Monotremata, Marsupialia and Placentalia: Inferring Phylogenetic Relationships From Molecular and Morphological Data

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Abstract

Living mammals are divided into three groups, Monotremata, Marsupialia and Placentalia. Complete mitochondrial (mt) genome sequencing has provided a large dataset for inferring phylogenetic relationships between these groups and within Placentalia. Marsupials however, are underrepresented, with only mt genomes from an opossum (*Didelphis virginiana*) and a wallaroo (*Macropus robustus*) having been published prior to this study. Herein I report complete mt genome sequences for two further marsupials, a northern brown bandicoot (*Isoodon macrourus*) and a common brushtail possum (*Trichosurus vulpecula*). Phylogenetic analysis of the protein-coding and RNA-coding mtDNA sequences provides the first statistically robust support for placing bandicoots within the cohort Australidelphia (Australasian marsupials plus Microbiotheria).

Examination of vertebrate mtDNA sequences revealed two major problems for phylogeny reconstruction, (a) nucleotide (particularly cytosine versus thymine) compositional bias among taxa and (b) differences in substitution processes between data partitions (concatenation bias). Both biases enhanced the signal for Marsupionta, a grouping of marsupials and monotremes. When the DNA sequence was RY-coded (lumping C and T, and lumping A and G) and the maximum-likelihood analysis partitioned over subsets of similarly evolving sites the traditional morphology-based Theria hypothesis (marsupials plus placentals) was recovered.

The relationships of monotremes, marsupials and placentals to Mesozoic (65-250 mybp) fossil taxa that are known from near-complete skeletons were tested with dental, mandibular, vertebral, basicranial and upper and lower appendicular characters. The null hypothesis for the phylogeny of Mesozoic mammals that retained essentially ancestral mammalian niches (morganucodontids, (eutriconodonts, (spalacotheriids, (eupantotheres, (eutherians, metatherians))))) is strongly supported and congruent with respect to the above anatomical region partitions. The inclusion of the fossorial (echidnas) and semi-aquatic (platypuses) monotremes induces extreme and topologically complex incongruence between the anatomical region partitions. A novel incompatibility analysis for examining signal and conflict among characters indicates that homoplasy affecting monotreme placement evolved non-independently among characters, essentially at the level of the anatomical regions. After consideration of monotreme shoulder girdle and forelimb traits, in view of trends among the background phylogeny, it is apparent that correlated reversal of upper appendicular characters has occurred along the monotreme stem lineage. This is consistent with phylogenetic inference from both mtDNA and the other anatomical regions, which together suggest a close relationship between monotremes and therians.

As with the molecular data, the major problem affecting phylogeny reconstruction from morphological data is non-stationarity in evolutionary processes, such as functional convergence. Monotreme affinities appear to be affected by a less recognizable non-stationarity problem, in that niche-related modification results in attraction towards outgroup taxa. Comparison of morphological and molecular trees suggests that outgroup-attraction of "morphological long-branches" may be a common problem. Incorporating information on morphological evolutionary processes will be crucial for inferring the relationships of fossil taxa to extant mammals. Nevertheless, it is encouraging that analysis of RY-coded mtDNA data resulted in a placental tree, that unlike previous mtDNA trees, is entirely consistent with interordinal relationships inferred from long nuclear sequences. Furthermore, divergence dates inferred from the mtDNA analysis suggest that as well as monotremes, at least two marsupial and six placental lineages diverged prior to the Cretaceous-Tertiary boundary. However, it seems likely that the phyletic and ecological diversification of modern mammals was not tightly coupled, with the later diversification being largely restricted to the Tertiary period.

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Whether it was climbing the kindergarten fence to get a closer look at the water dragons, or crossing the Tasman sea to study mammalian evolution, I've always been keen to expand my horizons in order to better understand nature. Dad, thanks for encouraging my interests with hikes and camping trips and also for passing on your "I need to know how everything works" gene. Mum, thanks for caring and for tempering my enthusiasm with sensible judgment. Two people I am especially grateful to for inspiring my interest in the biological sciences are my grandfathers, Stan (for walks to the creek and the wildlife heritage books) and Wilf (for the fishing and prawning trips).

Oddly enough, my PhD project was born out of an email I sent to David Penny about rates of evolution in plants. That a thesis on the early evolution of mammals resulted is an indication of the eclectic nature of the research interests that David and I share. As well as his encouragement for interdisciplinary studies, many forms of support and ability to evade being hemmed in by orthodoxy, I thank David for putting together and inspiring a stimulating and diverse research group. My other supervisors, Pete Lockhart and Mike Hendy have taught me much about phylogenetics and obediently signed all of the doctoral research committee forms. Cheers!

Studying (predominantly) Australian animals in what was a botany department in New Zealand has not been as lonely a scientific endeavour as might be expected. Numerous people deserve credit for the contributions they made to this work. Yu-Hsin (Owen) Lin's labours in the lab produced over 60% of the marsupial DNA sequences. Trish McLenachan provided the bandicoot control region sequence, technical support, proofreading services and wise counsel. Kerryn Slack and Abby Harrison had the tasks of further proofreading and instructing me on lab protocols. Like that song, "Thank-you…for loving me at my worst" (and sometimes my best), both of you. Thanks also to Robert Gemmell for the bandicoot tissue samples (obtained with permission from both the University of Queensland and Massey University animal ethics committees). I am also grateful for the Marsden grant that financed my project and the Massey University doctoral scholarship that financed me.

The first section of Chapter 5 came out of discussions with Lindell Bromham and David Penny, which Lindell and I fashioned into a "Trends in Ecology and Evolution" paper. I am also grateful to Lindell for giving me a home in her lab (and an abundance of good advice) while I was in Brisbane. With a degree in botany, there was a lot to catch-up on in the field of mammalian evolution. I am in debt to the many who helped to correct the situation, especially Guillermo

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It was a great privilege to kick off the writing of this thesis at the shed on Stradbroke Island in the middle of the whale-watching season. Many thanks to the Hines family for that. Trips to Australia allowed me to avoid a couple New Zealand winters and examine museum collections (thanks to Sandy Ingleby at the Australian Museum and Andrew Amey at the Queensland Museum, for organizing loans of mammal specimens). These visits home also provided opportunities to recharge for the challenges that lay ahead. My family and friends in Brisbane have no idea how important their roles were in this. I am particularly grateful to Barney, Courtney, Scott, Terry, Steve, Glen, James, Ben and Robbie, Misser, Suzie, Andrew Yeh and most of all, my brothers, Ben and Andrew.

After isolating myself during the writing of this thesis, I look forward to re-establishing many friendships and my interests outside of science. I also look forward taking my next steps in attempting to reconstruct evolutionary history.

Contents

Papers included in this thesis

- Phillips, M. J., Lin, Y. -H., Harrison, G. L. and Penny, D. (2001) Mitochondrial genomes of a bandicoot and a brushtail possum confirm the monophyly of australidelphian marsupials. *Proceedings of the Royal Society of London B* **268**, 1533-1538. Reprinted (as Chapter 1) with permission from The Royal society of London.
- Bromham, L., Phillips, M. J. and Penny, D. (1999) Growing up with dinosaurs: molecular dates and the mammalian radiation. *Trends in ecology and Evolution* **14**, 113-118. Reprinted (as Chapter 5.1) with permission from Elsevier Science.

Related papers not included in this thesis

- Phillips, M. J. and Penny, D. (1998) Mass survival of tetrapods across the Cretaceous-Tertiary boundary. In *Geology and Genes* (R. A. Cooper and C. M. Jones, eds.), pp. 63-65. Geological Society of New Zealand, Wellington.
- Lin Y. -H., McLenachan, P. A., Gore, A. R., Phillips, M. J., Ota, R., Hendy, M. and Penny, D. (2002) Four new mitochondrial genomes, and the increased stability of evolutionary trees of mammals from improved taxon sampling. *Molecular Biology and Evolution.* In Press.
- Poole, A. M., Phillips, M.J. and Penny, D. Prokaryote and eukaryote evolvability. *Biosystems.* In Press.

Abbreviations and symbols

A	adenine
aa	amino acid
AIC	Akaike information criterion
C	cytosine
CMI	character-map incompatibility
CMI_O	observed character-map incompatibility
CMI_E	expected character-map incompatibility
CMI_R	relative character-map incompatibility
df	degrees of freedom
G	guanine
GTR	general time-reversible (model of nucleotide substitution)
H_0	null hypothesis
H-strand	heavy-strand (mtDNA)
kb	kilo base(s)
LB	lower appendicular and basicranial meta-region
L-strand	light-strand (mtDNA)
LCA	last common ancestor
ln*L*	log-likelihood
m.	muscle
mm.	muscles
ME	minimum-evolution
ML	maximum-likelihood
MP	maximum-parsimony
mt	mitochondrial
mtDNA	mitochondrial DNA
mybp	millions of years before present
MVD	mandibular, vertebral and dental meta-region
NJ	neighbour-joining
OTU	operational taxonomic unit
PHT	partition homogeneity test
PTN	protein
R	purine (adenine and guanine)
RASA	relative apparent synapomorphy analysis
RCV	relative compositional variability
RNA	ribonucleic acid
rRNA	ribosomal RNA
T	thymine
ti	transitions
tRNA	transfer RNA
tv	transversions
U	upper appendicular characters
Y	pyrimidine (cytosine and thymine)
$\times g$	centrifugal force (relative to gravity)
χ^2	chi-square
∞	infinity
Γ	gamma-model
α	gamma shape parameter

Terminology

abduction Movement of a body part away from the midline of the body (e.g. the elbow moving laterally, further from the sagittal plane of the body).
adduction Movement of a body part toward the midline of the body (e.g. the elbow moving medially, closer to the sagittal plane of the body).
advanced / primitive In the context of this work, the words primitive and advanced describe relative placements or character state conditions with respect to the mammalian backbone lineage, rather than suggesting that some character states or taxa are intrinsically more advanced than others. **antebrachium** The lower forelimb, from elbow to hand.
apparent phylogenetic signal Character covariance above "noise" in a data matrix that may or may not result from common ancestry (i.e. may be convergence etc.). I often refer to this simply as signal.
basicranium The base of the skull
buccal In dental morphology, the aspect of teeth that faces the cheek or the outside of the mouth.
condyle A rounded projection at the end of a bone, usually as part of a bone to bone joint.
cursorial Behaviour of (and adaptation for) running.
crown group A taxonomic grouping that includes the last common ancestor (LCA) of a given set of (often extant) taxa and all living and extinct descendents of that LCA.
crus The lower hindlimb, from knee to foot.
distal In anatomy, away from the centre of the body or away from the end that is attached to the body (e.g. the knee is distal to the hip).
dorsal The back, or upper side (in vertebrates, the surface closest to the notochord).
foramen A hole, usually for the passage of blood vessels and nerves through bone or cartilage.
fossorial Digging adaptation and/ or behavior.
gamma distribution In molecular phylogenetics, a continuous or discrete distribution of evolutionary rates across data (DNA or amino acid) sites.
glenoid (usually) ossified cup-shaped socket for another bone.
indel Insertion or deletion event, leaves a gap in a molecular sequence alignment.
lateral Pertains to the side of the body, or as a relative term, the part furthest from the sagittal plane (e.g. the cheeks are lateral to the tongue).
lingual In dental morphology, the aspect of teeth that faces the tongue or the inside of the mouth.
manus Carpus (=wrist) and forefoot (=hand)
medial Pertains to the middle of the body, or as a relative term, the part closest to the sagittal plane (e.g. the tongue is medial to the cheeks).
NT-coding Standard coding for DNA (A, C, G, T).
paradentary bones Plate-like bones attached to the dentary, these are splenial and coronoid that occur in many Mesozoic mammals
parasagittal 1. posture, with feet placed directly under their proximal attachments and the elbow or knee in approximately the same plane as these, 2. locomotion, with limbs swinging through an arc that is approximately parallel to the sagittal plane (the vertical plane through the midline of the body). I consider posture and locomotion that has the manus and pes almost vertically aligned with their proximal attachments, but with the knees and/or elbows somewhat lateral to these (due to humeral/femoral abduction) to be near-parasagittal.
pes hind foot (including the ankle)
phylogenetic signal Character covariance above "noise" in a data matrix that results or is inferred to result from common ancestry. I refer to character covariance above "noise" in a data matrix that may, or may not, result from common ancestry (i.e. may be convergence etc.) as apparent phylogenetic signal, or simply as signal.

phylogenetic trend (or evolutionary trend) The tendency for a character or complex of characters to undergo directional evolution, along a lineage, or within a group of taxa. This relates to gradual (as opposed to punctuated) evolution under selection pressures that remain similar along a lineage, or among members of a clade. As such, in the context of this work, evolutionary trends are not orthogenesis, which implies the maintenance of directional evolution, independently of selection.
plesiomorphy An ancestral character state.
postdentary bones Three associated bones that are lodged in the postdentary trough on the medial surface of the dentary of mammal-like reptiles and early mammals such as *Morganucodon*, and form the primitive jaw-joint (articular-quadrate). Of these bones, the articular and prearticular were (phylogenetically) transformed into the malleus in modern mammals, while the angular become the tympanic bone, which supports the tympanic membrane of the ear.
proximal In anatomy, towards the centre of the body or the end that is attached to the body (e.g. the hip is proximal to the knee).
purine bias Nucleotide compositional bias in which the relative frequency of the two purines (adenine versus guanine) differs between taxa. Indicated by variability (or the standard deviation) across taxa in a dataset for the frequency difference between A and G (A–G).
pyrimidine bias Nucleotide compositional bias in which the relative frequency of the two pyrimidines (thymine versus cytosine) differs between taxa. Indicated by variability (or the standard deviation) across taxa in a dataset for the frequency difference between T and C (T–C).
RY-coding Coding nucleotides as either purines (A and G = R) or pyrimidines (T and C = Y).
sagittal plane A vertical plane through the midline of the body, in most bilaterally symmetrical animals (including all tetrapods).
scansorial Behaviour including (and adaptation for) both climbing and terrestrial locomotion.
signal Character covariance above "noise" in a data matrix that may or may not result from common ancestry (i.e. may result from correlated homoplasy etc.). I also refer to this as apparent phylogenetic signal.
Stem taxa Organisms excluded from a specified crown group, but more closely related to that crown group than to another crown group. For example, *Archaeopteryx* is considered to be a stem bird.
stemminess A relative measure of phylogenetic structure, defined here as the percentage of uncorrected minimum-evolution tree distance attributed to internal (as opposed to external) branches.
synapomorphy A derived character state that is (or is inferred to be) shared between taxa as a result of common ancestry.
Synapsida Taxonomic group that includes mammals and taxa more closely related to mammals than are sauropsid reptiles (such as birds, turtles and lizards). The name Synapsida refers to the presence of a single opening in the skull (behind the eye socket).
synonymous substitution A nucleotide substitution that does not result in an amino acid replacement. In contrast, a non-synonymous substitution does result in an amino acid replacement.
transition In molecular biology, a nucleotide substitution from one purine to another purine (A↔G) or from one pyrimidine to another pyrimidine (T↔C).
transversion In molecular biology, a nucleotide substitution from a purine to a pyrimidine (e.g. A→T) or from a pyrimidine to a purine (e.g. T→A).
trochlea A pulley-like articular surface.
ventral The undersurface (in vertebrates, the surface furthest from the notochord).

Candidate's note

This thesis is a collection of published papers and extensions of manuscripts in preparation for submission, to international journals. Each chapter has an introduction and can be read on its own. The intention for the following introductory notes is to provide additional background information and a rationale for the current examination of relationships among mammals.