biases toward high AT. Whilst reptiles and amphibians have DNA with approximately equal proportions of the different bases, homeotherms have a biased distribution, with high levels of adenine- and thymine-derived nucleotides (high AT). The connection with high metabolism that is suggested by the homeotherm-reptile comparison is further supported by the fact that even higher levels of AT are found in the most metabolically-active mammals, such as shrews and bats. Similarly, bee DNA has a higher AT level than is found in the DNA of the metabolically less-active ant. The highest AT levels in vertebrates are found in the megabats. An exception that may prove this rule is the very high AT content of DNA in Dicyostelium.

The occurrence of high AT in DNA from such a wide variety of unrelated taxa suggests that it could have arisen independently in the two kinds of flying mammals. Normal AT values are found in many microbats, and megabat AT values are all outside the microbat range. Neither of these facts can be easily reconciled with the assumption of high AT in a common ancestor of megabats and microbats. The most parsimonious explanation for the AT bias in the substitution shared by microbats and megabats is therefore that AT bias has been acquired independently in the two lineages, as a metabolic consequence of the demands of flight.
Resolution of conflicting data sets: total evidence v. consensus

There are two different approaches to the resolution of conflict between data sets used in phylogenetic reconstruction: total evidence (Kluge 1989) and consensus (Lanyon 1993). The difference between these two approaches can be illustrated by an example taken from the present tight funding situation for research proposals. Suppose that an investigator under review receives, from each of two reviewers, acclaim and the maximum possible scores for track record, for personal qualifications and for the scientific qualities of the proposal. A third reviewer gives very low marks for both the investigator and the proposal. The total evidence approach would pool all the data, with the result that the average final score obtained would be unlikely to gain funding in these stringent times. The consensus approach would seek to understand the gross discrepancy between the first two reviewers and the third. If a committee of scholars could resolve the discrepancy, for example in terms of a consistent negative bias on the part of reviewer 3, then the outcome might be a decision to accept the opinions of reviewers 1 and 2, with a greater chance of the meritorious proposal being funded.

Apart from the inherent problem of smearing that is evident from the hypothetical example just given, the total evidence approach has a number of severe limitations when one tries to put it into practice. In the case of the bat controversy, it can be a difficult problem to join DNA sequence data to morphological data in the same data matrix. First, the sizes of the conflicting matrices may be very different, raising the possibility that one data set will swamp the other. Second, when there are uncertainties about alignment, the number of possible data matrices to be tested can be so large as to preclude testing. This is the case for the e-globin intron studied by Bailey et al. (1992) and used to reject the flying primate hypothesis. This intron was a different length in every taxon for which a sequence was obtained, with the result that hundreds of gaps had to be inserted to achieve alignment. There were very few conservative sites to aid alignment, with the result that there are many equally acceptable alternative alignments. Alternative alignments can even be found that contradict the major conclusions of the study! (Fig. 4). The positions of most of the gaps can be altered, giving an unmanageable number of alternative possible data matrices to be combined with the morphological data matrix. Pairwise alignment algorithms to choose the 'best' alignments for analysis are not necessarily a solution to the problem in the present case because of the base compositional bias in bat DNA. Since a base compositional bias may increase similarity scores between two unrelated sequences, algorithms using similarity measures, such as Clustal, may not provide a rigorous alignment. Apart from compromising the alignment process, the base compositional biases found in bat DNA suggest that it is unwise to use the raw DNA data without correcting for the effects of the bias. While techniques for dealing with such biases are beginning to appear, such as the four-taxon test developed by Steel et al. (1993), they cannot yet be applied to a DNA data matrix that is being joined to a matrix of other data.

A system-by-system consensus approach gives a majority of systems in favour of the flying primate hypothesis, with three systems in opposition (patagium, mtDNA, nDNA). There is strong motivation to put more weight on the DNA data because of the perception that they are superior in their objectivity and their avoidance of problems such as the determination of homology. In the present case, these advantages of DNA data are more apparent than real, since subjectivity played a role in alignment of one data set that appeared in an influential journal and since many of the sequence data are derived from non-coding sequences where homology between sites cannot be as rigorously determined as for coding sequences.

Apart from these reasons for being cautious about the DNA data, there is another consideration with the potential to resolve the conflict completely. The AT bias in all the DNA sets is the only source of conflict. A consideration of GC substitutions from the same data set actually provides support for flying primates. Given the plausible connection between AT bias and the metabolic demands of flight, the consensus approach could resolve all conflicts between the systems. Since the flying primate hypothesis includes a dual origin for flight, it can encompass dual origins of the corollaries of flight such as wing anatomy and AT bias. Seen in this overall context, it is unparsimonious to propose that AT bias and wing anatomy were inherited from a common flying ancestor.

Future investigations

A large proportion of mammalian genes are located in the H3 isochore, a small GC-rich fraction of the genome (Bernardi 1993). Since this isochore is relatively ‘protected’ from the AT mutational bias that reaches such extremes in megabats, future DNA sequence data could profitably be collected from this part of the genome rather than the L isochores where AT bias is maximal.
addition, DNA data already collected could be reanalysed with the AT bias in mind, along with amino acid sequence data where one could possibly correct for those shared amino acid substitutions that might be coincidentally related to the AT bias.

Megabats, and some microbats, have remarkable changes in genomic organization that are arguably related to flight. Further work on bats could therefore illuminate the currently obscure origins and mechanisms of features of genomic organization such as isochores (that are present in all mammals but dramatically shifted in their distribution in megabats), C-value reduction and AT increase. The flying primate hypothesis therefore has the potential to be a powerful heuristic stimulus for cellular and molecular biology as well as for neurobiology.

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References


Flying primates: crashed, or crashed through?

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Synopsis

The flying primate hypothesis originated from the finding that megabats shared a number of advanced visual pathway characters with primates that were not found in any other mammalian order, nor in microbats. This hypothesis indicates that primates, colugos and megabats share a common ancestor with each other more recent than any shared with microbats. The hypothesis has found increasing support from other sources of evidence. Examples reviewed here include further derived brain features, both visual and non-visual, immunological studies of serum proteins with monoclonal antibodies and analysis of restriction sites and protein sequences (globoh and α-crystallin). DNA sequence data, while supporting the colugo-primate association, have been used to reject a primate-megabat connection, even though the total evidence for a colugo-megabat link is better than the generally accepted evidence for a colugo-primate link, and even though DNA sequence data and protein sequence data on the same genes give conflicting phylogenies. A resolution to this conflict is suggested by a bias in all the published DNA sequence data on bats. The shared substitutions claimed in support of bat monophyly are mostly of adenine (A) or thymine (T), in the same direction as the bias that exists in the overall base composition of DNA from metabolically-active, volatil organisms. If the AT content of DNA is taken into account by using the NZ algorithm, the much-vaunted claims for bat monophyly based on DNA sequences are not supported. It is more parsimonious to assume that the AT bias responsible for the claimed association arose independently in the two lines of flying mammals.

Introduction

The ‘flying primate’ hypothesis arose from the unexpected finding that megabats shared a number of derived brain features with primates that were not shared with other mammals, particularly not with microbats (Pettigrew 1986). Of the many controversial aspects of this hypothesis, perhaps the most contentious is its corollary: that powered mammalian flight has evolved more than once (see the four-part debate on this topic; Pettigrew 1991a, b;