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Base-compositional biases and the bat problem. III. The question of microchiropteran monophyly

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Using single-copy DNA hybridization, we carried out a whole genome study of 16 bats (from ten families) and five outgroups (two primates and one each dermopteran, scandentian, and marsupial). Three of the bat species represented as many families of Rhinolophoidea, and these always associated with the two representatives of Pteropodidae. All other microchiropterans, however, formed a monophyletic unit displaying interrelationships largely in accord with current opinion. Thus noctilionoids comprised one clade, while vespertilionids, emballonurids, and molossids comprised three others, successively more closely related in that sequence. The unexpected position of rhinolophoids may be due either to the high AT bias they share with pteropodids, or it may be phylogenetically authentic. Reanalysis of the data with varying combinations of the five outgroups does not indicate a rooting problem, and the inclusion of many bat lineages divided at varying levels similarly discounts long branch attraction as an explanation for the pteropodid–rhinolophoid association. If rhinolophoids are indeed specially related to pteropodids, many synapomorphies of Microchiroptera are called into question, not least the unitary evolution of echolocation (although this feature may simply have been lost in pteropodids). Further, a rhinolophoid–pteropodid relationship—if true—has serious implications for the classification of bats. Finally, among the outgroups, an apparent sister-group relation of Dermoptera and Primates suggests that flying lemurs do not represent the ancestors of some or all bats; yet, insofar as gliding of the type implemented in dermopterans is an appropriate model for the evolution of powered mammalian flying, the position of *Cynocephalus* in our tree indirectly strengthens the argument that true flight could have evolved more than once among bats.

Keywords: Archonta; bat monophyly; bat phylogeny; Microchiroptera; molecular evolution

1. INTRODUCTION

The monophyly versus diphyly of bats remains a contentious issue, despite much marshalling of evidence on both sides of the question (e.g. Smith & Madkour 1980; Pettigrew 1986, 1991*a*,*b*, 1995; Pettigrew *et al.* 1989; Baker *et al.* 1991*b*; Simmons *et al.* 1991; Thewissen & Babcock 1991; Simmons 1994). Significantly, trees based on DNA data are always at least consistent with a monophyletic Chiroptera (Adkins & Honeycutt 1991; Baker *et al.* 1991*a*; Mindell *et al.* 1991; Ammerman & Hillis 1992; Bailey *et al.* 1992; Kilpatrick & Nuñez 1993; Stanhope *et al.* 1993; Kirsch *et al.* 1995*b*; Porter *et al.* 1996). While the prestige of molecular techniques may seem to render such results decisive, the sequencing and DNA hybridization studies conducted to date all suffer from inadequate sampling of the diversity of Chiroptera, leaving open the possibility that algorithmic artefacts (such as the 'attraction of long branches' (Felsenstein 1978; Swofford & Olsen 1990)) may be responsible for the joining of microchiropterans and megachiropterans in most DNA based trees. Alternatively, Pettigrew (1994, 1995) has pointed out that many, but not all, microbats share with pteropodids (and some other mammals) a notably elevated AT content, which could also conceivably bias DNA based topologies in favour of bat monophyly.

In the two companion papers to this one, Pettigrew & Kirsch (1998) and Kirsch & Pettigrew (1998) have considered the bias question, respectively presenting DNA hybridization elution curves and trees which were based not only on whole genome single-copy DNAs but also on labelled fractions 'enriched' for either AT or GC content. While the trees in Kirsch & Pettigrew (1998) may be regarded as indecisive with respect to bat monophyly, they all show, or are consistent with, a remarkable association of the representative microchiropteran rhinolophoid (*Rhinolophus philippinensis*) and megachiropteran pteropodid (*Pteropus vampyrus*) exclusive of other microbats (the noctilionoids, *Noctilio albiventris* or *N. leporinus*, and *Pteronotus parnellii*), regardless of which type of label was used. As rhinolophoids are amongst the most AT rich of microchiropterans, with up to 70% AT content as compared to the mammalian average of about 60% (Pettigrew 1995), one interpretation of Pettigrew & Kirsch's results is that, indeed, such a bias may affect apparent relationships among microbats, if not necessarily between these, megabats, and other orders of mammals.

However, the issue of taxonomic sampling remains unaddressed by Pettigrew & Kirsch's experiments: only four bat species were included in even the largest of their matrices. Therefore, we undertook to extend their whole genome study (Kirsch & Pettigrew 1998, table 1) to a

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total of 21 species, including five outgroups and representatives of ten bat families. In every case, we were able to subdivide each putative bat lineage at least once, and usually near its midpoint. In this way we hoped to avoid the possibility of long branch attraction; and, by inclusion of many outgroups (four of which apparently constitute a subdivided clade), to reach a reliable conclusion about the position of the root among bats (if, that is, bats are indeed monophyletic). Further, by including several bats whose phyletic positions are fairly well known, any discrepancies from received opinion would be highlighted and signal the need to consider spurious reasons (such as base-composition bias) for the topology obtained.

In the event, our trees did not differ from Kirsch & Pettigrew's (1998) as to the position of rhinolophoids, though in all other respects, except possibly the placement of emballonurids, they were consistent with current views about microchiropteran relationships. Additionally, the trees suggest that dermopterans are the sister group of primates rather than of some or all bats; primates are also excluded from the special relationship with megabats. However, because our trees only included putative archontans as eutherian outgroups, we do not claim to have resolved the question of bat monophyly. Nevertheless, the results with respect to rhinolophoid microbats mean that either the effect of high AT content on molecular trees is real, or a radical rethinking of bat relationships is in order—in particular, with respect to the monophyly of Microchiroptera and the characters which define that taxon. If microbats are indeed paraphyletic, as our trees suggest, then the present subordinal dichotomy within Chiroptera should be abandoned.

2. MATERIALS AND METHODS

Methods for purification of DNA, preparation of extracts for radiolabelling with ^{125}I and hybridization, and evaluation of hybrids were as outlined in earlier papers (Kirsch *et al.* 1990; Bleiweiss *et al.* 1994), except that the single-copy fractions were separated at a higher Equivalent-C_0t (1980–2260 rather than 1130), and amounts of driver DNA were reduced to 25 or 13 μg from 50 μg. Eighteen of the 21 species examined were labelled and over 1000 hybrids were prepared, with tracer:driver ratios of *ca.* 1:500.

A matrix was assembled from two or more 'runs' of up to 25 hybrids with each of the 18 tracers, the differences (Δ's) in melting temperatures being calculated from 56 °C with reference to 2–8 homoduplexes per label (averaged across runs) and indexed as ΔT_{mode}s. Modes are not ordinarily considered appropriate for very distant comparisons among mammals due to a marked low temperature peak with which the true mode may be conflated, and which may be caused by the presence of many poorly matched paralogous sequences (Fox & Schmid 1980): usually the height, but not location, of this secondary peak is correlated with distance (Kirsch *et al.* 1995*a*). However, bat hybrids do not seem to show such a peak, or only rarely, and empirically a single peak of variable position was found in its place when plotting curves involving distantly related taxa; apparently this is the true mode, as it seemed to be in Pettigrew & Kirsch's (1998) experiments. Moreover, Kirsch & Pettigrew's (1998) trees employing the very different ΔNPH index gave results parallel to those using the ΔT_{mode}, so we consider it sufficient to present just results obtained with the latter index here.

Reciprocal comparisons were corrected for asymmetry by the method of Sarich & Cronin (1976) to obviate systematic experimental error (the 'compression effect' of Springer & Kirsch (1991), which is mainly due to modal differences in fragment sizes among tracers), and missing values were then reflected from their known counterparts. Where both members of a pair of reciprocals were unmeasured (ten cases), the entries were estimated by the additive procedure of Landry *et al.* (1996). Trees were then calculated for 21- and 18-taxon subsets of the data, using the FITCH program in Felsenstein's (1993) PHYLIP package, employing the global branchswapping, subreplicate, and Cavalli-Sforza & Edwards options and randomly varying the input order of taxa 100 times; reflected or estimated cells were conservatively considered as measured once in the tree computations. Fitted pathlengths were correlated with the measured distances in order to obtain an estimate of how well the data conformed to the assumption of additivity. The 21- and 18-taxon trees were validated by Krajewski & Dickerman's (1990) adaptation of bootstrapping for distance data (a technique for exploring measurement error), generating a consensus of 1000 pseudoreplicate trees in each case; and the 21- and 18-taxon topologies were further tested for stability of the underlying matrices by the jackknife on taxa for weighted trees of Lapointe *et al.* (1994). For the jackknives, both 'all single' and '500 random' deletions (drawn proportionately from among the possible combinations of single and multiple deletions) were performed, calculating the average pathlengths recovered over the pseudoreplicates in each case. For the 21-taxon tree, we also jackknifed separately on all possible combinations of the five outgroups (including none), with the suite of bats held constant; an average consensus was then calculated from the pathlengths on FITCH trees corresponding to all such deletions, as for the jackknives on the entire matrix or the 18-taxon subset, omitting the three unlabelled taxa. For both the bootstraps and jackknives, each pseudoreplicate matrix was separately symmetrized and completed prior to tree construction.

Finally, the data were tested for phylogenetic (or other) structure by a randomization test (Kirsch *et al.* 1995*a*). This test produces a '*z* score,' which essentially states how many standard deviations from the mean sum-of-squares for FITCH trees based on randomized data lies the sum-of-squares for the tree calculated from the unrandomized matrix. Because outgroups may render this test too liberal, we performed this test at two levels: on the full matrix (but holding the outgroup opossum values constant) and on the matrix without any outgroups.

3. RESULTS

Figure 1 shows representative thermal elution curves obtained with the labelled rhinolophoid *Hipposideros galeritus*, and illustrates the level of discrimination generally found in our experiments. *Rhinolophus philippinensis* (a member of the same microchiropteran superfamily as the homologous species) is approximately intermediate between the homologues and the depicted molossid microbat *Molossus sinaloae*;

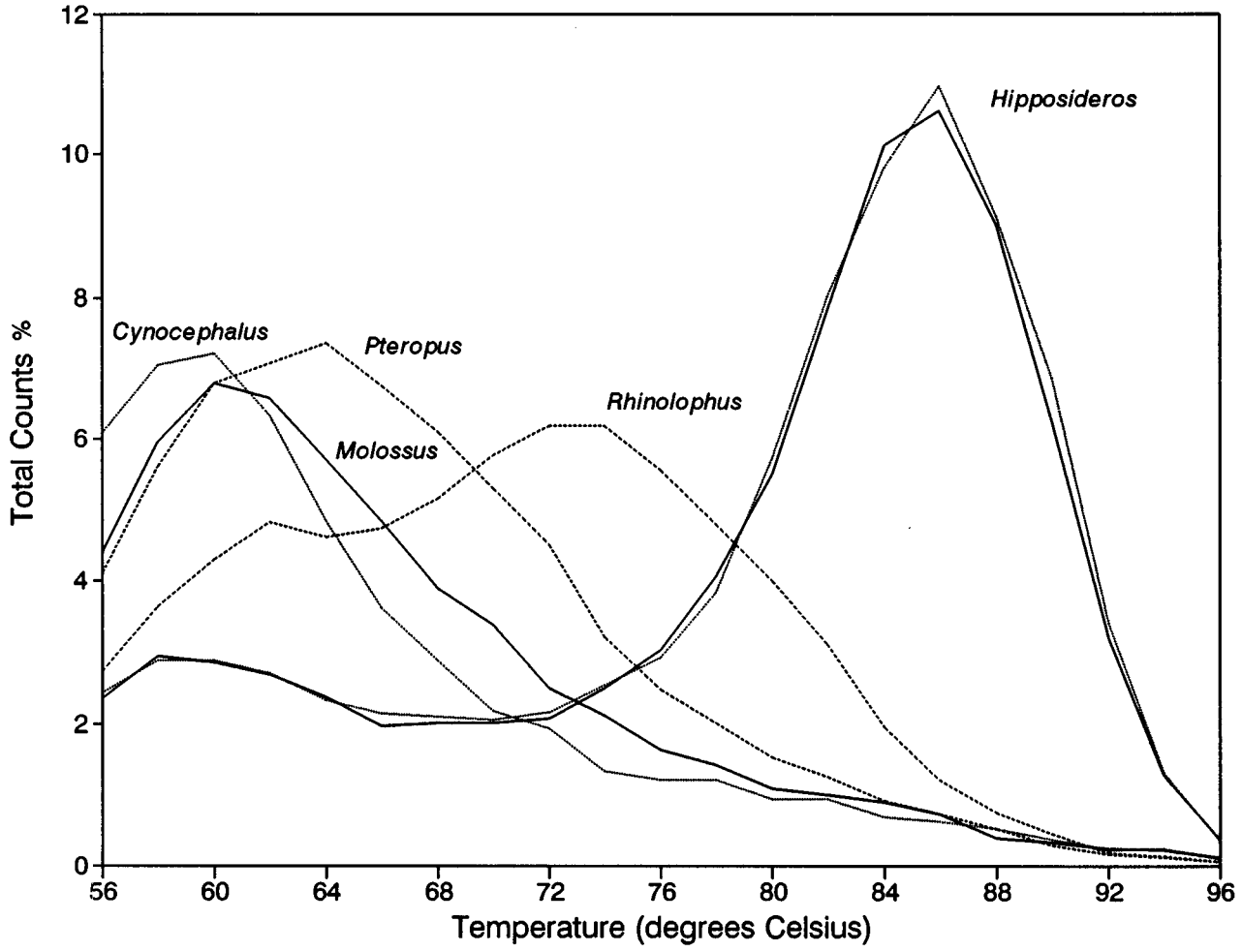

Figure 1. Representative thermal elution curves for hybrids with the labelled rhinolophoid microbat *Hipposideros galeritus* (replicate homologues at right). Each elution has been divided by total counts and expressed as a percentage of the total eluted for that hybrid to show the extent of hybridization as well as distribution of counts. The *Hipposideros* tracer was one of the few bat labels to show any evidence of a low temperature peak.

most other non-rhinolophoid microbats were about as distant from rhinolophoids as was *Molossus*, but the outgroups (such as the dermopteran *Cynocephalus variegatus*, shown here) were still more distant. However, pteropodids (e.g. *Pteropus vampyrus* in figure 1) were markedly closer to the homologues than were non-rhinolophoid microchiropterans, and reciprocal experiments were always consistent with the ordering depicted in figure 1.

Table 1 presents the raw ΔT_{mode}s for the 18 labels, with corrections for asymmetry listed at the bottom; and also (on the third lines of cells) the values used to construct the trees of figures 2 (of all 21 taxa) and 3 (of the 18 labelled species only) after symmetrization, reflection of 94 cells, and estimation of ten missing pairs (i.e. 26% of the possible comparisons were reflected or estimated for calculation of figure 2). The correlation (r) of figure 2 fitted pathlengths with these distances is 0.96, indicating near perfect additivity of the table 1 values; $r=0.98$ for the 18-taxon subset of figure 3. Both correlations are highly significant ($p<0.01$) even with a very conservative estimate of degrees of freedom (10 and 9, respectively). In both figures 2 and 3, bootstrap numbers have been shown at the nodes (except for the root node, which was fixed by definition). The two thin lines in figure 3 indicate discrepancies between the FITCH and jackknife trees (see below).

Figure 2 confirms the association (here, with 100% bootstrap support) of *Rhinolophus* and *Pteropus* found by Kirsch & Pettigrew (1998), with representation of their lineages here increased from one rhinolophoid species to three (representing as many families) and from one to two pteropodids. All other microchiropterans group together with high bootstrap support (88%), with the three noctilionoids separated from the rest (100% support). Emballonurids are the sister group of molossids, with moderate (83%) support; and vespertilionids are more weakly positioned (69% support) as part of a larger group including emballonurids and molossids. In figure 3, based on just the 18 labelled taxa (47 cells or 15% of the total comparisons were reflected after symmetrization), the molossid–emballonurid relationship has 91% support). However, the relationship of vespertilionoids with molossids-plus-emballonurids in figure 3 is poorly supported by the bootstrap (at 48%) and not by the average consensus on the 500 random deletion jackknives (where noctilionoids occupy that position; the intervening internode is therefore shown as a thin line). Within any familial or superfamilial group of bats, recovered relationships are as currently accepted (e.g. Koopman 1994), and bootstrap support for each node is 98% or better in both figures 2 and 3. Moreover, comparisons using additional

Table 1. *Matrix of* ΔT_{mode}s (*number of hybrids*=1028) *for* 18 *tracer- and* 21 *driver-species*

(Columns are tracers, designated by the first four letters of genus-names and first letters of specific epithets, given in rows. First lines of cells give average Δs, except that actual mean melting temperatures (rather than zeroes) are given for homologous modes to permit comparison of labels. Second lines give standard deviations (s.d.s; not counting missing cells or those with only one measurement) and numbers of replicates, separated by slashes. Third lines give values after symmetrization, reflection, and estimation of missing pairs. Boldfaced numbers are reflected values and numbers, both boldfaced and underlined are estimated values. Reflected or estimated values were considered to have been measured once in tree computations. Average table-wide s.d. is 0.71; correlation of s.d.s with distance, 0.19. 'Corrections' at feet of columns are initial column multipliers (row : column ratios) used to effect symmetrization by method of Sarich & Cronin (1976); iterations (multiplication of column values followed by recalculation of row : column ratios) were continued until ratios reached unity. Asymmetries before correction (filled cells only) and after were 6.00 & 1.51 for the full table. Abbreviation: na, not applicable.)

	DideM	*TupaM*	*CynoV*	*LemuC*	*NyctC*	*DobsM*	*PterV*	*MacrG*	*HippG*	*RhinP*	*EpteH*	*MyotL*	*ScotK*	*NoctA*	*PterP*	*MoloS*	*RhynN*	*TaphM*	*SaccB*	*MimoC*	*ChaeP*
Didelphis marsupialis	83.46	20.34	25.08	26.75	18.20	27.82	26.02	26.52	26.77	25.77	26.65	25.70	24.88	na	26.75	25.89	25.34	26.16	na	na	na
	0.34/2	na/1	1.19/2	1.01/4	2.18/2	0.04/2	1.47/4	2.09/2	1.49/3	2.13/4	0.62/2	1.64/2	0.83/4		na/1	1.23/2	0.13/2	na/1			
	0	24.28	24.46	24.97	25.50	24.47	24.27	25.99	25.03	24.59	25.70	25.62	24.64	**24.42**	23.90	25.51	27.82	27.34	**22.85**	**23.07**	**22.87**
Tupaia montana	22.58	82.18	23.52	24.85	17.56	26.76	25.26	24.32	26.32	23.80	25.59	24.66	23.11	26.77	26.27	25.32	22.71	23.81	na	na	na
	0.33/2	0.68/4	0.90/2	0.81/2	1.57/2	na/1	0.66/2	0.06/3	0.04/2	0.37/2	0.18/2	0.62/2	0.75/2	na/1	0.52/2	0.27/2	0.66/3	0.56/5			
	26.00	0	22.94	23.20	24.61	23.54	23.56	23.84	24.61	22.71	24.68	24.58	22.88	25.06	23.47	24.95	24.93	24.89	**21.52**	**23.80**	**25.43**
Cynocephalus variegatus	21.20	20.74	85.34	23.60	17.60	25.73	24.50	23.28	25.71	24.18	24.58	23.71	23.78	25.61	25.99	23.89	22.59	23.79	na	na	na
	0.63/4	1.63/3	0.18/8	0.17/4	0.59/2	0.64/5	1.30/7	0.39/3	1.08/8	0.79/5	0.33/3	0.21/4	0.37/8	1.05/5	1.18/4	0.35/4	0.77/4	0.47/3			
	24.41	24.75	0	22.03	24.66	22.64	22.85	22.82	24.04	23.08	23.70	23.63	23.55	23.98	23.22	23.54	24.80	24.87	**24.31**	**24.30**	**23.87**
Lemur catta	21.72	19.67	22.02	84.86	16.14	25.80	24.30	22.99	24.38	24.71	24.44	23.75	23.21	26.25	25.46	24.02	21.50	22.76	na	na	na
	0.35/2	1.02/3	0.64/4	0.29/4	0.40/2	0.07/4	0.80/3	0.25/3	0.59/4	0.45/4	0.42/2	0.30/2	0.18/2	na/1	0.20/2	0.69/2	0.86/3	0.54/3			
	25.01	23.48	21.48	0	22.62	22.70	22.67	22.53	22.80	23.58	23.57	23.67	22.98	24.57	22.74	23.66	23.60	23.79	**20.19**	**21.86**	**21.47**
Nycticebus coucang	22.43	20.33	23.99	21.53	82.26	27.78	27.31	25.04	26.17	26.01	na	na	25.07	26.98	na	na	24.98	24.93	na	na	na
	1.23/2	0.11/2	0.52/2	0.56/2	0.43/4	0.45/2	3.07/2	0.10/2	0.63/2	0.55/2			0.19/2	na/1			2.34/2	0.47/3			
	25.83	24.26	23.40	20.10	0	24.44	25.47	24.54	24.47	24.82	**24.07**	**21.97**	24.82	25.26	**24.99**	**24.62**	27.42	26.06	**21.86**	**25.00**	**25.25**
Dobsonia magna	na	20.43	24.04	na	16.57	86.29	7.20	22.00	22.09	22.51	23.69	22.28	22.98	23.50	24.55	23.10	21.38	21.26	na	na	na
		0.74/2	0.69/4		0.88/2	0.14/4	0.47/4	0.86/4	0.69/4	0.88/4	0.62/4	0.42/4	0.36/4	0.43/2	0.95/4	0.47/4	1.62/5	0.20/3			
	24.47	24.38	23.45	**22.70**	23.22	0	6.72	21.56	20.66	21.48	22.85	22.21	22.75	22.00	21.93	22.76	23.47	22.22	**22.72**	**22.43**	**21.63**
Pteropus vampyrus	21.14	19.90	24.28	24.97	17.43	6.98	84.69	21.44	21.36	21.29	23.09	22.00	22.97	24.10	23.65	22.25	20.83	21.39	na	na	na
	0.59/2	0.55/2	0.74/7	1.92/4	0.98/2	0.17/5	0.16/7	0.43/4	1.10/8	1.25/8	0.32/4	0.17/4	0.91/8	0.79/6	0.32/4	0.56/4	0.51/4	0.48/3			
	24.34	23.75	23.68	23.31	24.42	6.14	0	21.01	19.97	20.32	22.27	21.93	22.74	22.56	21.13	21.92	22.87	22.36	**22.08**	**21.91**	**21.78**
Macroderma gigas	21.66	20.03	23.90	25.66	17.56	na	23.00	85.42	21.68	20.20	na	na	23.50	25.57	na	na	20.99	22.62	na	na	na
	0.03/2	0.11/2	1.12/4	0.19/2	0.92/2		1.20/4	0.28/3	1.44/4	2.24/4			0.55/4	1.52/4			0.82/2	0.63/3			
	24.94	23.91	23.31	23.95	24.61	**21.56**	21.45	0	20.27	19.28	**23.28**	**22.85**	23.27	23.94	**22.37**	**22.15**	23.04	23.64	**23.97**	**22.86**	**21.82**
Hipposideros galeritus	21.72	20.34	24.02	24.64	18.05	24.38	22.47	21.13	85.66	13.00	24.09	22.77	22.99	24.78	24.63	23.25	22.00	22.42	na	na	na
	na/1	0.67/2	0.76/6	0.08/2	0.90/2	0.75/3	2.64/5	1.31/3	0.18/8	0.65/6	0.49/4	0.61/4	0.36/6	1.23/4	0.66/3	0.59/4	0.85/4	0.27/3			
	25.01	24.28	23.43	23.00	25.29	21.45	20.96	20.71	0	12.41	23.23	22.70	22.76	23.20	22.00	22.91	24.15	23.43	**23.60**	**23.13**	**22.08**

Continued

Table 1. *Continued*

Rhinolophus philippinensis	20.84 0.71/2 24.00	19.31 0.04/2 23.05	23.46 1.04/7 22.88	24.46 0.48/4 22.83	17.70 1.09/2 24.80	23.80 0.11/2 20.94	21.52 1.27/8 20.07	21.00 0.52/4 20.58	13.39 0.56/8 12.52	86.06 0.53/8 0	22.92 0.92/4 22.10	21.84 0.64/4 21.77	22.20 0.39/7 21.98	25.43 1.69/6 23.81	24.25 0.27/3 21.66	22.72 0.67/4 22.38	20.99 0.51/3 23.04	21.54 0.17/3 22.52	na **23.68**	na **22.28**	na **20.77**
Eptesicus hottentotus	na **25.70**	20.49 1.50/3 24.46	24.55 0.33/4 23.94	na **23.57**	17.18 0.84/3 24.07	25.44 0.66/2 22.38	23.02 1.44/4 21.47	23.75 0.67/3 23.28	25.99 1.01/4 24.30	23.15 2.48/4 22.09	85.88 0.18/4 0	6.67 0.33/4 6.65	9.33 0.60/4 9.24	23.91 1.25/3 22.38	23.73 1.94/4 21.20	22.10 0.75/4 21.77	21.43 0.34/3 23.53	22.33 0.56/3 23.34	na **22.41**	na **22.63**	na **20.50**
Myotis lucifugus	21.86 na/1 25.17	19.77 0.42/2 23.60	25.02 0.98/6 24.40	25.88 0.33/2 24.16	15.68 2.26/2 21.97	25.72 0.10/2 22.63	23.30 1.95/6 21.73	23.31 0.48/4 22.85	24.37 0.76/5 22.79	22.28 1.53/5 21.26	6.47 0.26/4 6.24	86.08 0.20/4 0	9.55 0.51/6 9.46	24.88 1.27/6 23.29	24.89 0.47/4 22.24	21.16 1.59/4 20.85	21.12 0.30/2 23.19	22.10 0.11/2 23.10	na **22.61**	na **22.06**	na **20.87**
Scotophilus kuhli	na **24.64**	19.01 0.33/2 22.69	23.85 0.76/4 23.26	25.69 0.35/4 23.98	17.54 1.13/2 24.58	25.51 0.60/5 22.44	24.00 2.01/8 22.39	23.22 0.11/2 22.76	24.27 0.88/7 22.70	na **21.98**	9.24 0.16/2 8.91	10.05 0.12/2 10.02	85.17 0.32/7 0	25.41 0.77/3 23.79	24.17 0.64/2 21.59	22.36 0.27/2 22.03	22.16 1.34/3 24.33	22.98 0.58/3 24.02	na **23.07**	na **22.73**	na **21.52**
Noctilio albiventris	21.21 0.22/2 24.42	20.23 na/1 24.15	24.18 0.97/4 23.58	25.58 0.51/2 23.88	17.30 0.74/2 24.24	26.33 0.27/2 23.16	24.57 0.81/4 22.92	24.07 0.01/3 23.59	25.51 0.47/2 23.86	24.50 1.44/4 23.38	23.65 0.03/2 22.81	22.30 0.40/2 22.23	22.98 0.34/4 22.75	84.85 0.55/4 0	22.59 0.53/2 20.18	22.85 0.27/2 22.51	21.90 0.30/3 24.04	23.05 0.36/3 24.09	na **22.99**	na **18.55**	na **23.42**
Pteronotus parnellii	na **23.90**	19.76 0.74/2 23.58	24.61 0.09/4 24.00	na **22.74**	17.83 1.22/2 24.99	25.19 0.23/2 22.16	23.10 0.95/4 21.55	22.83 0.25/3 22.37	24.00 0.12/4 22.44	23.54 0.82/4 22.46	22.57 0.82/3 21.77	21.18 0.96/4 21.11	22.69 0.24/4 22.47	18.28 1.64/3 17.11	85.57 0.26/3 0	21.94 0.46/4 21.62	20.97 0.22/3 23.02	21.58 0.66/3 22.56	na **23.53**	na **15.03**	na **20.60**
Molossus sinaloae	na **25.51**	21.31 2.36/2 25.43	25.31 0.41/2 24.68	na **23.66**	17.57 1.28/2 24.62	25.56 0.24/4 22.49	22.97 1.48/2 21.43	22.60 0.32/4 22.15	24.44 0.66/2 22.85	22.98 0.41/2 21.93	22.13 0.02/2 21.34	21.76 1.29/2 21.69	21.65 1.47/2 21.44	23.81 0.20/2 22.29	24.24 0.66/2 21.65	85.23 0.40/2 0	19.41 0.79/4 21.31	21.19 0.42/3 22.15	na **22.61**	na **22.41**	na **14.70**
Rhynchonycteris naso	na **27.82**	na **24.93**	na **24.80**	na **23.60**	17.20 0.30/2 24.10	na **23.47**	na **22.87**	24.07 0.73/2 23.59	na **24.15**	na **23.04**	na **23.53**	na **23.19**	na **24.33**	na **24.04**	na **23.02**	na **21.31**	83.46 0.37/4 0	20.75 na/1 21.69	na **7.18**	na **23.42**	na **22.24**
Taphozous mauritianus	na **27.34**	21.34 0.85/2 25.47	na **24.87**	na **23.79**	20.15 0.11/2 28.24	na **22.22**	na **22.36**	24.14 na/1 23.66	na **23.43**	na **22.52**	na **23.34**	na **23.10**	na **24.02**	na **24.09**	na **22.56**	na **22.15**	17.23 3.13/2 18.92	84.39 0.42/5 0	na **23.12**	na **23.19**	na **21.85**
Saccopteryx bilineata	na **22.85**	18.03 na/1 21.52	24.93 0.09/2 24.31	na **20.19**	na **21.86**	25.83 na/1 22.72	23.67 1.46/2 22.08	24.46 na/1 23.97	25.24 0.35/2 23.60	24.81 1.05/2 23.68	23.24 1.07/2 22.41	22.68 1.31/2 22.61	23.30 0.30/2 23.07	24.56 0.36/2 22.99	26.34 2.59/2 23.53	22.95 0.21/2 22.61	6.54 na/1 7.18	22.12 na/1 23.12	na 0	na **20.00**	na **18.83**
Mimon cozumelae	na **23.07**	19.94 0.52/2 23.80	24.92 0.16/2 24.30	na **21.86**	17.84 1.03/2 25.00	25.50 na/1 22.43	23.49 1.60/2 21.91	23.32 0.46/3 22.86	24.73 0.22/2 23.13	23.35 1.79/2 22.28	23.47 1.09/2 22.63	22.13 0.91/2 22.06	22.95 0.88/2 22.73	19.82 na/1 18.55	16.82 0.40/2 15.03	22.75 0.30/2 22.41	21.33 0.95/2 23.42	22.19 0.50/3 23.19	na **20.00**	na 0	na **20.61**
Chaerephon plicata	na **22.87**	21.31 na/1 25.43	24.47 0.37/4 23.87	na **21.47**	18.02 0.79/2 25.25	24.59 0.01/2 21.63	23.35 1.24/3 21.78	22.26 0.18/2 21.82	23.61 0.22/4 22.08	21.76 1.34/4 20.77	21.26 0.53/2 20.50	20.94 0.81/2 20.87	21.73 0.34/4 21.52	25.02 0.21/4 23.42	23.06 0.20/2 20.60	14.92 0.91/2 14.70	20.26 0.31/3 22.24	20.90 0.47/3 21.85	na **18.83**	na **20.61**	na 0
correction	1.133	1.209	0.979	0.911	1.409	0.875	0.925	0.947	0.932	0.952	1.002	1.024	1.002	0.946	0.918	1.021	0.981	0.900	na	na	na

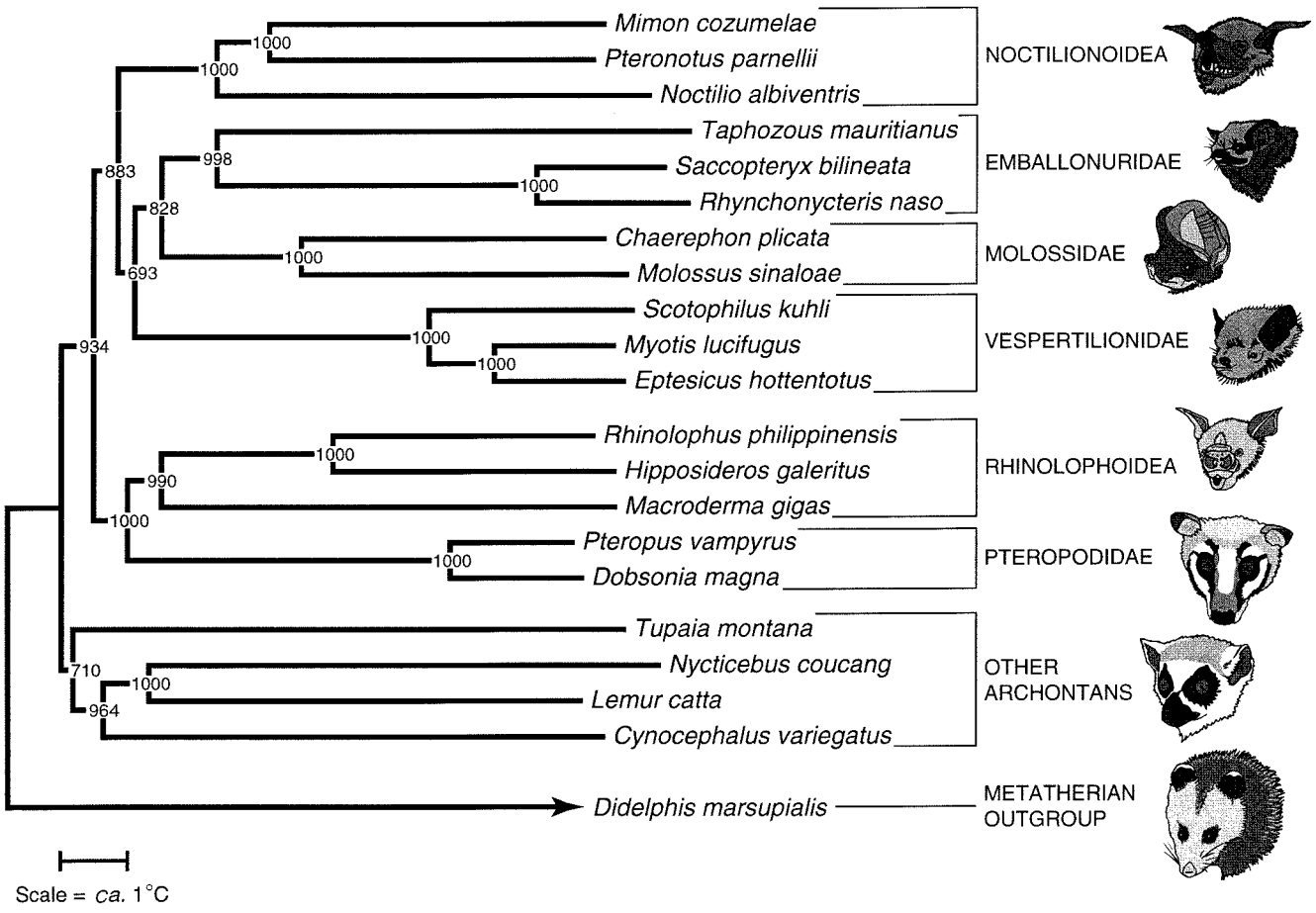

Figure 2. FITCH tree of relationships among 16 species of bats and five outgroups, from data of table 1 (third lines of cells). Numbers at nodes (except for the root) are bootstrap replicates supporting those clades. Branch lengths are drawn approximately to scale, and are based on the averages observed over 1000 bootstrap pseudoreplicate trees. Animal figures redrawn after several sources, but primarily Hill & Smith (1980).

species as drivers only (data not shown) always gave distances from a confamilial tracer which were less than those to other families; the use of single species to represent families or subfamilies therefore appears justified.

Didelphis generally rooted the trees between bats and the four non-bat eutherians. As expected, the two primates were the closest pair among these latter four; less expected was the sister-group relation of *Cynocephalus* to the primates, but *Tupaia* was only very weakly associated with the other three, judging by the bootstrap percentages (in fact, support for placing *Tupaia* with the bats is 51% for the 18-taxon matrix). The last node is also the single outgroup relationship which was unstable in the jackknives, though only on the 18-taxon subset of table 1. Both jackknife average consensus trees on the 21-taxon set placed *Tupaia* with non-bats, as did the average consensus of single deletions on the 18-taxon subset; random deletions among the 18 taxa put the scandentian with bats, however. Thus, with respect to the eutherian taxa studied here, neither Primates nor the flying lemur appear to be more nearly related to some or all bats than the latter are among themselves (a result consistent with fig. 7*a* in Kirsch *et al.* (1995*b*), where bats grouped together and with ungulates rather than with *Perodicticus*, *Rattus*, or *Scalopus*); and while *Tupaia* is clearly not a primate, its position should be considered unresolved by our experiments.

Because determination of the intrachiropteran root is critical to the question of the affinity of rhinolophoids, we also carried out a jackknife on the full (21 taxon) data set involving deletions just of outgroups. Trees calculated from a series of subsets of the data where, collectively, all possible combinations of the five non-bats were excluded (30 trees), did not materially alter intrabat relationships. Elimination of all five outgroups similarly gave the same topology among bats alone as is shown in figure 2, although obviously the root of this outgroupless tree could not be determined. However, we note again that bootstrap support for the placement of *Tupaia* on figures 2 and 3 is low, and that the average consensus tree for random deletions on the 18-taxon subset placed the scandentian as sister to the chiropterans.

Results of the randomization tests were highly significant: the z score for the full table 1 matrix (with the outgroup opossum distances held constant) was 11.30; for the 16-taxon matrix lacking all outgroups it was 9.76. A z score of about 2 would be considered significant at $p < 0.05$, assuming a normal distribution of the randomized sums-of-squares, which they have. Therefore, these results strongly suggest that the ΔT_{mode} index provides discrimination among the studied taxa (i.e. that the data have phylogenetic or other structure).

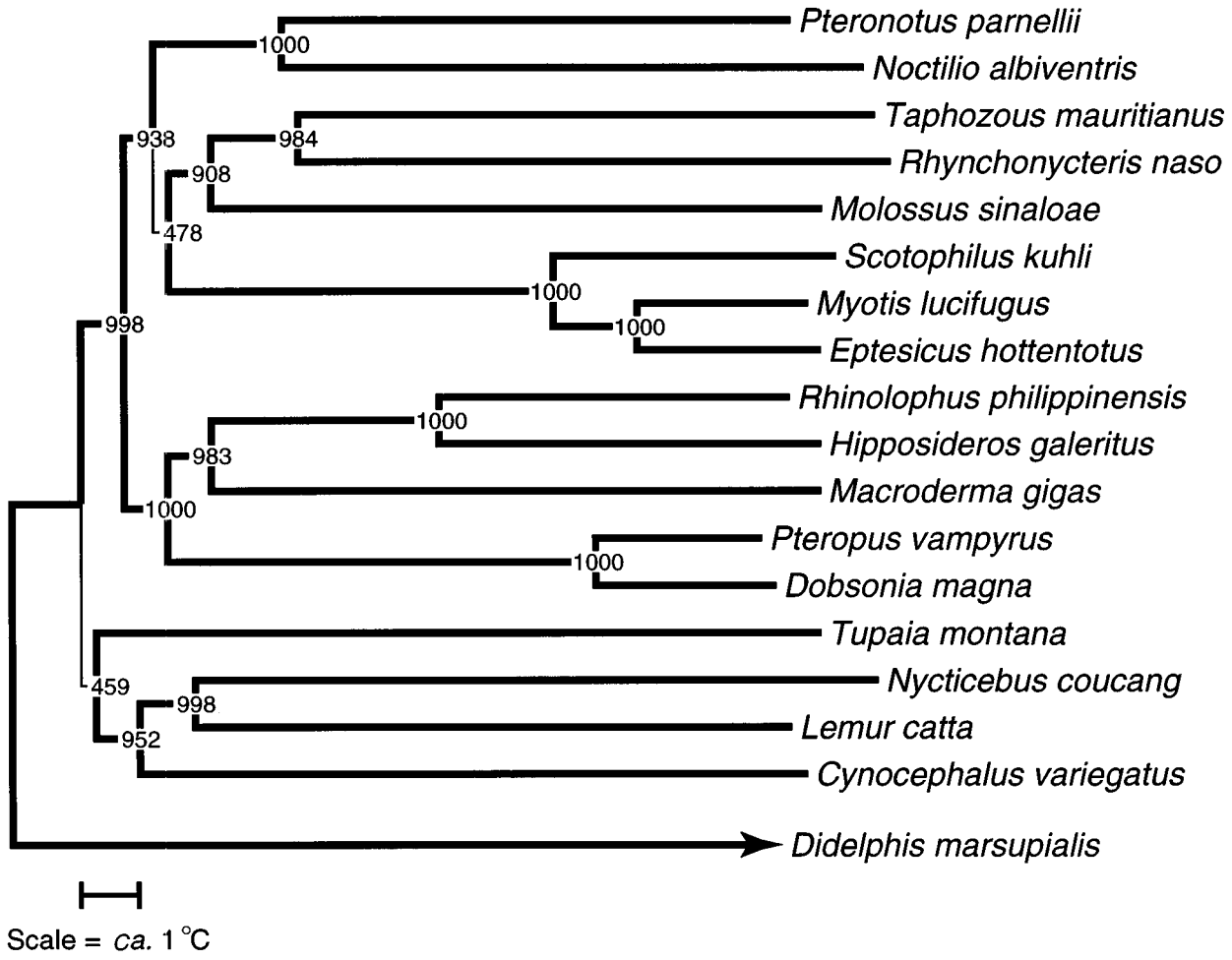

Figure 3. FITCH tree of relationships among 13 species of bats and five outgroups, from table 1 data on labelled taxa only. Numbers at nodes (except for the root) are bootstrap replicates supporting those clades. Thin lines indicate relationships not supported in average consensus trees of both single and random deletion jackknives: single deletions put *Tupaia* with non-bat eutherians (as shown), while random deletions placed the scandentian with bats and interchanged the positions of vespertilionids and noctilionoids. Branch lengths are drawn approximately to scale, and are based on the average consensus single-deletion jackknife tree.

4. DISCUSSION

(a) *Analytical considerations*

Our results raise several issues of an analytic as well as a phylogenetic nature. First, it no longer appears possible to attribute the linking of the rhinolophoid and pteropodid found by Kirsch & Pettigrew (1998) solely to long branch attraction—or at least, if it is due to such an algorithmic artefact, there is little more that can be done to alleviate that problem. *Dobsonia* and *Pteropus* are about as numerically distant from each other as are any two pteropodids examined (Kirsch *et al.* 1995*b*), even though the relatively slow evolving *Nyctimene* and *Paranyctimene* together seem to comprise the sister group to other pteropodids (Kirsch *et al.* 1995*b*, figs 2, 3, and 6); inclusion of additional pteropodids would not provide much by way of divisions further down the branch connecting *Dobsonia* and *Pteropus* to the node joining them with rhinolophoids. In fact, when we 'sutured' the table 1 data with a matrix among 19 pteropodids (Kirsch *et al.* 1995*b*, table 2 in that paper), the resulting FITCH tree did not differ for shared taxa from figure 2, except that *Tupaia* was sister to the bats. Inclusion of craseonycterids, nycterids, or rhinopomatids (which are other taxa sometimes placed with Emballonuroidea or Rhinolophoidea (Koopman 1994)), on the other hand, might provide additional basal chiropteran subdivisions; but the rhinolophoid branch is already fairly evenly subdivided in figures 2 and 3, as are all familial or superfamilial lineages within the non-rhinolophoid microbats. Moreover, in a recent DNA hybridization study of 26 hummingbirds, Bleiweiss *et al.* (1997) showed that the basic structure of their tree was unaltered even when the number of ingroup taxa was reduced to six. For FITCH analysis of DNA hybridization data, at least, the long branch attraction problem may have been overstated.

The position of the root within Chiroptera recovered here is thus more than an artefact of poor taxonomic sampling among bats; but as Marshall (1991) and Kirsch *et al.* (1995*c*) have shown, when ingroup taxa are separated by short internodes, experimental error among ingroup–outgroup distances may significantly distort ingroup relationships. Here, a shift of the intrabat root by only one node is all that would be required to restore microchiropteran monophyly. Yet we took care to include a suite of outgroup species, most of which were themselves likely to be interrelated apart from the ingroup, as seems good practice in molecular systematics (Smith 1994). The single and random deletion jackknife analyses on table 1 (21- and

18-taxon subsets) show that incorrect rooting due to short and uncertain basal internodes is probably not the explanation for our surprising phylogeny, and the more explicit outgroup-deletion experiments—sequentially removing all possible combinations of the five outgroups from table 1—also failed to change the position of the intrabat root. Moreover, with all outgroups eliminated, ingroup topology remained unaltered, although of course the root among bats was then undetermined. Nevertheless, inclusion of representatives from additional eutherian orders could conceivably produce different relationships among the taxa of figure 2, but then problems of resolution would doubtless be exacerbated due to the subtleties of partitioning already very short basal internodes. However, our results are not likely to be incorrect because of poor discrimination by the thermal stability index chosen: randomization tests on the matrix were highly significant for structure. It may be objected that the subdivision of bat clades subverts this conclusion with respect to the intrachiropteran root because the tests are therefore too liberal, but we performed another randomization test on single representatives of each major chiropteran lineage (six taxa), leaving only very short basal internodes and no subdivisions along the bat lineages, and still obtained a highly significant result (z score = 5.03).

It is, however, possible that no combination of outgroups would produce a different placement of the rhinolophoids. If it is correct that a shared AT bias accounts for the observed affinity of rhinolophoids with pteropodids (or, alternatively, the 'repulsion' of pteropodids from proximity to Primates), and that a bias is likely to be manifested as an apparently slower rate of evolution (Pettigrew & Kirsch 1998), then AT-biased taxa will inevitably terminate relatively short branches and may be segregated apart from other taxa even by a FITCH computation, despite the fact that FITCH is relatively forgiving of apparent rate nonuniformities. Interestingly, in Pierson's fig. 3 (1986, opposite p. 21), a midpoint rooted FITCH tree based on microcomplement-fixation data, *Pteropus* formed a cluster with both emballonuroids and rhinolophoids. Another, more recent, serological study (Schreiber *et al.* 1994) seems to align both megadermatids and phyllostomids with megabats, but this investigation was based entirely on one-way comparisons using anti-human sera. Thus, the attempt to fix a root among bats may be intrinsically doomed (here, because of the attraction of short (not long!) branches), and in general we do not see that the situation is much better for parsimony as opposed to distance analyses (cf. Janke *et al.* (1994, 1996) for examples of unlikely associations among the mammalian infraclasses and cohorts).

(b) *Phylogeny and character evolution*

Whatever the veracity of the within-bat root, our trees do appear to support bat monophyly, but it should be emphasized that only a few eutherian outgroups were included; the results really say nothing even about the monophyly of Cohort Archonta, much less indicate the results which would be forthcoming with respect to bats had representatives of additional orders been included and controls for base composition bias been used (e.g. had the experiments been repeated with GC-enriched labels; but cf. figs 1E–H in Kirsch & Pettigrew (1998)). While figures 2 and 3 do seem to exclude the possibility that flying lemurs are by themselves sister to either group of bats, we caution that the internodes among the four eutherian outgroups are very short, the lineages are not generally subdivided, and some of our experiments with fractionated DNAs (Kirsch & Pettigrew 1998; Pettigrew & Kirsch 1998) support a different interpretation where megabats and the colugo are much closer; many morphological and neural data also support the latter association (Pettigrew 1995). There has, however, been some dispute recently regarding the association of Dermoptera with other presumptive archontans. Beard (1993) noted that superficial morphological similarities based primarily on flying/gliding adaptations lead to the 'unnatural assemblage' of Volitantia, a cohort comprised of all bats and colugos. Beard argued that the patagia of these two taxa are, in fact, convergent and that dermopterans are connate with primates. Several palaeontological and molecular studies (see Beard 1993), as well as a number of neural apomorphies that are uniquely shared between primates and colugos (Pettigrew *et al.* 1989) or specifically between tarsiers and colugos (Rosa *et al.* 1996), have also suggested this relationship, which if true has interesting implications for the evolution of flight; and, indirectly, for the question of bat monophyly.

Most current scenarios for the evolution of chiropteran flight suggest a move from an arboreal, insectivorous progenitor, through a gliding phase, to true powered flight (cf. Smith 1977; Hill & Smith 1980; Norberg & Rayner 1987). None of the presumed transitional stages have yet been demonstrated in the fossil record, and the earliest known chiropteran fossils with reasonably complete postcranials appear to have been wholly capable of powered flight (e.g. Jepsen 1966, 1970). However, assuming that the gliding-to-flight narrative is correct, and that the particular implementation of gliding in *Cynocephalus* (i.e. all limbs, the fingers, and the tail being involved in providing a lifting surface) provides a good model for the early stages of evolution of powered flight, then the molecular evidence for removal of flying lemurs from proximity to bats suggests that true flight could have evolved more than once among mammals. That is to say, because no living glider seems to represent the ancestor of some or all bats, gliding itself (and of the dermopteran variety) must have evolved at least twice; and if twice, then why not three times? For proponents of bat diphyly, this argument should be encouraging, because it removes some of the stigma of positing dual evolution of the complex flight mechanisms of Chiroptera.

What also seems clear from our results is that the rhinolophoid–pteropodid association is 'real,' whether because these taxa share a high AT : GC ratio or because they are in fact specially related. Concerning the first possible cause, once again we must stress that only additional tests with GC rich labels extending Pettigrew & Kirsch's (1998) experiments to fractions which would completely obviate the AT bias, but including a wider range of species (as here), can determine if the rhinolophoid–pteropodid relationship is entirely due to a biochemical artefact. In this regard, we note that Porter *et al.* (1996) found a particular association of the rhinolophoid *Megaderma* with pteropodids, a result reminiscent of ours.

On the other hand, anatomical studies suggest that emballonuroids are phyletically near Rhinolophoidea, while our DNA hybridization trees place Emballonuridae nearest to molossids; Koopman (1994), for example, considers Emballonuroidea and Rhinolophoidea to constitute his microchiropteran infraorder Yinochiroptera, although Simmons (1998) questions the monophyly of emballonuroids, and this is also an implication of Pierson's (1986) data. Otherwise, all interbat relationships shown in figures 2 and 3 herein agree closely with conventional views, a circumstance which makes the anomalous placement of rhinolophoids (and, to a lesser extent, of emballonurids) the more striking.

It is possible to make a case in support of our association between pteropodids and rhinolophoids on zoogeographic grounds, as outlined below, but there are numerous anatomical characters that must have undergone homoplastic changes to explain this association. Most obviously, uniting rhinolophoids and pteropodids implies either the separate evolution of echolocation in rhinolophoids and other microbats or the loss of this adaptation in Pteropodidae. Laryngeal sonar is a sophisticated form of echolocation found only in Microchiroptera (in all 17 extant families as well as in some extinct taxa, the latter inference being based upon radiographic evidence of the associated cochlear specialization (Novacek 1985)). This adaptive complex is lacking in pteropodids, although one genus (*Rousettus*) has developed a primitive form of echolocation involving tongue clicking, so it is necessary to explain the absence of such a valuable ability in the pteropodids (apparent close relatives of the echolocating rhinolophoids in our DNA hybridization trees), which have comparable navigational requirements in a nocturnal or crepuscular volant niche. The lack of sonar in pteropodids is particularly difficult to explain if one considers the arguments of Speakman *et al.* (1989). These authors suggest that volant organisms have a special advantage in the energetically costly production of sonar pulses, because they can achieve efficiencies not available to terrestrial organisms by co-opting the same muscles for use both in flight and in sonar production. If one posits that pteropodids are ancestral to rhinolophoids, then rhinolophoid sonar must have evolved independently of laryngeal sonar in other microbats, a remarkable parallelism given the extraordinary sophistication of the processing machinery required and the unique elaboration of the cochlea found in all microbats. On the other hand, if pteropodids are derived from rhinolophoids, then one has to explain the loss of sonar and its subsequent reacquisition (in *Rousettus)* in a primitive and vastly simplified form.

Since much is known about microbat sonar, it is possible to argue for and against these different scenarios for the evolution of sonar in pteropodids and rhinolophoids in a cogent way that emphasizes the difficulties facing our attempts to reconcile the phylogenetic conflict demonstrated here. But other systems also show marked differences in pteropodids when compared with rhinolophoids. It is worth pointing out that examination of these systems gives rise to a long list of fundamental differences between pteropodids and microbats, each of which would have to be reversed were our pteropodid–rhinolophoid association real. Space does not permit discussion of all of these differences, but the rhinolophoid–pteropodid alliance would require the reversal (or independent acquisition) of more than 50 characters at the node joining these taxa, in systems ranging widely from brain to skin to reproduction (see Pettigrew 1995, table 1). In the case of the brain characters, one can make functional arguments for and against losses or shared gains, like those made in the case of sonar; while in other cases, such as the striated pilo-erector muscle fibres of rhinolophoids compared with the smooth pilo-erector muscle fibres of the pteropodids, the needed reversal is puzzling whether one can provide an explanation or not. A general treatment of the scenarios that could have given rise to a monophyletic clade including both pteropodids and (all or some) microbats must include the following two broad possibilities: (1) where the microbat general condition is ancestral, or (2) where the pteropodid condition is thought to be ancestral ('deaf fruit bat' and 'blind cave bat' scenarios respectively, as discussed in Pettigrew *et al.* (1989)).

Regarding more classical craniodental and skeletal characters, we note here only that at least one student of bat anatomy (Sigé 1993) has suggested a phylogeny broadly similar to that implied in our figures 2 and 3, deriving pteropodids along with rhinolophoids and some other microchiropterans from archaeonycterids, separately from a clade that includes vespertilionoids and which apparently originated from paleochiropterygids. However, some aspects of Sigé's phylogram (e.g. inclusion of noctilionoids in a clade with megabats) are falsified by our data (see also fig. 6 in Kirsch *et al.* (1995*b*)).

Finally, a weak case that our tree might be phyletically true could be made from distributional data. It appears probable that megachiropterans originated in Asia or the Australo-Pacific region (Ducrocq *et al.* 1993), and Bogdanowicz & Owen (1992) concluded that rhinolophids *sensu stricto* likewise had a southeast Asian origin. In a later study, Bogdanowicz & Owen (1998) were unable to decide about the centre of origin of hipposiderids; but Hand & Kirsch (1998) concluded from a mapping of geographic distributions on their cladogram of Hipposideridae that Australasia was a likely ancestral area for that family, a result we have since confirmed with Bremer (1992) ancestral area reconstruction (J. M. Hutcheon, unpublished data). Of course, all such historical–zoogeographic conclusions depend greatly on the suite of taxa examined, and even more on the reliability of trees relating those taxa; but more broadly based considerations of bat distributions (Hershkovitz 1972; Pierson 1986) have also led to the conclusion that some at least of the extant microbat families originated in Gondwanaland. As the case for a southeast Asian or Gondwanan origin seems strongest for rhinolophoids and pteropodids, and some of the included taxa have similar current distributions as well as congruent subsidiary geographic moieties (cf. Bogdanowicz & Owen 1992; Kirsch *et al.* 1995*b*), it is perhaps not entirely fanciful to adduce historical zoogeography in support of a rhinolophoid–pteropodid relationship. However, the same sort of argument might be made for a proposed megabat–colugo-primate association.

As for the classificatory and nomenclatural implications of a pteropodid–rhinolophoid association, these would most obviously include submergence of Megachiroptera

within a subordinal (or ordinal, if bat monophyly be disproved) group that includes at least Pteropodidae and Rhinolophoidea. Koopman's (1994) Yinochiroptera is an available name, although that taxon might need to be further redefined to exclude Emballonuridae (Pierson 1986; Simmons 1998; this paper). However, as we have not yet examined other putative yinochiropteran families (i.e. Craseonycteridae, Nycteridae, and Rhinopomatidae), and because a tree as startling as ours obviously must be verified by additional studies, it is clearly premature to make such nomenclatural recommendations.

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REFERENCES

Adkins, R. M. & Honeycutt, R. L. 1991 Molecular phylogeny of the superorder Archonta. *Proc. Natn. Acad. Sci. USA* **88**, 10 317–10 321.

Ammerman, L. K. & Hillis, D. M. 1992 A molecular test of bat relationships: monophyly or diphyly? *Syst. Biol.* **41**, 222–232.

Bailey, W. J., Slightom, J. L. & Goodman, M. 1992 Rejection of the 'flying primate' hypothesis by phylogenetic evidence from the ϵ-globin gene. *Science* **256**, 86–89.

Baker, R. J., Honeycutt, R. L. & Van Den Bussche, R. A. 1991*a* Examination of monophyly of bats: restriction map of the ribosomal DNA cistron. In *Contributions to mammalogy in honor of Karl F. Koopman. Bull. Am. Mus. Nat. Hist.* **202**, 42–53.

Baker, R. J., Novacek, M. J. & Simmons, N. B. 1991*b* On the monophyly of bats. *Syst. Zool.* **40**, 216–231.

Beard, K. C. 1993 Origin and evolution of gliding in early Cenozoic Dermoptera (Mammalia, Primatomorpha). In *Primates and their relatives in phylogenetic perspective* (ed. R. D. E. MacPhee), pp. 63–90. New York: Plenum.

Bleiweiss, R., Kirsch, J. A. W. & Matheus, J. C. 1994 DNA hybridization evidence for subfamily structure among hummingbirds. *Auk* **111**, 8–19.

Bleiweiss, R., Kirsch, J. A. W. & Matheus, J. C. 1997 DNA hybridization evidence for the principal lineages of hummingbirds (Aves: Trochilidae). *Molec. Biol. Evol.* **14**, 325–343.

Bogdanowicz, W. & Owen, R. D. 1992 Phylogenetic analyses of the bat family Rhinolophidae. *Z. Für Zool. Syst. Evol.* **30**, 142–160.

Bogdanowicz, W. & Owen, R. D. 1998 In the Minotaur's labyrinth: the origin of the Hipposideridae. In *Bats: phylogeny, morphology, echolocation, and conservation biology* (ed. T. H. Kunz & P. A. Racey). Washington, DC: Smithsonian Institution Press. (In the press.)

Bremer, K. 1992 Ancestral areas: a cladistic reinterpretation of the centre of origin concept. *Syst. Biol.* **41**, 436–445.

Cavalli-Sforza, L. L. & Edwards, A. W. F. 1967 Phylogenetic analysis: models and estimation procedures. *Am. J. Hum. Genet.* **19**, 233–257.

Ducrocq, S., Jaeger, J.-J. & Sigé, B. 1993 Un mégachiropterère dans l'Eocerène supérior de Thaïlande incidence dans la discussion phylogénique du groupe. *N. Jb. Geol. Paläont. Mh.* **1993**, 561–575.

Felsenstein, J. 1978 Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* **27**, 401–410.

Felsenstein, J. 1993 *PHYLIP, phylogenetic inference package, program and documentation, version 3.5c.* Seattle, WA: University of Washington.

Fox, G. M. & Schmid, C. W. 1980 Related single copy sequences in the human genome. *Biochim. Biophys. Acta* **609**, 349–363.

Hand, S. J. & Kirsch, J. A. W. 1998 A southern origin for the Hipposideridae (Microchiroptera)? Evidence from the Australian fossil record. In *Bats: phylogeny, morphology, echolocation, and conservation biology* (ed. T. H. Kunz & P. A. Racey). Washington, DC: Smithsonian Institution Press. (In the press.)

Hershkovitz, P. 1972 The recent mammals of the neotropical region: a zoogeographic and ecological review. In *Evolution, mammals, and southern continents* (ed. A. Keast, F. C. Erk & B. Glass), pp. 311–431. Albany, NY: State University of New York Press.

Hill, J. E. & Smith, J. D. 1980 *Bats: a natural history.* Austin, TX: University of Texas Press.

Janke, A., Feldmaier-Fuchs, T., Thomas, W. K., von Haeseler, A. & Pääbo, S. 1994 The marsupial mitochondrial genome and the evolution of placental mammals. *Genetics* **137**, 243–256.

Janke, A., Gemmell, N. J., Feldmaier-Fuchs, G., von Haeseler, A. & Pääbo, S. 1996 The mitochondrial genome of a monotreme—the platypus (*Ornithorhynchus anatinus*). *J. Molec. Evol.* **42**, 153–159.

Jepsen, G. L. 1966 Early Eocene bat from Wyoming. *Science* **154**, 1333–1339.

Jepsen, G. L. 1970 Bat origins and evolution. In *Biology of bats* (ed. W. A. Wimsatt), pp. 1–64. New York: Academic Press.

Kilpatrick, C. W. & Nuñez, P. E. 1993 Monophyly of bats inferred from DNA–DNA hybridization. In *Abstracts sixth international theriological congress*, p. 155. Sydney: University of New South Wales.

Kirsch, J. A. W. & Pettigrew, J. D. 1998 Base-compositional biases and the bat problem. II. DNA hybridization trees based on AT and GC-enriched tracers. *Phil. Trans. R. Soc. Lond.* B **353**, 381–388.

Kirsch, J. A. W., Springer, M. S., Krajewski, C., Archer, M., Aplin, K. & Dickerman, A. W. 1990 DNA/DNA hybridization studies of carnivorous marsupials. I. The intergeneric relationships of bandicoots (Marsupialia: Perameloidea). *J. Molec. Evol.* **30**, 434–448.

Kirsch, J. A. W., Dickerman, A. W. & Reig, O. A. 1995*a* DNA/DNA hybridization studies of carnivorous marsupials. IV. Intergeneric relations among opossums (Didelphidae). *Marmosiana* **1**, 57–78.

Kirsch, J. A. W., Flannery, T. F., Springer, M. S. & Lapointe, F. -J. 1995*b* Phylogeny of the Pteropodidae (Mammalia: Chiroptera) based on DNA hybridisation, with evidence for bat monophyly. *Aust. J. Zool.* **43**, 395–428.

Kirsch, J. A. W., Lapointe, F. -J. & Foeste, A. 1995*c* Resolution of portions of the kangaroo phylogeny (Marsupialia: Macropodidae) using DNA hybridization. *Biol. J. Linn. Soc.* **55**, 309–328.

Koopman, K. F. 1994 *Chiroptera: systematics. Handbook of zoology, VIII, 60, Mammalia* 1–217. Berlin: Walter de Gruyter.

Krajewski, C. & Dickerman, A. W. 1990 Bootstrap analysis of phylogenetic trees derived from DNA hybridization distances. *Syst. Zool.* **39**, 383–390.

Landry, P.-A., Lapointe, F.-J. & Kirsch, J. A. W. 1996 Estimating phylogenies from lacunose distance matrices: additive is superior to ultrametric estimation. *Molec. Biol. Evol.* **13**, 818–823.

Lapointe, F.-J., Kirsch, J. A. W. & Bleiweiss, R. 1994 Jackknifing of weighted trees: validation ofphylogenies reconstructed from distance matrices. *Molec. Phylogenet. Evol.* **3**, 256–267.

Marshall, C. R. 1991 Statistical tests and bootstrapping: assessing the reliability of phylogenies based on distance data. *Molec. Biol. Evol.* **8**, 386–391.

Mindell, D. P., Dick, C. W. & Baker, R. J. 1991 Phylogenetic relationships among megabats, microbats, and primates. *Proc. Natn. Acad. Sci. USA* **88**, 10 322–10 326.

Norberg, U. M. & Rayner, J. M. V. 1987 Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond.* B **316**, 335–427.

Novacek, M. J. 1985 Evidence for echolocation in the oldest known bats. *Nature* **315**, 140–141.

Pettigrew, J. D. 1986 Flying primates? Megabats have the advanced pathway from eye to midbrain. *Science* **231**, 1304–1306.

Pettigrew, J. D. 1991*a* Wings or brain? Convergent evolution in the origins of bats. *Syst. Zool.* **37**, 199–216.

Pettigrew, J. D. 1991*b* A fruitful, wrong hypothesis? Response to Baker, Novacek and Simmons. *Syst. Zool.* **40**, 321–239.

Pettigrew, J. D. 1994 Flying DNA. *Curr. Biol.* **4**, 277–280.

Pettigrew, J. D. 1995 Flying primates: crashed, or crashed through? In *Ecology, evolution and behaviour of bats* (ed. P. A. Racey & S. M. Swift), pp. 3–26. Oxford: Clarendon Press.

Pettigrew, J. D. & Kirsch, J. A. W. 1998 Base-compositional biases and the bat problem. I. DNA hybridization melting curves based on AT and GC-enriched tracers. *Phil. Trans. R. Soc. Lond.* B **353**, 369–379.

Pettigrew, J. D., Jamieson, B. G. M., Robson, S. K., Hall, L. S., McAnally, K. I. & Cooper, H. M. 1989 Phylogenetic relations between microbats, megabats and primates (Mammalia: Chiroptera, Primates). *Phil. Trans. R. Soc. Lond.* B **325**, 489–559.

Pierson, E. D. 1986 Molecular systematics of the Microchiroptera: higher taxon relationships and biogeography. Unpublished PhD thesis, University of California, Berkeley, CA, USA.

Porter, C. A., Goodman, M. & Stanhope, M. J. 1996 Evidence on mammalian phylogeny from sequences of exon 28 of the von Willebrand factor gene. *Molec. Phylogenet. Evol.* **5**, 89–101.

Rosa, M. G. P., Pettigrew, J. D. & Cooper, H. M. 1996 Unusual pattern of retinogeniculate terminations in the controversial primate, *Tarsius*. *Brain Behav. Evol.* **48**, 121–129.

Sarich, V. M. & Cronin, J. E. 1976 Molecular systematics of the primates. In *Molecular anthropology, genes, and proteins in the evolutionary ascent of the primates* (ed. M. Goodman & R. E. Tashian), pp. 141–170. New York: Plenum Press.

Schreiber, A., Bauer, D. & Bauer, K. 1994 Mammalian evolution from serum protein epitopes. *Biol. J. Linn. Soc.* **51**, 359–376.

Sigé, B. 1993 Toward a phylogeny of bats. In *Paleobiology and evolution of early Cenozoic mammals. A symposium in honor of D. E. Russell.* Poster; Fourth Congress of the European Society for Evolutionary Biology, Montpellier, France.

Simmons, N. B. 1994 The case for chiropteran monophyly. *Am. Mus. Novitates* **3103**, 1–54.

Simmons, N. B. 1998 Higher-level relationships of microchiropteran bats. In *Bats: phylogeny, morphology, echolocation, and conservation biology* (ed. T. H. Kunz & P. A. Racey). Washington, DC: Smithsonian Institution Press. (In the press.)

Simmons, N. B., Novacek, M. J. & Baker, R. B. 1991 Approaches, methods, and the future of the chiropteran monophyly controversy. *Syst. Zool.* **40**, 239–243.

Smith, A. B. 1994 Rooting molecular trees: problems and strategies. *Biol. J. Linn. Soc.* **51**, 279–292.

Smith, J. D. 1977 Comments on flight and the evolution of bats. In *Major patterns in vertebrate evolution* (ed. M. K. Hecht, P. C. Goody & B. M. Hecht), pp. 427–438. New York: Plenum.

Smith, J. D. & Madkour, G. 1980 Penial morphology and the question of chiropteran phylogeny. In *Proceedings fifth international bat research conference* (ed. D. E. Wilson & A. L. Gardner), pp. 347–365. Lubbock, TX: Texas Tech Press.

Speakman, J. R., Anderson, M. E. & Racey, P. A. 1989 The energy cost of echolocation in pipistrelle bats (*Pipistrellus pipistrellus*). *J. Comp. Phys.* A **165**, 679–686.

Springer, M. S. & Kirsch, J. A. W. 1991 DNA hybridization, the compression effect, and the radiation of diprotodontian marsupials. *Syst. Zool.* **40**, 131–151.

Stanhope, M. J., Bailey, W. J., Czelusniak, J., Goodman, M., Si, J.-S., Nickerson, J., Sgouros, J. G., Singer, G. A. M. & Kleinschmidt, T. K. 1993 A molecular view of primate supraordinal relationships from the analysis of both nucleotide and amino acid sequences. In *Primates and their relatives in phylogenetic perspective* (ed. R. D. E. MacPhee), pp. 251–292. New York: Plenum Press.

Swofford, D. L. & Olsen, G. J. 1990 Phylogeny reconstruction. In *Molecular systematics* (ed. D. M. Hillis & G. Moritz), pp. 411–501. Sunderland, MA: Sinauer Associates, Inc.

Thewissen, J. G. & Babcock, S. K. 1991 Distinctive cranial and cervical innervation of wing muscles: new evidence for bat monophyly. *Science* **252**, 934–936.