

Volume 12 Number 7 • July 2001

Mammalian Genome

Incorporating *Mouse Genome*



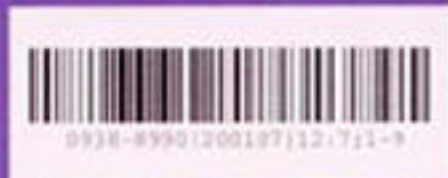
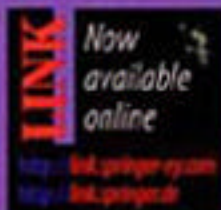
Official Journal of the
International Mammalian Genome Society

Sponsored by the
International Committee for Standardized Genetic Nomenclature

Editors
Lee M. Silver • Joseph H. Nadeau • Steve D.M. Brown



Springer



335 MAMGEC 12(7) 485-574 (2001)
ISSN 0938-8990

COVER LEGEND

North America's only living native marsupial, the Virginia Opossum, is portrayed by San Francisco Bay artist Pat Sherwood. Marsupials lay at the center of a fundamental problem in mammalian taxonomy-- the sister relations of egg-laying, marsupial, and placental mammals. In this issue, Killian *et al.* provide the first sequence data from a large nuclear gene that reconcile genetic, epigenetic, and morphology based predictions of higher-order mammalian phylogeny.

Marsupials and Eutherians reunited: genetic evidence for the Theria hypothesis of mammalian evolution

J. Keith Killian,¹ Thomas R. Buckley,² Niall Stewart,³ Barry L. Munday,³ Randy L. Jirtle¹

¹Departments of Radiation Oncology and Pathology, Duke University Medical Center, Box 3433, Durham, North Carolina 27710, USA

²Department of Biology, Duke University, Durham, North Carolina, 27708, USA

³School of Biomedical Science, University of Tasmania, Launceston, Tasmania 7250, Australia

Received: 8 December 2000 / Accepted: 01 February 2001

Abstract. The three living monophyletic divisions of Class Mammalia are the Prototheria (monotremes), Metatheria (marsupials), and Eutheria ('placental' mammals). Determining the sister relationships among these three groups is the most fundamental question in mammalian evolution. Phylogenetic comparison of these mammals by either anatomy or mitochondrial DNA has resulted in two conflicting hypotheses, Theria and Marsupionta, and has fueled a "genes versus morphology" controversy. We have cloned and analyzed a large nuclear gene, the mannose 6-phosphate/insulin-like growth factor II receptor (*M6P/IGF2R*), from representatives of all three mammalian groups, including platypus, echidna, opossum, wallaby, hedgehog, mouse, rat, rabbit, cow, pig, bat, tree shrew, colugo, ringtail lemur, and human. Statistical analysis of this nuclear gene unambiguously supports the morphology-based Theria hypothesis that excludes monotremes from a clade of marsupials and eutherians. The *M6P/IGF2R* was also able to resolve the finer structure of the eutherian mammalian family tree. In particular, our analyses support sister group relationships between lagomorphs and rodents, and between the primates and Dermoptera. Statistical support for the grouping of the hedgehog with Ferungulata and Chiroptera was also strong.

Introduction

The Class Mammalia includes the egg-laying monotremes, as well as the more familiar live-birthing members. The viviparous mammals are further divided into marsupials and eutherians, depending on reproductive parameters such as placentation and the extent of intrauterine gestation of the developing offspring. Marsupial young generally exit the womb at a significantly premature stage of development relative to eutherians (Griffiths 1999; Renfree and Shaw 1999). A sister relationship between the marsupials and eutherians, exclusive of monotremes, was deduced from anatomical data (Marshall 1979). Morphological synapomorphies that distinguish marsupials and eutherians from monotremes include: olfactory bulb mitral cell organization, braincase architecture, cranial nerve distribution, inner ear cochlear architecture, mammary glands with teats, tooth enamel, molar dentition, and foot anatomy (Lewis 1983; Marshall 1979).

Recent analyses of tribosphenic molar fossils concluded that monotremes and marsupials/eutherians descended from ancestors in geographically distinct areas: living monotremes are descendants of an ancient mammalian clade endemic to Gondwanan land

The *M6P/IGF2R* nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers: AF339157-163 (bat, rabbit, wallaby, lemur, tree shrew, hedgehog, colugo), AF339885 (pig), AF342813 (opossum), and AF342814 (echidna).

Correspondence to: Randy L. Jirtle; E-mail: jirtle@radonc.duke.edu

masses, whereas marsupials and eutherians shared a common ancestor upon Laurasian continents (Luo et al. 2001). In accord with the conflict hypothesis for the origin of genomic imprinting (Moore and Haig 1991), the differential expression of parental alleles of certain fetal growth-regulatory genes is another therian apomorphy shown to not be present in Prototherian ancestors (Killian et al. 2000). Furthermore, delineation of retroposon elements in the three mammalian groups supports the earlier divergence of Prototheria (Gilbert and Labuda 2000).

The value and accuracy of decades of morphological study have been discounted recently by mitochondrial DNA inference, which has reinvigorated Gregory's claim that monotremes are highly-derived marsupials (Gregory 1947; Janke et al. 1997; Penny et al. 1999). The accurate resolution of the mammalian family tree is essential to determining whether the unique physical attributes of marsupials and eutherians, as well as the imprinting of certain genes, have evolved once or numerous times. If the Marsupionta hypothesis proves correct, then either these characteristics represent phenotypic and epigenetic convergences in marsupials and eutherians, or else they were gained and then lost in the monotreme lineage. Substantiation of the Theria hypothesis would implicate a more parsimonious single origin for these features.

The incongruent mammalian family trees resulting from mitochondrial sequence analysis and morphological characters have contributed to a divisive debate over their utilities in phylogenetic inference (Gura 2000). Thus far, only small nuclear genes have been applied to the Theria/Marsupionta question (i.e., <1.5 kb open reading frame; Kullander et al. 1997; Toyosawa et al. 1999), and they have failed to convincingly validate either hypothesis. In an effort to resolve the higher order structure of the mammalian family tree, we have performed phylogenetic inference based on the mannose-6-phosphate/insulin-like growth factor II receptor (*M6P/IGF2R*) gene from representatives of all three mammalian groups, using chicken and fish homologs as outgroups.

The 48 exons of *M6P/IGF2R* produce a large 9-kb transcript, which is translated into a protein of over 2400 amino acids (Jirtle 1999). Studies in mice have established that the *M6P/IGF2R* is an essential, single-copy nuclear gene that is critically involved in embryogenesis (Jirtle 1999). The *M6P/IGF2R* has a long and well-established evolutionary history in the animal kingdom, with definitive homologs identified in fish, aves, and mammals (Jirtle 1999; Killian et al. 2000; Nadimpalli et al. 1999). Biochemical evidence also indicates the presence of a homologous gene in invertebrates (Lakshmi et al., 1999). We have isolated and sequenced novel *M6P/IGF2R* homologs from monotremes, marsupials, and eutherians, including echidna, wallaby, hedgehog, pig, bat, tree shrew, colugo, and ringtail lemur. To generate the mammalian phylogeny, these sequences were used in addition to previously characterized *M6P/IGF2R* homologs from chicken, platypus, opossum, mouse, rat, cow, and human (Jirtle 1999; Killian et

Table 1. Cross-species *M6P/IGF2R* PCR primers.

Forward Primers 5' to 3'		Reverse primers 5' to 3'	
F primer ^a	Sequence	R primer ^a	Sequence
311F	CTGTGCAGTTACACATGGGAAGC	1078R	GGCATACTCAGTGATCCACTC
589F	GGAACTCCTGAATTTGAACTGCCACAG	1498R	GTAGGTGCAGTCCACCTC
617F	TGTGTGCATTAACCTTTGAATGGAGGAC	1505R	GTGAAGAAGTAGGTGCAATC
1428F	GGCTTTCAGCGGATG	1642R	CCATCCACTGCTTCCCA
1450F	ATGAGTGCATAAACTTTGAGTGC	1921R	GCACTTTCTAGATCACCTGG
1451F	TGTCATAAACTTTGAGTGCAA	2043R	TCAAAGAAAAACCTGCCTG
1650F	GAACAGAATTGGGAAGC	4225R	GTCATTGAAAGGTGGGAGGC
1655F	CAGAATCAGAACAGAATTGGGAAGCTGTGG	5153R	AAGCCTCATAACCACCACTGCG
1657F	TTGGGAAGCAGTGGATG	6018R	CTCCCAATGCCTCATCTTTCATCAC
1929F	CCAGGTGATCTAGAAAAGTGC	6534R	CCATTCCACTCTGCACCTCCTG
2410F	TACCTACAACCTCCGGTGG	6877R	GGGTACAGTGGAAAAAGAT
2415F	AACTCTACTACAACCTCCGGTGGTACAC	7250R	ATCTCTCCATCAGCCACTC
2883F	CAACTGGGAGTGTGTGGTC	7252R	CTGGATTCTTCCATAAGCCA
4230F	TTTGAGTGGCGAACCCAGTATGCCCTG	7588R	GTCCTCGTGCCTGCTGCATG
5834F	GTGAATGGTGTATCGTTGGCCTCCAG	oligo 8	GACCACGCGTATCGATGTCGACT ₁₆ V
5886F	CCCTTCATATTCAATGGGAAGAGC	oligo 9	GACCACGCGTATCGATGTCGAC
6129F	CCTCCAAAGAAGATGGAGTGC		
7263F	GAGAATGAAACGGAGTGGCTTATGGA		

^a Oligonucleotides are numbered relative to the 9090-bp human *M6P/IGF2R* transcript (GenBank Accession number: NM_000876). PCR primer use is described in Materials and methods. Oligo 8 and oligo 9 are commercially available (Roche Boehringer Mannheim, Indianapolis, Ind.).

al. 2000). Inclusion of these novel sequences allowed us to split long branches in the phylogeny and to test the ability of the *M6P/IGF2R* to resolve the interordinal relationships among mammals.

Materials and methods

Tissue samples. Short-beaked echidna (*Tachyglossus aculeatus*) samples were obtained from animal victims of car accidents. Samples of the Tasmanian subspecies of *Macropus rufogriseus*, the Bennett's wallaby, were obtained from Lenah Game Meats, Launceston, Tasmania. European hedgehog (*Erinaceus europaeus*) tissue was obtained postmortem from an ailing animal euthanized by a veterinarian at Duke University Medical Center Vivarium. New Zealand white rabbit tissue was obtained from the Duke University Medical Center Vivarium. Domestic pig (*Sus scrofa*) muscle was obtained from Neese's Sausage, Burlington, North Carolina, USA. Brown bat (*Myotis lucifugus*) samples were provided by the North Carolina Wildlife Commission. Tree shrew (*Tupaia glis*) tissue was donated from the archives of David Fitzpatrick, Duke University Medical Center. Philippine flying lemur (*Cynocephalus volans*) tissue was a kind gift from the Field Museum, Chicago, USA. Ringtail lemur (*Lemur catta*) tissues were from the archives of the Duke University Primate Facility. In most cases, tissues were dispatched to Duke University Medical Center in RNALater (Ambion, Austin, TX) until DNA and RNA extraction.

***M6P/IGF2R* cloning.** Total RNA was isolated from 50 mg of animal tissue by homogenization in RNA-Stat 60 (Tel-Test, Friendswood, Texas). First-strand cDNA was synthesized from 1–5 µg of total RNA by using SuperScript II (Life Technologies, Baltimore, Md.) and oligo 8 (Roche Boehringer Mannheim, Indianapolis, Ind.) as the primer for reverse transcription (Table 1). We have previously described the design and implementation of a battery of non-degenerate PCR primers for cross-species *M6P/IGF2R* amplification of opossum and platypus orthologues (Killian et al. 2000). Briefly, the use of primers 311F, 589F, and/or 617F with 1078R yielded a correct amplicon for all species tested and allowed us to obtain species-specific *M6P/IGF2R* sequence (Table 1). To reduce the complexity of the PCR template, we then performed 3'-RACE according to the manufacturer's protocol (Life Technologies) with a species-specific *M6P/IGF2R* primer and oligo 9 (Table 1) (Roche Molecular Biochemicals, Indianapolis, Ind.). Typical PCR reactions used 1% of the RNA-to-cDNA RT products, 1.5 U Expand Long Template DNA polymerase mix (Roche Molecular Biochemicals) 1 µM of each primer, and 500 µM dNTPs in a 50 µl PCR reaction volume (94°C × 20 s, 55°C × 5 s, and 68°C × 3 min for 35 cycles). Unpurified 3'-RACE product (1 µl) was then used as template in additional cross-species *M6P/IGF2R* PCR amplifications by using various combinations of non-degenerate cross-species *M6P/IGF2R* oligos and identified species-specific oligos; the PCR reaction parameters were identical to those previously described. PCR products were analyzed by agarose gel

electrophoresis, and appropriately sized fragments were excised, purified (GenElute, Sigma Chemical Co., St. Louis, Mo.), and PCR sequenced. A partial fish (*Xiphophorus maculatus* × *Xiphophorus helleri*) *M6P/IGF2R* sequence was obtained from GenBank (Accession Number AJ278449).

Phylogenetic methods. *M6P/IGF2R* amino acid sequences were aligned using ClustalX (Higgins et al. 1992), with regions of ambiguous homology excluded prior to phylogenetic analysis. The assumption of amino acid frequency stationarity among taxa (i.e., constant amino acid frequencies among lineages) was evaluated using χ^2 tests in Tree-Puzzle 5.0 (Strimmer and von Haeseler 1996). Phylogenetic trees were constructed by using maximum parsimony (MP), maximum likelihood (ML), minimum evolution (ME), and split decomposition (reviewed by Swofford et al. 1996). We performed the MP analyses in PAUP*4.0b4a (Swofford 1998), using equal weights and the Blosum 80 step matrix (Henikoff and Henikoff 1992). All MP analyses employed a heuristic search strategy with start trees obtained via stepwise addition with 10 random addition replicates followed by TBR branch swapping. ML trees were constructed with a global search by using Proml from the Phylip 3.6a package (Felsenstein 1993) under the Dayhoff et al. (1978) model with rate categories constrained to fit a discrete gamma distribution (Yang 1994). The gamma distribution was estimated by using Tree-Puzzle 5.0. ME trees were constructed in PAUP*4.0b4a from distances estimated in Protdist from Phylip 3.6a under the Dayhoff et al. (1978) model with a gamma correction as described above. Log Determinant (Lockhart et al. 1994) distances with conserved site removal were estimated in SplitsTree 3.0 (Huson 1998). Shimodaira-Hasegawa (1999) tests were performed with PAML 3.0 (Yang 1997) under the Dayhoff et al. (1978) and JTT-F (Jones et al. 1992) models with among-site rate variation described by using a discrete gamma distribution. The test statistic for the Shimodaira-Hasegawa (1999) test was obtained by comparing the ML topology with the most likely topology under the constraint that the marsupial and monotreme taxa form a monophyletic group.

Results and discussion

Alignment of the *M6P/IGF2R* genes yielded a data set containing 2257 amino acid sites from each of the 15 mammals and the chicken outgroup. Of these sites, 1408 were varied and 973 were parsimony informative. We report herein only the analyses of the amino acid sequences that contained sufficient variation to generate robust estimates of the mammalian phylogeny. All of our phylogenetic analyses strongly support the grouping of the eutherian mammals with the marsupials, consistent with the traditional. Theoria hypothesis, but not with the Marsupionta hypothesis (Fig. 1). In particular, MP, ML, and ME analyses of *M6P/IGF2R* sequences resulted in 100% bootstrap support for the grouping of marsupials

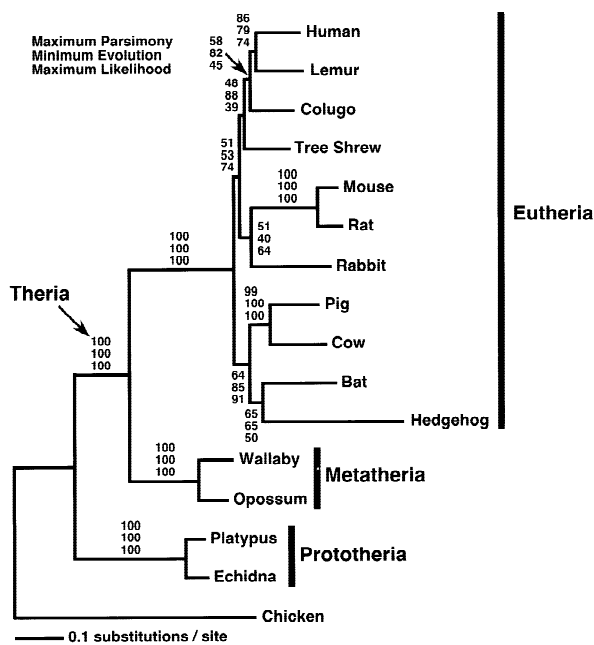


Fig. 1. Phylogenetic tree constructed by maximum likelihood from *M6P/IGF2R* amino acid sequences. Bootstrap values showing statistical support are given adjacent to each node. From the top: weighed maximum parsimony, minimum evolution [Dayhoff et al. (1978) model with a Γ -correction], and maximum likelihood [Dayhoff et al. (1978) model with distributed rates].

and eutherian mammals (Fig. 1). Additionally, using the conservative Shimodaira-Hasegawa (1999) test, we were able to reject ($P < 0.001$) the null hypothesis that there is no difference in likelihood between the ML topology and the constrained Marsupionta tree. Therefore, the results of this test indicate that the data are inconsistent with the Marsupionta hypothesis.

Because the χ^2 tests for amino acid frequency stationarity indicated that the marsupials and the chicken had deviating amino acid compositions, we constructed minimum evolution trees and split decomposition networks from log Determinant distances (Lockhart et al. 1994) with conserved site removal (Penny et al. 1999; Waddell et al. 1999a). These analyses (data not shown) still supported the Theria clade with 97% bootstrap support under split decomposition. In addition, to test the sensitivity of our analyses to the outgroup selected, we restricted our alignment to 764 amino acid sites in order to use a partial fish *M6P/IGF2R* sequence. The addition of the fish sequence had no effect on the relationships among the three major mammalian lineages, with the Theria hypothesis receiving 97% bootstrap support under weighted maximum parsimony, and 87% bootstrap support under minimum evolution. This observation indicates that our results are insensitive to outgroup selection. Therefore, support for the Theria hypothesis is strongly independent of the method of phylogenetic reconstruction employed and does not stem from an incorrect rooting of the mammalian tree.

Our phylogenetic analyses also support (64% to 91%) the grouping of the hedgehog with the Feruungulata. This relationship was predicted by Waddell et al. (1999b), but contradicts that of some analyses of whole mitochondrial genomes (Mouchaty et al. 1999). We also note that our grouping of hedgehog with the Feruungulata is consistent with other nuclear gene analyses (Stanhope et al. 1998) and with maximum likelihood analyses of 1st and 2nd codon positions from whole mitochondrial genomes (Sullivan and Swofford 1997). The estimates of bootstrap support are moderate (50%–65%) for the grouping of the hedgehog with the bat sequence. This relationship is not surprising, given that Mouchaty et al. (2000) observed a sister group relationship between another

insectivore, the mole, and the bat. The observation that phylogenetic analyses of a number of nuclear genes support the affinity of the hedgehog with the Feruungulata and/or Chiroptera (Stanhope et al. 1998; Springer et al. 1999; Waddell et al. 1999b; this study) indicates that the hedgehog is almost certainly misplaced in most studies of whole mitochondrial genomes.

All of our analyses support the Euarchonta clade (Waddell et al. 1999b), containing the orders Primates, Dermoptera, and Scandentia. Estimates of bootstrap support are low under MP (48%) and ML (39%), yet they are high under ME (88%). This grouping appears to conflict with the analyses of whole mitochondrial genomes described by Schmitz et al. (2000); however, the bootstrap support values they obtained for a Lagomorph + Scandentia clade are low, and a Scandentia + Primate grouping could not be rejected when the complete data set was used. The phylogenetic analyses of four nuclear genes and three mitochondrial genes described by Teeling et al. (2000) also yielded a Dermoptera + Primate clade with moderate bootstrap support, consistent with the results of our study.

Interestingly, all of our phylogenetic analyses support the grouping of rodents and lagomorphs in the Glires clade. The Glires grouping is somewhat controversial, although support from morphological (Liu and Miyamoto 1999), paleontological (Meng et al. 1994), and embryological (Luckett and Hartenberger 1993) studies is strong. Estimates of statistical support from various molecular data sets (Halanych 1998; Huchon et al. 1999; Waddell et al. 1999a; Robinson-Rechavi et al. 2000) have typically been moderate, as is the bootstrap estimate we have obtained under ML (64%). We suspect that the generally low level of support for the Glires clade observed in all molecular studies to date stems in part from poor taxon sampling of both lagomorphs and rodents.

The phylogenetic relationships among Eutherian mammals that we have recovered are largely consistent with the predictions of Waddell et al. (1999b). These observations are important because they indicate that the *M6P/IGF2R* gene provides a good source of characters for resolving the higher-order mammalian phylogeny. Additionally, these results indicate that the *M6P/IGF2R* gene is a promising candidate for further resolving relationships among other vertebrate groups.

The strong genetic support that we have obtained for the Theria hypothesis strikingly contradicts the mammalian family tree supported by statistical analyses of whole mitochondrial genomes (Janke et al. 1996; Penny and Hasegawa 1997). Interestingly, Janke et al. (1997) were able to reject the Theria hypothesis only by using the *Xenopus laevis* mitochondrial sequence as an outgroup, and not with the much more appropriate chicken sequence. The statistical tests of topology (Kishino and Hasegawa 1989), implemented by Janke et al. (1997), also used substitution models that assumed equal substitution rates among sites. This assumption is known to seriously bias tests of phylogenetic hypotheses in many situations (e.g., Sullivan and Swofford 1997). Waddell et al. (1999a) observed that support for the Marsupionta hypothesis was very poor when conserved mitochondrial tRNA sequences were analyzed, especially under ML. Furthermore, Penny et al. (1999) determined with the use of the distance Hadamard method that there was strong conflict from within the mitochondrial data for grouping monotremes with the marsupials.

Our findings also contradict the DNA hybridization studies of Kirsch and Mayer (1998). Their results supported the Marsupionta hypothesis; however, they cautioned that they could be biased by shifts in GC nucleotide content over the tree. There is also debate concerning the utility of DNA hybridization data for divergences older than 50 million years (Hillis et al. 1996). Thus, the relationships among the three major mammalian lineages appear not to be definitely resolved by either the mitochondrial or DNA hybridization studies.

The results presented herein provide the first genetic sequence data from a large nuclear gene that reconcile genes, epigenetics,

and morphology in understanding the mammalian family tree, and they unambiguously support the accuracy of the Theria hypothesis. In the Triassic period, monotreme ancestors diverged from the mammalian ancestors that in the late Jurassic/early Cretaceous periods diverged to give rise to marsupial and eutherian mammals. It is significant that apomorphies of the therian ancestors, such as the braincase, cranial nerve architecture, and reproductive physiology do not need to be reclassified as convergences, a problem created previously by both mitochondrial DNA and DNA hybridization studies. Furthermore, our findings support the postulate that the imprinting of growth-regulatory genes, such as *M6P/IGF2R* is an apomorphy of viviparous mammals, further distinguishing them from oviparous taxa.

Acknowledgments. The authors thank the following for kindly providing tissues: Steven Atkins, Lenah Game Meats, Animals Exotique, Neese's Sausage, Wild Kingdom Animal Removal, David Fitzpatrick, The Field Museum, and the Duke University Primate Center. We further wish to thank the Duke University DNA analysis facility for DNA sequencing, and Kay Nolan for critical review of the manuscript. This study was supported by National Institutes of Health grants CA25951 and ES08823, Department of Defense grant DAMD17-98-1-8305, Sumitomo Chemical Company, Ltd., and AstraZeneca Pharmaceuticals, Ltd. T.R. Buckley thanks Clifford Cunningham and the Duke University Cancer Center for support.

References

- Dayhoff M, Schwartz R, Orcutt B (1978) A model of evolutionary change in proteins. In *Atlas of Protein Sequence and Structure*, Dayhoff MO, ed. (Washington, DC: National Biomedical Research Foundation), pp 345–352
- Felsenstein J (1993) PHYLIP: phylogeny inference package, version 3.6a, Department of Genetics, Univ. of Washington, Seattle
- Gilbert N, Labuda D (2000) Evolutionary inventions and continuity of CORE-SINEs in mammals. *J Mol Biol* 298, 365–377
- Gregory WK (1947) The monotremes and the palimpsest theory. *Bull Am Mus Nat Hist* 88, 1–52
- Griffiths M (1999) Monotremes. In *Encyclopedia of Reproduction*, Knobil E, Neill JD, eds. (San Diego, Calif.: Academic Press), pp 295–302
- Gura T (2000) Bones, molecules . . . or both? *Nature* 406, 230–233
- Halanych KM (1998) Lagomorphs misplaced by more characters and fewer taxa. *Syst Biol* 47, 138–146
- Henikoff S, Henikoff JG (1992) Amino acid substitution matrices from protein blocks. *Proc Natl Acad Sci USA* 89, 10915–10919
- Higgins D, Bleasby A, Fuchs R (1992) Improved software for multiple sequence alignment. *Comput Appl Biosci* 8, 189–191
- Hillis DM, Mable BK, Moritz C (1996) Applications of molecular systematics. In *Molecular Systematics*, 2nd ed, Hillis DM, Moritz C, Mable BK, eds. (Sunderland, Mass.: Sinauer Associates) pp 515–543
- Huchon D, Catzeflis FM, Douzery EJP (1999) Molecular evolution of the nuclear von Willebrand factor gene in mammals and the phylogeny of rodents. *Mol Biol Evol* 16, 577–589
- Huson D (1998) SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14, 68–73
- Janke A, Gemmell NJ, Feldmaier-Fuchs G, von Haeseler, Paabo S. (1996) The mitochondrial genome of a monotreme. The platypus (*Ornithorhynchus anatinus*). *J Mol Evol* 42, 153–159
- Janke A, Xu X, Arnason U (1997) The complete mitochondrial genome of the wallaroo (*Macropus robustus*) and the phylogenetic relationship among Monotremata, Marsupialia, and Eutheria. *Proc Natl Acad Sci USA* 94, 1276–1281
- Jirtle RL (1999) Mannose 6-phosphate receptors. In *Encyclopedia of Molecular Biology*, Creighton TE, ed. (New York, NY: Wiley-Liss, Inc.), pp 1441–1447
- Jones D, Taylor W, Thornton J (1992) The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* 8, 275–282
- Killian JK, Byrd JC, Jirtle JV, Munday BL, Stoskopf MK et al. (2000) *M6P/IGF2R* imprinting evolution in mammals. *Mol Cell* 5, 707–716
- Kirsch JAW, Mayer GC (1998) The platypus is not a rodent: DNA hybridization, amniote phylogeny and the palimpsest theory. *Philos Trans R Soc Lond Biol Sci* 353, 1221–1237
- Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequences, and the branching order of Hominoidea. *J Mol Evol* 29, 170–179
- Kullander K, Carlson B, Hallbook F (1997) Molecular phylogeny and evolution of the neutrophins from monotremes and marsupials. *J Mol Evol* 45, 311–321
- Lakshmi YU, Radha Y, Hille-Rehfeld A, von Figura K, Kumar NS (1999) Identification of the putative mannose 6-phosphate receptor protein (MPR 300) in the invertebrate unio. *Biosci Rep* 19, 403–409
- Lewis OJ (1983) The evolutionary emergence and refinement of the mammalian pattern of foot architecture. *J Anat* 137, 21–45
- Liu F-G, Miyamoto (1999) Phylogenetic assessment of molecular and morphological data for Eutherian mammals. *Syst Biol* 48, 54–64
- Lockhart PJ, Steel MA, Hendy MD, Penny D (1994) Recovering evolutionary trees under a more realistic model of sequence evolution. *Mol Biol Evol* 11, 605–612
- Lockett WP, Hartenberger J-L (1993) Monophyly or polyphyly of the order rodentia: possible conflict between morphological and molecular interpretations. *J Mammal Evol* 1, 127–147
- Lockett WP, Hong N (1998) Phylogenetic relationships between the orders Artiodactyl and Cetacea: a combined assessment of morphological and molecular evidence. *J Mamm Evol* 5, 127–182
- Luo Z-X, Cifelli RL, Kielan-Jaworowska Z (2001) Dual origin of tribosphenic mammals. *Nature* 409, 53–57
- Marshall LG (1979) Evolution of metatherian and eutherian (mammalian) characters: a review based on cladistic methodology. *Zool J Linn Soc* 66, 369–410
- Meng J, Wyss AR, Dawson MR, Zhai R (1994) Primitive fossil rodent from Inner Mongolia and its implications for mammalian phylogeny. *Nature* 370, 134–136
- Moore T, Haig D (1991) Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet* 7, 45–49
- Mouchatry SK, Gullberg A, Janke A, Arnason U (2000) The phylogenetic position of the Talpidae within the Eutheria based on analysis of complete mitochondrial sequences. *Mol Biol Evol* 17, 60–67
- Nadimpalli SK, Yerramalla UL, Hille-Rehfeld A, von Figura K (1999) Mannose 6-phosphate receptors (MPR 300 and MPR 46) from a teleostean fish (trout). *Comp Biochem Physiol B Comp Biochem Mol Biol* 123, 261–265
- Penny D, Hasegawa M (1997) Molecular systematics. The platypus put in its place. *Nature* 387, 549–550
- Penny D, Hasegawa M, Waddell PJ, Hendy MD (1999) Mammalian evolution: timing and implications from using the log determinant transform for proteins of differing amino acid composition. *Syst Biol* 48, 76–93
- Renfree MB, Shaw G (1999) Marsupials. In *Encyclopedia of Reproduction*. Knobil E, Neill JD, eds (San Diego: Academic Press), pp 104–114
- Robinson-Rechavi M, Ponger L, Mouchiroud D (2000) Nuclear gene LCAT supports rodent monophyly. *Mol Biol Evol* 17, 1410–1412
- Schmitz J, Ohme M, Zischler H (2000) The complete mitochondrial genome of *Tupaia belangeri* and the phylogenetic affiliation of Scandentia to other Eutherian orders. *Mol Biol Evol* 17, 1334–1343
- Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol Biol Evol* 16, 1114–1116
- Springer MS, Amrine HM, Burk A, Stanhope MJ (1999) Additional support for Afrotheria and Paenungulata, the performance of mitochondrial versus nuclear genes, and the impact of data partitions with heterogeneous base composition. *Syst Biol* 48, 65–75
- Stanhope MJ, Waddell VG, Madsen O, de Jong W, Hedges SB et al. (1998) 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
- Strimmer K, von Haeseler A (1996) Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Mol Biol Evol* 13, 964–969
- Sullivan J, Swofford D (1997) Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. *J Mamm Evol* 4, 77–86
- Swofford D (1998) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. (Sunderland, Mass.: Sinauer Associates).
- Swofford D, Olsen G, Waddell P, Hillis D (1996) In *Molecular System-*

- atics*, 2nd ed., Hillis DM, Moritz C, Mable BK, eds. (Sunderland, Mass.: Sinauer Associates), pp 407–514
- Teeling EC, Scally M, Kao DJ, Romagnoli ML, Springer MS et al. (2000) Molecular evidence regarding the origin of echolocation and flight in bats. *Nature* 403, 188–192
- Toyosawa S, O’Huigin C, Klein J (1999) The dentin matrix protein 1 gene of prototherian and metatherian mammals. *J Mol Evol* 48, 160–167
- Waddell PJ, Cao Y, Hauf J, Hasegawa M (1999a) Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid-invariant sites-logDet plus site stripping, to detect internal conflicts in the data, with special reference to the positions of hedgehog, armadillo, and elephant. *Syst Biol* 48, 31–53
- Waddell P, Okada N, Hasegawa M (1999b) Towards resolving the interordinal relationships of placental mammals. *Syst Biol* 48, 1–5
- Yang Z (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J Mol Evol* 39, 306–314
- Yang Z (1997) Phylogenetic analysis by maximum likelihood (PAML). Version 3.0. (London, UK: University College London).