From Clone to Bone

The Synergy of Morphological and Molecular Tools in Palaeobiology

EDITED BY

Robert J. Asher
Museum of Zoology,
University of Cambridge, UK

Johannes Müller
Museum für Naturkunde/Humboldt
University of Berlin, Germany

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town,
Singapore, São Paulo, Delhi, Mexico City

Cambridge University Press
The Edinburgh Building, Cambridge CB2 8RU, UK

Published in the United States of America by Cambridge University Press, New York

www.cambridge.org
Information on this title: www.cambridge.org/9781107003262

First published 2012
Printed in the United Kingdom at the University Press, Cambridge

A catalogue record for this publication is available from the British Library

Library of Congress Cataloging-in-Publication Data

From clone to bone : the synergy of morphological and molecular tools in palaeobiology /
edited by Robert J. Asher, Johannes Müller.
pages cm. – (Cambridge studies in morphology and molecules ; 4)
Includes bibliographical references and index.
ISBN 978-1-107-00326-2 (Hardback) – ISBN 978-0-521-17676-7 (Paperback)
1. Evolutionary paleobiology. 2. Paleobiology–Methodology. 3. Morphology (Animals)
4. Morphogenesis. 5. Molecular biology. I. Asher, Robert J. II. Müller, Johannes, 1973–
QE721.2.E85F76 2012
560–dc23

2012014611

ISBN 978-1-107-00326-2 Hardback
ISBN 978-0-521-17676-7 Paperback

3

Rocking clocks and clocking rocks: a critical look at divergence time estimation in mammals

OLAF R. P. BININDA-EMONDS, ROBIN M. D. BECK AND ROSS D. E. MACPHEE

Introduction

Much has been written about the molecular revolution in phylogenetics and the ongoing conflict between molecules and morphology (Hillis 1987; Patterson 1987; Springer *et al.* 2004). With reference to therian mammals at least, the supposed conflict has been largely overblown: there is in fact general agreement between the two data sources, something unfortunately overshadowed by a handful of persistent 'problem children'. The taxonomic content of most mammalian orders and other traditional higher-level taxa originally proposed purely on the basis of morphology has remained unscathed by the application of molecular sequence analysis. Even within these taxa, conflicts between molecular and morphological hypotheses of relationships are comparatively rare and usually relatively minor. For instance, a comparative study within Carnivora (Bininda-Emonds, 2000) revealed that most data sources and methods of analysis pointed at the same general solution, a few admittedly problematic taxa (e.g. Felidae) notwithstanding. In the end, the frequency and nature of disagreements over tree topology is arguably of the same order of magnitude within the separate spheres of molecular and morphological systematics as it is between them (Patterson *et al.* 1993). In many ways, the situation in mammals parallels that in vertebrates, where a fairly robust tree including gnathostomes, actinopterygians, sarcopterygians, tetrapods, amniotes and diapsids (among many other groups) has been supported by comparative anatomy since the 1800s (Asher and Müller, this volume).

Instead, many of the more celebrated conflicts in mammals tend to represent a lack of information, especially on the morphological side. The evolutionary

From Clone to Bone: The Synergy of Morphological and Molecular Tools in Palaeobiology, ed. Robert J. Asher and Johannes Müller. Published by Cambridge University Press.

tree of eutherian mammals presented by Novacek (1992), which exemplified the state-of-the-art morphological opinion at the time, is conspicuous today not for being very wrong (although some clades within it have been overturned by molecular information), but for its lack of resolution. Insectivora was long recognized to be a taxonomic wastebasket for any small brown mammal with sharp teeth that wasn't a rodent and couldn't fly. With time, the application of ever more detailed morphological data to the problem resulted in the exclusion of Macroscelidea, Scandentia and a host of early Cenozoic clades better placed elsewhere, but ultimately it just wasn't possible to tease more out of the data. Molecular data, however, revealed the non-monophyly of the remaining, 'lipotyphlan' insectivores, now allocated to two different mammalian superorders (see Springer *et al.* 2004).

In many cases, new information or improved analyses resolved apparent conflicts. For instance, Cetartiodactyla was first proposed based on molecular data (Graur and Higgins, 1994), but subsequently received strong morphological support with the discovery of fossils of early, terrestrial whales that preserved features previously thought diagnostic of non-cetacean artiodactyls (e.g. *Pakicetus*; Geisler and Uhen, 2003; Gingerich *et al.* 2001; Thewissen *et al.* 2001). On the flip side, the morphologically strongly supported grouping Glires, which unites rodents and lagomorphs and was rejected by several early molecular analyses (e.g. Graur *et al.* 1996; Misawa and Janke 2003), is now consistently recovered by most molecular studies with sufficient taxon sampling (see Springer *et al.* 2004). More recently, the morphological study of Wible *et al.* (2007) recovered a monophyletic Euarchontoglires (a placental superorder first recognized on the basis of molecular data; Murphy *et al.* 2001) and clades that are similar (although not identical) in taxonomic content to the molecularly supported placental superorders Laurasiatheria (Waddell *et al.* 1999) and Afrotheria (Stanhope *et al.* 1998).

At higher taxonomic levels at least, arguably the only major phylogenetic conflict between molecules and morphology is the monophyly of Afrotheria, which unites paenungulates, aardvark, elephant shrews and afrosoricidan 'insectivores'. Despite extensive research (Asher 1999, 2001, 2007; Whidden 2002; Asher *et al.* 2003; Sánchez-Villagra *et al.* 2007; Seiffert 2007b; Wible *et al.* 2007; Asher and Lehmann, 2008; Seiffert, 2010), strong morphological support (at least with afrosoricidans included) for this placental superorder is still scarce. The failure of morphology to recognize the monophyly of Afrotheria (and the non-monophyly of Lipotyphla) means that many of the groups that are accepted today, like Laurasiatheria, were not strictly recovered in the past, thereby giving the appearance of conflict. However, a more historically accurate view reveals that the content of the higher-level taxa, albeit not identical, is often very similar between morphological and molecular studies.

Instead of conflict, therefore, the combination of molecules and morphology is achieving a robust consensus with respect to mammalian higher-level relationships, with a recent study (Lee and Camens 2009) showing that hidden support for relationships originally founded on molecular grounds exist within morphological data sets. The same general lack of conflict, however, cannot be said to be true for estimates of divergence times within Mammalia and for the origin and initial radiations of the ordinal crown groups in particular. Here the conflict is real and continuing, and plays in the expected direction: molecular-based estimates are consistently older than fossil-based ones (although not universally; see Douzery *et al.* 2003; Kitazoe *et al.* 2007). What makes this conflict particularly interesting is both the scale of the difference and the implied relative importance of the Cretaceous–Palaeogene (K–Pg; traditionally known as the Cretaceous–Tertiary or KT) mass extinction event for basal divergences within Placentalia. Whereas fossil-based estimates (as exemplified by Wible *et al.* 2007) typically place both the origins and the basal diversifications of the crown groups close to the K–Pg boundary, most molecular-based estimates (as exemplified by Bininda-Emonds *et al.* 2007, 2008) indicate that the crown groups both originated as well as began radiating well within the Late Cretaceous.

In this review, our goal is not to find the answer to the problem of when existing mammalian (primarily therian) crown groups evolved, but instead to determine where the difficulties might lie in finding such an answer. The use of fossil, molecular, or both kinds of data for estimating divergence times is coupled with any number of crucial assumptions, many of which are hardly ever mentioned explicitly. Very often, the 'blame' for any conflict is simply passed to the other side, but it could equally be the case that both data sources are wanting and providing flawed estimates. Here, with particular reference to examples from the mammalian radiation, we elucidate some of the assumptions and potential sources of error underlying each data type and describe their respective strengths and weaknesses. The hope is that the increased awareness of both sides of the discussion will help bring fossil- and molecular-based date estimates closer together with time.

Of rocks: assumptions underlying fossil-based date estimates

The fossil record, whether directly in the form of fossil taxa or indirectly through inferred palaeobiogeographic or stratigraphic information, ultimately represents the single (and only!) data source against which all our divergence time estimates are calibrated. It is accepted that individual fossils can, at best, be as old as their associated nodes on a phylogeny, and that they will often moderately to severely underestimate nodal age. This discrepancy often derives

simply from sampling issues, with fossils of the oldest members of particular lineages not yet having been discovered (or possibly not even preserved in the first place). Thus, whether any given fossil has any real relevance for dating actual divergence times is often questionable when there is no basis for estimating how well fossil members of the focal lineages have been sampled. Such factors undoubtedly play a role in debates about the validity of molecular-based versus fossil-based dates for the deeper mammalian divergences, including those of the ordinal crown groups. Indeed, the incompleteness of the fossil record arguably represents the default explanation in any cases of severe conflict.

In the case of placental mammals, it remains possible that the oldest members of the extant superorders and orders might have been present in regions that are as yet poorly sampled for particular intervals (e.g. some parts of Gondwana). However, there are other, less appreciated factors involving the interpretation of fossil data (e.g. our ability to recognize basal members of the mammalian superorders and orders in the fossil record) that may be equally if not more important and whose examination might lead to new insights concerning the interpretation of morphological data in a phylogenetic context. In the following sections we examine a wide range of issues associated with interpreting fossil evidence, including the epistemological nature of characters and character definition, the significance of gaps in the fossil record, how the phylogenetic affinities of fossils are established, and the contingent relevance of biogeography in interpreting evolutionary history.

Defining and using morphological characters in phylogenetics: the funnel of induction and the ratchet

Even without limitations incurred by the incompleteness of the fossil record, our interpretation of fossil material and its taxonomic affinities relies on a fundamental aspect of modern systematics, the definition and analysis of discrete morphological characters. Certainly, the process of character description has intensified greatly over the past 35–40 years, largely as a result of the search for characters useful for making phylogenetic inferences. In the case of mammalian systematics, this activity has produced a number of important insights into morphological evolution, such as the likely primitive therian premolar number (Giallombardo and AToL Mammal Morphology Team 2010; Kielan-Jaworowska *et al.* 2004) and mammalian cochlear form and mechanics (Luo *et al.* 2011a).

In working with morphological characters, we need to be aware of the distinction drawn by Hennig (1966), who recognized that semaphoronts – the 'holders' of characters, whether individuals or groups, adults or juveniles – are

empirically real; whereas character phylogenies are abstractions based on characters detected on semaphoronts and interpreted in the context of a given study. Within the fossil record, semaphoronts are often temporally clumped and are separated (in the simplest case) by intervals in which nothing very much like them may occur. Coeval semaphoronts can often be defined in terms of characters apparently unique to them, as well as in terms of characters that link them monophyletically with other groups up or down the tree. The end result is, metaphorically, a series of beads on a string, each bead more or less different from, but still related to, those above or below; what keeps them all together in the mind of the systematist are the successively derived character states of features they purportedly hold in common.

Several points concerning this procedure need to be examined. First, and most obviously, is that the entire exercise, from drafting definitions of characters to interpreting a final tree, proceeds entirely inductively (Bryant 1989, 1991). Semaphoronts do not advertise their diagnostic characters; these need to be selected by an informed mind in which prior inductive knowledge plays a substantial role. The teeth of fossil mammals are usually heavily sampled for characters, both because they are among the most readily fossilized elements of the mammalian skeleton and because the dentition is often a very good (if imperfect) guide to relationships. Another issue concerns how morphological character states are defined, counted, or measured; these decisions operate with few constraints, especially compared with the restricted state space for molecular data (i.e. nucleotide bases or amino acids, or perceived gaps between them). The choice of descriptor language used in character definition may differ sharply between systematists, even when describing the same thing, with consequences for how characters may be scored across taxa and, possibly, the form of the final tree (Cartmill 1981; MacPhee 1994). This is not a minor problem. The current drive toward comparatively large morphological character sets and more control over descriptors arising from community efforts like MorphoBank (www.morphobank.org; O'Leary 2011; also Novacek and AToL Mammal Morphology Team 2008; Vogt *et al.* 2010) is welcome at one level (e.g. access to information, ability to recognize poor-quality characters), but because the characters are still generated in much the same way as before, nothing has changed procedurally.

Another serious problem is the general tendency, in most real records, for the quality of the information extractable from semaphoronts to decline as one goes backward in time (Benton and Donoghue 2007; Donoghue and Purnell 2009; Sansom *et al.* 2010). This is not only because the fossils themselves may become less complete or adequately preserved, as already noted, but because candidate derived characters, based on prior experience and used to operationally define a

given group of interest, are either not represented in the preserved anatomy or are no longer interpretable as part of the same character-state complex. This circumstance – the 'character funnel' – is commonly observed in many kinds of inductive processes in which some set of initial propositions about a phenomenon is serially depleted (in this case, across time) until the phenomenon can no longer be stipulated on the basis of the propositions that remain, reducing that its likelihood can be characterized as either true or false, or perhaps present versus absent (Hacking 2001; Vickers 2010). Incidentally, the possibility that morphological characters ever disappear in a formal sense need not detain us; certainly, the recognition criteria for such characters do, which ultimately terminates any chain of induction about character 'states'.

A good example of the funnel in action is the result achieved when only characters present in existing members of the crown group are utilized to determine group membership for fossil candidates. If at some point character states antecedent to the crown group's defining synapomorphies cannot be recognized, the funnel empties and the chain of induction terminates with the last member in which the derived character(s) can be recognized. Of course, in any real situation there will be more characters in play and very probably considerable variation as to when individual characters drop out. However, the end result is the same: analysis stops with the earliest definable member of a group, and its age provides the (minimum) constraint on divergence time.

As we note later on, a danger when relying solely on diagnostic characters for the placement of fossils is that the process can easily stray into the realm of typology rather than phylogenetics. It is curious that, in securing ingroup placement for a fossil, we often demand that the diagnostic features of the clade be identifiable even when such features may not be universally present in the extant members due to loss or additional character modification. For example, the presence of limbs is diagnostic for (fossil) tetrapods, although they are lost or highly modified in a variety of extant forms. By contrast, the use of a phylogenetic perspective in combination with fossil evidence can often overturn or amend hypotheses based on extant taxa only. If extant crown-group morphology were the sole basis for investigating the systematics of mammals, our understanding of their evolution would be meagre, if not flawed, indeed. For example, careful study of fossils reveals that epipubic bones, which are diagnostic for extant marsupials, are actually plesiomorphous at a much higher level (Mammaliaformes) and therefore of little cladistic utility for defining groups at lower hierarchical levels (Novacek *et al.* 1997).

In practice, fossils can play an important role in determining the content of monophyletic groups, particularly for the purpose of defining stem membership. Specifically, character evidence from fossils provides basic phylogenetic

structure that would be impossible to induce merely from extant crown-group morphology. It acts in much the same way that a ratchet provides control over an object while work is performed on it, thus helping to ensure that the job will be completed satisfactorily. Perhaps the most compelling example of the induction ratchet at work – in which something other than mere data reshuffling occurred – is the paper by Luo *et al.* (2001a) in which critical features of tribosphenic mammals from the Mesozoic of northern and southern continents were compared (cf. Rich *et al.* 1997; Flynn *et al.* 1999; Rauhut *et al.* 2002; Rougier *et al.* 2007). Earlier, the fact that all such taxa had superficially similar eutherian-style molars was taken as evidence that they had to be related as eutherians (Rich *et al.* 1997). Luo *et al.* (2001a) instead argued that the southern forms are better regarded as highly derived monotreme relatives, grouped as australosphenidans, in which a descriptively tribosphenic cusp pattern was independently evolved. As a result of this analysis, much more was learned about the groups concerned and new phylogenetic relationships suggested. Our point here is not whether the australosphenidan/boreosphenidan dichotomization is correct – something that can only be justified with still more inductively based propositions about molar evolution – but rather that palaeontological evidence should not be thought of as a source of evolutionary knowledge that is somehow less privileged compared with neontological evidence. The two are, in fact, properly conceived as reciprocally illuminating the understanding of systematists as they set about trying to interpret the fragments of evolutionary history on which they work.

Of course, the implied promise here is that more description of more fossils will always yield better phylogenies. Actual cases reveal that 'better' (that is, more resolved or more compatible) phylogenies often do result when fossils are included (Donoghue *et al.* 1989), but such improvements to systematic knowledge are not necessarily helpful for determining divergence dates. Thus, the recent large increase in the number of named taxa of plesiadapiforms has greatly improved our understanding of the morphology, adaptations and within-group relationships of these stem primates (e.g. Bloch *et al.* 2007). However, perhaps because of the countervailing effect of the character funnel (combined with the difficulty in scoring potentially diagnostic characters in available fossils; see above), this effort has not, so far, resulted in any consensus regarding how such taxa are related to the extant crown group beyond the mere fact that they reside somewhere near it on the primate tree trunk.

If even the best-researched groups suffer from such uncertainties, definitive statements about poorly known groups are clearly premature. The widely cited Explosive Model of placental evolution (Archibald and Deutschmann 2001) in its strictest form denies the existence of Cretaceous members of the extant

modern orders, either because they had not evolved to that time point or, less restrictively, because none are yet known. But how representative are the 40-odd recognized genera of Cretaceous eutherians of the diversity that existed on the planet as a whole 65 to 100 million years (Ma) ago? The majority of these taxa are known solely from highly incomplete dentitions, and all but three are from northern continents. If the loss of information through the funnel were commonly offset by the information gained via the ratchet, it is possible that the status of possible Cretaceous antecedents of crown-group orders might need to be re-evaluated. It is relevant to note that the problem is not unique to the placental/eutherian distinction, but instead occurs at all higher-level positions along the hierarchy. A good example is the paucity of characters that appear to distinguish reliably the molars of basal eutherians and metatherians (Rougier *et al.* 1998; Luo *et al.* 2002, 2003; Averianov *et al.*, 2003; Wible *et al.* 2005). On the whole, therefore, although additional fossil evidence bearing on these problems would be very welcome, it may never be sufficient to stipulate divergence dates with any greater certainty than at present.

Gaps in the fossil record and their interpretation

Gaps in the fossil record can occur if the relevant taxa are restricted to biogeographical regions that are poorly sampled (or completely unsampled) for fossils, for which fossil-bearing strata of the appropriate age are unavailable, or for which the original conditions for fossil preservation were poor. Recent discoveries have partially 'filled in' some gaps in the fossil record of therian mammals, but it is clear that major lacunae remain. How we interpret these gaps can impinge greatly on our views of the likely time and place of origin of the modern mammalian orders and superorders.

Based on the results of their comprehensive morphological analysis of Cretaceous eutherian mammals and representatives from many placental crown groups, Wible *et al.* (2007) favoured a Laurasian origin for all placental higher taxa at or near the K–Pg boundary, with explosive divergence and extension of clade ranges onto southern terranes occurring thereafter. Certainly, this Laurasia-first interpretation finds better support in the fossil record than does any other interpretation: the oldest generally accepted stem and crown members of the extant placental orders are almost exclusively from northern continents. However, the overall accuracy of this view depends on the likelihood that the evidentiary gaps are meaningful and accurate, and that true placentals have not been missed due to insufficient prospecting or, possibly, misinterpretation of existing fossils (see below).

Of particular interest at the moment are the allegedly euarchontan-like *Deccanolestes* and the possible 'condylarth' *Kharmerungulatum*, both from the Maastrichtian of India. Regardless of the precise affinities of either fossil (which are disputed), both minimally demonstrate the presence of eutherians during the Late Cretaceous in this as yet relatively poorly sampled region. However, if either or both taxa are indeed placentals (as has been argued for *Deccanolestes*; cf. Boyer *et al.* 2010; Prasad *et al.* 2010; Seiffert 2010), at least some divergences within Placentalia must have occurred before the K–Pg boundary, a possibility in fact allowed by Wible *et al.* (2007). In addition, accepting this conclusion would require that one either reject exclusively Laurasian origins for the modern placental orders (see below) or invoke palaeogeographically unlikely early contacts between northward-moving India and either Africa or Asia (see Krause 1986; Rose *et al.*, 2008, 2009; Boyer *et al.* 2010; Prasad *et al.* 2010; Smith *et al.* 2010). It would also support the hypothesis that India (and possibly other poorly sampled southern landmasses such as Africa and Antarctica) may have played a 'Garden of Eden' role (*sensu* Foote *et al.* 1999) for placental mammals during the Cretaceous (see also Krause and Maas 1990).

Even if both taxa are stem eutherians rather than placentals, their presence in the Late Cretaceous of India is still biogeographically surprising given that India was an island continent by about 80 Ma ago and experienced its maximum degree of isolation during the latest Cretaceous (Ali and Aitchison 2008). This raises questions as to how and when eutherians reached India, and whether eutherians were distributed more widely in southern continents during the Late Cretaceous. Providing confident answers to these questions is probably impossible given the paucity of the Late Cretaceous and early Palaeogene fossil record for most southern continents. For example, the Cretaceous fossil record for Africa lacks any verified, published examples of eutherians, but the late Mesozoic terrestrial record for that continent is generally so poor that it can be called into question whether the observed absence of evidence is likely to be real evidence of absence. Similarly, there is no Late Cretaceous fossil record whatsoever of mammals in Australasia: there is only a single site containing mammals (the early Eocene Tingamarra Local Fauna; Godthelp *et al.* 1992) for the time period spanning ~110 to 25 Ma ago. However, the presence of a probable terrestrial eutherian at Tingamarra (*Tingamarra porterorum*; Godthelp *et al.* 1992) hints at a wider distribution of Eutheria within Gondwana than is perhaps generally assumed.

Even the South American fossil record, which is the best of all the major southern landmasses, is puzzling in many ways. Pre- and post-K–Pg mammal records for this continent are staggeringly different, implying that a major biogeographical shift had to have occurred there at the close of the Mesozoic

(Pascual and Ortiz-Jaureguizar 1992, 2007). The therian groups characteristic of South America during the Cenozoic (metatherians, eutherian 'condylarths', 'ungulates' and xenarthrans) were seemingly absent during the Late Cretaceous, with the continent populated instead by a variety of non-therian groups ('symmetrodonts', dryolestoids, monotremes and gondwanatherians), most or all of which had disappeared completely by the end of the Palaeocene. At about this time (i.e. middle to late Palaeocene), therians make their first appearance in the South American fossil record (de Muizon 1991; de Muizon *et al.* 1998; de Muizon and Cifelli 2000; Marshall and de Muizon 1988). In the case of the metatherians and 'condylarths', their arrival in South America was almost certainly the result of dispersal from North America, possibly via a proto-Antillean connector inferred to have existed for a brief period around the end of the Cretaceous (Gayet 2001; Iturralde-Vinent 2006). The origins of xenarthrans are far more puzzling. The earliest record of Xenarthra is from the late Palaeocene or early Eocene Itaborai fauna in Brazil (Bergqvist *et al.* 2004; Gelfo *et al.* 2009). The Itaboraian xenarthrans are relatively derived and include probable crown-group forms (cingulates; Bergqvist *et al.* 2004), suggesting a considerable period of unsampled prior evolution in South America or elsewhere. However, fossils of Xenarthra, which should be easily identifiable based on the presence of osteoderms (a distinctive xenarthran apomorphy rarely found in any other therian clade), are as yet unknown from older sites in South America or indeed from any other continent (MacPhee and Reguero 2010). The same is true for putative xenarthran relatives, which are either lacking entirely from Late Cretaceous (and later) fossil records of other Gondwanan fragments (e.g. Africa and Australia) or do not display expected diagnostic traits such as xenarthry (e.g. the Laurasian palaeanodonts).

The nature of gaps, however, is that they can be filled by new material, and often substantially so. If correct, the suggestion of Seiffert *et al.* (2007) and Seiffert (2010) that *Widanelfarasia bowni* and *Dilambdogale gheerbranti* from late Eocene localities in the Fayum area of northern Egypt are possible stem members of a tenrec–golden mole clade, pushing the first split within that clade to a minimum of 37 Ma ago. Previously, the oldest known fossils relevant to this divergence were the tenrecoids *Protenrec*, *Erythrozootes* and *Parageogale* and the chrysochlorid *Prochrysochloris* from the early Miocene of East Africa (Butler 1984), some 17 Ma younger. Moreover, the latter fossils are at least zalambdodont in aspect, a distinctive dental morphology of extant tenrecs and golden moles, which the Fayum fossils are not. This new minimum is, in any case, still at least 25–30 Ma younger than the presumed divergence predicted by most molecular studies (e.g. Douady and Douzery 2003; Poux *et al.* 2008).

Some first appearance records, already reasonably ancient, have been pushed back even more, but only modestly. For example, recent discoveries have pushed evidence for both Proboscidea (*Eritherium azzouzorum* from Morocco; Gheerbrant 2009) and Cetartiodactyla (*Ganungulatum xincunliense* from the Nongshanian of China; Ting *et al.* 2007; Clyde *et al.* 2010) into the middle Palaeocene (both ~60 Ma ago). (The cetartiodactylan affinities of *Ganungulatum* admittedly are not certain, however; see below.) Although only slightly older, these two new discoveries require us to re-evaluate the evolutionary scenarios attached to them given that both orders are well nested within their respective superorders (Afrotheria and Laurasiatheria): their earlier, middle Palaeocene appearance leaves only 5 Ma for the deeper, superordinal splits to have occurred under an the Explosive Model of placental origins (see Wible *et al.* 2007).

Whether future finds will actually push the inferred origins of placental orders over the K–Pg boundary, as predicted by most molecular studies, remains to be seen. A recent discovery bearing on this issue is the recovery of a single upper premolar referred to *Protungulatum*, an archaic 'ungulate' sometimes regarded as an early placental (e.g. Spaulding *et al.* 2009), in rocks of unquestionably latest Cretaceous age in Montana (Archibald *et al.* 2011). This is perhaps not that surprising because this taxon is already well known from the earliest Palaeocene and the new fossil is thought to be only a few hundred thousand years earlier than the K–Pg boundary. Although Archibald *et al.* (2011; also Wible *et al.* 2007) discount a placental affinity for *Protungulatum*, this discovery remains important because it raises two critical questions. First, what else is being missed and why? Second, in addition to problems associated with apomorphy lag (see below) and the character funnel, can we place the often highly fragmentary Cretaceous and Palaeocene mammal fossils within a phylogenetic framework robustly enough to be confident of their relationships to the extant taxa? In short, can we ever hope to recognize the earliest putative members of a group for what they are?

Establishing the phylogenetic affinities of fossils

Whether fossil data are used to infer divergence times in isolation or as calibration points for molecular-based analyses, there are two crucial prerequisites for their use: these data must be (1) associated accurately with particular nodes on the phylogenetic tree for which we want to derive divergence time information and (2) appropriate to act as estimators for the divergence date of the node in question *even if* they are properly placed. These issues are intertwined, logically as well as empirically, and become increasingly important and problematic the further back in time we go, when both character information

and taxonomic sampling become increasingly limited and limiting. Using current concepts and analyses, we shall concentrate on some of the associated epistemological and empirical issues that complicate the search for evolutionary origins and diversifications within mammals.

Associating a fossil taxon with a particular node on a phylogenetic tree requires both that (1) the taxon possesses one or a combination of candidate apomorphies enabling it to be plausibly referred to one of the lineages descending from that node (preferably demonstrated via formal phylogenetic analysis) and (2) its available fossil remains actually do preserve at least one *diagnostic* apomorphy that can be recognized for what it is. The first condition is largely definitional under a cladistic framework. Without the possession of attendant apomorphies, no matter how weakly buttressed, we have no basis for associating any taxon (fossil or extant) with a particular clade except at the most uninformative level (e.g. 'cf. Mammalia'). Instead, the second condition is the more problematic given that missing information can prevent the accurate placement of fossil material when the first condition is fulfilled.

In this regard, data completeness for fossil material is frequently a problem. In mammals, the majority of fossil taxa are known only from partial dental remains (usually molars of adult individuals). Thus, information from the fossil specimens alone is often insufficient to confidently resolve their phylogenetic affinities. For example, *Eomaia* and *Sinodelphys* from the 125-Ma-old Yixian Formation of China represent among the oldest known generally accepted members of Eutheria and Metatheria, respectively, and therefore represent critical data points for inferring the age of the placental–marsupial split. However, the determination of their taxonomic affinities was based partly on morphological features that are only identifiable because of the exceptional preservation of the Yixian specimens (Ji *et al.* 2002; Luo *et al.* 2003). If both taxa were known only from the more typical fragmentary dental remains, it is questionable whether either could be confidently distinguished from tribosphenic stem therians (e.g. both retain the plesiomorphic condition of eight upper post-canines, rather than seven as in crown-group placentals and marsupials).

An example of the more typical difficulty associated with assigning mammalian taxa known only from fragmentary dental remains is provided by the Middle Jurassic (Bathonian) Malagasy fossil *Ambondro mahabo* (Flynn *et al.* 1999), known only from three lower teeth in a jaw fragment. On the basis of this relatively meagre evidence, it has been serially associated with monotremes (Luo *et al.* 2001b), eutherians (Woodburne *et al.* 2003) or therians as a whole (Rowe *et al.* 2008) in robust cladistic analyses. Such differences in opinion are, in fact, typically encountered in the interpretative history of many Mesozoic mammal fossils. A comparison of phylogenies by Luo and co-workers (e.g. Ji *et al.* 2002;

Luo *et al.* 2002, 2003, 2007; Kielan-Jaworowska *et al.* 2004; Luo and Wible 2005) with those of Woodburne *et al.* (2003), Rougier *et al.* (2007) and Rowe *et al.* (2008) reveals major differences in how these authors portray the relationships of key taxa such as monotremes, Mesozoic tribosphenic forms from Gondwana (including *Ambondro*), allotherians (multituberculates, haramiyidans and gondwanatherians) and the various groups of triconodonts and symmetrodonts. In turn, these differences in opinion will have a major impact on the choice of fossil taxa suitable for dating the monotreme–therian split and thus the root of crown-group Mammalia. For instance, the choice of *Ambondro* by Bininda-Emonds *et al.* (2007) for this purpose might turn out to be unduly conservative, particularly if the controversial hypothesis that haramiyidans, the first record of which is from the Late Triassic, are indeed crown-group mammals (Luo *et al.* 2002, 2007; Luo and Wible 2005; Rowe *et al.* 2008). This would mean that the deepest divergences within living mammals might be even older than estimated by Bininda-Emonds *et al.* (2007, 2008). The recent discovery of the putative eutherian *Juramaia sinensis* (contemporaneous with *Ambondro* at ~160 Ma; Luo *et al.*, 2011b) would strongly hint that this is indeed the case.

More generally, the crucial structures needed to place a given fossil are often not preserved in the fossil record. For instance, arguably the most distinctive morphological apomorphy of Cetartiodactyla is their apparently uniquely derived 'double-pulleyed' astragalus (Schaeffer 1947; Rose 1996; Luckett and Hong 1998). However, determining the presence or absence of this feature in fossil taxa requires the discovery of post-cranial material, which is comparatively much rarer than dental material. Thus, *Ganungulatum xincunliense* from the middle Palaeocene (Nongshanian) of China might be the oldest known cetartiodactyl (as suggested by Ting *et al.* 2007), but, in the absence of potentially definitive post-cranial evidence, this identification rests on relatively minor dental features. Instead, the oldest cetartiodactyl known to have a double-pulleyed astragalus, the early Eocene *Diacodexis*, is at least 4 Ma younger. Similarly, the early Eocene Australian marsupial *Djarthia murgonensis* was originally described based on dental material that was insufficient to determine its higher-level relationships (Godthelp *et al.* 1999); it was only after the subsequent referral of tarsal remains preserving diagnostic apomorphies that *Djarthia* could be identified as the oldest unequivocal member of the crown-group marsupial clade Australidelphia (Beck *et al.* 2008).

A similar problem arises when the potentially diagnostic morphological apomorphies occur early in ontogeny. In such cases, adult material, even when abundantly available, can be uninformative with regard to the question at hand. Unfortunately, well-preserved fossils of juvenile mammals are comparatively rare (but see Rougier *et al.* 1998; Shoshani *et al.* 2006) because their hard parts

are usually much less robust than those of adults. Thus, unequivocally documenting the presence of a petrosal-derived bulla (a widely accepted diagnostic apomorphy of crown-group primates; MacPhee 1981) in a given case requires young specimens of the taxon to show that no suture ever intervenes between the bulla and promontorium. When this criterion is applied to Plesiadapiformes, none could currently qualify as crown-group primates simply because there are no known taxa in which bullar composition can be fully resolved (Boyer 2009, but for a contrary opinion regarding *Ignacius graybullianus*, see Kay *et al.* 2001). Similarly, determining the presence or absence of the highly derived, potentially diagnostic pattern of tooth replacement seen in living marsupials (in which replacement is restricted to the last premolar; Luo *et al.* 2004) in fossils requires adequate juvenile material that is only rarely available (Cifelli and de Muizon 1998).

Finally, correctly placing fossils in a phylogeny can also prove difficult when there are few or no genuinely diagnostic apomorphies for the group of interest. The latter is the case for all placental superorders except for the morphologically highly derived xenarthrans. For example, putative morphological synapomorphies of Afrotheria, such as an increased number of thoracolumbar vertebrae (Sánchez-Villagra *et al.* 2007) or delayed eruption of the permanent dentition (Asher and Lehmann 2008), show considerable homoplasy both within Afrotheria and between afrotherians and other placentals (see Asher and Lehmann 2008: their fig. 3). Thus, confidently assigning fossils to Afrotheria might be expected to be extremely difficult; indeed, the 'pseudoextinction' analyses of Springer *et al.* (2007) argue that morphological data alone are often insufficient to correctly place extant placentals in their appropriate superorder (but see Asher and Hofreiter 2006; Asher *et al.* 2008; Lee and Camens 2009).

This situation raises the possibility that the earliest members of each of the placental superorders were morphologically little different from stem eutherians. Potential support for this hypothesis is provided by a series of recent papers (Hooker 2001; Boyer *et al.* 2010; Prasad *et al.* 2010; Seiffert 2010; Smith *et al.* 2010) that highlight close dental and post-cranial similarities between *Deccanolestes*, the adapisoriculids *Adapisoriculus*, *Bustylus* and *Remiculus* from the Palaeocene of Europe, and *Afrodon*, a taxon originally known from dental specimens from the latest Palaeocene and earliest Eocene of North Africa but subsequently also identified in the early Palaeocene of Europe. Adapisoriculids have been identified as plesiadapiforms (Storch 2008; Smith *et al.* 2010) and hence members of the placental supraordinal clade Euarchonta. *Deccanolestes* may be a euarchontan (Hooker 2001; Boyer *et al.* 2010; Smith *et al.* 2010), an afrotherian (Goswami *et al.* 2010a), or a stem eutherian (Wible *et al.* 2007). Finally, *Afrodon* may be an adapisoriculid (and hence a possible euarchontan; Smith *et al.* 2010) or an afrotherian (Seiffert 2010). It seems probable that at

least one of these taxa is indeed a crown-group placental, yet lacks diagnostic apomorphies that would unequivocally identify it as such; the position of *Purgatorius* (widely thought to be a euarchontan) outside Placentalia in the analysis of Wible *et al.* (2007) may represent another example of this (see Boyer *et al.* 2010). If *Deccanolestes* is a stem eutherian (Wible *et al.* 2007), adapisoriculids are euarchontans (Boyer *et al.* 2010; Smith *et al.* 2010) and *Afrodon* is an afrotherian (Seiffert 2010), then their close morphological similarities are presumably plesiomorphic retentions. This, in turn, raises the possibility that the earliest members of the four superorders may have been 'adapisoriculid-like' in dental, and possibly also post-cranial, morphology (Goswami *et al.* 2010b; Seiffert 2010).

Fossils as divergence date estimators

A traditional palaeontological view is that a lineage begins with the 'first' or earliest taxon that can be assigned to that lineage on the basis of shared derived characters. In deference to evolutionary theory, an indeterminate (but usually small) amount of time is often allowed for 'prior evolution' to take care of the problem that a fossil taxon that already possesses the earmark apomorphies of a monophyletic group must itself descend from an ancestor that lacked those apomorphies (or expressed them differently), yet nevertheless also belonged to that group. Looked at in this way, the independent history of the modern placental orders has usually been assumed by palaeontologists to begin within a few million years of the K–Pg boundary for most groups because this is as far back as the 'first taxa + prior evolution' estimate allows one to push the available data without interpolating the lengthy ghost lineages (e.g. of up to 20–30 Ma, if not more) many molecular-based results would require.

However, the true period of time between a lineage's origin and the acquisition of its first diagnostic apomorphies is probably unknowable, and there seems no a-priori reason why it could not be quite lengthy. In the absence of compelling evidence for a 'morphological clock' (see Larsson *et al.*, this volume), it seems probable that it varies considerably both between different clades and between lineages within the same clade. If this period is long, then even a perfect fossil record will considerably underestimate the time of origin because the oldest fossil taxon with diagnostic apomorphies will be (much) younger than its parent node. Verifying the existence of such 'apomorphy lag' is difficult, because its most obvious manifestation will be a large discrepancy between (older) molecular and (younger) fossil estimates of the age of the node in question, for which several other explanations are possible (as discussed elsewhere in this chapter), and because, by definition, it cannot be identified

by phylogenetic analyses of morphological data. As such, it represents a convenient, but possibly inherently untestable, ad hoc explanation that can be invoked whenever molecular and fossil divergence dates are incongruent.

Reconciliation with palaeobiogeography

Phylogenetic trees that include information on nodal divergence dates – such as the ones discussed in this chapter – have implications. Palaeobiogeographical interpretations of the distributional history of Late Cretaceous and earliest Cenozoic eutherians, for example, can be strongly influenced by one's preferred reconstruction of their phylogeny. Thus, a clear implication of the Explosive Model (like that favoured by Wible *et al.* 2007) is that post-divergence distributions must have been largely accomplished by over-water transport of propagules rather than dispersal across terrestrial portals (e.g. pre-rift conjugate terranes or land bridges).

Recent plate-tectonic and palaeogeographic reconstructions for the interval 150–50 Ma ago (e.g. Aitchison *et al.* 2007; Eagles 2007, 2010; Whittaker *et al.* 2007; Ali and Huber 2010; Ali and Krause, 2011;) indicate that, with the exception of southern South America/West Antarctica (via the Antarctic Peninsula) and probably also eastern Antarctica/Australia (via the South Tasman Rise), the major terranes comprising Gondwana did not remain in proximate contact after the close of the Mesozoic. Dispersal of the ancestors of australidelphian marsupials into Australia before the final, apparently gradual separation of the latter from East Antarctica (Woodburne and Case 1996; Whittaker *et al.* 2007; Beck 2008) could have involved an all-terrestrial route any time from the late Mesozoic until the earliest Eocene. Evans *et al.* (2008) make a case for several terrestrial vertebrate taxa (but no eutherians) dispersing across Antarctica via a dry-land route joining India/Madagascar to southern South America.

These points are of interest here because the lack of portals among Gondwanan fragments by the early Cenozoic should have acted as a very strong constraint on mammalian movements. Yet this factor is either ignored or side-stepped by studies advocating an Explosive Model. The necessary corollary to this argument is that nothing except non-placental eutherians (and other kinds of mammaliaforms) could have occupied Gondwanan daughter terranes until the start of the Cenozoic, when the modern orders came into existence. This is effectively a Palaeogene version of the Sherwin–Williams Effect (Hershkovitz 1968; Clemens 1986) that divergences of major mammalian lineages are always assumed to have occurred on northern landmasses, with movement to the south only taking place significantly later. The alternative is that initial differentiation of Placentalia not only began well within the Cretaceous, but also occurred

among clades already occupying Gondwanan terranes whose fossils are as yet unknown. Is this plausible? Fossil taxa such as the Maastrichtian *Deccanolestes* and *Kharmerungulatum* from India hint that it might be (see also Krause and Maas 1990), although the generally poor Cretaceous fossil record for Africa, India and Australasia (see above) prevents a definite assessment of this hypothesis.

And clocks: assumptions underlying molecular-based date estimates

The molecular-clock hypothesis

All molecular-based methods of divergence time estimates rely crucially on two factors: (1) calibration data in the form of fossil and/or biogeographic events and (2) the assumption that molecular data evolve in a more-or-less clock-like fashion (the molecular-clock hypothesis). We have already dealt with the data and inference issues underlying the first factor and will only add here that the fact that poor calibration data can cause problems should be obvious (although the scale of any errors is often unknown). Indeed, in cases of conflict, molecular phylogeneticists typically question the quality of the calibration data in the first instance (e.g. Near and Sanderson 2004; Inoue *et al.* 2010; Pyron 2010). But what of the other side of the equation?

The molecular-clock hypothesis dates to the early 1960s when Zuckerkandl and Pauling (1962, 1965) noted that, unlike morphological evolution (but see Polly 2001), amino-acid changes accumulated at a relatively constant rate over the long term in haemoglobin. This hypothesis, in turn, formed an important cornerstone of the neutral theory of evolution first proposed by Kimura (1983). In many ways, however, the molecular-clock hypothesis has been misinterpreted to imply an absolutely constant rate of molecular evolution or a strict molecular clock. Even from the earliest days, however, it was realized that there was no universal clock. In generalizing their observations, Zuckerkandl and Pauling (1962, 1965)were careful to make it protein specific. Today, we know that different proteins run according to different clocks in line with the degree of inferred functional constraint they are under (more constrained genes tend to evolve more slowly; see data in Nei 1987; Ohta 1995) and differences between the faster mitochondrial and slower nuclear genomes are also apparent. In addition, it was noted that the clock could fluctuate, often greatly, over the short term. It was only over evolutionary time spans that a clock-like behaviour became apparent.

It is well accepted today that the rate of molecular evolution also varies between organismal groups (Britten 1986; Drake *et al.* 1998) and often

dramatically so (e.g. viruses, the extreme speedsters, juxtaposed against the more stately mammals) because of differences in the accuracy of the DNA replication machinery and/or the inverse relationship between substitution rate and body size, whether directly or indirectly through generation time. This rate variation can also occur on a more taxonomically restricted scale and also within the same gene (heterotachy). Among mammals, for instance, rodents have a comparatively high rate of molecular evolution, whereas the clock is appreciably slower in the great apes (the 'hominid slowdown') and cetaceans; other rate differences across mammals are also apparent (see Bininda-Emonds 2007).

Although the idea of a global clock has largely been discredited for the reasons provided above, it is unfortunately still commonly applied. For instance, a substitution rate of 2% per million years for mitochondrial DNA has been applied for primates, birds and arthropods, among other groups (see Brown *et al.* 1979; Brower 1994; Ho 2007)! In addition to the improbability of such diverse groups all possessing the same rate of evolution, the use of such a value also ignores the documented rate variation among mtDNA genes (e.g. estimated as 19.2× across genes for mammals in Bininda-Emonds 2007).

Given that the molecular clock is not so much of a Rolex as it is a Timex, what hope is there then for molecular-based date estimates? Fortunately, clock-like evolution does appear to occur on more restricted timescales (and thus also taxonomic scales) before heterotachy acts to change the rate of evolution significantly. In fact, there might be a surprising amount of (local) clock-like activity. For instance, the comprehensive study of Bininda-Emonds (2007) revealed very few mammalian clades (42 of 1282) or individual branches in the mammalian tree (74 of 3332) where the rate of evolution differed significantly from the overall mammalian average. Significant changes in rate compared with an ancestral node or branch were even more rare (38 and 20, respectively). Mapping of the cytochrome b (*MT-CYB*) data set from Bininda-Emonds *et al.* (2007) onto the topology of the mammalian supertree (Bininda-Emonds *et al.* 2007) reveals more surprises. Using ModelTEST v3.7 (Posada and Crandall 1998), PAUP* v4.0b10 (Swofford 2002), and a likelihood ratio test to examine each clade in turn, a clock-like rate of evolution could not be rejected at the 0.05 level (corrected for multiple comparisons using a Holm–Bonferroni correction; Holm 1979) for 237 of the 561 clades in total (= 42.2%) (red + green lineages in Figure 3.1)! As would be expected with the idea of a local clock, the clock-like clades were both significantly younger (16.5 ± 13.7 versus 22.1 ± 20.2 Ma (mean ± SD); Mann–Whitney $U = 3.348 \times 10^4$; $z = -2.593$, $P = 0.009518$) and smaller in size (5.6 ± 3.6 versus 25.9 ± 34.6 species; $U = 1.674 \times 10^4$; $z = -11.5$, $P = 1259 \times 10^{-30}$) than the non-clock-like clades. However, the oldest clock-like clade, which links the three representatives in the tree for Afrosoricida and

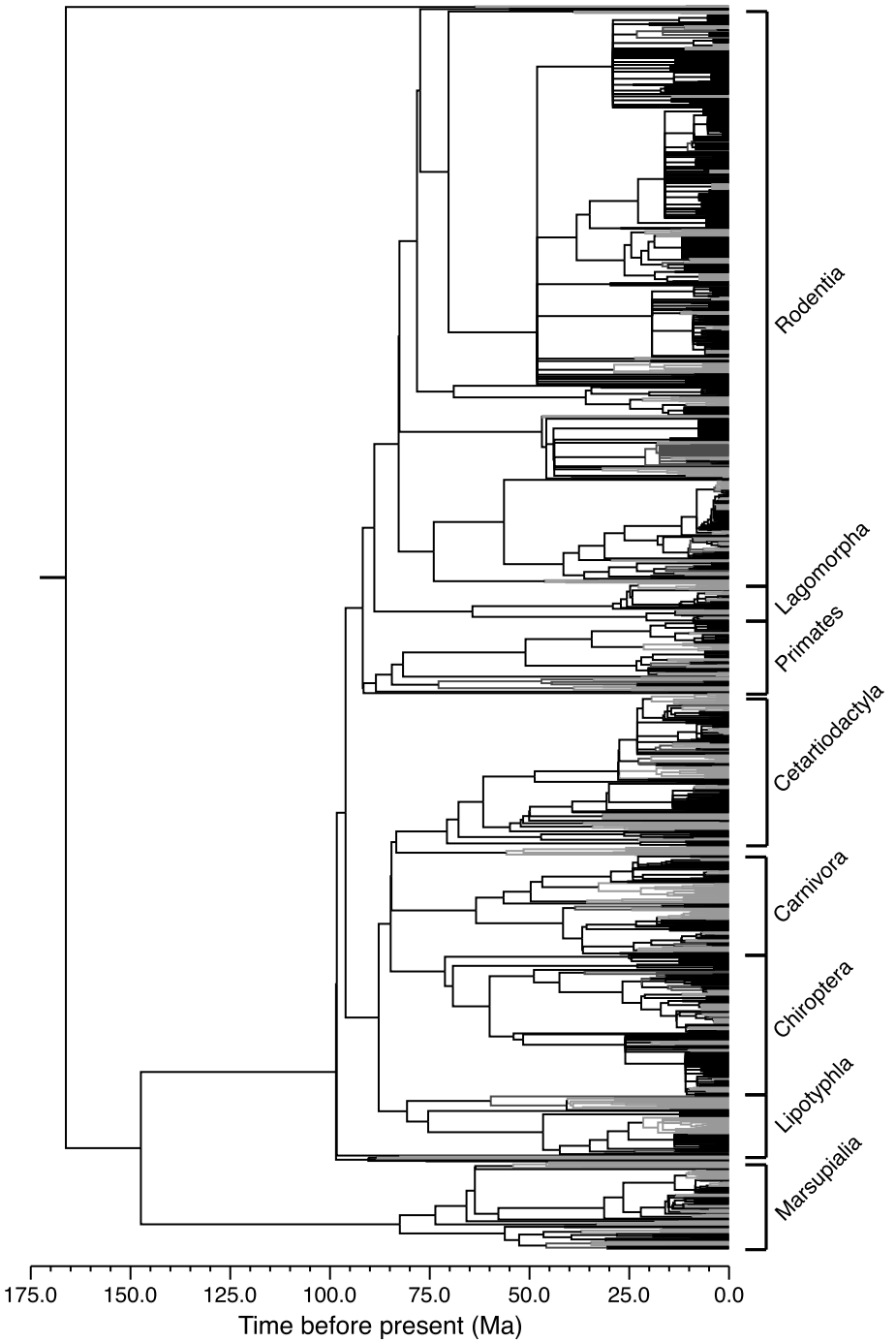

Figure 3.1 The mammal supertree of Bininda-Emonds *et al*. (2007, 2008) restricted to those species for which *MT-CYB* data were available. Clades that evolve in a clock-like fashion according to a likelihood ratio test with a nominal *P*-value of 0.05 are highlighted in either green (clade and all its descendant clades are clock-like) or red (only the focal clade is clock-like). (See also colour plate.)

Macroscelidea, is some 88.3 Ma old, a much longer timescale for clock-like behaviour than perhaps expected by many. (The largest clock-like clade comprised 22 rodent species, spanning from Echimydae plus *Capromys pilorides*, and was 21 Ma old.)

Admittedly, the previous comparisons run into a statistical problem of non-independence given the hierarchical structure of the tree, meaning that clock-like clades can contain other clock-like clades, therefore overestimating their frequency. However, this need not be the case, and there are several instances where a clock-like status emerged despite not being present in the subclades. Moreover, the non-independence problem only occurs because we have examined all the constituent clades in turn: the clock-like clades would retain this characteristic if they were examined in isolation. If, however, we apply the more conservative criterion that all subclades in a clade must evolve in a clock-like fashion for the clade to recognized as such, then 96 such clades still remain (green lineages in Figure 3.1), with no appreciable difference in average size (5.0 ± 2.8 species; maximum = 17 species within the shrew genus *Sorex*) or age (17.9 ± 1.53 Ma; maximum = 88.3 Ma for the Afrosoricida + Macroscelidea clade) than before.

Thus, although the idea of a global clock is largely untenable, local clocks do appear to exist and might, in fact, be more widespread than is commonly assumed. In fact, local clocks play a crucial role in molecular dating studies given that they underlie most modern methods in some form. True local clock methods such as non-parametric rate smoothing (NPRS; Sanderson 1997), PATHd8 (Britton *et al.* 2007), or relDate (Purvis 1995; Bininda-Emonds *et al.* 2007) allow for different rates of molecular evolution among different lineages, but assume the rate to be constant within each lineage. Relaxed clock methods such as penalized likelihood (PL; Sanderson 2002) and those implemented in PhyBayes (Aris-Brosou and Yang 2002) and MULTIDIVTIME (Thorne and Kishino 2002) model rates as being autocorrelated along branches, essentially assuming that the rate of molecular evolution itself is a heritable character that can evolve over time. Yet another solution is provided by BEAST (Drummond and Rambaut 2007), which, in addition to a strict molecular clock, also implements uncorrelated relaxed clock models in which the rates of molecular evolution are drawn from an underlying rate distribution (e.g. exponential or log normal distributions). These two categories of methods represent the extremes of a continuum: as the lineages for local clock methods become increasingly restricted taxonomically, the methods will increasingly resemble relaxed clock methods (albeit without any model for the rate of molecular evolution). Even a global clock is simply an extreme version of a local clock method, with only a single lineage being specified.

Revisiting the use of fossils as calibration points in molecular dating analyses

In molecular dating analyses, fossils (and biogeographic or stratigraphic data) not only act as calibration points to indicate how fast the molecular clock is ticking, but are often also used to constrain the range of divergence date for the same nodes as well. Because fossils can, at best, only be as old as their associated nodes, they have typically been used to provide minimum age constraints for that node. A frequent criticism of molecular studies then was that although many nodes possessed lower bounds, comparatively few possessed upper bounds, potentially contributing to the inflation of molecular divergence time estimates compared with strictly fossil based ones. This criticism could apply to the mammal supertree. As noted above, only the root was fixed at 166.2 Ma (based on the Middle Jurassic Malagasy fossil *Ambondro mahabo*); all other fossil calibrations were minimal age estimates and upper bounds were not specified. Thus, although the maximum age of the entire tree was capped, no such restriction existed for nodes within the tree. That being said, there is no evidence for the divergence times bunching up against the 166.2 Ma ceiling, which would indicate potential age inflation among the internal nodes.

The use of maximal age constraints is becoming increasingly common (see Benton *et al.* 2009; Phillips *et al.* 2009) and is usually associated with range constraints. Indeed, the case could be made within extant mammals that at least some of the fossil calibrations for the ordinal crown groups could approximate maximal age constraints for particularly well sampled and morphologically distinct taxa. For example, the teeth of crown-group rodents are highly diagnostic (Meng and Wyss 2005) and, of the hard anatomy, teeth are the most commonly preserved mammalian fossils. Yet, no single rodent cheek tooth has ever been identified from Cretaceous strata, suggesting strongly that the crown-group rodents are restricted to the Cenozoic. A similar case (albeit not based on tooth characters exclusively) could be made for crown-group primates with their well-researched fossil record (Bloch *et al.* 2007, but see Tavaré *et al.* 2002; Martin *et al.* 2007). Any alternative explanations (e.g. preservation bias) seem dubious, as they would have to explain why there is no trace of the Cretaceous members of these two clades that are otherwise well represented in the Cenozoic fossil record. In the case of rodents, the presence in Cretaceous sediments of probable ecological equivalents, namely multituberculates (Krause 1986), also require that such hypothetical preservation biases would be driven by phylogeny rather than by ecology, which seems unlikely.

Such upper bounds, however, remain inherently problematic in that they can easily be overturned by the old saw of a single new discovery. Unlike lower

bounds, reasonable upper bounds are also more difficult to specify robustly, the primate and rodent examples above being the possible exceptions. Methods including the application of phylogenetic bracketing (Reisz and Müller 2004; Hug and Roger 2007) and stochastic modelling (Tavaré *et al.* 2002) do exist for this purpose, but have yet to find wide use and also have specific shortcomings (see Ho and Phillips 2009). Moreover, a molecular overestimate may indicate that a given calibration is too young and that new fossil material is waiting to be discovered. An excellent example here is the recent discovery of crown-group strepsirhine primates from the middle Eocene of Egypt (Seiffert *et al.* 2003; Seiffert 2007a). These fossils are both relatively congruent with prior molecular studies that indicated a 50–62 Ma date for the first splits within crown-Strepsirhini and also supported the prevailing contention among palaeontologists that the previous earliest fossil record for the group (some 20 Ma younger from the early Miocene) were too young.

More recently, an important development in molecular dating methods has been the modelling of uncertainty in calibration data via the use of soft bounds and/or a variety of probabilistic distributions (Drummond *et al.* 2006; Yang and Rannala 2006), thereby causing calibration data to become prior probabilities instead of point estimates. These same methods can be used to specify (probabilistic) upper constraints for any calibration point as well. As important as these advances have been, it still must be realized that the validity of the (many) available models remains to be established, as do reasonable parameters for them. Most represent standard statistical models (e.g. normal, log-normal, exponential or gamma), but this does not automatically guarantee that they represent an accurate portrayal of modelling the uncertainty in calibration data. For instance, the discoveries of *Murtoilestes abramovi* (Averianov and Skutschas 2001) at ~120–128 Ma and *Eomaia scansoria* (Ji *et al.* 2002) at ~125 Ma pushed back the evidence for the origin of eutherian mammals by some 13% from previous estimates (Cifelli 1999), probably well outside the upper bounds many users would specify for the statistical models. More recently, the description of *Juramaia sinensis* (Luo *et al.* 2011b) at ~160 Ma adds another astonishing 28% to these two estimates. Actual research to quantify exactly how uncertain fossil calibrations might be is rare (but see Tavaré *et al.* 2002). In any case, the use of fuzzy calibrations/constraints does seem to improve and/or focus molecular date estimates (Inoue *et al.* 2010) and the use of some vaguely realistic bounds has to be preferable to the open-ended scenario that was in play before. The changes brought about by using bounded estimates can sometimes be dramatic, as shown by Ho and Phillips (2009). In their study, the use of bounded (including distributional) calibrations shifted the inferred origin of Neoaves across the K–Pg boundary into the Cretaceous, in contrast to the use

of point estimates, which instead inferred a Palaeogene origin. Underlining this difference is that the 95% HPD (highest posterior density) intervals for the respective estimates did not overlap at all.

In the future, additional improvements to model accuracy could be achieved by the modelling of clade diversification and variation in fossil recovery potential in time and space (Inoue *et al.* 2010; Marjanović and Laurin 2008). Such parameters are not incorporated currently in existing molecular-dating software.

Modelling the rate of molecular evolution

Modern molecular dating methods are invariably model driven, usually in a likelihood framework. Minimally, this involves likelihood estimates of the amount of molecular evolution, but can also include modelling how these data translate into actual divergence times for the relaxed clock methods. The use of models can help us to represent the evolutionary process more precisely, thereby improving our estimates, but they are simultaneously problematic in that they are simple, tractable implementations of what are probably extremely complicated processes. Both the simplification itself and choosing which simplification is the right one can have important consequences.

Likelihood-derived branch lengths are actually rates measured in average number of substitutions per site per unit time, therefore confounding the amount of evolutionary change with the time in which it has taken place. Thus, whereas a long branch means that a lot of evolution has occurred, it does not reveal whether it has occurred over a long time span at a slow rate, over a short time span at a fast rate, or something in between. However, given that modern molecular dating methods necessarily assume some form of relative rate constancy, long branches default to meaning more evolution over longer times. Thus, without the appropriate calibration data, changes in the rate of molecular evolution will be difficult to detect (although less so for large changes) and, in fact, will tend to be smoothed out by the different methods (but see Kitazoe *et al.* 2007).

Evolutionary scenarios do exist, however, where a dramatic fluctuation in the rate of molecular evolution can be imagined, if not likely, to occur. One example is adaptive radiations, where a high rate of phenotypic evolution is coupled with a burst of speciation in a short time span. It seems likely that the rate of molecular evolution, which ultimately underlies the other two phenomena, would also spike during these intervals (e.g. Kitazoe *et al.* 2007), even in the apparent absence of a long-term relationship between the rates of phenotypic and molecular evolution (Davies and Savolainen 2006; Bromham *et al.* 2002, but see Seligmann 2010). Exactly such an event is inferred by

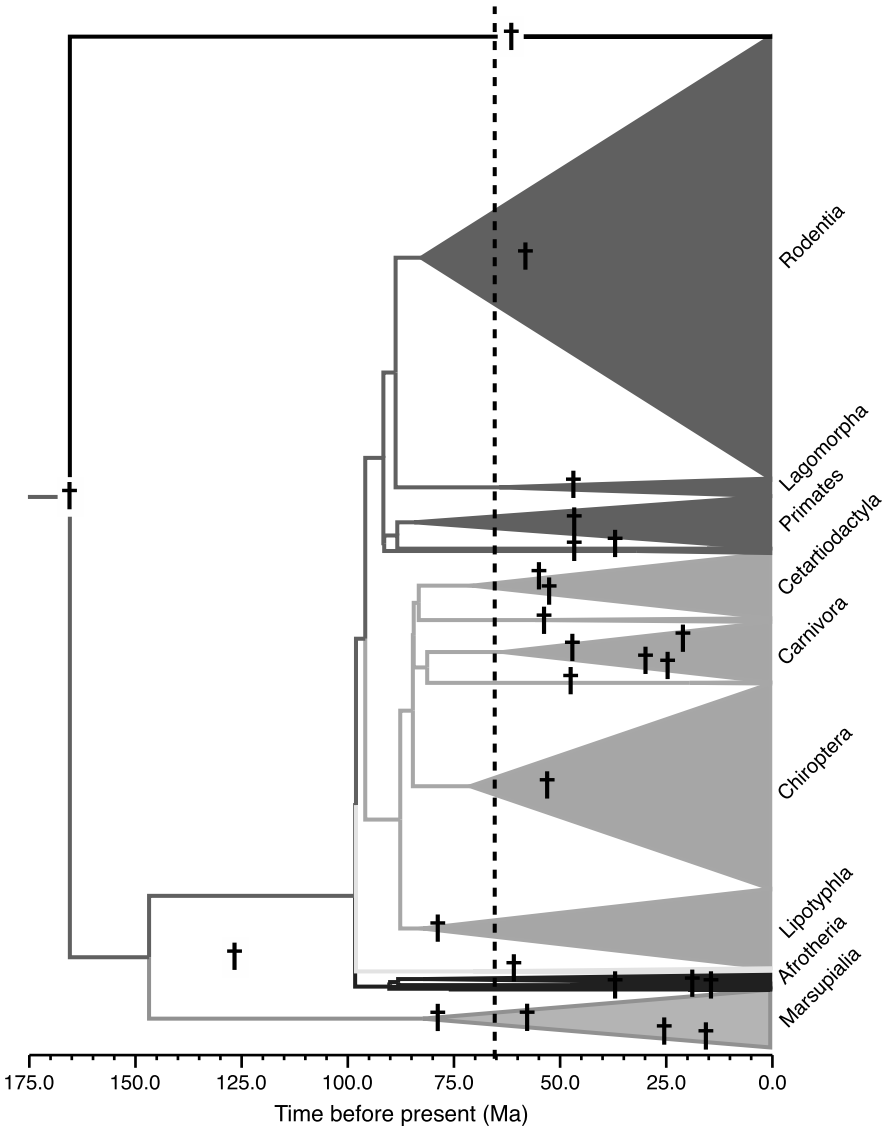

Figure 3.2 Simplified representation of the mammal supertree (Bininda-Emonds *et al.* 2007, 2008) showing the approximate temporal and phylogenetic position of the 30 fossil calibration points used (marked with †). The K–Pg boundary is indicated by the dashed vertical line. (See also colour plate.)

palaeontologists to have occurred immediately after the K–Pg boundary with the demise of the non-avian dinosaurs opening up new niches for the surviving forms (including mammals). However, given the paucity of suitable fossils before this time to recalibrate the clock (see Figure 3.2), such an adaptive

radiation for mammals could be easily missed by molecular methods. Curiously, some molecular studies do detect an adaptive radiation of the placental mammal orders, but place it well in advance of the K–Pg boundary at 80 Ma or older (Springer *et al.* 2003; Bininda-Emonds *et al.* 2007). Could this be the missing post-K–Pg radiation of mammals?

The effect on the rate of molecular evolution of the restricted time span of an adaptive radiation can be modelled by constraining the basal divergences for all ordinal crown groups of the mammal supertree to occur between 62.5 and 65.5 Ma (which is the extreme case and admittedly unrealistic for more recent crown groups such as Hyracoidea) and redating the rest of the tree. Doing so results in no significant difference across all node ages compared with the original ages, but differences for those ages associated with each of the nodes linking the ordinal crown groups, the basal-most nodes within the crown groups, and the remainder of the crown-group nodes (Table 3.1; Figure 3.3). The change for the basal crown-group nodes, which all bunch up under the K–Pg boundary, is especially apparent, as is the greater variation for the remaining crown-group nodes. More importantly, significant differences in the inferred rates of evolution occur, with branch specific rates of evolution being significantly increased for the basal and remaining crown-group branches and the degree of local rate shifts being significantly decreased for the branches linking the crown groups (Table 3.1; Figure 3.4). Again, without the necessary calibration data, such increased rates would likely be smoothed out by most programs.

In addition, even gradual, directed changes in the rate of molecular evolution could prove to be problematic for most molecular methods, which generally model rate evolution as a stochastic and/or autocorrelated process (see Drummond *et al.* 2006). Again, in the absence of appropriate calibration data, it might not be possible to account for concerted changes in the clock (e.g. either a continual speedup or slowdown with time). It is known, for example, that Cretaceous mammals were, on average, smaller than Palaeogene and Recent forms (Alroy 1999) and also that body size tends to cluster among mammals (e.g. rodents being generally small, cetaceans being large). It is also well established that the rate of molecular evolution in mammals correlates strongly and inversely with body size (Martin and Palumbi 1993; Bromham *et al.* 1996; Lanfear *et al.* 2010), meaning that there might have been a gradual, possibly lineage-specific, overall slowdown in the mammalian clock over time. Correcting for this artifact by incorporating body size or generation time information into the models, however, would have the effect of drawing the deeper mammalian divergences even farther back in time given that the inferred amounts of molecular evolution would have actually occurred in a shorter timeframe than has been reconstructed currently. Indirect evidence here is provided by the

Table 3.1 Statistical comparison of branch-specific rates of evolution and shifts in the rate of evolution compared with an ancestral node for the mammal supertree when the basal divergences of all ordinal crown groups are constrained to occur between 62.5 and 65.5 Ma, thereby mimicking the Explosive Model of placental evolution. All comparisons used a Wilcoxon signed rank test; *P*-values with an asterisk indicate significant differences at a nominal *P*-value of 0.05 corrected for multiple comparisons (Holm 1979). Values were obtained using the Perl script moleRat.pl v1.0 following the procedure in Bininda-Emonds (2007). Taxonomic partitions were defined as follows: stem – all branches basal to the ordinal crown groups; basal – basal node of the ordinal crown groups and all immediate daughter and granddaughter nodes; crown group – all remaining nodes in the crown group. Only nodes that were older than 35 Ma according to Bininda-Emonds *et al.* (2008) were included.

Variable	Partition	*n*	Median of original value	Median value under Explosive Model	Trend relative to original values	*W*	*z*	*P*
Node age	All	119	54.9	62.5	+	3861	0.7717	0.4403
	Stem	24	86.3	86.2	−	280	3.714	0.0002*
	Basal	49	56.5	62.5	+	1091	4.76	1.94×10^{-6}*
	Crown	46	46.2	50.9	+	958	4.561	5.08×10^{-6}*
Branch-specific rate of evolution (normalized)	All	118	−3.90	−2.24	+	4131	2.807	0.0050*
	Stem	24	−3.16	−1.98	+	195	1.286	0.1985
	Basal	49	−5.39	−0.37	+	1053	4.382	1.18×10^{-5}*
	Crown	45	−2.68	−2.59	+	813.5	3.341	0.0008*
Rate shift relative to an ancestral node (normalized)	All	133	−0.10	−0.23	−	4805	0.7849	0.4325*
	Stem	23	0.45	−0.08	−	237	3.011	0.0026*
	Basal	50	−0.46	3.08	+	732	0.9122	3.62×10^{-1}*
	Crown	60	−0.11	−1.56	−	1106	1.406	0.1597

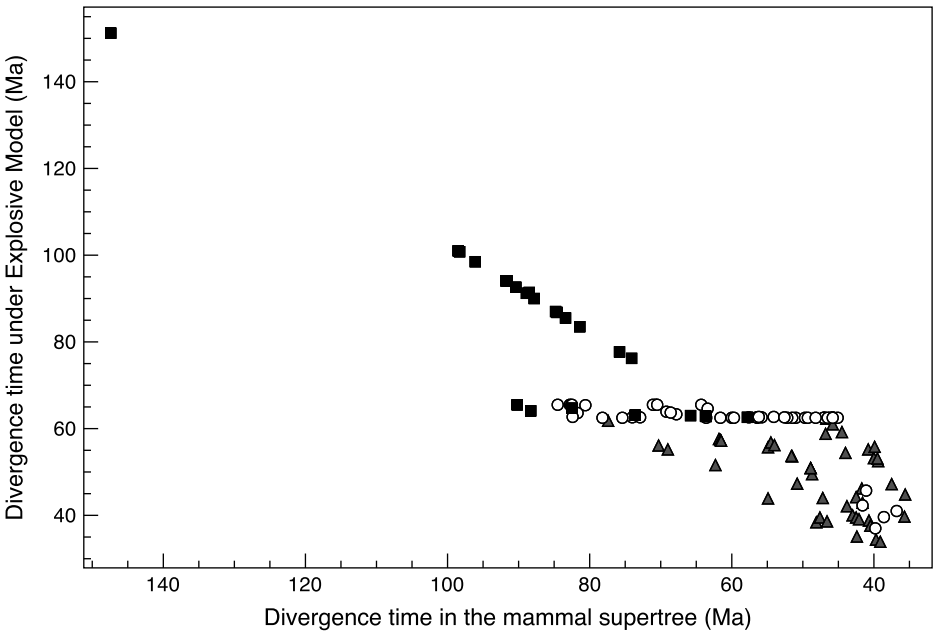

Figure 3.3 Graphical comparison of node ages from the mammal supertree (Bininda-Emonds *et al.* 2007, 2008) and a redating that models the Explosive Model of placental evolution. Nodes are partitioned taxonomically as (1) all branches linking the ordinal crown groups (black squares), (2) basal node of the ordinal crown groups and all immediate daughter and granddaughter nodes (white circles), and (3) all remaining nodes in the crown group (grey triangles). Only nodes that were older than 35 Ma according to Bininda-Emonds (2008) were included.

mammal supertree (Bininda-Emonds *et al.* 2007, 2008), where the molecular date estimates for the origin of the Cetacea, a taxon characterized by large body sizes and slow generation times (and therefore a slow rate of molecular evolution), severely underestimate the fossil calibration (22.9 versus 52.2 Ma, respectively). By contrast, when Springer *et al.* (2003) restricted their analyses to species that appear to have maintained (small) body sizes similar to those of Cretaceous mammals, divergence time estimates compared with the full analysis were largely unchanged, if not slightly younger.

Even in the absence of such scenarios as above, it remains that the application of a single, simple model of evolution often will not reflect biological reality. Most of the models being used currently can account for rate variation between sites in a given sequence (rate heterogeneity as modelled using gamma distribution), but not between clades in a tree despite there being good evidence for heterotachy in many groups. Indeed, it has been shown that accounting for

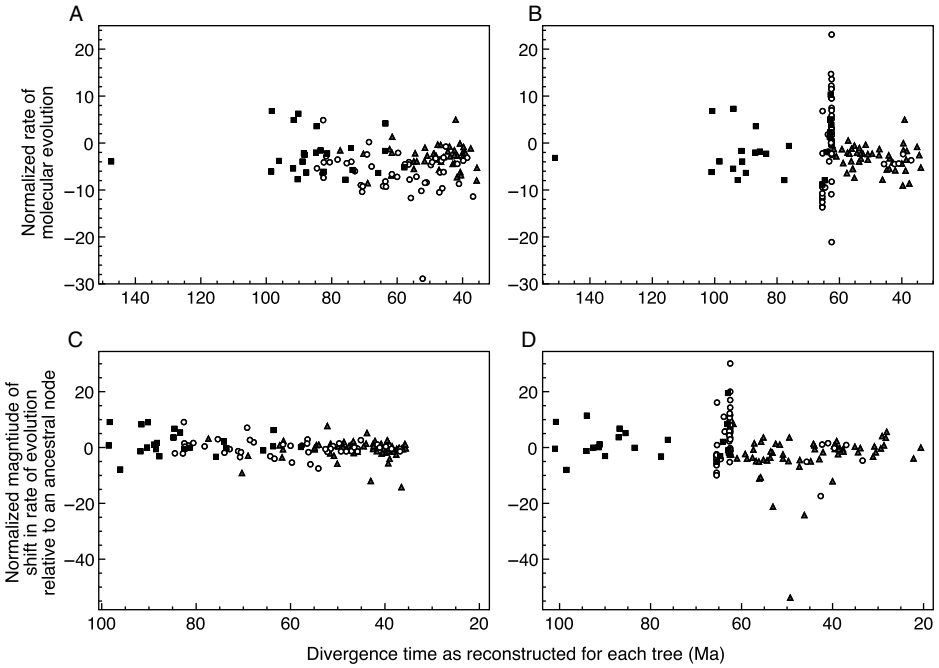

Figure 3.4 Graphical comparison of rates of molecular evolution inferred from the mammal supertree (A, C) and a redating that models the Explosive Model of placental evolution (B, D). Comparisons are made for both normalized branch-specific rates of evolution (A, B) and normalized relative shifts in the rate of evolution compared with an ancestral node (C, D) (following Bininda-Emonds 2007). Taxonomic partitions are as in Figure 3.3.

the latter might dramatically impact our molecular-based divergence time estimates. In using a model to correct for heterotachy within placental mammals (and particularly for speedups in the lineages leading to Euarchontoglires and Laurasiatheria and a subsequent slowdown within the latter), Kitazoe *et al.* (2007) reconstructed divergence time estimates for the ordinal crown groups that more closely agreed with those from the palaeontological Explosive Model (although a number of lineages still crossed into the Cretaceous). In so doing, however, it should be noted that their model of rate variation favours relatively recent radiations. They also did not use any calibration data older than 65 Ma, which might otherwise have pulled the divergence times older, and also used some maximal age constraints. Thus, although they constrained the divergence between the rabbit and the pika to 36–55 Ma, their molecular estimate for this node at ~55 Ma (i.e. at the upper limit of their constraint) suggests that this fossil calibration may be an underestimate (see Arnason *et al.* 2008). An

outstanding problem, however, is recognizing heterotachy a priori or during the course of the analysis. Here the application of relative rates tests (Sarich and Wilson 1973; Tajima 1993), which appear to have fallen somewhat out of favour, might be a viable solution.

In a related vein, choosing the right model of evolution and the right parameters for it are also important considerations. There has been much work into the modelling of mtDNA data, with several studies showing that the use of purine-pyrimidine (RY) coding for the third positions often improves the accuracy of phylogenetic estimates from these data (Phillips and Penny 2003; Phillips *et al.* 2004). Phillips (2009) has extended this research to show that conventional coding for mtDNA leads to proportionately longer branches deep in the vertebrate tree compared with RY-coding when using deep calibration points. This, in turn, leads to proportionately older divergence time estimates, which again might help to explain the discrepancy between molecular- and fossil-based dates for the placental orders.

Complicating this story is the question of whether only a single model across the tree (as opposed to the sequence data) should be used. Analysis of the *MT-CYB* data for the mammal supertree also reveals that the use of a single substitution model across the tree is unrealistic. Of the 561 nodes for which an optimal model could be estimated using ModelTEST, 6 of the 14 base models in ModelTEST were indicated (HKY, TrN, K81uf, TIM, TVM, GTR) and 23 of the 56 models incorporating correction for invariant sites (+I) and rate heterogeneity (+G). Thus, analogous to the relationship between heterotachy and gamma-corrected rate heterogeneity, a tree-based counterpart to partition-specific substitution models is arguably needed as well.

In estimating (molecular) divergence times, we are chasing an unknown, or worse, compounding two unknowns: the topology of the phylogenetic tree and its branch lengths. However, unlike the case with the pattern of relationships, we often have no good a-priori idea of what a reasonable divergence time estimate should be. That living primates form a single clade is a reasonable expectation, as borne out by application of phylogenetic methods. But, we have little to no intuitive idea precisely how old crown-group primates should be beyond a rough estimate of between 55 (Bloch *et al.* 2007) and, at the outside, 80–100 Ma (Tavaré *et al.* 2002; Martin *et al.* 2007).

Thus, in the absence of a largely complete fossil record, it is often difficult to assess the accuracy of our estimates. Simulation studies can always be undercut by the argument that the parameters used are not realistic. In the end, we are left with a congruence-based approach, both with fossil data as well as with other molecular-based estimates. But, analogous to the conflict with fossil data, even different molecular-based estimates often differ widely. Some of the

earliest molecular studies argue for a 'Short-fuse Model' (*sensu* Archibald and Deutschmann 2001) of placental evolution, whereby both the origins and basal diversifications of many of the ordinal crown groups appear well within the Cretaceous (Springer 1997; Kumar and Hedges 1998; Penny *et al.* 1999). More recent studies favour a 'Long-fuse' Model (*sensu* Archibald and Deutschmann 2001), where only the origins occur in the Cretaceous, but the radiations of the crown groups primarily occur in the Cenozoic in agreement with conventional interpretations of the fossil evidence (Springer *et al.* 2003). Still other results argue for an intermediate scenario between these extremes. So, although there is agreement among most studies that placental mammals are older than their first appearances in the fossil record, there is little consensus on just how much older this might be.

In many cases, the different estimates arise because of the different assumptions and models being used by the molecular methods. A cogent example is provided by Welch *et al.* (2005), who examined why analysis of the same data set by two different methods could give wildly divergent estimates for the timing of the divergence between protostomes and deuterostomes. Whereas an earlier local clock-based study (Bromham *et al.* 1998) placed this split at no earlier than 680 Ma, a relaxed-clock study (Aris-Brosou and Yang 2003) indicated a more recent date of 582 ± 112 Ma in line with the Cambrian explosion hypothesis. The difference, Welch *et al.* (2005) concluded, was due in part to the statistically tractable, but probably unrealistic, assumptions being made in the relaxed-clock analysis: a constant net speciation rate through time and a random sampling of the sequences at the tips. Even between comparable methods like the two relaxed-clock methods MCMCTREE (Yang and Rannala 2006; Rannala and Yang 2007) and MULTIDIVTIME, numerous, often subtly different assumptions are being made (see Inoue *et al.* 2010), with no clear indication of which are the more realistic. Molecular dating methods then are arguably even more 'black box' than are phylogenetic inference methods, where different implementations tend to differ more with respect to the search algorithms used rather than in the underlying assumptions being made.

Issues of data quality

As with any other scientific exercise, the quality of the results hangs upon the quality of the data and the methods used to analyze them. Interestingly, issues of data quality in molecular dating studies typically tend to focus on the quality of the calibration points. This is not without justification given that the molecular divergence time estimates directly depend on these data. A badly chosen calibration point, therefore, can have far-reaching effects.

As a result, there has been a reasonable amount of attention paid to the calibration data, including how to counteract any potential problems, to account for uncertainty in them (see above), and/or to assess their influence on the results. For instance, a general, common-sense guideline is that as many calibration points that are spread out across the phylogeny should be used as far as is possible. This strategy both minimizes the potential negative impact of any single questionable calibration point as well as regularly resets the clock to prevent having to interpolate rates of evolution from distant calibration points. Indeed, this point arguably represents a potential weakness of the dating for the mammal supertree in that the large majority of the calibration data used were necessarily from Cenozoic fossils (see Figure 3.2), meaning that all divergence events up to and including the K–Pg boundary hang primarily on a few, very old calibration points.

In addition, it is naturally important that the calibration data be as robust as possible and associated with the proper node on the tree (Reisz and Müller 2004). As discussed earlier, only fossils where the fossil record is reasonably extensive should be used (or where there is otherwise no concern about substantially underestimating the oldest known member of a given group) and fossils should be placed within their focal taxon using robust, explicit criteria (e.g. possessing at least one synapomorphy in common with the taxon; cf. Bininda-Emonds *et al.* 2007). For several mammalian clades (notably bats, which appear to have particularly low preservation potential; Teeling *et al.* 2005), however, the first of these desiderata may be unattainable. Finally, there have been several suggestions regarding sensitivity analysis involving the calibration data. These range from the informal usage of several different calibration dates for any given node to assess the overall stability of the molecular dates (e.g. Springer *et al.* 2003) to more formal methods such as fossil cross-validation (Near and Sanderson 2004) or the likelihood method of Pyron (2010) that can help to identify calibration data that disagree strongly with the remainder of the data set. The principle behind the Near and Sanderson (2004) method is to fix the dates for those nodes with calibration data singly in turn and then to assess how the dates for the remaining nodes agree with their (unused) calibration data. A variant on this procedure (fossil-based model cross-validation) is also used to help shape the parameters of the rate-smoothing model used in PL (Near and Sanderson 2004). The method of Pyron (2010) goes a step beyond this to compare possible placements for fossil taxa where their phylogenetic affinity is ambiguous, thereby potentially providing novel, molecular-based information as to the placement of a given fossil.

What has received surprisingly little attention, however, is the quality (or suitability) of the molecular data being used in the analyses. Although the

dating methods and the models behind them are attracting increasing interest, the raw sequence data themselves have largely been ignored. This is surprising precisely because so much attention has been paid to sequence data quality and suitability in the related area of phylogenetic analysis. It is well accepted that genes evolve at different rates and are maximally informative at different phylogenetic levels: conservative genes are preferentially used to infer relationships between higher-level taxa whereas more variable genes are used closer to the species level. Hundreds of papers have also addressed the problems of saturation/multiple hits and its role in long-branch attraction (for a comprehensive review, see Bergsten 2005). Yet in cases where methods indicate a conflict between one or more calibration points and the molecular dates, it is usually the calibration points that are called into question when they could instead actually be informing models of sequence evolution that are being misled by model misspecification or rate heterogeneity (including heterotachy). So why are the sequence data typically taken for granted in molecular-dating analyses?

Perhaps the answer to the contradiction lies with the fact that the same genes are often used to derive the divergence times as were used to obtain the phylogeny in the first place, meaning that the sequence data have already been vetted to some degree. Indeed, programs like BEAST can derive the topology and date estimates for a given data set simultaneously. It remains, however, that the taxonomic and genomic scope of our phylogenetic analyses is increasing rapidly. For instance, the dating of the mammal supertree required a multigene data set comprising 68 genes of different rates to adequately cover all 4554 species and the 160+ Ma time span. The same is true of other, similar large-scale studies, which are becoming increasingly common. However, not all genes in such data sets will be optimally informative throughout the tree and some might be positively misinformative in places (e.g. saturated genes or sites for deep divergences). This problem can be ameliorated to a certain extent by the use of likelihood-based models of sequence evolution that can account for phenomena like saturation, particularly those where the models can be fitted individually to different partitions (e.g. genes or codons). However, as shown by the study of Phillips (2009; see above), the efficacy of this correction depends crucially on using the right model.

Although Bayesian methods can account for uncertainty in the molecular data, they still must use all the molecular data provided, even when parts of the data set appear to be positively misleading or disruptive. An unexplored line of research lies in developing the molecular counterpart of fossil cross-validation, where 'gene cross-validation' would identify and outright eliminate or

otherwise downweight outlier genes (or, more generally, partitions) that conflict strongly with the remainder of the data set.

Conclusions

Although our focus here has been largely restricted to the mammalian radiation, it is important to note that the issues we have identified herein go well beyond this taxonomic group. Analogous conflicts between fossil- and molecular-based date estimates have been noted for many other groups, with apparent explosive radiations of the animal phyla (Precambrian versus Cambrian) and birds (K–Pg boundary) being contradicted by molecular data (Cooper and Fortey 1998). Reconciling the disparate divergence time estimates within mammals, as well as for these other groups, will require a critical assessment of the data source and assumptions being made on both sides of the equation and thus the active collaboration of palaeontologists and molecular systematists. Hopefully, this strategy will prove to be an important step in achieving a consensus as to when mammals (and other taxa) evolved, analogous to the growing agreement regarding their relationships to one other.

Summary

A consensus is emerging on phylogenetic relationships within Mammalia, one contributed to, in hindsight, by both morphological and molecular data. This state of general agreement, however, does not extend to divergence time estimates within the group: fossil- and molecular-based dates tend to differ considerably, especially as regards the origins of and initial diversifications within the ordinal crown groups. In this chapter, we take a critical look at both fossil- and molecular-based frameworks for divergence time estimation, with a particular focus on the situation as it affects placental mammals. Our goal was not to determine when these (or any other) mammals evolved, but rather to highlight the assumptions underlying the analysis of each type of data. This exercise provided some insights into how fossil and molecular date estimates can disagree so profoundly as well as suggestions for achieving a better consensus than exists at present.

Acknowledgements

We thank Rob Asher and Johannes Müller for organizing an interesting and diverse symposium at SVP 2009 and for inviting us to present our views there. We thank Andres Giallombardo for discussion of the topics raised in this chapter as well as two anonymous reviewers and Rob Asher for their helpful

comments. Funding for Robin Beck was provided by NSF grant DEB-0743039 (in collaboration with Rob Voss at the AMNH) and for the research of Ross MacPhee on Gondwanan mammalian evolution by NSF grant OPP-0636639.

REFERENCES

Aitchison, J. C., Ali, J. R. and Davis, A. M. (2007). When and where did India and Asia collide? *Journal of Geophysical Research*, **112**, 1–19.

Ali, J. R. and Aitchison, J. C. (2008). Gondwana to Asia: plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166–35 Ma). *Earth-Science Reviews*, **88**, 145–66.

Ali, J. R. and Huber, M. (2010). Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature*, **463**, 653–6.

Ali, J. R. and Krause, D. W. (2011). Late Cretaceous bioconnections between Indo-Madagascar and Antarctica: refutation of the Gunnerus Ridge causeway hypothesis. *Journal of Biogeography*, **38**(10), 1855–72.

Alroy, J. (1999). The fossil record of North American mammals: evidence for a Paleocene evolutionary radiation. *Systematic Biology*, **48**, 107–18.

Archibald, J. D. and Deutschmann, D. H. (2001). Quantitative analysis of the timing of the origin and diversification of extant placental orders. *Journal of Mammalian Evolution*, **8**, 107–24.

Archibald, J. D., Zhang, Y., Harper, T. and Cifelli, R. L. (2011). *Protungulatum*, confirmed Cretaceous occurrence of an otherwise Paleocene eutherian (placental?) mammal. *Journal of Mammalian Evolution*, **18**, 153–61.

Aris-Brosou, S. and Yang, Z. (2002). Effects of models of rate evolution on estimation of divergence dates with special reference to the metazoan 18S ribosomal RNA phylogeny. *Systematic Biology*, **51**, 703–714.

Aris-Brosou, S. and Yang, Z. (2003). Bayesian models of episodic evolution support a late Precambrian explosive diversification of the Metazoa. *Molecular Biology and Evolution*, **20**, 1947–54.

Arnason, U., Adegoke, J. A., Gullberg, A., *et al.* (2008). Mitogenomic relationships of placental mammals and molecular estimates of their divergences. *Gene*, **421**, 37–51.

Asher, R. J. (1999). A morphological basis for assessing the phylogeny of the 'Tenrecoidae' (Mammalia, Lipotyphla). *Cladistics*, **15**, 231–52.

Asher, R. J. (2001). Cranial anatomy in tenrecid insectivorans: character evolution across competing phylogenies. *American Museum Novitates*, **3352**, 1–54.

Asher, R. J. (2007). A web-database of mammalian morphology and a reanalysis of placental phylogeny. *BMC Evolutionary Biology*, **7**, 108.

Asher, R. J. and Hofreiter, M. (2006). Tenrec phylogeny and the noninvasive extraction of nuclear DNA. *Systematic Biology*, **55**, 181–194.

Asher, R. J. and Lehmann, T. (2008). Dental eruption in afrotherian mammals. *BMC Biology*, **6**, 14.

Asher, R. J., Novacek, M. J. and Geisler, J. H. (2003). Relationships of endemic African mammals and their fossil relatives based on morphological and molecular evidence. *Journal of Mammalian Evolution*, **10**, 131–94.

Asher, R. J., Geisler, J. H. and Sanchez-Villagra, M. R. (2008). Morphology, paleontology, and placental mammal phylogeny. *Systematic Biology*, **57**, 311–17.

Averianov, A. O. and Skutschas, P. P. (2001). A new genus of eutherian mammal from the Early Cretaceous of Transbaikalia, Russia. *Acta Palaeontologica Polonica*, **46**, 431–6.

Averianov, A. O., Archibald, J. D. and Martin, T. (2003). Placental nature of the alleged marsupial from the Cretaceous of Madagascar. *Acta Palaeontologica Polonica*, **48**, 149–51.

Beck, R. M. D. (2008). Form, function, phylogeny and biogeography of enigmatic Australian metatherians. PhD dissertation, University of New South Wales, Australia.

Beck, R. M. D., Godthelp, H., Weisbecker, V., Archer, M. and Hand, S. J. (2008). Australia's oldest marsupial fossils and their biogeographical implications. *PLoS One*, **3**, e1858.

Benton, M. J. and Donoghue, P. C. J. (2007). Paleontological evidence to date the tree of life. *Molecular Biology and Evolution*, **24**, 26–53.

Benton, M. J., Donoghue, P. C. J. and Asher, R. J. (2009). Calibrating and constraining molecular clocks. In *The Timetree of Life*, ed. S. B. Hedges and S. Kumar. Oxford, UK: Oxford University Press.

Bergqvist, L. P., Abrantes, E. A. L. and Avilla, L. (2004). The Xenarthra (Mammalia) of São José de Itaboraí Basin (upper Paleocene, Itaboraian), Rio de Janeiro, Brazil. *Geodiversitas*, 323–37.

Bergsten, J. (2005). A review of long-branch attraction. *Cladistics*, **21**, 163–93.

Bininda-Emonds, O. R. P. (2000). Factors influencing phylogenetic inference: a case study using the mammalian carnivores. *Molecular Phylogenetics and Evolution*, **16**, 113–26.

Bininda-Emonds, O. R. P. (2007). Fast genes and slow clades: comparative rates of molecular evolution in mammals. *Evolutionary Bioinformatics Online*, **3**, 59–85.

Bininda-Emonds, O. R. P., Cardillo, M., Jones, K. E., *et al.* (2007). The delayed rise of present-day mammals. *Nature*, **446**, 507–12.

Bininda-Emonds, O. R. P., Cardillo, M., Jones, K. E., *et al.* (2008). Corrigendum: The delayed rise of present-day mammals. *Nature*, **456**, 274.

Bloch, J. I., Silcox, M., Boyer, D. M. and Sargis, E. J. (2007). New Paleocene skeletons and the relationship of plesiadapiforms to crown-clade primates. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1159–64.

Boyer, D. M. (2009). New cranial and postcranial remains of late Paleocene Plesiadapidae ('Plesiadapiformes', Mammalia) from North America and Europe: description and evolutionary implications. PhD thesis, Stony Brook University, New York.

Boyer, D. M., Prasad, G. V. R., Krause, D. W., *et al.* (2010). New postcrania of *Deccanolestes* from the Late Cretaceous of India and their bearing on the evolutionary and biogeographic history of euarchontan mammals. *Naturwissenschaften*, **97**, 365–77.

Britten, R. J. (1986). Rates of DNA sequence evolution differ between taxonomic groups. *Science*, **231**, 1393–8.

Britton, T., Anderson, C. L., Jacquet, D., Lundqvist, S. and Bremer, K. (2007). Estimating divergence times in large phylogenetic trees. *Systematic Biology*, **56**, 741–52.

Bromham, L., Rambaut, A. and Harvey, P. H. (1996). Determinants of rate variation in mammalian DNA sequence evolution. *Journal of Molecular Evolution*, **43**, 610–21.

Bromham, L., Rambaut, A., Fortey, R., Cooper, A. and Penny, D. (1998). Testing the Cambrian explosion hypothesis by using a molecular dating technique. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 12 386–9.

Bromham, L., Woolfit, M., Lee, M. S. and Rambaut, A. (2002). Testing the relationship between morphological and molecular rates of change along phylogenies. *Evolution*, **56**, 1921–30.

Brower, A. V. (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 6491–5.

Brown, W. M., George, M., Jr. and Wilson, A. C. (1979). Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 1967–71.

Bryant, H. N. (1989). An evaluation of cladistic and character analyses as hypothetico-deductive procedures, and the consequences for character weighting. *Systematic Zoology*, **38**, 214–27.

Bryant, H. N. (1991). The polarization of character transformations in phylogenetic systematics: role of axiomatic and auxiliary assumptions. *Systematic Zoology*, **40**, 433–45.

Butler, P. M. (1984). Macroscelidea, Insectivora and Chiroptera from the Miocene of east Africa. *Palaeovertebrata*, **14**, 117–200.

Cartmill, M. (1981). Hypothesis testing and phylogenetic reconstruction. *Journal of Zoological Systematics and Evolutionary Research*, **19**, 73–96.

Cifelli, R. L. (1999). Tribosphenic mammal from the North American Early Cretaceous. *Nature*, **401**, 363–6.

Cifelli, R. L. and de Muizon, C. (1998). Tooth eruption and replacement pattern in early marsupials. *Comptes Rendus de l'Académie des Sciences IIA–Earth and Planetary Science*, **326**, 215–20.

Clemens, W. (1986). On Triassic and Jurassic mammals. In *The Beginning of the Age of Dinosaurs: Faunal Change across the Triassic-Jurassic Boundary*, ed. K. Padian. Cambridge, UK: Cambridge University Press.

Clyde, W. C., Ting, S., Snell, K. E., *et al.* (2010). New paleomagnetic and stable-isotope results from the Nanxiong Basin, China: implications for the K/T boundary and the timing of Paleocene mammalian turnover. *Journal of Geology*, **118**, 131–43.

Cooper, A. and Fortey, R. (1998). Evolutionary explosions and the phylogenetic fuse. *Trends in Ecology and Evolution*, **13**, 151–5.

Davies, T. J. and Savolainen, V. (2006). Neutral theory, phylogenies, and the relationship between phenotypic change and evolutionary rates. *Evolution*, **60**, 476–83.

de Muizon, C. (1991). La fauna de mamíferos de Tiupampa (Paleoceno Inferior, Formación Santa Lucía), Bolivia. In *Fósiles y Facies de Bolivia*, Vol. 1 *Vertebrados*, ed. R. Suárez-Soruco. Santa Cruz, Bolivia: Revista Téchnica de Yacimientos Petrolíferos Fiscales Bolivianos.

de Muizon, C. and Cifelli, R. L. (2000). The 'condylarths' (archaic Ungulata, Mammalia) from the early Palaeocene of Tiupampa (Bolivia): implications on the origin of the South American ungulates. *Geodiversitas*, **22**, 47–150.

de Muizon, C., Cifelli, R. L. and Bergqvist, L. P. (1998). Eutherian tarsals from the early Paleocene of Bolivia. *Journal of Vertebrate Paleontology*, **18**, 655–63.

Donoghue, M. J., Doyle, J. A., Gauthier, J., Kluge, A. G. and Rowe, T. (1989). The importance of fossils in phylogeny reconstruction. *Annual Review of Ecology and Systematics*, **20**, 431–60.

Donoghue, P. C. J. and Purnell, M. A. (2009). Distinguishing heat from light in debate over controversial fossils. *BioEssays*, **31**, 178–89.

Douady, C. J. and Douzery, E. J. P. (2003). Molecular estimation of eulipotyphlan divergence times and the evolution of 'Insectivora'. *Molecular Phylogenetics and Evolution*, **28**, 285–96.

Douzery, E. J. P., Delsuc, F., Stanhope, M. J. and Huchon, D. (2003). Local molecular clocks in three nuclear genes: divergence times for rodents and other mammals and incompatibility among fossil calibrations. *Journal of Molecular Evolution*, **57**, S201–13.

Drake, J. W., Charlesworth, B., Charlesworth, D. and Crow, J. F. (1998). Rates of spontaneous mutation. *Genetics*, **148**, 1667–86.

Drummond, A. J. and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Drummond, A. J., Ho, S. Y. W., Phillips, M. J. and Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, e88.

Eagles, G. (2007). New angles on South Atlantic opening. *Geophysical Journal International*, **168**, 353–61.

Eagles, G. (2010). The age and origin of the central Scotia Sea. *Geophysical Journal International*, **183**, 587–600.

Evans, S. E., Jones, M. E. H. and Krause, D. W. (2008). A giant frog with South American affinities from the Late Cretaceous of Madagascar. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 2951–6.

Flynn, J. J., Parrish, J. M., Rakotosamimanana, B., Simpson, W. F. and Wyss, A. R. (1999). A new Middle Jurassic mammal from Madagascar. *Nature*, **401**, 57–60.

Foote, M., Hunter, J. P., Janis, C. M. and Sepkoski, J. J., Jr. (1999). Evolutionary and preservational constraints on origins of biologic groups: divergence times of eutherian mammals. *Science*, **283**, 1310–14.

Gayet, M. (2001). A review of some problems associated with the occurrences of fossil vertebrates in South America. *Journal of South American Earth Sciences*, **14**, 131–45.

Geisler, J. H. and Uhen, M. D. (2003). Morphological support for a close relationship between hippos and whales. *Journal of Vertebrate Paleontology*, **23**, 991–6.

Gelfo, J. N., Goin, F. J., Woodburne, M. O. and de Muizon, C. (2009). Biochronological relationships of the earliest South American Paleogene mammalian faunas. *Palaeontology*, **52**, 251–69.

Gheerbrant, E. (2009). Paleocene emergence of elephant relatives and the rapid radiation of African ungulates. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 10 717–21.

Giallombardo, A. and AToL Mammal Morphology Team (2010). Postcanine homologies in Mammalia. *Journal of Vertebrate Paleontology*, **30**, 96A.

Gingerich, P. D., Haq, M., Zalmout, I. S., Khan, I. H. and Malkani, M. S. (2001). Origin of whales from early artiodactyls: hands and feet of Eocene Protocetidae from Pakistan. *Science*, **293**, 2239–42.

Godthelp, H., Archer, M., Cifelli, R. L., Hand, S. J. and Gilkeson, C. F. (1992). Earliest known Australian Tertiary mammal fauna. *Nature*, **356**, 514–16.

Godthelp, H., Wroe, S. and Archer, M. (1999). A new marsupial from the Early Eocene Tingamarra local fauna of Murgon, southeastern Queensland: a prototypical Australian marsupial? *Journal of Mammalian Evolution*, **6**, 289–313.

Goswami, A., Prasad, G. V. R., Upchurch, P., *et al.* (2010a). The phylogenetic affinities of the enigmatic mammal *Deccanolestes* from the Late Cretaceous of India and implications for the origin of crown placental mammals. Paper presented at Third International Palaeontological Congress, held London, UK, no. 183.

Goswami, A., Prasad, G. V. R., Upchurch, P., Boyer, D. M. and Seiffert, E. R. (2010b). The phylogenetic affinities of the enigmatic mammal *Deccanolestes* from the Late Cretaceous of India and implications for eutherian mammal evolution. *Journal of Vertebrate Paleontology*, **30**, 98A.

Graur, D. and Higgins, D. G. (1994). Molecular evidence for the inclusion of cetaceans within the order Artiodactyla. *Molecular Biology and Evolution*, **11**, 357–64.

Graur, D., Duret, L. and Gouy, M. (1996). Phylogenetic position of the order Lagomorpha (rabbits, hares, and allies). *Nature*, **379**, 333–5.

Hacking, I. (2001). *An Introduction to Probability and Inductive Logic*. Cambridge, UK: Cambridge University Press.

Hennig, W. (1966). *Phylogenetic Systematics*. Urbana, IL: University of Illinois Press.

Hershkovitz, P. (1968). The recent mammals of the neotropical regions: a zoogeographic and ecological reivew. In *Evolutions, Mammals, and Southern Continents*, ed. A. Keast, F. C. Erk and B. Glass. Albany, NY: State University of New York Press.

Hillis, D. M. (1987). Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics*, **18**, 23–42.

Ho, S. Y. W. (2007). Calibrating molecular estimates of substitution rates and divergence times in birds. *Journal of Avian Biology*, **38**, 409–14.

Ho, S. Y. W. and Phillips, M. J. (2009). Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology*, **58**, 367–80.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65–70.

Hooker, J. J. (2001). Tarsals of the extinct insectivoran family Nyctitheriidae (Mammalia): evidence for archontan relationships. *Zoological Journal of the Linnean Society*, **132**, 501–29.

Hug, L. A. and Roger, A. J. (2007). The impact of fossils and taxon sampling on ancient molecular dating analyses. *Molecular Biology and Evolution*, **24**, 1889–97.

Inoue, J., Donoghue, P. C. J. and Yang, Z. (2010). The impact of the representation of fossil calibrations on Bayesian estimation of species divergence times. *Systematic Biology*, **59**, 74–89.

Iturralde-Vinent, M. A. (2006). Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review*, **48**, 791–827.

Ji, Q., Luo, Z.-X., Yuan, C.-X., *et al.* (2002). The earliest known eutherian mammal. *Nature*, **416**, 816–822.

Kay, R. F., Thewissen, J. G. M. and Yoder, A. D. (2001). Cranial anatomy of *Ignacius graybullianus* and the affinities of the Plesiadapiformes. *American Journal of Physical Anthropology*, **89**, 477–98.

Kielan-Jaworowska, Z., Cifelli, R. L. and Luo, Z.-X. (2004). *Mammals from the Age of Dinosaurs: Origins, Evolution, and Structure*. New York: Columbia University Press.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge, UK: Cambridge University Press.

Kitazoe, Y., Kishino, H., Waddell, P. J., *et al.* (2007). Robust time estimation reconciles views of the antiquity of placental mammals. *PLoS One*, **2**, e384.

Krause, D. W. (1986). Competitive exclusion and taxonomic displacement in the fossil record: the case of rodents and multituberculates in North America. In *Vertebrates, Phylogeny and Philosophy: A Tribute to George Gaylord Simpson*, ed. K. M. Flanagan and J. A Lillegraven. Laramie, WY: University of Wyoming.

Krause, D. W. and Maas, M. C. (1990). The biogeographic origins of late Paleocene– early Eocene mammalian immigrants to the Western Interior of North America. In *Dawn of the Age of Mammals in the Northern Part of the Rocky Mountain Interior, North America*, ed. T. M. Brown and K. D. Rose. Boulder, CO: Geological Society of America.

Kumar, S. and Hedges, S. B. (1998). A molecular timescale for vertebrate evolution. *Nature*, **392**, 917–20.

Lanfear, R., Welch, J. J. and Bromham, L. (2010). Watching the clock: studying variation in rates of molecular evolution between species. *Trends in Ecology and Evolution*, **25**, 495–503.

Lee, M. S. and Camens, A. B. (2009). Strong morphological support for the molecular evolutionary tree of placental mammals. *Journal of Evolutionary Biology*, **22**, 2243–57.

Luckett, W. P. and Hong, N. (1998). Phylogenetic relationships between the orders Artiodactyla and Cetacea: a combined assessment of morphological and molecular evidence. *Journal of Mammalian Evolution*, **5**, 127–82.

Luo, Z.X. and Wible, J. R. (2005). A Late Jurassic digging mammal and early mammalian diversification. *Science*, **308**, 103–7.

Luo, Z.-X., Cifelli, R. L. and Kielan-Jaworowska, Z. (2001a). Dual origin of tribosphenic mammals. *Nature*, **409**, 53–7.

Luo, Z.-X., Crompton, A. W. and Sun, A.-L. (2001b). A new mammaliaform from the early Jurassic and evolution of mammalian characteristics. *Science*, **292**, 1535–40.

Luo, Z.-X., Kielan-Jaworowska, Z. and Cifelli, R. L. (2002). In quest for a phylogeny of Mesozoic mammals. *Acta Palaeontologica Polonica*, **47**, 1–78.

Luo, Z.-X., Ji, Q., Wible, J. R. and Yuan, C.-X. (2003). An Early Cretaceous tribosphenic mammal and metatherian evolution. *Science*, **302**, 1934–40.

Luo, Z.-X., Kielan-Jaworowska, Z. and Cifelli, R. L. (2004). Evolution of dental replacement in mammals. *Bulletin of the Carnegie Museum of Natural History*, **36**, 159–75.

Luo, Z.-X., Chen, P., Li, G. and Chen, M. (2007). A new eutriconodont mammal and evolutionary development in early mammals. *Nature*, **446**, 288–93.

Luo, Z.-X., Ruf, I., Schultz, J. A. and Martin, T. (2011a). Fossil evidence on evolution of inner ear cochlea in Jurassic mammals. *Proceedings of the Royal Society B*, **278**, 28–34.

Luo, Z.-X., Yuan, C.-X., Meng, Q.-J. and Ji, Q. (2011b). A Jurassic eutherian mammal and divergence of marsupials and placentals. *Nature*, **476**, 442–5.

MacPhee, R. D. E. (1981). Auditory regions of primates and eutherian insectivores: morphology, ontogeny, and character analysis. *Contributions to Primatology*, **18**, 1–282.

MacPhee, R. D. E. (1994). Morphology, adaptations, and relationships of *Plesiorycteropus*, and a diagnosis of a new order of eutherian mammals. *Bulletin of the American Museum of Natural History*, **220**, 1–214.

MacPhee, R. D. E. and Reguero, M. A. (2010). Reinterpretation of a Middle Eocene record of Tardigrada (Pilosa, Xenarthra, Mammalia) from La Meseta Formation, Seymour Island, West Antarctica. *American Museum Novitates*, **3689**, 1–21.

Marjanović, D. and Laurin, M. (2008). Assessing confidence intervals for stratigraphic ranges of higher taxa: the case of Lissamphibia. *Acta Palaeontologica Polonica*, **53**, 413–32.

Marshall, L. G. and de Muizon, C. (1988). The dawn of the age of mammals in South America. *National Geographic Research*, **4**, 23–55.

Martin, A. P. and Palumbi, S. R. (1993). Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 4087–91.

Martin, R. D., Soligo, C. and Tavaré, S. (2007). Primate origins: implications of a Cretaceous ancestry. *Folia Primatologica*, **78**, 277–96.

Meng, J. and Wyss, A. R. (2005). Glires (Lagomorpha, Rodentia). In *The Rise of Placental Mammals: Origins and Relationships of the Major Extant Clades*, ed. K. D. Rose and J. D. Archibald. Baltimore, MD: Johns Hopkins University Press.

Misawa, K. and Janke, A. (2003). Revisiting the Glires concept – phylogenetic analysis of nuclear sequences. *Molecular Phylogenetics and Evolution*, **28**, 320–7.

Murphy, W. J., Eizirik, E., Johnson, W. E., *et al.* (2001). Molecular phylogenetics and the origins of placental mammals. *Nature*, **409**, 614–18.

Near, T. J. and Sanderson, M. J. (2004). Assessing the quality of molecular divergence time estimates by fossil calibrations and fossil-based model selection. *Philosophical Transactions of the Royal Society of London B–Biological Sciences*, **359**, 1477–83.

Nei, M. (1987). *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Novacek, M. J. (1992). Mammalian phylogeny: shaking the tree. *Nature*, **356**, 121–5.

Novacek, M. J. and AToL Mammal Morphology Team (2008). A team-based approach yields a new matrix of 4,500 morphological characters for mammalian phylogeny. *Journal of Vertebrate Paleontology*, **28**, 121A.

Novacek, M. J., Rougier, G. W., Wible, J. R., *et al.* (1997). Epipubic bones in eutherian mammals from the Late Cretaceous of Mongolia. *Nature*, **389**, 483–6.

O'Leary, M. A. (2011). MorphoBank: collecting and storing phenomic data for phylogenetic research in the 'cloud'. *American Journal of Physical Anthropology*, **144**, 228–9.

Ohta, T. (1995). Synonymous and nonsynonymous substitutions in mammalian genes and the nearly neutral theory. *Journal of Molecular Evolution*, **40**, 56–63.

Pascual, R. and Ortiz-Jaureguizar, E. (1992). Evolutionary pattern of land mamal faunas during the Late Cretaceous and Paleocene in South America: a comparison with the North American pattern. *Annales Zoologici Fennici*, **28**, 245–52.

Pascual, R. and Ortiz-Jaureguizar, E. (2007). The Gondwanan and South American episodes: two major and unrelated moments in the history of the South American mammals. *Journal of Mammalian Evolution*, **14**, 75–137.

Patterson, C. (ed.) (1987). *Molecules and Morphology in Evolution: Conflict or Compromise?*. Cambridge, UK: Cambridge University Press.

Patterson, C., Williams, D. M. and Humphries, C. J. (1993). Congruence between molecular and morphological phylogenies. *Annual Review of Ecology and Systematics*, **24**, 153–88.

Penny, D., Hasegawa, M., Waddell, P. J. and Hendy, M. D. (1999). Mammalian evolution: timing and implications from using the LogDeterminant transform for proteins of differing amino acid composition. *Systematic Biology*, **48**, 76–93.

Phillips, M. J. (2009). Branch-length estimation bias misleads molecular dating for a vertebrate mitochondrial phylogeny. *Gene*, **441**, 132–40.

Phillips, M. J. and Penny, D. (2003). The root of the mammalian tree inferred from whole mitochondrial genomes. *Molecular Phylogenetics and Evolution*, **28**, 171–85.

Phillips, M. J., Delsuc, F. and Penny, D. (2004). Genome-scale phylogeny and the detection of systematic biases. *Molecular Biology and Evolution*, **21**, 1455–8.

Phillips, M. J., Bennett, T. H. and Lee, M. S. Y. (2009). Molecules, morphology, and ecology indicate a recent, amphibious ancestry for echidnas. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 17 089–94.

Polly, P. D. (2001). On morphological clocks and paleophylogeography: towards a timescale for *Sorex* hybrid zones. *Genetica*, **112–113**, 339–57.

Posada, D. and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–18.

Poux, C., Madsen, O., Glos, J., De Jong, W. W. and Vences, M. (2008). Molecular phylogeny and divergence times of Malagasy tenrecs: influence of data partitioning and taxon sampling on dating analyses. *BMC Evolutionary Biology*, **8**, 102.

Prasad, G. V. R., Verma, O., Gheerbrant, E., *et al.* (2010). First mammal evidence from the Late Cretaceous of India for biotic dispersal between India and Africa at the KT transition. *Comptes Rendus Palevol*, **9**, 63–71.

Purvis, A. (1995). A composite estimate of primate phylogeny. *Philosophical Transactions of the Royal Society of London B*, **348**, 405–21.

Pyron, R. A. (2010). A likelihood method for assessing molecular divergence time estimates and the placement of fossil calibrations. *Systematic Biology*, **59**, 185–94.

Rannala, B. and Yang, Z. (2007). Inferring speciation times under an episodic molecular clock. *Systematic Biology*, **56**, 453–66.

Rauhut, O. W. M., Martin, T., Ortiz-Jaureguizar, E. and Puerta, P. (2002). A Jurassic mammal from South America. *Nature*, **416**, 165–8.

Reisz, R. R. and Müller, J. (2004). Molecular timescales and the fossil record: a paleontological perspective. *Trends in Genetics*, **20**, 237–41.

Rich, T. H., Vickers-Rich, P., Constantine, A., *et al.* (1997). A tribosphenic mammal from the Mesozoic of Australia. *Science*, **278**, 1438–42.

Rose, K. D. (1996). On the origin of the order Artiodactyla. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 1705–9.

Rose, K. D., Deleon, V. B., Missiaen, P., *et al.* (2008). Early Eocene lagomorph (Mammalia) from Western India and the early diversification of Lagomorpha. *Proceedings of the Royal Society B*, **275**, 1203–8.

Rose, K. D., Rana, R. S., Sahni, A., *et al.* (2009). Early Eocene primates from Gujarat, India. *Journal of Human Evolution*, **56**, 366–404.

Rougier, G. W., Wible, J. R. and Novacek, M. J. (1998). Implications of *Deltatheridium* specimens for early marsupial history. *Nature*, **396**, 459–63.

Rougier, G. W., Martinelli, A. G., Forasiepi, A. M. and Novacek, M. J. (2007). New Jurassic mammals from Patagonia, Argentina: a reappraisal of australosphenidan morphology and interrelationships. *American Museum Novitates*, **3566**, 1–54.

Rowe, T., Rich, T. H., Vickers-Rich, P., Springer, M. S. and Woodburne, M. O. (2008). The oldest platypus and its bearing on divergence timing of the platypus and echidna clades. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 1238–42.

Sánchez-Villagra, M. R., Narita, Y. and Kuratani, S. (2007). Thoracolumbar vertebral number: the first skeletal synapomorphy for afrotherian mammals. *Systematics and Biodiversity*, **5**, 1–7.

Sanderson, M. J. (1997). A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution*, **14**, 1218–31.

Sanderson, M. J. (2002). Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, **19**, 101–9.

Sansom, R. S., Gabbott, S. E. and Purnell, M. A. (2010). Non-random decay of chordate characters causes bias in fossil interpretation. *Nature*, **463**, 797–800.

Sarich, V. M. and Wilson, A. C. (1973). Generation time and genomic evolution in primates. *Science*, **179**, 1144–7.

Schaeffer, B. (1947). Notes on the origin and function of the artiodactyl tarsus. *American Museum Novitates*, **1356**, 1–24.

Seiffert, E. R. (2007a). Early evolution and biogeography of lorisiform strepsirrhines. *American Journal of Primatology*, **69**, 27–35.

Seiffert, E. R. (2007b). A new estimate of afrotherian phylogeny based on simultaneous analysis of genomic, morphological, and fossil evidence. *BMC Evolutionary Biology*, **7**, 224.

Seiffert, E. R. (2010). The oldest and youngest records of Afrosoricida (Placentalia, Afrotheria) from the Fayum Depression of northern Egypt. *Acta Palaeontologica Polonica*, **55**, 599–616.

Seiffert, E. R., Simons, E. L. and Attia, Y. (2003). Fossil evidence for an ancient divergence of lorises and galagos. *Nature*, **422**, 421–4.

Seiffert, E. R., Simons, E. L., Ryan, T. M., Bown, T. M. and Attia, Y. (2007). New remains of Eocene and Oligocene Afrosoricida (Afrotheria) from Egypt, with implications for the origin(s) of afrosoricid zalambdodonty. *Journal of Vertebrate Paleontology*, **27**, 963–72.

Seligmann, H. (2010). Positive correlations between molecular and morphological rates of evolution. *Journal of Theoretical Biology*, **264**, 799–807.

Shoshani, J., Walter, R. C., Abraha, M., *et al.* (2006). A proboscidean from the late Oligocene of Eritrea, a 'missing link' between early Elephantiformes and Elephantimorpha, and biogeographic implications. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 17 296–301.

Smith, T., De Bast, E. and Sigé, B. (2010). Euarchontan affinity of Paleocene Afro-European adapisoriculid mammals and their origin in the late Cretaceous Deccan Traps of India. *Naturwissenschaften*, **97**, 417–22.

Spaulding, M., O'leary, M. A. and Gatesy, J. (2009). Relationships of Cetacea (Artiodactyla) among mammals: increased taxon sampling alters interpretations of key fossils and character evolution. *PLoS One*, **4**, e7602.

Springer, M. S. (1997). Molecular clocks and the timing of the placental and marsupial radiations in relation to the Cretaceous-Tertiary boundary. *Journal of Mammalian Evolution*, **4**, 285–302.

Springer, M. S., Burk-Herrick, A., Meredith, R., *et al.* (2007). The adequacy of morphology for reconstructing the early history of placental mammals. *Systematic Biology*, **56**, 673–84.

Springer, M. S., Murphy, W. J., Eizirik, E. and O'Brien, S. J. (2003). Placental mammal diversification and the Cretaceous-Tertiary boundary. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 1056–61.

Springer, M. S., Stanhope, M. J., Madsen, O. and de Jong, W. W. (2004). Molecules consolidate the placental mammal tree. *Trends in Ecology and Evolution*, **19**, 430–8.

Stanhope, M. J., Waddell, V. G., Madsen, O., *et al.* (1998). Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 9967–72.

Storch, G. (2008). Skeletal remains of a diminutive primate from the Paleocene of Germany. *Naturwissenschaften*, **95**, 927–30.

Swofford, D. L. (2002). *PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4*. Sunderland, MA: Sinauer Associates.

Tajima, F. (1993). Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics*, **135**, 599–607.

Tavaré, S., Marshall, C. R., Will, O., Soligo, C. and Martin, R. D. (2002). Using the fossil record to estimate the age of the last common ancestor of extant primates. *Nature*, **416**, 726–9.

Teeling, E. C., Springer, M. S., Madsen, O., *et al.* (2005). A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science*, **307**, 580–4.

Thewissen, J. G., Williams, E. M., Roe, L. J. and Hussain, S. T. (2001). Skeletons of terrestrial cetaceans and the relationship of whales to artiodactyls. *Nature*, **413**, 277–81.

Thorne, J. L. and Kishino, H. (2002). Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51**, 689–702.

Ting, S.-Y., Meng, J., Qian, L., *et al.* (2007). *Ganungulatum xincunliense*, an artiodactyl-like mammal (Ungulata, Mammalia) from the Paleocene, Chijiang Basin, Jiangxi, China. *Vertebrata PalAsiatica*, **45**, 278–86.

Vickers, J. (2010). The problem of induction. In *The Stanford Encyclopedia of Philosophy*, ed. E. N. Zalta. Stanford, CA: Stanford University.

Vogt, L., Bartolomaeus, T. and Giribet, G. G. (2010). The linguistic problem of morphology: structure versus homology and the standardization of morphological data. *Cladistics*, **26**, 301–25.

Waddell, P. J., Okada, N. and Hasegawa, M. (1999). Towards resolving the interordinal relationships of placental mammals. *Systematic Biology*, **48**, 1–5.

Welch, J. J., Fontanillas, E. and Bromham, L. (2005). Molecular dates for the 'Cambrian Explosion': the influence of prior assumptions. *Systematic Biology*, **54**, 672–8.

Whidden, H. P. (2002). Extrinsic snout musculature in Afrotheria and Lipotyphla. *Journal of Mammalian Evolution*, **9**, 161–84.

Whittaker, J. M., Muller, R. D., Leitchenkov, G., *et al.* (2007). Major Australian-Antarctic plate reorganization at Hawaiian-Emperor bend time. *Science*, **318**, 83–6.

Wible, J. R., Rougier, G. W. and Novacek, M. J. (2005). Anatomical evidence for superordinal/ordinal eutherian taxa in the Cretaceous. In *The Rise of Placental Mammals: Origins and Relationships of the Major Extant Clades*, ed. K. D. Rose and J. D. Archibald. Baltimore, MD: Johns Hopkins University Press.

Wible, J. R., Rougier, G. W., Novacek, M. J. and Asher, R. J. (2007). Cretaceous eutherians and Laurasian origin for placental mammals near the K/T boundary. *Nature*, **447**, 1003–6.

Woodburne, M. O. and Case, J. A. (1996). Dispersal, vicariance, and the late Cretaceous to early Tertiary land mammal biogeography from South America to Australia. *Journal of Mammalian Evolution*, **3**, 121–61.

Woodburne, M. O., Rich, T. H. and Springer, M. S. (2003). The evolution of tribospheny and the antiquity of mammalian clades. *Molecular Phylogenetics and Evolution*, **28**, 360–85.

Yang, Z. and Rannala, B. (2006). Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. *Molecular Biology and Evolution*, **23**, 212–26.

Zuckerkandl, E. and Pauling, L. (1962). Molecular disease, evolution, and genetic heterogeneity. In *Horizons in Biochemistry*, ed. M. Kasha and B. Pullman. New York: Academic Press.

Zuckerkandl, E. and Pauling, L. (1965). Evolutionary divergence and convergence in proteins. In *Evolving Genes and Proteins*, ed. V. Bryson and H. J. Vogel. New York: Academic Press.