9. Gardiner, B. G. The relationships of the palaeoniscid fishes, a review based on new specimens of *Mimia* and *Moythomasia* from the Upper Devonian of Western Australia. *Bull. Brit. Mus. Nat. Hist. (Geol.)* **37,** 173–428 (1984).
10. Young, G. C. New information on the structure and relationships of *Buchanosteus* (Placodermi: Euarthrodira) from the Early Devonian of New South Wales. *Zool. Jour. Linn. Soc.* **66,** 307–352 (1979).
11. Young, G. C. A new early Devonian placoderm from New south Wales, Australia, with a discussion of placoderm phylogeny. *Palaeontographica* **167A,** 10–76 (1980).
12. Young, G. C. The relationships of placoderm fishes. *Zool. Jour. Linn. Soc.* **88,** 1–57 (1986).
13. Goujet, D. Placoderm interrelationships: a new interpretation, with a short review of placoderm classification. *Proc. Linn. Soc. NSW* **107,** 211–243 (1984).
14. Goujet, D. Les affinités des Placoderms, une revue des hypothèses actuelles. *Géobios, Mém. Spéc.* **6,** 27–38 (1982).
15. Gardiner, B. G. The relationships of placoderms. *J. Vert. Paleont.* **4,** 379–395 (1984).
16. Gross, W. Das Kopfskelett von *Cladodus wildungensis* Jaekel. *Senckenbergiana* **19**, 80–107 (1937).
17. Schaeffer, B. & Dalquest, W. W. A palaeonisciform braincase from the Permian of Texas, with comments on cranial fissures and the posterior myodome. *Am. Mus. Novit.* **2658,** 1–15 (1937).
18. de Beer, G. R. *The Development of the Vertebrate Skull* (Oxford Univ. Press, Oxford, 1937).
19. Coates, M. I. & Sequeira, S. E. K. The braincase of a primitive shark. *Trans. R. Soc. Edinb., Earth Sci.* **89,** 63–85 (1998).
20. Miles, R. S. in *Interrelationships of Fishes* (eds Greenwood, P. H., Miles, R. S. & Patterson, C.) *Zool. J. Linn. Soc.* **53** (suppl. 1), 63–103 (1973).
21. Chang, M. M. The braincase of *Youngolepis*, a Lower Devonian crossopterygian from Yunnan, south-western China. Thesis, Univ. Stockholm (1982).
22. Swofford, D. L. PAUP: phylogenetic analysis using parsimony, version 3.1.1. (Illinois Natural History Survey, Champaigne, Illinois, 1993).

Acknowledgements

We thank D. Goujet and C. Poplin for helpful discussion. A.B. thanks the Australian Museum for use of facilities, and acknowledges support from an Australian Postgraduate Award and Macquarie University Postgraduate Fund. M.I.C. acknowledges support from the Biotechnology and Biological Sciences Research Council UK.

Correspondence and requests for materials should be addressed to A.B. (e-mail: abasden@laurel.ocs.mq.edu.au).

Molecular evidence regarding the origin of echolocation and flight in bats

Emma C. Teeling*†, Mark Scally*†, Diana J. Kao‡, Michael L. Romagnoli‡, Mark S. Springer‡ & Michael J. Stanhope*†

**Queen's University of Belfast, Biology and Biochemistry, 97 Lisburn Road, Belfast BT9 7BL, UK*
‡ *Department of Biology, University of California, Riverside, California 92521, USA*

Bats (order Chiroptera) are one of the few orders of mammals that echolocate and the only group with the capacity for powered flight. The order is subdivided into Microchiroptera and Megachiroptera, with an array of characteristics defining each group[1], including complex laryngeal echolocation systems in microbats and enhanced visual acuity in megabats. The respective monophylies of the two suborders have been tacitly assumed, although microbat monophyly is uncorroborated by molecular data. Here we present a phylogenetic analysis of bat relationships using DNA sequence data from four nuclear genes and three mitochondrial genes (total of 8,230 base pairs), indicating that microbat families in the superfamily Rhinolophoidea are more closely related to megabats than they are to other microbats. This implies that echolocation systems either evolved independently in rhinolophoids and other microbats or were lost in the evolution of megabats. Our data also reject flying lemur (order Dermoptera) as the bat sister group, indicating that presumed shared derived characters for flying lemurs and bats[2] are convergent features that evolved in association with gliding and flight, respectively.

Bat species were chosen to include at least one representative from each microbat superfamily[3] as well as representative megabats. Because the choice of outgroup can have a major influence on reconstructed ingroup topologies, and because the sister group to bats is controversial[4,5], we included four diverse outgroup taxa. We obtained new sequences and/or extracted sequences from GenBank for four nuclear genes and three mitochondrial genes. The nuclear genes were exon 11 of BRCA1 (breast cancer susceptibility gene; 2.8 kilobases (kb)), exon 28 of vWF (von Willebrand factor; 1.2 kb), RAG1 (recombination activating gene 1; 1.1 kb), and RAG2 (recombination activating gene 2; 0.8 kb). The mitochondrial genes were the complete 12S and 16S ribosomal RNA genes and the intervening valine transfer RNA gene. Independent mitochondrial and nuclear loci were chosen to index different types of DNA sequence information.

None of our phylogenetic analyses supported the monophyly of microbats. We found that *Megaderma* and *Hipposideros*, two microbat species from different families within the superfamily Rhinolophoidea, grouped as the sister clade to the megabats (Fig. 1). Individual loci did not provide decisive bootstrap support for this association, but all loci provided higher support for Rhinolophoidea plus megabats than for microbat monophyly. For example, with neighbour-joining and logdet distances (all sites included), the bootstrap support deriving from independent genes for Rhinolophoidea plus megabats and microbat monophyly, respectively, was as follows: 12S–16S: 55 and 10; RAG1: 54 and 0; RAG2: 37 and 15; vWF: 25 and 11; BRCA1: 24 and 1. Because none of the strongly supported clades based on individual data sets were mutually incompatible, we concatenated the sequences into combined nuclear and mitochondrial-plus-nuclear data sets. The main principle behind combining data is that it allows for amplification of phylogenetic signal, and increased resolving power, in cases where signal is masked by homoplasy among the individual data sets. Analyses based on concatenated nuclear and mitochondrial-plus-nuclear data sets provided much higher bootstrap support for Rhinolophoidea plus megabats (Fig. 1; Table 1) and statistical tests with both parsimony and maximum likelihood rejected microbat monophyly in favour of the rhinolophoid–megabat alliance (Table 2).

Furthermore, using the variable-length bootstrap[6,7], support for Rhinolophoidea plus megabats increased as a function of the number of resampled base pairs for each locus (Fig. 2a), except for BRCA1. Removing third positions of BRCA1 resulted in the same pattern of increasing support for the Rhinolophoidea–megabat hypothesis (Fig. 2a). Third positions of BRCA1 exhibit two characteristics, base compositional heterogeneity and lineage specific rate variation, that may confound phylogenetic estimations. With or without third positions, BRCA1 did not support microbat monophyly. Bootstrap support with the concatenated data sets increased as a function of the number of re-sampled base pairs, much as for the individual genes (Fig. 2b), but was always higher with similar numbers of nucleotides sampled from the concatenated alignment compared to the individual genes (compare Fig. 2a and b, respectively). This finding parallels results[8] wherein individual mitochondrial genes did not perform as well as random samples of equivalent size that were drawn from the pooled data set. Also, much of the increased resolving power of the pooled data relative to the individual data sets is because of the increased number of base pairs in the pooled data. For many problems in the molecular phylogenetic analysis of mammalian evolutionary history, it is likely that adequate resolving power will only be achieved through data set concatenation; the present finding is simply one example.

Phylogenetic analyses can yield results that are artefacts of the choice of outgroup, long-branch attraction, heterogeneous base composition and site-specific rate variation[9]. We therefore investi-

†Present addresses: Department of Biology, University of California, Riverside, California 92521, USA (E.C.T., M.S.); Bioinformatics, SmithKline Beecham Pharmaceuticals, 1250 South Collegeville Road, UP1345, Collegeville, Pennsylvania 19426-0989, USA (M.J.S.).

gated whether any of these factors contributed to the phylogenetic association of rhinolophoids and megabats. Outgroup choice sensitivity analyses consistently resulted in strong bootstrap support for rhinolophoids plus megabats, irrespective of which taxon was chosen as the outgroup (57%–100% bootstrap support, with 10 of the 16 different analyses performed in excess of 85%). Phylogenetic analyses were also performed with a 10-taxon data set that excluded *Tonatia* and *Mus* because our examination of these data indicated potential problems with long edges and/or base composition involving these two taxa (Table 1). As before, the association of rhinolophoids and megabats was strongly supported (Table 1). Statistical tests with the 10-taxon data set rejected microbat monophyly (Table 2). Finally, maximum likelihood bootstrap analyses that accounted for site-specific rate variation with a gamma distribution of rates provided 100% bootstrap support for the rhinolophoid–megabat association with both the 12-taxon and 10-taxon concatenations of mitochondrial and nuclear genes (Table 1).

Our phylogenetic result is also supported by other types of data. Hutcheon *et al.*[10] suggested that rhinolophoids and megabats are sister taxa based on single-copy DNA hybridization results. Rhinolophoids and megabats also share a high AT bias and an alternative interpretation of the hybridization data was that rhinolophoids and megabats clustered together as an artefact of base composition[10]. For the genes that we sequenced, we did not find evidence for high AT content in megabats and rhinolophoids relative to other chiropterans. For example, among informative sites in our alignment, the percentage of A + T was higher in vespertilionoids (53.5%–56.4%), megabats (52.9%–55.3%) and the emballonurid (55.3%) than in rhinolophoids (48.9%–52.3%) or the phyllostomid (44.2%). These results are inconsistent with an association of

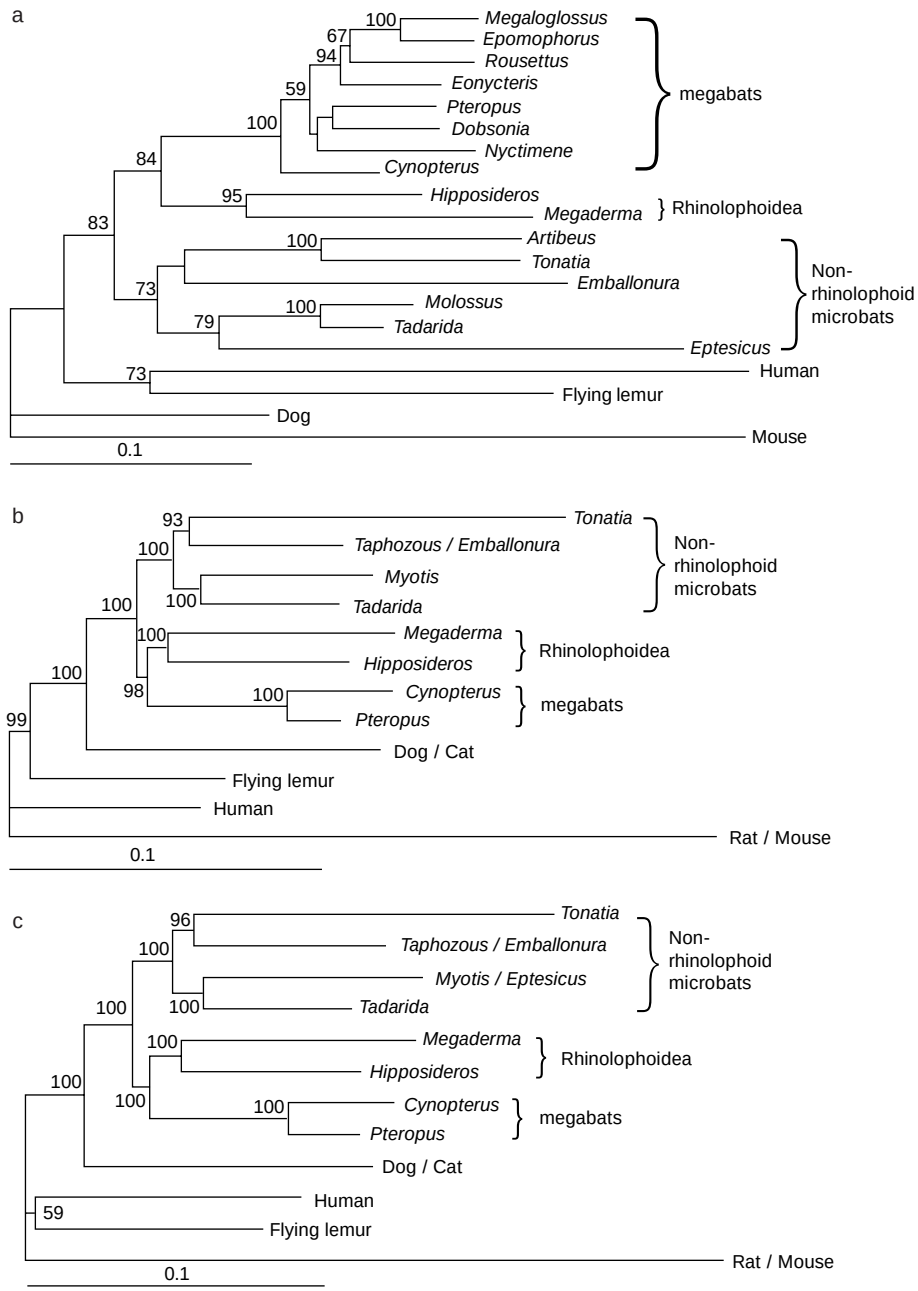

Figure 1 Maximum likelihood trees with branch lengths drawn proportional to amount of sequence change and with maximum likelihood bootstrap figures indicated. **a,** 12S–16S; **b,** combined nuclear genes; **c,** combined nuclear and mitochondrial sequences. **b,c,** A solidus separating two taxonomic names indicates a hybrid sequence involving those two taxa.

Table 1 Bootstrap support for Rhinolophoidea-megabat and microbat monophyly using indicated phylogenetic methods and data positions

	Parsimony		Transversion parsimony		Minimum evolution-Logdet		Minimum evolution-maximum likelihood		Maximum likelihood with gamma		Maximum likelihood	
	RM	MM	RM	MM	RM	MM	RM	MM	RM	MM	RM	MM
12 taxa, nuclear-plus-mitochondrial	98	2	90	1	99	1	100	0	100	0	100	0
20 taxa, mitochondrial	73	11	44	3	58	9	68	5	89	6	77	9
12 taxa, nuclear	90	3	89	9	97	1	98	1	98	1	96	1
10 taxa, mitochondrial	66	8	42	0	50	7	53	4	94	0	79	5
10 taxa, nuclear	62	3	91	8	92	5	89	9	99	0	88	12
10 taxa, nuclear-plus-mitochondrial	91	1	70	1	98	2	97	2	100	0	100	0

RM, Rhinolophoidea–megabats; MM, microbat monophyly. The rationale behind conducting a 10-taxon analysis involved the following: Relative Apparent Synapomorphy Analysis (RASA)[26] indicated lack of significant phylogenetic signal for the 12-taxon, mitochondrial-plus-nuclear data set; such a result can be an indicator of long-branch attraction[26]. Removal of *Tonatia* and *Mus*, the ingroup and outgroup taxa with the longest edges, respectively, resulted in significant phylogenetic signal at $P = 0.001$ (tRASA = 4.699). Also, a chi-square test of homogeneity in base composition (variable sites only) was significant when all 12 taxa were included ($P = 0.00000000$), but not significant when *Tonatia* was eliminated ($P = 0.13$). Logdet values shown above are based on analyses with both variable and constant sites. With the removal of constant sites in the logdet analyses, bootstrap support for the Rhinolophoidea–megabat association with the combined nuclear-plus-mitochondrial data sets was 96% for both the 12-taxon and 10-taxon analyses.

rhinolophoids and megabats that is merely an artefact of high A + T content. Also, results obtained with the logdet method, which addresses potential concerns with heterogeneity of base composition[9], agree with results for other methods in supporting a rhinolophoid–megabat alliance (Table 1). With logdet, bootstrap support for Rhinolophoidea plus megabats with the combined data sets always exceeded 90%, even with the removal of constant sites (Table 1).

Beyond molecular evidence favouring a rhinolophoid–megabat alliance, there are other considerations that support this hypothesis. First, a sister-group relationship between rhinolophoids and megabats, rather than between megabats and all other chiropterans, reduces the duration of the implied ghost lineage for megabats from the early Eocene (the holotype of the earliest bat, *Icaronycteris*, is dated at 53 million years ago[11]) to the late Eocene (there are fossil rhinolophoids at 37–34 million years ago[4,12]). Another non-molecular consideration that supports a rhinolophoid–megabat association is that both groups have distributions that are restricted to the Old World.

Our results have implications for the evolution of both flight and echolocation in bats. First, bats and flying lemurs did not share a flying common ancestor as has been suggested in various morphological analyses conducted over the course of the last century[13,14]. None of our analyses support a sister-group relationship between bats and flying lemur, and indeed this hypothesis is rejected in statistical tests involving the concatenated genes (Table 2). Instead, we find, in agreement with other molecular studies[5], that bats are not part of a monophyletic group that includes other archontans (such as primates and flying lemurs). Although it is likely that the ancestor of bats progressed through an intermediate flying stage[12], we suggest that this must have occurred independently of the evolution of flight in flying lemurs. Numerous presumed synapomorphies for bats and flying lemurs[2], including markedly elongated forelimbs, presence of a humeropatagialis muscle and fusion of several carpal elements into the scaphocentralunate, are convergent features that evolved in association with gliding or flight. Such an interpretation would concur with another morphological analysis[15] which argued against Volitantia and favoured a Primates/Dermoptera clade.

Second, our phylogeny indicates that echolocation either evolved in the ancestor of crown group bats and was then lost in Old World fruitbats or evolved independently in rhinolophoids versus other microchiropterans. Regarding the first scenario, some workers have argued for the implausibility of loss of laryngeal echolocation because echolocation has obvious benefits, with only minimal cost, when coupled with flight[3,16]. We suggest that changes in roosting locations and foraging habits may have obviated the need for echolocation. Also, laryngeal echolocation systems may limit body size because echolocation pulse frequency is coupled to wingbeat rate and ventilation rate. If wingbeat rate, ventilation rate, and pulse frequency became too low, selection for increased body size in an ancestral megabat may have compromised the selective advantage of echolocation. Laryngeal echolocation may have been selected against in favour of enhanced visual acuity and larger body size.

As for the second scenario, several lines of evidence suggest that echolocation may have evolved independently among different

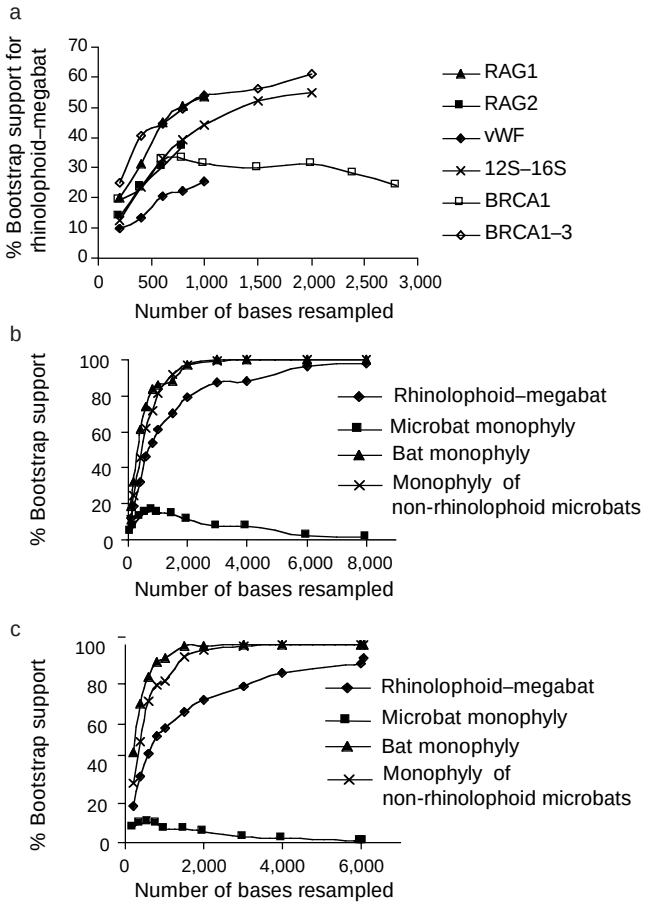

Figure 2 Bootstrap support using neighbour-joining with logdet distances, for various phylogenetic associations. Percentage values are given as a function of the number of resampled nucleotides, and there are various data partitions. **a,** Rhinolophoidea–megabat support for each of the individual loci. **b,** Support for various possible associations involving the bats, using the concatenated nuclear gene data set. **c,** Support for bat phylogenetic associations involving the nuclear-plus-mitochondrial data set.

Table 2 Results of statistical tests on microbat monophyly and the Volitantia concept

	Additional steps to maximum parsimony tree*	Decrease in likelihood to maximum likelihood tree†	Kishino Hasegawa test parsimony	Templeton test parsimony	Kishino Hasegawa test maximum likelihood
Microbat monophyly					
12-taxon analyses	+15	24.511; s.d. = 9.445	0.0394	0.0394	0.0095
10-taxon analyses	+14	36.528; s.d. = 13.592	0.0433	0.0433	0.0072
Volitantia					
12-taxon analyses	+65	106.063; s.d. = 19.355	0.0001	0.0001	<0.0001
10-taxon analyses	+82	154.461; s.d. = 23.638	<0.0001	<0.0001	<0.0001

* Score is 7,128 for 12 taxa and 5,189 for 10 taxa.
† − lnL = 44,397.611 for 12 taxa and 36,346.401 for 10 taxa.
s.d. standard deviation.

microbats. Echolocation is known to occur among other mammalian groups including cetaceans and insectivores, as well as the megabat Rousettus[12,17–19]. The cost of laryngeal echolocation is relatively low when coupled with flight[20]. Microbat echolocation systems are highly variable, but nonetheless taxon-specific, illustrating the evolutionary flexibility of bat echolocation. Some bats emit echolocation pulses orally whereas in other taxa they are emitted through the nasal cavity. In addition, echolocation pulses range from frequency-modulated to constant-frequency[12]. There are numerous putative morphological synapomorphies for microbats. However, many such presumed synapomorphies of microchiropterans, including features related to flight and ventilation, are either directly related to echolocation or are functionally correlated with the echolocation system. Thus, they do not represent independent evolutionary events in support of microbat monophyly. For example, the enlarged orbicular process on the malleus, common to all microbats, may help to avoid self-deafening and/or improve the ability of the middle-ear ossicles to transmit high-frequency sounds with minimal delay time[4]. Features of the postcranial skeleton in microbats include anterior laminae and enlarged posterior laminae on the ribs. Rib laminae stiffen the ribcage and provide a greater area for muscle origins, for example, muscles of the serratus anterior complex. In microbats, there is a close association between wingbeat and sound emission[21]; Simmons and Geisler[4] hypothesized that the anterior and posterior laminae on the ribs, along with the expanded anterior serratus, increase the efficiency of the flight mechanism, ventilation and the production of echolocation calls. Rather than being synapomorphic for all microchiropterans, features such as expanded rib laminae may have evolved independently in rhinolophoids and other microbats in conjunction with the evolution of laryngeal echolocation. □

Methods

Data collection and taxa representation

Taxon sampling was designed to include at least one representative (in some cases two) of the four proposed microbat superfamilies[3], as well as megabat representatives, combined with diverse outgroups, for all loci. The microbat taxa and their loci were: (1) superfamily Emballonuroidea, family Emballonuridae: *Taphozous* sp. (BRCA1, RAG1, RAG2); *Emballonura atrata* (12S–16S, vWF). (2) Rhinolophoidea, Megadermatidae: *Megaderma lyra* (all loci); Hipposideridae: *Hipposideros commersoni* (all nuclear loci), *Hipposideros galeritus* (12S–16S). (3) Phyllostomoidea, Phyllostomidae: *Tonatia bidens* (all loci). (4) Vespertilionoidea, Vespertilionidae: *Myotis daubentoni* (BRCA1, RAG1, RAG2), *Myotis velifer* (vWF), *Eptesicus fuscus / capensis* (12S–16S); Molossidae: *Tadarida brasiliensis* (all loci), *Molossus sinaloae* (12S–16S). Megabats included the following taxa: Pteropodidae: *Cynopterus sphinx* (all nuclear loci), *Cynopterus brachyotis* (12S–16S), *Pteropus rayneri* (BRCA1, RAG1, RAG2), *Pteropus hypomelanus* (12S–16S, vWF) and 12S–16S from *Nyctimene robinsoni, Dobsonia moluccensis, Megaloglossus woermanni, Epomophorus wahlbergi, Rousettus amplexicaudatus* and *Eonycteris spelaea.* Outgroup taxa included the following: flying lemur (*Cynocephalus variegatus*, Dermoptera; all loci), human (*Homo sapiens*, Primates; all loci), mouse (*Mus musculus*, Rodentia; all loci except vWF), rat (*Rattus norvegicus*, Rodentia; vWF), dog (*Canis familiaris*, Carnivora; all loci except RAG1 and RAG2) and cat (*Felis catus*, Carnivora; RAG1 and RAG2). Accession numbers for the 10 mitochondrial and 32 nuclear sequences new to this study are: AF203737–AF203778. To obtain the complete alignments these sequences were combined with those already available—mitochondrial: AF203726, AF17929, AF179288, AF061340, U61082, U93053–5, U93059, U93061, U93064–5, U93068, U93070, U97073, AF069536–8, AF044606–7, AF044610–11, AF044620, AF044626, J01420, U96639, J01415; nuclear: M29474–5, M94633, M64796, U31616, U31622, AF061061, U31605–6, L76227, M25851, AJ224673, U68174, AF019081, U50709, L78833.

Polymerase chain reaction (PCR) and sequencing for 12S–16S and vWF were as described elsewhere[22,23]. Primers for RAG1, RAG2 and BRCA1 were designed on the basis of conserved regions of sequence alignments involving the few mammalian taxa available in GenBank. BRCA1 PCR primers were the following (numbers refer to the location of the 5′ end of the primer sequence in human exon 11): BRCA1F126: 5′-GTTTCAAACTTG-CATGTGGAGCC-3′; BRCA1R3012: 5′-GTTGGAAGCAGGGAAGCTCTTCATC-3′. PCR primers for RAG genes were the following (location of the 5′ end of the primer sequence, in the single human exon of both genes, is indicated by the number included in the primer label): RAG1: RAG1F1705: 5′-GCTTTGATGGACATGGAAGAAGACAT-3′; RAG1R2864: 5′-GAGCCATCCCTCTCAATAATTTCAGG-3′; RAG2: RAG2F220 5′-GATTCCTGC-TA(CT)CT(TC)CCTCCTCT-3′; RAG2R995 5′-CCCATGTTGCTTCCAAACCATA-3′.

Phylogenetic analysis

Data sets for individual loci and combined data sets were all analysed with maximum parsimony, transversion parsimony, minimum evolution with maximum likelihood and logdet distances, and maximum likelihood. Individual data sets, for both 10 and 12 taxa, provided strong bootstrap support (that is, > 90% in parsimony analyses) for at least one clade (RAG2; 12 taxa) and as many as eight different clades (BRCA1; 12 taxa). In all parsimony analyses gaps were treated as missing data. Both parsimony and minimum evolution analyses employed tree bisection-reconnection branch swapping. In parsimony searches 10 random input orders were used. Parsimony and minimum evolution bootstrap analyses involved 500 replicates. Maximum likelihood trees were obtained with heuristic searches (10 random input orders) under the HKY-85 model of sequence evolution with a 2 to 1 transition to transversion ratio and an assumption of equal rates across sites for the mitochondrial, nuclear and mitochondrial-plus-nuclear data sets. For each of the resulting trees, we then obtained maximum likelihood estimates of the transition to transversion ratio (20 taxa: mitochondrial(m) = 3.29; 12 taxa: nuclear(n) = 2.47; mitochondrial-plus-nuclear (m+n) = 2.52; 10 taxa: m = 3.30; n = 2.45; m+n = 2.56) and the shape parameter for the gamma distribution (20 taxa: m = 0.25; 12 taxa: n = 0.77; m+n = 0.53; 10 taxa: m = 0.25; n = 61; m+n = 0.44). These values were used in subsequent maximum likelihood analyses, including bootstrap resampling (200 replications). Phylogenetic methods that were employed in outgroup sensitivity analyses included quartet puzzling, minimum evolution with maximum likelihood and logdet distances, and maximum parsimony. Kishino and Hasegawa[24] and Templeton[25] tests were used to evaluate *a priori* hypotheses. All analyses were performed using PAUP* 4.0b2 (ref. 7).

Received 20 October; accepted 15 November 1999.

1. Novacek, M. J. in *Proceedings of the 5th International Bat Research Conference* (eds Wilson, D. E. & Gardner, A. L.) 317–330 (Texas Tech, Lubbock, 1980).
2. Simmons, N. B. *Bat relationships and the origin of flight. Symp. Zool. Soc. London* **67,** 27–43 (1995).
3. Hill, J. E. & Smith, J. D. in *Bats: A Natural History* (Heffers, England, 1984).
4. Simmons, N. B. & Geisler, J. H. Phylogenetic relationships of *Icaronycteris*, *Archaeonycteris*, *Hassianycteris* and *Palaeochiropteryx* to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. *Bull. Am. Mus. Nat. Hist.* **235,** 1–182 (1998).
5. Pumo, D. E. *et al.* Complete mitochondrial genome of a neotropical fruit bat, *Artibeus jamaicensis*, and a new hypothesis of relationships of bats to other Eutherian mammals. *J. Mol. Evol.* **47,** 709–717 (1998).
6. Springer, M. S., Amrine, H. M., Burk, A. & Stanhope, M. J. Additional support for Afrotheria and Paenungulata, the performance of mitochondrial versus nuclear genes, and the impact of data partitions with heterogenous base composition. *Syst. Biol.* **48,** 65–75 (1999).
7. Swofford, D. L. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. (Sinauer, Sunderland, MA, 1998).
8. Cummings, M. P., Otto S. P. & Wakeley, J. Sampling properties of DNA sequence data in phylogenetic analysis. *Mol. Biol. Evol.* **12,** 814–822 (1995).
9. Swofford, D. L., Olsen, G. J., Waddell, P. J. & Hillis, D. M. in *Molecular Systematics* (eds Hillis, D. M., Moritz, C. & Mable, B. K.) 407–514 (Sinauer, Sunderland, MA, 1996).
10. Hutcheon, J. M., Kirsh, J. A. W & Pettigrew, J. D. Base-compositional biases and the bat problem. III.The question of microchiropteran monophyly. *Phil. Trans. R. Soc. Lond. B* **353,** 607–617 (1998).
11. Woodburne, M. O. & Swisher, C. C. in *Geochronology, Time Scales and Global Stratigraphic Correlation* (eds Beggren, W. A., Kent, D. V., Aubry, M. & Hardenbol, J.) *Special Publ. Soc. Sedim. Geol.* **54** 335–364 (Tulsa, Oklahoma, 1995).
12. Altringham, J. D. *Bats: Biology and Behaviour.* (Oxford Univ. Press, New York, 1996).

13. Miller, G. S. The families and genera of bats. *Bull. US Natl Mus.* **57,** 1–282 (1907).
14. Novacek, M. J. & Wyss, A. R. Higher-level relationships of the recent eutherian orders: morphological evidence. *Cladistics* **2,** 257–287 (1986).
15. Beard, K. C. in *Primates and their relatives in phylogenetic perspective* (ed. MacPhee, R. D. E.) 63–90 (Plenum, New York, 1993).
16. Rayner, J. M. V. The cost of being a bat. *Nature* **350,** 383–384 (1991).
17. Gould, E. Evidence for echolocation in the Tenrecidae of Madagascar. *Proc. Am. Phil. Soc.* **109,** 352–360 (1965).
18. Tomasi, T. E. Echolocation by the short-tailed shrew *Blarina brevicauda*. *J. Mammal.* **60,** 751–759 (1979).
19. Forsman, K. A. & Malmquist, M. G. Evidence for echolocation in the common shrew, *Sorex araneus*. *J. Zool. Lond.* **216,** 655–662 (1988).
20. Speakman, J. R & Racey P. A. No cost of echolocation for bats in flight. *Nature* **350,** 421–423 (1991).
21. Kalko, E. K. V. Coupling of sound emission and wing-beat in naturally foraging European pipistrelle (*Microchiroptera: Vespertilionidae*). *Folia Zool.* **43,** 363–376 (1994).
22. Springer, M. S. *et al.* Endemic African mammals shake the phylogenetic tree. *Nature* **388,** 61–64 (1997).
23. Stanhope, M. J. *et al.* Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. *Proc. Natl Acad. Sci. USA* **95,** 9967–9972 (1998).
24. Kishino, H. & Hasegawa, M. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order within Hominoidea. *J. Mol. Evol.* **29,** 170–179.
25. Templeton, A. R. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37,** 221–244 (1983).
26. Lyons-Weiler, J., Hoelzer, G. A. & Tausch, R. J. Relative Apparent Synapomorphy Analysis (RASA) I: the statistical measurement of phylogenetic signal. *Mol. Biol. Evol.* **13,** 749–757 (1996).

Acknowledgements

This work was supported by grants from the Training and Mobility of Researchers programme of the European Commission to M.J.S., and the National Science Foundation to M.S.S.

Correspondence and requests for materials should be addressed to M.J.S. (e-mail: Michael_J_Stanhope@sbphrd.com) or M.S.S. (e-mail: mark.springer@ucr.edu).

Human cerebellar activity reflecting an acquired internal model of a new tool

Hiroshi Imamizu*, Satoru Miyauchi†, Tomoe Tamada*, Yuka Sasaki†‡, Ryousuke Takino§, Benno Pütz*‡, Toshinori Yoshioka* & Mitsuo Kawato*‖

** JST/ERATO Kawato Dynamic Brain Project, 2-2 Hikaridai, Seika-cho, Soraku-gun, Kyoto 619-0288, Japan*
† Communications Research Laboratory, 588-2, Iwaoka, Nishi-ku, Kobe, Hyogo 651-2401, Japan
§ Shiraume Gakuen College, 1-830, Ogawa-cho, Kodaira-shi, Tokyo 1878570, Japan
‖ ATR Human Information Processing Research Laboratories, 2-2 Hikaridai, Seika-cho, Soraku-gun, Kyoto 619-0288, Japan

Theories of motor control postulate that the brain uses internal models of the body to control movements accurately. Internal models are neural representations of how, for instance, the arm would respond to a neural command, given its current position and velocity[1–6]. Previous studies have shown that the cerebellar cortex can acquire internal models through motor learning[7–11]. Because the human cerebellum is involved in higher cognitive function[12–15] as well as in motor control, we propose a coherent computational theory in which the phylogenetically newer part of the cerebellum similarly acquires internal models of objects in the external world. While human subjects learned to use a new tool (a computer mouse with a novel rotational transformation), cerebellar activity was measured by functional magnetic resonance imaging. As predicted by our theory, two types of activity were observed. One was spread over wide areas of the cerebellum and was precisely proportional to the error signal that guides the acquisition of internal models during learning. The other was confined to the area near the posterior superior fissure and remained even after learning, when the error levels had been equalized, thus probably reflecting an acquired internal model of the new tool.

Most neuroimaging studies have found that the regional blood flow in the human cerebellum increases significantly at the beginning of learning for a new motor or cognitive task and decreases as the learning proceeds[16–19]. These results are often interpreted as meaning that the cerebellum is involved only in the early phase of learning and is not a memory site, that is, it does not store internal models. Here we present a different interpretation (see also ref. 15) based on our computational theory and experimental results.

Previous cerebellar learning theories[20–22] make no specific predictions about the activity of internal models (see Supplementary Information for details). We have proposed that multiple internal models exist and that they compete to learn new environments and tools[23]. During the learning, all of these multiple internal models receive a copy of the error signal and only one or a few learn the new transformation, thereby reducing the error signal and localizing the new activity to a distinct region of the cerebellum. The two types of

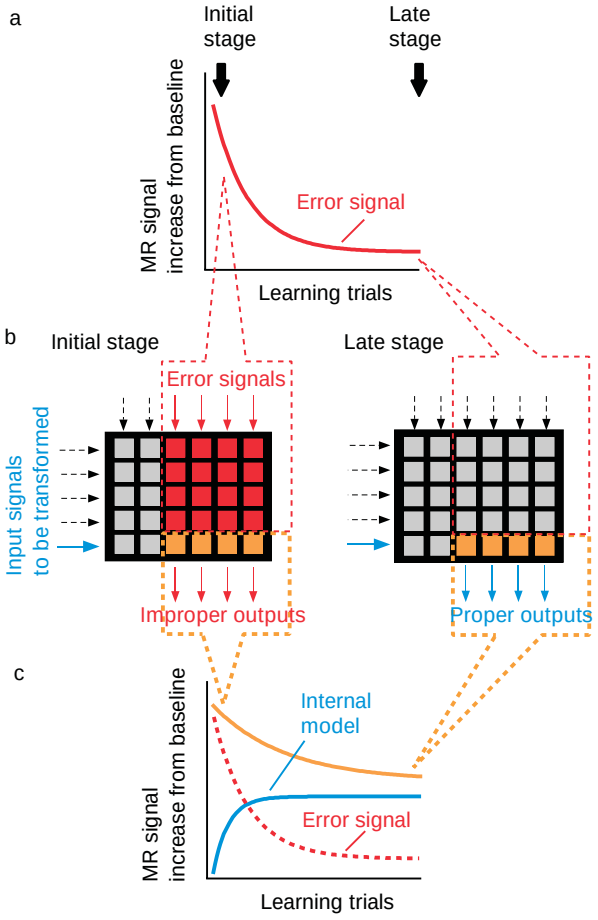

Figure 1 Changes in cerebellar activity predicted by the learning theory of internal models. **a**, Signal intensity change caused by error signals (red curve). **b**, Activity maps of the initial and late stages of learning. Each small square represents a unit of multiple internal models. Orange squares indicate regions where the internal model is acquired. **c**, Signal intensity change in the regions where the internal model is acquired. Orange curve indicates activity in the orange squares (the sum of activity reflecting error signals (red curve) and that reflecting the acquired internal model (cyan curve)).

‡ Present addresses: Massachusetts General Hospital NMR Center, 149, 13th Street, Charlestown, Massachusetts 02129, USA (Y.S.); Kernspintomographie, Max-Planck-Institut fur Psychiatrie, Kraepelinstrasse 10, 80804 Muenchen, Germany (B.P.).