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Approaches, Methods, and the Future of the Chiropteran Monophyly Controversy: A Reply to J. D. Pettigrew

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It is clear that data now being generated in neuroscience laboratories around the world provide an important new dimension to our understanding of mammalian evolution. Pettigrew and his colleagues (e.g., Pettigrew et al., 1989; Pettigrew, 1991b [this volume]) have undeniably demonstrated that neural data must be considered in any discussion of bat relationships. Nevertheless, we are disturbed by several as-

pects of Pettigrew's approach to this problem.

The manner in which questions are framed constrains not only the methods used to find answers but also the nature of potential solutions. The title of Pettigrew's latest paper ("Wings or brain? Convergent evolution in the origins of bats," 1991b) betrays a preconceived notion that convergence must have occurred in one or the

other of these systems in bats. Rather than addressing the issue of bat relationships within Eutheria, Pettigrew (1991b:200) sought to answer a somewhat different question:

Have the primate brain features evolved twice, once in primates and once within a part of the "bat" lineage? Or have wings evolved twice, once within the primate lineage and once in the line giving rise to microbats? A clear-cut choice between these two exclusive possibilities has not yet been made by systematists.

By framing the question in this manner, Pettigrew effectively eliminated at least two other possibilities: (1) convergence in *both* systems (admittedly unlikely) and (2) homology of the derived features in both systems (brain traits evolving once in an ancestor of primates + bats, wings evolving once in an ancestor of bats), with subsequent reversal of brain traits in Microchiroptera.

It is not clear to us why Pettigrew apparently rejects the possibility of reversal of neural characters in the lineage leading to Microchiroptera. Evolutionary reversal—secondary evolution of conditions similar to those of ancient ancestors—has played a significant role in many evolutionary explanations of differences among taxa. The developmental mechanisms that may produce reversals have been investigated in a variety of vertebrates (e.g., Hall, 1984), and the genetic basis of reversal has been experimentally demonstrated in at least one mammal (i.e., in Sewall Wright's experiments with digit numbers in *Cavia*; see review by Wright [1977]). Cladistic analyses of relationships among mammals have frequently concluded that reversals must have occurred during mammalian evolution (e.g., Rowe, 1988; Wyss, 1988). Recognition of homoplasy requires comparison of organisms within a phylogenetic framework; a hypothesis of relationships is essential before one can hope to identify instances of either reversal or convergence. Neither reversal nor convergence should be ruled out a priori before a thorough phylogenetic analysis has been conducted.

By rejecting the possibility of reversal,

Pettigrew misleadingly reduces the issue to determining which of two systems (musculoskeletal or neural) is most likely to have undergone convergence in primates and bats. For various reasons outlined in his paper, Pettigrew prefers to accept convergence in the evolution of wings rather than convergence in brain traits. We have outlined many of our arguments with Pettigrew's assumptions and methods elsewhere (see Baker et al., 1991 [this volume]), so we will limit our comments here to a few points: (1) There is no justification for an a priori claim that functionless characters are more informative in phylogenetic analyses than characters of obvious functional significance. (2) There is no reason to believe that the neural characters cited by Pettigrew are functionless. (3) There is no evidence that neural characters are less subject to homoplasy than are musculoskeletal characters (see Northcutt [1984] for several examples of homoplasy in the vertebrate central nervous system). (4) Megachiropteran and microchiropteran wings exhibit many derived similarities (including relative proportions of most limb elements), but only a few differences. (5) There is no reason to believe that any mammal evolving membranous wings must use four long digits to support a wing membrane (three might function equally well).

It is clear that a comprehensive phylogenetic analysis of primates and bats—in the context of a broad set of eutherian outgroups—must be conducted before drawing inferences about character conservatism, homoplasy, and evolutionary relationships. Widening a data base to include characters from different functional systems is important when character conflicts exist. We agree that penis morphology (Smith and Madkour, 1980) offers two characters that appear to support the hypothesis of bat diphyly. In contrast, we strongly disagree with Pettigrew's dismissal of five cranial characters (supporting bat monophyly) described by Wible and Novacek (1988). We also believe that far fewer than the 10 characters of the visual neural system cited by Pettigrew (1991b: 202) actually distinguish Megachi-

roptera and Microchiroptera, a difference of opinion that involves methods of coding nonindependent characters rather than the validity of the observational data themselves (see discussion in Baker et al., 1991).

The nonvisual neural characters cited by Pettigrew (1991b) in support of the bat diphyly hypothesis are tantalizing, but we cannot evaluate most of them because full descriptions have not yet been published. Three corticospinal pathway characters were cited from an abstract (Kennedy et al., 1987) and a dissertation study that has not yet been completed (W. D. Kennedy, in prep.). Similarly, the hippocampal characters have been reported only in one abstract (Buhl and Dann, 1989); two papers that apparently describe these features in more detail were still in press at the time of our writing (Buhl and Dann, 1991, in press). Finally, the features of the ankle joint cited as suggestive of bat diphyly have been mentioned only in one quote that was published in a popular article (Litos, 1988). Given the current disagreements concerning character interpretation, we reserve judgment until the data on these characters have been fully reported.

Molecular studies provide another arena for studies of mammalian relationships. Unfortunately, none of the molecular data now available provide unequivocal resolution of the problem of bat relationships. We and Pettigrew (1991b) seem to agree that the mitochondrial DNA study by Bennet et al. (1988) and the hemoglobin data presented in Pettigrew et al. (1989) are inconclusive. We are disturbed, however, by Pettigrew's claim that data from the 18S-28S ribosomal gene complex recently studied by Baker et al. (in press) support the bat diphyly hypothesis. Restriction site #26 occurs only in dermopterans and megachiropterans out of the many taxa studied; Pettigrew (1991b:211-213) stated:

... the consistency index of site #26 was unity on the most parsimonious tree generated from Baker et al.'s data, in contrast to all the other restriction sites studied. I therefore interpret this synapomorphy (site #26) as another point for the "flying primate" hypothesis and against the monophyly of bats.

While site #26 does represent a possible synapomorphy of a Megachiroptera + Dermoptera clade, Pettigrew's assessment overlooked several significant points: (1) the bat diphyly tree was just one step shorter (45 steps) than the bat monophyly tree (46 steps); (2) the consistency index (CI) for site #26 was 1.000 only when measured on the bat diphyly tree; on the bat monophyly tree, the CI of this site was 0.500; (3) neither of these trees was actually the most parsimonious tree identified; that tree (a 43-step strict consensus tree constructed from 100 equally parsimonious trees) was largely unresolved and did not permit resolution of the evolutionary transformations of site #26 (Baker et al., in press). The topology of the consensus tree (which includes a clade containing only *Homo* and the microchiropteran *Rhinolophus*, to the exclusion of all other primates and bats) suggests that ribosomal restriction sites do not provide a particularly reliable guide to bat relationships.

RESOLVING THE CONTROVERSY: WHERE DO WE GO FROM HERE?

It is now apparent that the controversy over bat relationships will not be settled without more comprehensive analyses of morphology and molecular structures in a wide range of taxa. In addition to exploration of new character systems, more detailed information is clearly needed concerning many of the characters that have already been cited in support of various phylogenetic hypotheses. Much of this work is apparently already in progress, although in most cases only preliminary results have been reported (note that most of the references below are abstracts). As discussed above, several new neural characters are being studied by W. D. Kennedy (in prep.) and Buhl and Dann (1989, 1991, in press); additional studies of central nervous system characters are being conducted by Johnson and Kirsch (1991), Kaas (in press), Pettigrew (1991), and Preuss and Kaas (1991). Cranial and postcranial morphology of several relevant fossil taxa (ple-

siadapids, paromomyids, microsypids, mixodectids) is being studied by Gunnell (1989), Beard (1990, 1991), Kay et al. (1990), Szalay (pers. comm.), and others. In hope of identifying a new suite of morphological characters, Dumont (1990) is surveying enamel microstructure in various fossil and living archontan taxa. Details of cranial development, important in assessing several key characters, are being studied by Maier (1991) and J. R. Wible (Univ. Louisville, pers. comm.). Homologies of important palatal muscles are being investigated by Thewissen and Babcock (1991a, 1991b). W. P. Luckett (Univ. Puerto Rico) is continuing his work on fetal membranes and reproductive structures of archontan mammals (pers. comm.). Finally, one of us (Greenwald, 1990, 1991a, 1991b; Simmons, in prep.) is reassessing a wide range of morphological characters cited in previous studies of chiropteran relationships, and is studying several new systems (e.g., ear ossicle morphology).

In the molecular realm, serum protein immunological data are being reassessed by Sarich (1991). R. Benveniste and W. Modi (National Cancer Institute) and one of us (Baker) are examining archontan relationships using DNA-DNA hybridization. L. Ruedas and J. Bickham (Texas A&M Univ.) are doing a C-value nuclear study of DNA content in bats and other archontans (pers. comm.). L. Ammerman (Univ. Texas) is addressing bat phylogeny by sequencing the 12S gene for rRNA in mitochondria and the 28S gene for rRNA in the nucleus (pers. comm.). R. Atkins and R. L. Honeycutt (Texas A&M Univ.) are sequencing the cytochrome oxidase II gene in mitochondria from several mammalian orders, focusing on primates but including bats (pers. comm.). D. Mendell and C. Dick (Univ. Cincinnati) are sequencing the 12S gene for rRNA and the cytochrome oxidase I gene in mitochondria (pers. comm.). C. Moritz and J. D. Pettigrew (Univ. Queensland) are also sequencing parts of the mitochondrial genome (pers. comm.).

Results of studies such as those mentioned above could shift a critical case either for or against the concept of bat mono-

phyly. However, we feel that it is unlikely that anything will be resolved until workers can agree on (or at least discuss the implications of) basic issues concerning approach and methodology. When we are asked to outline the sort of evidence that would convince us that bats are diphyletic, we must reply in methodological rather than anatomical terms. We consider neither the relative ages of fossils nor a priori evolutionary scenarios to be relevant to the question of bat relationships. In our view, only demonstration of a substantial pattern of synapomorphies linking Megachiroptera and Microchiroptera with different eutherian clades would provide the evidence necessary to refute the hypothesis of bat monophyly. Relevant synapomorphies could be morphological or molecular, or could include both types of characters. Methodology is crucial here: results can only be considered conclusive when sufficient taxa (including outgroups) and characters are considered, the range of variation within each taxon is taken into account, all characters are appropriately scored, and the methods of analysis are appropriate and well documented. The difficulty, as always, comes with defining the terms "substantial," "sufficient," and "appropriate." Here and elsewhere (Baker et al., 1991), we have outlined the problems we see with the approach and methods (sampling, character coding, etc.) utilized by Pettigrew and his colleagues to study bat relationships (Pettigrew, 1986; Pettigrew et al., 1989). We find that most of these criticisms were not addressed in Pettigrew's latest contribution (1991b); accordingly, we still cannot accept his conclusions.

Given the conflicting data and the methodological disagreements described above, it appears that the chiropteran monophyly controversy will not be settled quickly. Much depends upon the outcome of studies currently in progress, but complete publication of data, methods, and results will be necessary before these studies can be fully evaluated. In the meantime, we caution against suggestions that the issue of bat relationships has been resolved.

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