Imperial College of London

Department of Earth Sciences and Engineering

An Appraisal of the 'Living Fossil' Concept

Dominic John Bennett

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Department of Earth Sciences and Engineering of Imperial College London

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“When I use a word,” Humpty Dumpty said, in rather a scornful tone, “it means just what I choose it to mean – neither more nor less.”

‘Through the Looking Glass’ by Lewis Carroll

ABSTRACT

Although the term 'living fossil' has been around for over 150 years, it remains scientifically undefined and contentious. Generally, it refers to any taxon that is evolutionarily unique, species-poor and exhibits traits closely resembling those of extinct taxa. This interpretation, however, is not universal. Other interpretations of the term include species that are evolutionary dead-ends, taxa that were first discovered in the fossil record and/or lineages that have undergone no (*sic*) morphological change. In addition to the confusion over definition, many have argued against the concept on the grounds that it is either not feasible, that it may not delineate a true category of biodiversity or that it recalls defunct Victorian scientific ideas. Despite these objections, it is evident that the concept of the living fossil can play an important role in our understanding of evolution over large timescales. In recent years there has been renewed interest in the concept of the living fossil thanks to the development of genomic techniques that are revealing previously hidden rates of molecular change in well-known living fossils. Additionally, many species that have been described as living fossils are threatened with extinction and there exists a risk that their unique evolutionary history is being lost. In this thesis, I attempt a re-appraisal of the living fossil concept. Firstly I test whether the concept of the living fossil can be made scientifically sound. Secondly I develop a new quantitative definition of the living fossil. I show that the concept is robust as it can be used to distinguish lineages that have experienced different rates of evolution. In addition I demonstrate that it is possible to devise a quantitative definition, which I use to identify living fossils and their traits across a wide range of taxonomic groups.

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FOREWORD

This thesis is entirely my own work but with substantial help and suggestions from my supervisors, Mark Sutton and Sam Turvey. Any content that is not my own is properly referenced. This thesis has been written as a single continuous piece of work, it should, however, be noted that large sections of chapters two and six have been published in peer-reviewed journals. Refer to references Bennett et al. (2017a) and Bennett et al. (2017b). Additionally, chapter four is currently under review for publication. The appendix contains supplementary materials in the form of tables, figures and additional text. Supplementary tables and figures are indicated with 'S' before the figure/table number. Supplementary text is indicated to the reader by the main document's text. Supplementary data too large for textual representation are stored electronically in the attached CD; see files 'chapter_4.zip' and 'chapter_6.zip'.

ABBREVIATIONS

AIC	.....	Akaike Information Criterion
ANOVA	.....	Analysis of Variance
DE	.....	Dead End Scenario
ED	.....	Evolutionarily Distinct
EDBMM	.....	Evolutionary Distinctness Biased Markov Model
Eph	.....	Ephemeral Scenario
EPI	.....	Evolutionary Performance Index
ERMM	.....	Equal Rates Markov Model
ES	.....	Equal Splits
FP	.....	Fair Proportion
GAM	.....	General Additive Model
GLS	.....	Generalised Least Squares
Hyd	.....	Hydra Scenario
IUCN	.....	International Union for the Conservation of Nature
LF	.....	Living Fossil
LMEM	.....	Linear Mixed Effects Model
MY	.....	Millions of Years
MYA	.....	Millions of Years Ago
NCBI	.....	National Center for Biotechnology Information
Pan	.....	Panchronic Scenario
PD	.....	Phylogenetic Diversity
PE	.....	Pendant Edge
pEPI	.....	Proximate Evolutionary Performance Index
PF	.....	Phylogenetic Fuse Scenario
PSV	.....	Phylogenetic Species Variability

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1.

Introduction

A STORY OF THE PALAEOBIOTIC

'Living fossil' is a catch phrase; like all catch phrases, it has a habit of sticking in our heads. Newspaper articles, web pages, books, T.V. … Few other terms from the palaeontological literature have so great a resonance in the popular mind-set (Burton, 1954). But also, like many other catch phrases, few people would be able to give its exact meaning. In its original conception, the term was used to describe a species or lineage that experienced a reduced rate of evolution in terms of both its levels of diversification and morphological and ecological innovations. Living fossils (LFs) would therefore be organisms that by rights should no longer exist; a palaeobiotic form that is less typical of our present era, and more typical of a period lost to deep time. Perhaps this tantalising prospect – that LFs, as we see them today, would have been indistinguishable from those that the dinosaurs encountered – is the reason the term continues to circulate.

It is, however, a term that attracts much controversy; a controversy that is as much to do with a serious image problem, as it is to do with genuine scientific concerns. It recalls the debunked *teleological* thinking of the Victorian age and too often it becomes mixed with the pseudo-science of creationists and their 'missing links'. In what respect might we then consider 'living fossil' a useful term for scientific discourse? Or is the term 'beyond the pale', suitable for expunging from the palaeontological lexicon?

For all its ills, the term may play an important role in two domains. Firstly, researchers who study evolution on a large scale are more inclined to aim their study towards the big changes in Earth's history: explosions in diversity and mass extinctions. Few have taken the time to consider the elements that keep lineages driving in the slow lane of evolution (TABLE 1.1). Developing a fuller picture of macroevolution requires a study of the complete spectrum of lineages. In this regard, I would consider that use of the term helps inspire broader research. Secondly, life on this planet is faced with an

extinction crisis. Conservation biologists have found that framing the loss of species as also a loss of evolutionary history has improved public understanding and participation in tackling biodiversity loss. A captivating phrase like 'living fossil' may better spark the imagination of what is at stake. In sum, there are good reasons for retaining the term, provided it can be shown to have a robust scientific meaning.

TABLE 1.1 LIVING FOSSILS ARE NOT WELL STUDIED

Institute of Scientific Information 'Web of Knowledge' searches for opposing (-) and equivalent (+) terms to the LF concept in order to gauge the asymmetric study of the spectrum of evolutionary performances. Records were searched between the years 1970-2017; search conducted 19/01/2017.

Search phrase		No. Records
"living fossil"	(-)	445
"evolutionary relict"	(-)	10
"adaptive radiation"	(+)	3,968
"bradytely"	(-)	7
"tacytely"	(+)	0
"evolutionary distinct(ive)?ness"	(-)	109
"mass extinction"	(+)	4,184
	+	*8,152*
	-	*571*

In this thesis, I attempt a fresh consideration of the LF concept. In this chapter I will introduce the reader to the background of the term by outlining its history and by describing the range of definitions that have been applied to it. I will describe the problems and arguments raised against it and its role in conservation. Finally, I will present a proposal for its greater clarification that will form the basis of this thesis.

Basic Terminology

First, however, to prevent confusion I wish to clarify the definitions of some technical terms that I use within this thesis and which are not often strictly defined. As will quickly become clear to the reader, the substantial methodologies of this thesis concern the phylogenetic tree: a hierarchical representation of the inter-relationships of species calibrated by time of separation. Other forms of calibration, such as the number of unique changes, are also possible but they are not often used in this thesis. I use phylogenetic tree, phylogeny and tree interchangeably. Despite the simple structure of a tree, multiple terms can be generated from it: branches, nodes, tips etc. (see FIGURE 1.1).

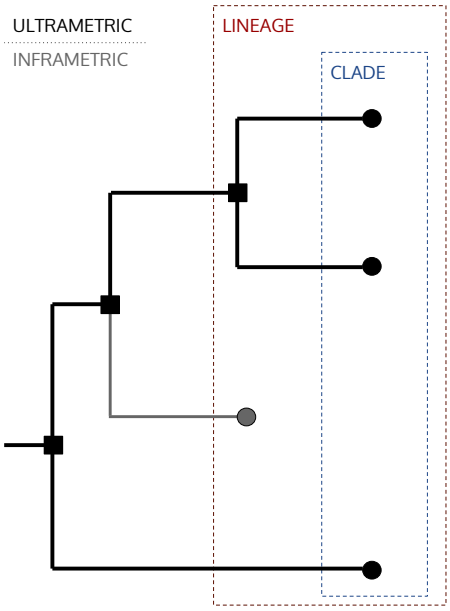


FIGURE 1.1 TERMINOLOGY OF THE TREE

Branches or edges are the lines that connect nodes and are measured in time. Tips (circles) are points at the end of a tree representing species. Nodes (squares) are points where branches split; they represent a speciation event in the past. A tree is ultrametric if all its tips are extant. A tree is inframetric if the tree contains extinct and extant tips. A clade is a group of extant species that share a common ancestor. A lineage is the set of extant and extinct species descending from a common ancestor and is often referred to as the unique set of named taxonomic ranks.

Thinking in tree terms, we may identify **species** as tips, **clades** as a group of tips that share a common node and **lineages** as all species that have ever descended from a node. Additionally, a lineage is often represented as a list of all the unique named taxonomic ranks that describe it. For example, the human lineage would be briefly described as Mammalia, Primates, Hominoidea, *Homo sapiens*. The important point to note is that all these terminologies refer to nodes in the phylogenetic tree. Groups that all uniquely share a common ancestor and thus a common node are said to be **monophyletic**. The difference between clade and species is in number, while the difference between lineage and clade is in perspective of time. There are cases, however, where we cannot be sure of the phylogeny or where groups have only ever been taxonomically defined or where we may be describing a mixture of species and clades. Thus, in addition, to these terms, I will use the more general terms of group or taxon (pl. taxa) to refer to any set of species.

Now that I have defined the basic definitions of my lexicon, we may move on to the topic of this thesis.

What is a Living Fossil?

As has been pointed out by many others before me, there is lack of a precise and agreed-upon definition for 'living fossil' (Schopf, 1984; Casane and Laurenti, 2013; Cavin and Guinot, 2014). Despite this circumstance, certain groups have been persistently labelled as LFs (TABLE 1.2, FIGURE 1.2). Naming a few examples, horseshoe crabs (Family: Limulidae), ginkgo (Species: *Ginkgo biloba*) and tuatara (Genus: *Sphenodon*), we can clearly see that they all first appeared a long time ago, have apparently changed little from their fossil ancestors and have few surviving species (Rudkin et al. 2008; Royer et al. 2003; Hay et al. 2008). Given these examples, can we then consider a LF to be a species or small clade that appears to have changed little for extended periods of time?

TABLE 1.2 EXAMPLES OF LIVING FOSSILS

Details of some common LFs. Common names, lineages of unique taxonomic groups with ranks that are named in square brackets, estimated number of species and description. Taxonomic information was taken from NCBI taxonomy (Federhen, 2012). Divergence dates were taken from TimeTree (Hedges et al. 2006).

Common name	Lineage	No. Sp.	Description
Coelacanths	Coelacanthimorpha Coelacanthidae *Latimeria* [genus]	2	The poster child of the LF. First recognised in fossil records, later found to be still extant. Currently there are two species that live in deep cavenous habitats. Group appeared early in the evolution of Sarcopterygia, around 500 MYA.
Egg-laying mammals	Prototheria Monotremata [order]	5	Sister to all placental and maruspial mammals, from which they split some 170 MYA. Ancestral mammalian characteristics suggested are laying eggs and milk sebaceous glands.
Horseshoe crabs	Merostomata [class] Xiphosura [order] Limulidae [family]	8	Appearing around 500 MYA, this group are survivors of an early offshoot of arthropods. There are eight extant species which are distributed throughout the world's oceans.
Lungfishes	Dipnoi Ceradontimorpha [superorder]	12	Aside from the Coelacanths, the lungfishes are considered the only other lobe-finned 'fish'. Also arising early in the evolution of Sarcopterygia, this group, with their primitive lungs, are thought to provide insight into early tetrapod evolution. There are twelve extant species.
Maidenhair tree	Ginkgoidae [subclass] Ginkgoales Ginkgoaceae [family] *Ginkgo* [genus]	1	The ginkgo is a non-flowering tree species and is the sole representative of the Ginkgoidae thought to have arisen in the Permian period, up to 300 MYA. The group was once widespread. Today however its native range is contained within China. It has been found to grow well in cities due to its ability to withstand car pollution, a feature that has been suggested for its survival through mass extinction events.
Sharks	Elasmobranchii [subclass]	1000	Sharks (and rays) are the living descendants of early cartilagenous fish, a group that emerged in the evolution towards bones around 400 MYA. It is primarily for this reason that they are described as LFs, due to their still exhbiting such a 'primitive' state. The group, however, has been very successful and today contains over one thousand species.
Tuatara	Sphenodontia [order] Sphenodotidae [family] *Sphenodon* [genus]	2	The reptile-like Tuatara is a distinct member of the sauropsida. Its nearest neighbours are the squamates from which it split approximately 200 MYA. There are 2 extant species living on islands off New Zealand. It features archaic traits thought to be akin to those of early diapsids, such as the parietal eye.



FIGURE 1.2 EXAMPLES OF LIVING FOSSILS

Representative species of various named LF examples, going clockwise from top-right: coelacanths (*Latimeria chalumnae*), Maidenhair tree (*Ginkgo biloba*), Tuatara (*Sphenodon punctatus*), sharks (*Rhincodon typus*), monotremes (*Tachyglossus aculeatus*), horseshoe crabs (*Limulus polyphemus*), lungfishes (*Protopterus annectens*). Photo credits, respectively, 'Mordecai 1998', 'Björn S...', Michal Klajban, Zac Wolf, Lyle Radford, Breese Greg and Loury Cédric (commons.wikimedia.org).

Taking a different set of examples, however, the common underlying attributes are not necessarily as clear-cut. For example, although 'sharks' are old, there are over a thousand extant species; other LFs are not nearly as species-rich. Equally, although there are only two species of *Sphenodon*, they are at the most 200 MY old; other LFs are up to 500 MY

old. Although we consider the monotremes 'primitive' in laying eggs, they have evolved innovations such as venom, electrolocation, spines and beaks, whereas, physically, the horseshoe crab is nearly indistinguishable from its fossil relatives. Evidently, there is no clear consensus when labelling a taxon as a LF. To understand this lack of consistency, we must look to its history to see how our predecessors used the term.

Origin and Popularisation

Darwin introduced the term 'living fossil'. It first appeared in print on two occasions in *On the Origin of Species* (1859).

> *(1) These anomalous forms may almost be called living fossils; they have endured to the present day, from having inhabited a confined area, and from having thus been exposed to less severe competition.*
>
> *(2) Species and groups of species, which are called aberrant, and which may fancifully be called living fossils, will aid us in forming a picture of the ancient forms of life.*

Through his first use of the term, Darwin was attempting to illustrate the relative differences in competitive ability of species. For example, in an earlier letter to Joseph Dalton Hooker in which he also uses the term, he notes that Asian and European plants are more successful when introduced into Australia than vice versa. He proposed that this may have been due to Australian plants occupying a small range, leading to a reduced rate of evolutionary change, making them less 'improved' (Darwin 1858). Despite what we may think of his theory of species range and rate of evolution, the key is his use of 'living fossil' for a group that has experienced a reduced rate of evolutionary change. In our second quotation, however, Darwin alludes to this potentially providing an insight into past life forms – presumably why he named them 'living fossils'. This is perhaps the meaning that most of us recognise: a species or clade that acts as a 'window

into the past'. In the latter part of the 19th century, before the development of Neo-Darwinism, evolutionary thinking was dominated by the idea of life being ranked between 'higher' and 'lower' forms (Haeckel, 1894; FIGURE 1.3). Unlike Darwin, no suggestions are made for what the underlying processes may have been for generating higher and lower forms; instead this *scala naturae* with 'Man' at the top may have merely acted as a comfort for a society still in shock at the revelations of evolution. Although the first lesson of any introductory class on evolutionary biology is that all living organisms – whether they be regarded as 'advanced' or 'primitive' – have evolved to meet the demands of their particular niche, the idea that evolution requires a trend towards increasing complexity still lingers. While it is true that early life was simple and that evolution has led to the development of complex traits and systems, modern levels of complexity occurred early on in Earth's history. For example, a high diversity of marine invertebrates occurred as early as the mid-Palaeozoic around 275 MYA (Alroy et al. 2008), the adaptive immune system arose around 500 MYA (Flajnik and Kasahara, 2009) and complex multicellular organisms arose over 600 MYA (Knoll, 2011). Complex organisms and ecosystems have been around for a long time and little 'improvement' has occurred since their inception. Furthermore, a teleological interpretation of evolution towards ever improvement ignores the advantages of the simple and basic. The 'primitive' bacteria are suspected of representing millions of species (Torsvik et al. 2002) – an order of magnitude more than the 'advanced' mammals or birds. This conceit of continuous improvement is particularly the case for creationists who use *so-called* LFs as evidence for the absence of evolution (Batten, 2011). No doubt some palaeontologists are loath to use the term by dint of this misapprehension.

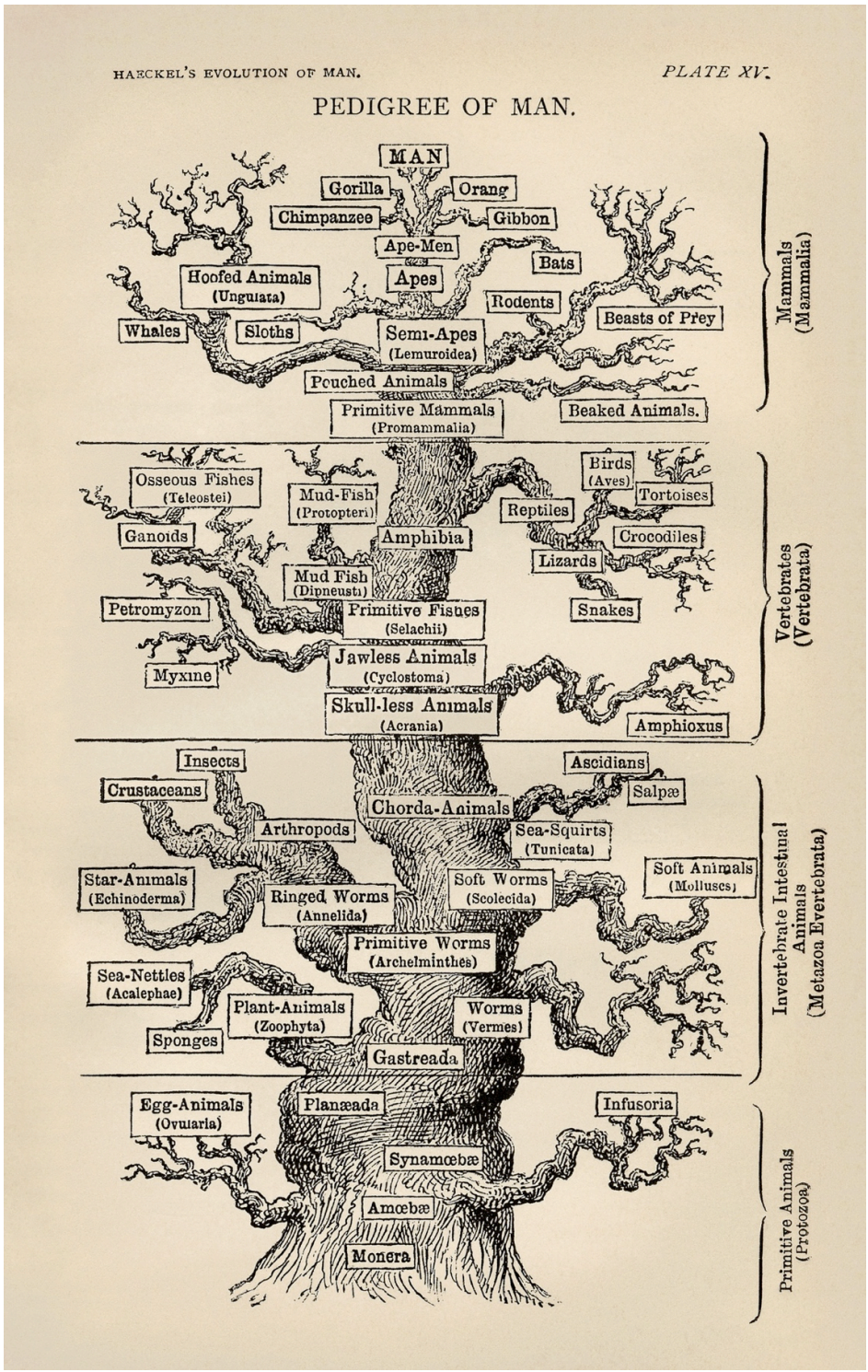


FIGURE 1.3 SCALA NATURAE

Ernst Haeckel's *scala naturae* originally published in *The Evolution of Man* (Published 1879). In this scheme, any organism that occupies the lowest ranks, e.g. the 'Primitive Animals', would have been considered a LF because by definition they are less advanced than the higher 'forms' (Source: American Philosophical Society Museum).

By the mid 20th century, however, the term had gained a new interpretation and an increased popularity. In 1938 a young museum curator called Marjorie Courtenay-Latimer based at the East London Museum in South Africa discovered a freshly caught specimen of coelacanth at a fish market (FIGURE 1.4).

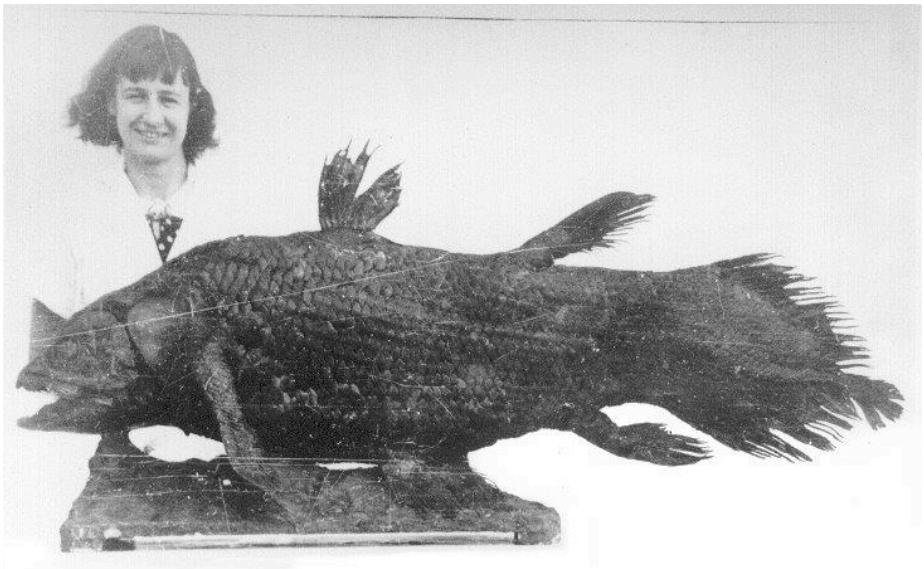


FIGURE 1.4 COURTENAY-LATIMER AND COELCANTH

Scientific discovery of a living coelacanth by Marjorie Courtenay-Latimer, South Africa, 1938. (Photo credit: South African Institute for Aquatic Biology)

Here was a fish, it was thought, that human eyes should never see. Prior to its discovery, it had been thought that coelacanths had become extinct in the Late Cretaceous (Smith 1939). This is an example of a clade that was first recognised in the fossil record, only subsequently found to be extant. As such, 'living fossil' has taken on the additional connotation of a group that was first known from the fossil record, and the coelacanth became one of the best-known LFs. The term 'Lazarus taxon' is, however, better suited to this case, and taxa of this type have not been consistently referred to as LFs. For example, lungfishes were also first recognised in the fossil record before any living taxa were discovered, and yet are not as often described as LFs (Forey 1984). Many other examples of this instance exist, even from zooarchaeological material and Quaternary fossil records. For example, Bulmer's fruit bat (*Aproteles bulmerae*, Hyndman and

Menzies, 1980), the Chacoan peccary (*Catagonus wagneri*, Wetzel, 1977), the grey whale (*Eschrichtius robustus*, Bryant, 1995), the false killer whale (*Pseudorca crassidens*, Owen, 1846), the bush dog (*Speothos venaticus*, Beisiegel and Zuercher, 2005) and the giant muntjac (*Muntiacus gigas* = *M. vuquangensis*, Turvey et al. 2016) were all first found as zooarchaeological remains and presumed extinct before extant species were discovered.

Living Fossil Definitions

This mix of interpretations for the LF was to persist into the post-war era, while additional connotations and slants have continued to be added to the term. There are now a number of alternative meanings of 'living fossil' and many related and overlapping concepts have been developed (TABLE 1.3). While this mixture of uses indicates the imprecision of the term, it also highlights the popularity of the overarching concept.

Many authors have devised their own working definitions of the LF based on different sets and combinations of factors, placing emphasises on various alternative aspects of perceived 'living fossil-ness'. Yoshida (2002) described LFs as "taxonomic groups surviving for a long time without any remarkable morphological change." Time was not important for Eldredge (1984), who defined them as "members of the recent biota whose external form, at least, has changed but little since the lineage's inception." For Fisher (1990) being relictual was important: they have "survived for relatively long intervals of geological time at low numerical diversity, often as the sole survivors of previously diverse taxa." Eisner (2003) indicated their distinctness by defining them as "organisms that, while still resembling their extinct progenitors in fundamental ways, have escaped the fate of these ancestors by specializing in ways that gave them an edge in survival." Conversely, Stanley (1998) defined them as "supra-specific taxa that show conservatism", therefore also excluding species-level taxa from the concept. These quotes demonstrate to what extent the 'living fossil' label can be arbitrary. Indeed, Schopf (1984)

identified seven general different definitions of the LF and argued that authors interpret each of these definitions in their own way. For example, he highlighted that for many authors the *Lingula* are LFs because they have existed since the Ordovician, yet as Schopf points out they have been excluded by some authors as "they contain several species today and may have undergone a rather large total number of speciation events during long intervals of persistence with little evolutionary change."

TABLE 1.3 LIVING FOSSIL SYNONYMS

Terms, synonyms and/or closely related concepts that are often confused, merged and substituted with 'living fossil'.

Terms	Description	References
Basal lineage	A clade that has split early and is distinct from the crown group	Liow (2006)
Bradytelic	A lineage that experiences low rates of diversification	Simpson (1944)
Evolutionary arrestment	A lineage that experiences reduced or halted change	Eldredge (1984)
Evolutionarily distinct	A species that has very few close relatives	Redding and Mooers, (2006) Isaac et al. (2007)
Evolutionary dead-end	A species or clade that has evolved into an unsustainable ecological niche	Parsons (1994)
Evolutionary relict	A clade that was once part of a much larger clade	Nagalingum et al. (2011)
Lazarus taxon	A species or clade that was first discovered in the fossil record and then found to be extant	Smith (1939)
Low ranked in *scala naturae*	A species or clade that sits on a low level of the "scales of evolution"	Haeckel (1894)
Morphologically conserved	A lineage displaying little morphological divergence in terms of extant or extinct species	Eldredge (1984)
Panchronic	A lineage that persists for an extended period of time	de Ricqles (1983)
Primitiveness	A species or clade that has features associated with much earlier forms of life	Emery and Thorington (1984)
Primitive traits	The persistent or re-emergence of simple or 'primitive' traits through a lineage	Schopf (1984)
Window into the past	An 'anomalous form' that offers an insight into past life	Darwin (1859)

The book *Living Fossils* (*Casebook in Earth Sciences*) (Eldredge and Stanley (eds), 1984) demonstrates well the problem of definition. Each chapter is written by a different expert who assesses the LF status of their specialist group. Among the most common qualifications mentioned by the authors are: existing for a long time; morphologically conserved; some other form of conservatism; having primitive features; phylogenetically distinct; a survivor of a once large clade; above the species-level; geographically isolated; or having a generalist niche. Each and all of these factors can be given a different emphasis to create a large number of possible definitions. This continual redefinition amongst authors studying the concept has led to the further complication of the term, rather than to its elucidation. All too frequently a LF group has been 'identified' by an independent researcher using an independent set of criteria; this researcher has then highlighted possible correlates for what makes a LF; and these correlates have in turn been appended to the concept of a LF. For example, geographical isolation as first proposed by Darwin and then by Eldredge (1979) and Stanley (1998), were once explanations for the 'living fossil' phenomenon but have since, at least implicitly, become part of the definition. The repetition of this process has created a protean and nebulous concept that can be interpreted in multiple ways.

A Dubious Idea

Without a strong definition the concept has found itself on hostile ground. New research in the fields of evolution and palaeontology find reasons to dismiss the label on both circumstantial and theoretical grounds.

Firstly, as pointed out above there is inconsistency in the common factors among the classic LFs. This is further exacerbated as the statuses of these palaeobiotic groups are questioned because of our improving understanding of their past and present evolution. New phylogenetic analyses have found that some clades, previously considered

depauperate, contain higher numbers of species and diversity than previously thought, e.g. tadpole shrimps (Mathers et al. 2013), horseshoe crabs (Obst et al. 2012), *Nautilus* (Wray et al. 1995) and monoplacophorans (Kano et al. 2012). Other clades that were thought to be ancient have been shown to have undergone recent diversifications, e.g. cycads (Nagalingum et al. 2011) (see FIGURE 1.5). Also, new fossil evidence is questioning the conventional stories of evolution, thus changing our perspective on how much change has occurred. For example, sharks (Elasmobranchii) were once thought to be a surviving lineage of a transitory group between placoderms and bony fish (Teleosteii), but the recently discovered *Entelognathus primordialis* fossil (Zhu et al. 2013), which features jaw traits from both sharks and bony fish, implies that sharks are not primitive replicas of early cartilaginous fishes but are instead as fully derived as the bony fish (Freidman and Brazeau, 2013). Another fossil recently identified as an early coelacanth has revealed the large diversity of early coelacanths (Friedman and Coates 2006). Crocodiles were thought to have maintained a constant body form since their evolutionary divergence, but the discovery of a highly derived fossil crocodile (*Simosuchus clarki*) undermines this view of an 'unchanging' crocodile lineage (Buckley et al. 2000). Similarly, analyses of fossil *Lingula* indicate previously undetected large morphological changes that question the view of stasis (Biernat and Emig 1993).

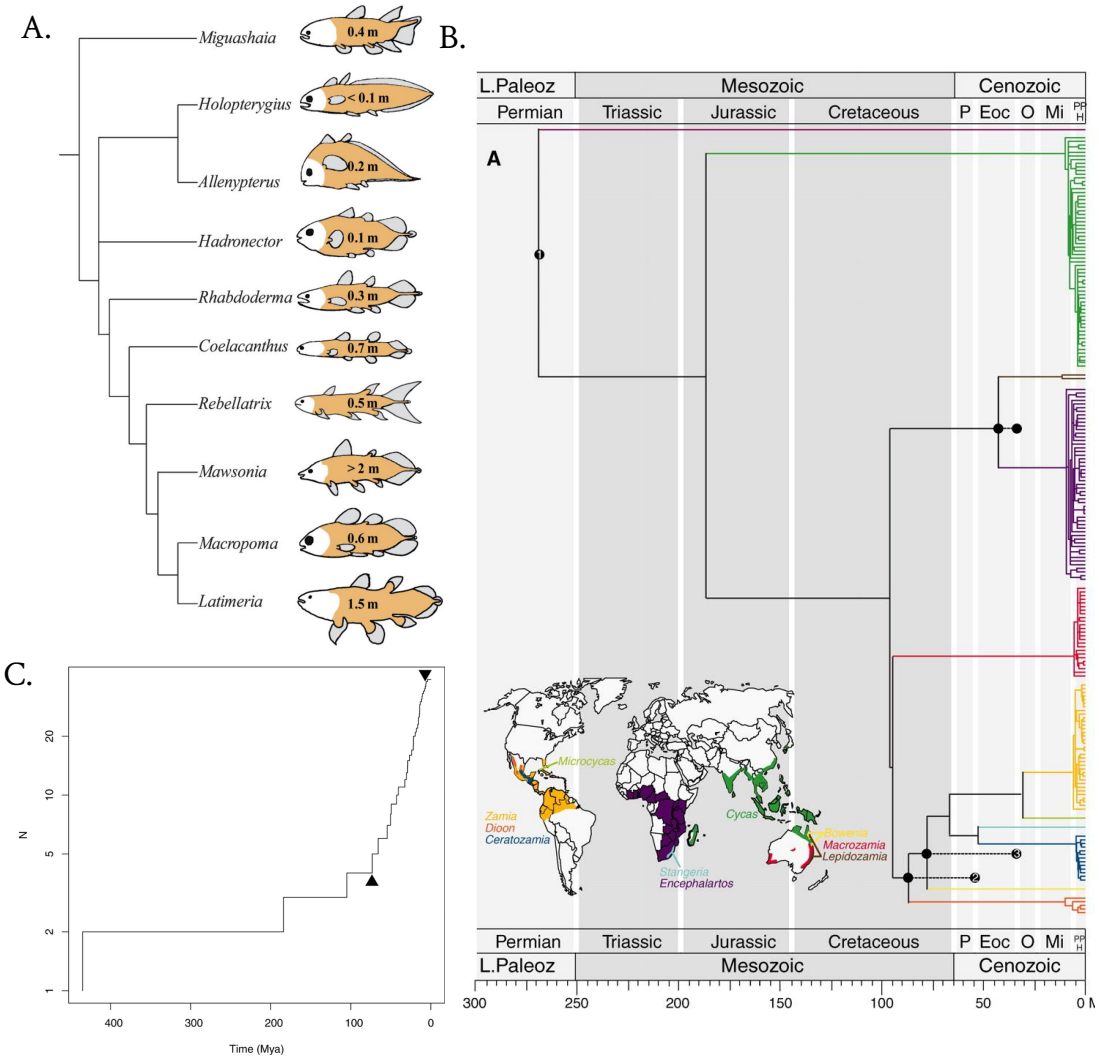


FIGURE 1.5 NEW EVIDENCE QUESTIONS THE LIVING FOSSIL

Questioning the 'living fossil' status of three well-known groups. A. Phylogeny and body-plans of coelacanths (FIGURE 1 from Casane and Laurenti, 2013) demonstrating morphological diversity of the clade in the past. B. Time-calibrated phylogeny and distribution of the cycads (Cycadophyta) (FIGURE 1 from Nagalingum et al. 2011), showing how the majority of the 300 species actually originated in the near recent. C. Diversification of tadpole shrimps (Notostraca) based on recent sequencing and phylogenetic modelling (FIGURE 2 from Mathers et al. 2013), highlighting the larger than expected number of potential species.

Secondly, others have rejected the concept on more theoretical grounds. 'Living fossil' is considered a phenomenon manifested at the level of species or above. Schopf (1984), however, argued that it should be considered at the level of the trait, such that 'living fossils' *per se* do not exist; instead only species that demonstrate the phenomenon of canalisation – the retention and stability of certain traits – can be said to exist. Thus, in this view 'living fossils' are only artefacts of the detection of stable characters in an uncertain and incomplete fossil record. Casane and Laurenti (2013) also argued against the term conceptually. They suggested that recent 'evidence' for the LF is the result of a biased interpretation where the identification of a group as a LF is a foregone conclusion. For example, they point out how many molecular studies exploring substitution rates in sections of coelacanth genomes argue implicitly in favour of coelacanths as LFs, even when evidence for it is not strong. Furthermore, upon considering the variety of morphological forms of the coelacanth lineage, they ask us whether we really think that the lineage has undergone no change. (See FIGURE 1.5)

Finally, as stated above, a tendency to consider evolution as a ladder of progress continues to persist and its influence is found in considering the 'primitiveness' of traits or organisms – an aspect often associated with the LF (Emery and Thorington, 1984; Schopf, 1984). This association led Casane and Laurenti (2013) to describe the LF as a product of "bad evolutionary thinking". The problem, however, is more than just bad thinking since connecting the LF to subjective assertions of primitiveness is *also* making the LF subjective. This is seen all the more so with the onset of new research. For example, the 'primitive' morphological traits of lungfishes are the result of paedomorphsis, not the result of direct inheritance (Bernis, 1984). Also, it was once thought that monotremes had lower body temperatures (~32°C) than other mammals because the Prototheria-Theria ancestor had low body temperature, making monotremes

closer to 'primitive' cold-blooded groups. Instead, cell-cycle evidence indicates lower body temperatures are in fact a derived trait (Watson and Graves, 2013).

Arguments for the Living Fossil

Casane and Laurenti (2013) took a strict view of the concept to be "evolutionary halting", the arrestment of any evolutionary change. Yet 'living fossil' status does not have to imply that no change has occurred: it can simply mean that a given group has changed less than others. Nor does the label have to imply that a group resembles in form or behaviour a now extinct set of organisms (Cavin and Guinot, 2014). Although this is possible, it is in many cases ultimately unknowable and distracts from any practical sense of the term. Those who would defend the use of primitive traits in defining LFs might argue it is simply the similarity of past traits to recent traits. This, however, can otherwise be termed as estimating the amount of change that has occurred, without the need of judgement on what constitutes an advanced or non-advanced trait.

Nor does the recent evidence that questions the status of various LFs mean the term is defunct. Inevitably new research will reveal previously unrecognised evolutionary change or patterns of past or present diversity, and cases of the misapplication of a label, due to flawed evidence, do not invalidate that label *per se*. Other studies provide evidence and argue in favour of the concept having a scientific meaning. Two large-scale studies that analysed the genomes of charismatic LFs, coelacanths and the elephant shark (*Callorhinchus milii*), both found evidence of slower than expected genome evolution. In the case of coelacanths this was through reduced substitution rates in protein coding sequences (Amemiya et al. 2013), while for the elephant shark this was due to significantly fewer insertions and deletions than would be expected for the clade's age (Venkatesth et al. 2014). Additionally, recent morphological studies have argued in support of two other well-known LFs: coelacanths and tuataras. By analysing the

number of derived unique traits that appear between three major vertebrates clades, Cavin and Guinot (2014) found that rates of change in the coelacanth lineage were significantly the slowest (FIGURE 1.6). Similarly, Herrera-Flores et al. (2017) found extant tuatara to have experienced unusually little morphological change.

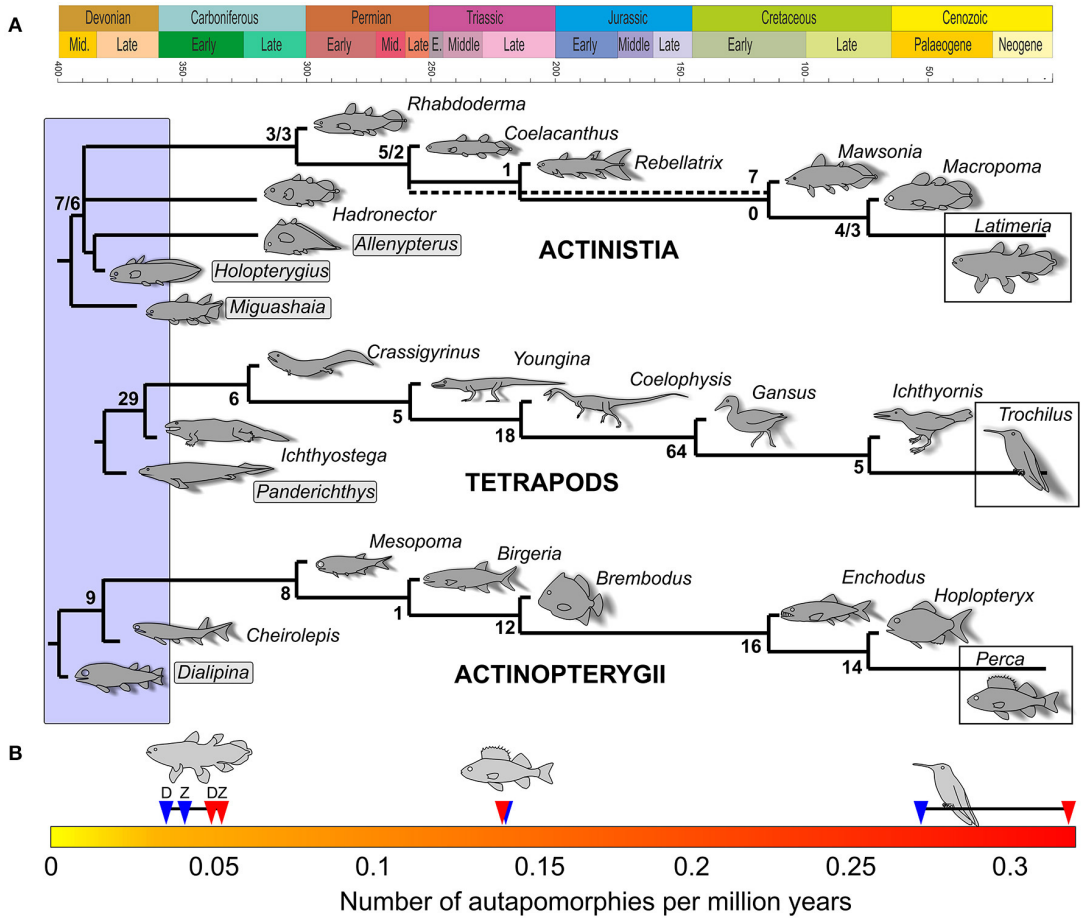


FIGURE 1.6 MORPHOLOGICAL CHANGE OCCURS AT DIFFERENT RATES

Different vertebrate lineages accrue new morphological innovations at different rates. A. Cladograms of three crown-vertebrate clades with numbers indicating the number of derived autapomorphies supporting each clade. B. Rate at which new traits appear for each of the clades. Actinistia, which include coelacanths, have a lower rate than comparative clades of equal ages. (Figure 2 from Cavin and Guinot, 2014)

Living Fossils and Models of Evolution

An additional complication for the definition of the LF are interpretations over how evolution has taken place, since the meaning of 'living fossil' also depends on one's model of macroevolution. Traditionally, there are two schools of thought for how LFs

arise. The first school of thought, propounded e.g. by Schopf (1984), considers there to be a single rate of evolutionary change, making LFs "nothing more than the tail end of a normal distribution" (Eldredge, 1984). In this 'neutral' model, LFs should not share any special features and their existence is purely a product of the roll of the die. Although proponents of this school reject the concept of the LF, this approach makes an important point. Proponents of the concept must design tests to ensure any identified LFs are the product other than chance alone.

The second school, states that diversification rates *do* differ significantly for different groups and has its origins in Simpson's (1944) three modes of evolutionary rates: bradytelic (slow), horotelic (normal) and tachytelic (fast). Simpson suggested that all clades can be grouped into one of these three groups of rates, and the causes for these rate differences can be shared between groups. For example, tachytelic groups, as Simpson suggested, are clades that experience adaptive radiations – a process that is often attributed to common factors such as an ecological opportunity (Yoder et al. 2010). In this framework, we can therefore consider LFs as groups or species that experience an unusually slow rate of evolutionary change due to a common set of factors. In the literature that predates the works of Gould, Eldredge and Stanley, these factors used to only concern species-level phenomena such as population genetic diversity and diverging or balancing selection (Eldredge 1984). Since then, however, extrinsic factors such as habitat types and life-history traits have been considered, see below.

Within the second school, the model of evolution also influences the interpretation of LF origins. Under a gradualist understanding of evolution the question of how a LF arises is essentially one of how rates of diversification are particularly low for certain clades, whereas under a punctuated equilibrium model, the question strictly concerns how speciation may be restrained in certain lineages (Stanley, 1979).

Factors Influencing Diversification

Proposed factors for how differences in speciation and extinction rates include niche breadth, the stability of the environment and geographical distribution. In some cases, however, these potential factors are apparently incompatible. For Parsons (1994), a highly varying or stressful environment is what creates LF species, as the evolutionary transition required for a species to survive in such environments would prevent it from returning to a less stressful environment. He provided the example of cave insects, which must adapt to tolerate a high concentration of CO_2. Once adapted, the cave insects are locked into the cave environment – leading to the accumulation of 'relict species'. Conversely, Delson and Rosenberger (1984) showed that LF species are more common in New World monkeys (Platyrrhini) than Old World monkeys (Catarrhini). New World monkeys live in the arguably evolutionarily more stable environments of Central and South America, while Old World monkeys have survived through the far more changeable Cenozoic ecosystems of Africa and Asia, which have experienced more severe periods of drying (Delson and Rosenberger 1984). As a result, Old World monkeys have experienced a greater turnover of species through time, preventing the development of LFs.

Eldredge (1979) and Stanley (1998) suggested that LF species are those that are more generalist than specialist, as this life-strategy reduces the chances for speciation to occur. This suggestion, however, is contested by the observation that generalist species tend to have broad distributions facilitating allopatric speciation (Jackson 1974). Furthermore, although it is often asserted that life-history specialisation should increase a lineage's risk of extinction, it cannot be determined from the outset. For example, Cieslak et al. (2014) demonstrated that cave beetles have in fact experienced multiple radiations since becoming specialised to a subterranean environment. From the literature alone it is not clear to what extent LFs can be considered specialist/generalist.

For a more detailed review of the factors that influence diversification refer to chapter five.

Turnover

New research on rates of evolutionary change in macroevolution has highlighted the importance of 'turnover' – the constant replacement of species and clades through speciation and extinction. Quental and Marshall (2013) analysed the speciation and extinction rates of selected extinct and declining families of mammals. Although each family had its own rate of diversification and extinction, they all followed the same trajectory – each experienced an increase in diversity until a peak was reached, after which there was a subsequent decrease until the (near-) extinction of the family. Quental and Marshall (2013) found that these rises and falls were shorter than expected if speciation and extinction were random – they were driven by changes in speciation and extinction at different stages of the trajectory (FIGURE 1.7). Simulations of adaptive radiations have found similar trends – termed 'overshooting' (Gavrilets and Losos 2009). These simulations have not detected extinction of a clade, but this may be due to their being performed on a non-macroevolutionary scale. One recent study found evidence for these peaks of diversity in extant mammals. By detecting step changes in diversification rates, Humphreys et al. (2014) could identify groups of mammals equivalent to families and genera, implying that these taxonomic groups are equivalent to the rise and fall of clades in the past. Today's genera could be tomorrow's orders.

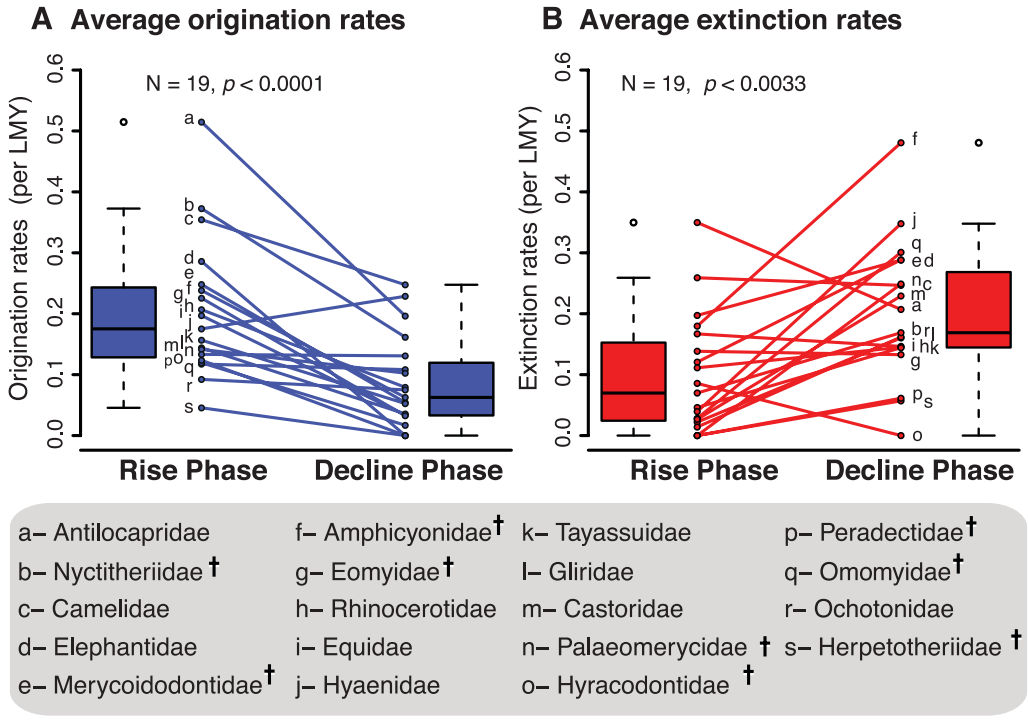


FIGURE 1.7 THE RISE AND FALL OF MAMMALIAN CLADES

Evidence from fossil mammalian orders for shifting diversification rates as clades rise to and fall from peak diversity. A. Origination (or 'speciation') rates are generally higher during the rising phase of a clade and lower during the decline. B. Extinction rates show a mirrored response. (Figure 2 from Quental and Marshall, 2013.)

On a smaller scale an analogous phenomenon has been described in islands – taxon cycling. This is the process where a new species arrives on an island; radiates into multiple new species; a new species arrives to inhabit the island; the original species become extinct; and the process is repeated. Although subject to much debate, a recent study using a large dataset and new computational methods found evidence for taxon cycles in passerine birds on Indo-Pacific islands (Jønsson et al. 2014).

Turnover could thus occur at multiple scales of biodiversity in space and time. If this were such a prevalent phenomenon, how would a 'living fossil' fit into this framework? Would it simply be a clade that has an unusually slow rate of turnover, in which case it would be bound for extinction? A large-scale analysis used to detect step changes in diversification across all jawed vertebrates (Alfaro et al. 2009) found the pattern of species distributions could be explained by three components: nine key

adaptive radiations, general turnover, and a few slowly evolving clades that the authors termed as 'living fossils'.

Quantifying the Living Fossil

As I have outlined above, it is difficult to gauge what the term 'living fossil' precisely means because of the many interpretations both in its definition and our understanding of evolution more generally. Before we can answer how LFs may arise or whether we can even think of it as a valid concept, we must pin down what we mean when we use the term. One solution would be to quantify the concept (Herrera-Flores et al. 2017). This would make the definition explicit and transparent, and would separate defining features from explanatory factors. It would create a continuous scale of "living-fossil-ness" that would allow a direct comparison with opposing phenomena such as adaptive radiations. It would allow for better testable exploration of what features may be more associated with LFs, giving insight into how they may arise. A simple metric might consider three simple and potentially measurable properties repeatedly associated with the LF concept: time, change and success (TABLE 1.4).

TABLE 1.4 SEPARATING THE PALAEOBIOTIC AND THE NEOBIOTIC

Comparing evolutionary performances between three LF (†) and three radiating clades (‡): *time* – estimated time since the clade emerged, *change* – nominal amount of evolutionary transitions since the clade emerged, and *success* – the number of species alive today.

Clade	Time (MY)	Change*	Success	References
Horseshoe crabs†	445	Low	8	Rudkin et al. (2008)
Ginkgo†	200-300	Low	1	Royer et al. (2003)
Tuatara†	220	Low	2	Hay et al. (2008)
Passerines‡	82	High	5,739	Barker and Cibois (2004)
Angiosperms‡	125	High	352,000	Berendse and Scheffer (2009), Kalwij (2012)
Aphids‡	80-150	Med	4,400	Von Dohlen (2000)

* 'low', 'med' and 'high' categories are based on my own comparisons between referenced descriptions of change

Monophyly

At this point a note should be made regarding the phylogenetic and taxonomic context of the LF. LF groups have sometimes been paraphyletic or have not been grounded in strict monophyly. For example, Emery and Thorington (1984) determined tree squirrels (*Sciurus*) to be LFs as they showed little morphological difference from the oldest known Sciuridae fossil, *Protosciurus*. While I understand that their focus was on establishing that tree squirrels exhibit the original squirrel morphology, in forming a group of an early fossil and a single extant genus of an entire family excludes a wide range of other squirrel genera (ground, flying, etc.), making it essentially non-monophyletic. Paraphyletic and polyphyletic groups are arbitrary because, as Cracraft (1984) put it, they have no "ontological status as a real historical entity; they are taxonomic artefacts." Using non-monophyletic groups also undermines any attempt in quantifying the LF, as robust values relating to time since the group split from other groups or number of species would not be computable. Additionally, some authors suggest that LFs may only be supraspecific (Stanley, 1998). Species, however, are monophyletic and can be the sole

surviving representatives of their clades, e.g. *Ginkgo biloba*. Their exclusion from consideration is an unnecessary complication that is in any case counter to most authors' usage of the term; their exclusion by Stanley will thus not be followed in this thesis.

Living Fossils and Conservation

One approach to devising a quantitative solution to the LF is in conservation biology. The LF concept plays an important role in framing conservation efforts. Because funds are limited not all species can be saved, therefore prioritisation is key to conservation strategy. The LF label is being used to justify conservation priority-setting both directly and indirectly. There are a few cases where conservationists seem to use the label to generate interest and funds (e.g. Celis-Diez et al. 2012; Li et al. 2012; Biton et al. 2013). To date, however, most species-level conservation prioritisation has been focussed on well-known charismatic taxa such as tigers, rhinos and polar bears (Mace and Collar, 2002; Entwistle and Dunstone 2000) as well as highly vulnerable species in urgent need of conservation (Brooks 2010). Efforts have been made to base conservation prioritisation on quantifiable values (human or otherwise) rather than subjective mass-appeal and popularism. One such effort has been to classify species by their Evolutionary Distinctiveness and Global Endangeredness (EDGE, 2017). This approach created a list of species ranked by how threatened they are (based on the IUCN Red List [2017]) and their isolation in the phylogenetic tree. We may think of this phylogenetic isolation or evolutionary distinctness (ED) as a putative correlate for the 'living fossil', as has been suggested by others (Isaac et al. 2007). Highly evolutionarily distinct species have few close species and have split from the tree of life a long time ago, much like many supposed LFs.

How Do We Measure Evolutionary Distinctness?

Unlike 'living fossil', evolutionary distinctness is measurable and has its roots in the increasing availability of phylogenetic trees in the early '90s. ED relies on time-calibrated phylogenetic trees to determine the uniqueness of a species measured in millions of years. The history of its computation arose from the conservation biologists' need to determine the relative amounts of biodiversity represented by different ecological communities. Considering two communities with the same number of species, one way to determine which of these we may consider to have greater biodiversity can be based on the amount of evolutionary history represented by each community, calculated by estimating the total branch lengths present in each community in the shared phylogenetic tree (FIGURE 1.8). This is termed phylogenetic diversity or PD (Faith, 1992).

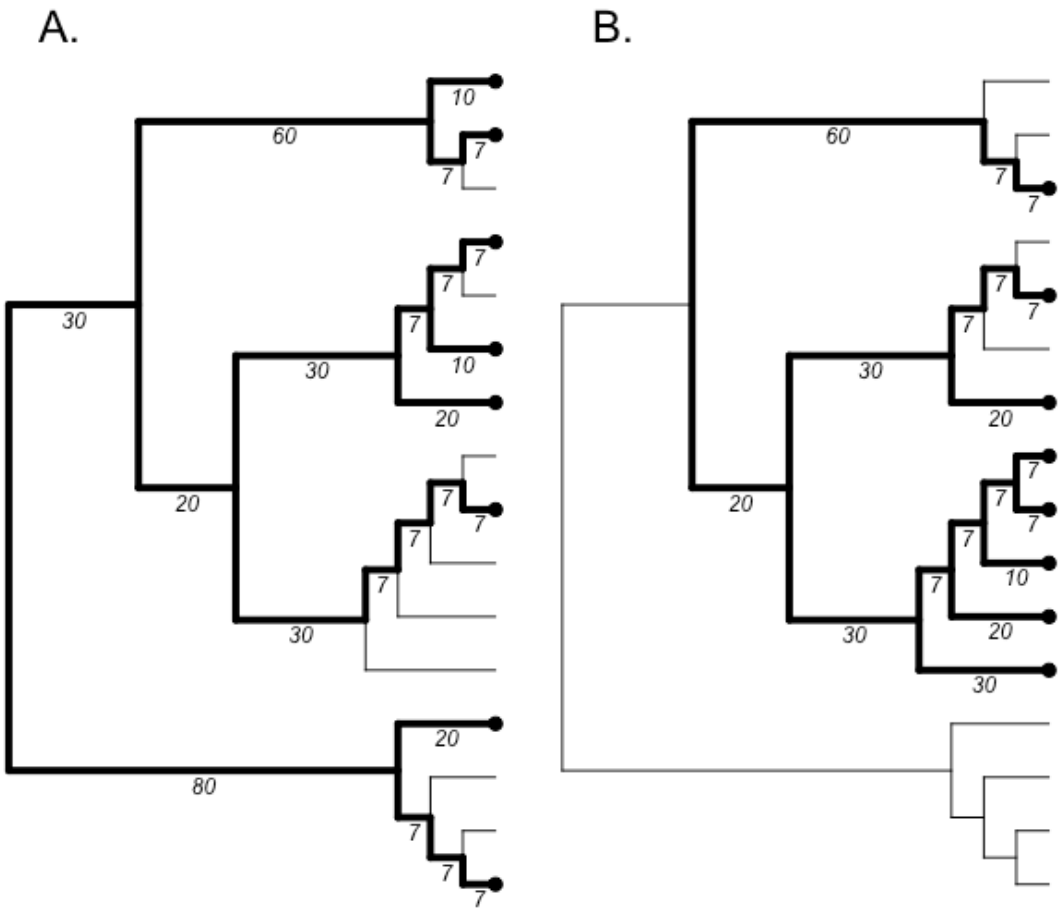


FIGURE 1.8 ILLUSTRATING PHYLOGENETIC DIVERSITY

Calculating phylogenetic diversity for two communities. Dots at the tips of trees indicate species' presence in communities. Community A contains the clade depicted at the bottom of the figure and has a much higher phylogenetic diversity (~400 MY). Community B lacks this clade, and although has the same number of species, it has a much lower phylogenetic diversity (~300 MY).

Once the PD method was established, the next question naturally followed: if a conservationist could only save one tip in the tree, which should it be? Which tip alone represents the greatest phylogenetic diversity? The most straightforward measure is equivalent to the time since the clade split from the rest of the tree of life – often termed 'pendant edge' (PE, Altschul and Lipman 1990). Although pendant edge has the benefit of simplicity, it does not take into account the rest of the tree structure. For example, two species that split at the same time would always be classed as representing the same amount of time for independent evolution, even if for one the sister clade contains 10,000 species while for the other it contains 1 species (FIGURE 1.9).

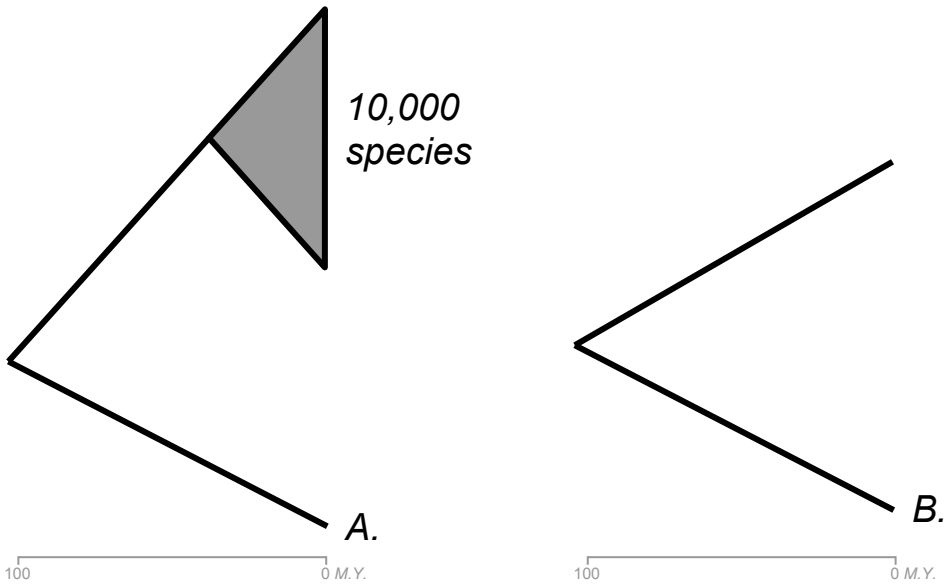


FIGURE 1.9 PHYLOGENETIC CONTEXT

FIGURE 1.4 The importance of phylogenetic context. Do A and B have the same evolutionary distinctness?

To account for such differences in phylogenetic context other measures were invented. 'Equal splits' (ES, Nee and May, 1997; Redding and Mooers 2006) apportions the evolutionary history represented in a phylogenetic tree between all species in the tree by sharing branch lengths equally between clades (Eq. 1). 'Fair proportion' (FP, Isaac et al. 2007) also apportions evolutionary history, but instead of sharing branch lengths equally between clades, it does so between descendants (Eq. 2, FIGURE 1.10).

$$ES = \sum_{j=1}^{r} \frac{B_j}{\prod_{k=1}^{j}(d(k-1)-1)} \quad (1)$$

$$FP = \sum_{j=1}^{r} \frac{B_j}{N_j} \quad (2)$$

Calculating evolutionary distinctness with ES and FP for a single species in a phylogenetic tree. Where *j* is the first internal node of the tip on the direct path to root (*r*), B_j is the branch length from the internal node *j* to *j-1*, *d(k)* is the degree (three for a

bifurcation) at node k, and N_j is the number of descendants of j (Redding and Mooers, 2006). By sharing between clades, equal splits weights isolated species more highly than fair proportion, whereas fair proportion focuses on the proportion of the tree that is lost if a species were to go extinct – which is equivalent to the proportion of evolutionary time that is unique to a species.

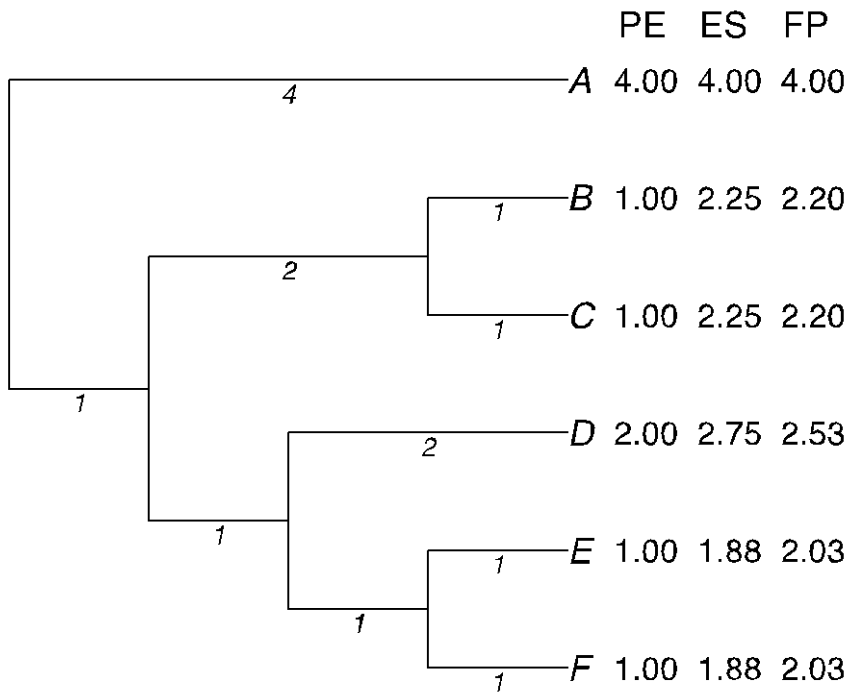


FIGURE 1.10 CALCULATING EVOLUTIONARY DISTINCTNESS

Comparing measures of evolutionary distinctness (time): pendant edge (PE), equal splits (ES) and fair proportion (FP). Numbers under each branch indicate branch length, which is equivalent to time in a time-calibrated ultrametric phylogenetic tree. ES weights early branching species (e.g. B and C) higher than FP because it distributes branch length between clades rather than single species.

Fair proportion has proved to be the most popular measure for evolutionary distinctness and has been suggested as a proxy for LF-ness (Cavin and Kemp, 2011). This metric, however, does not cover all aspects of the LF concept – most notably morphological change – and it requires large phylogenetic trees in order for it to be calculated. I can,

however, generate estimated ED values by dividing the age of a lineage by the number of extant descendants. Using these estimated ED values for groups that have been previously considered as LFs, I show that these groups do have higher ED when compared to non-living-fossils (TABLE 1.5).

TABLE 1.5 ESTIMATED EVOLUTIONARY DISTINCTNESSES

Estimated evolutionary distinctnesses using an approximate fair proportion (AFP) for commonly labelled LFs and exemplar sister taxa (in grey). AFP is calculated as the time since splitting (PE) divided by the number of extant species (N). Taxomnomic information was taken from NCBI taxonomy (Federhen, 2012). Divergence dates were taken from TimeTree (Hedges et al. 2006). (Numbers and sisters are for demonstrative purposes.)

Common name	Scientific name	N	PE (MY)	AFP (MY)
Coelacanths	*Latimeria*	2	500	250
Land animals	Tetrapoda	30,000	500	0.017
Egg-laying mammals	Monotremata	5	170	34
Therians	Theria	5,000	170	0.034
Horseshoe crab	Limulidae	8	500	63
Arthropods	Arthropoda	8,000,000	500	0.00006
Lungfishes	Dipnoi	12	500	42
Land animals	Tetrapoda	30,000	500	0.017
Maidenhair tree	*Ginkgo biloba*	1	300	300
Gymnosperms	Gymnospermae	1,000	300	0.3
Sharks	Elasmobranchii	1,000	400	0.4
Bony vertebrates	Teleostomi	50,000	400	0.008
Tuatara	*Sphenodon*	2	200	100
Lepidosaurs	*Lepidosauria*	7,000	200	0.03

ED as measured using the fair proportion metric is a useful proxy for living-fossil-ness. It is, however, not readily applicable for many of the most well-known LF species because of the lack of a fully resolved phylogenetic tree for the whole of life. As a measure, it can only be used as a comparator within taxonomic groups where phylogenetic trees are already available (e.g. mammals or birds), as is the case for the EDGE project (EDGE, 2017). There is, thus, room for an updated metric of LF-ness calculable independently of a phylogeny.

Conservation and the Living Fossil

As described in the section above, LFs are of interest to biologists forced to seek ways to prioritise the conservation of some species over others (i.e. the Noah's Ark problem). In this final section of the introduction I will outline the ways in which conservation is argued for and against conserving the evolutionarily distinct or living-fossil-like species.

Arguments for conserving evolutionarily distinct species can be found on both ethical and practical grounds. Ethically, these species represent a disproportionately large amount of evolutionary history, which can be interpreted as a general proxy for biodiversity *per se* (Isaac et al. 2007; Rosauer and Mooers 2013). Practically, ED has often been correlated with trait diversity and by preserving traits we may be preserving ecosystem functions and services (Millennium Ecosystem Assessment 2005). Evidence for the correlation between traits and ED, however, is mixed (Winter et al. 2013), and the link between trait diversity and ecosystem functions and services is untested. Furthermore, such pragmatic arguments cannot be held when conservation effort is targeted at single species (Rosauer and Mooers 2013). For community-level conservation alternative metrics exist that would be easier to measure and correlate better to ecosystems aspects we may wish to conserve e.g. evolutionary history using Faith's PD (1992).

Additionally, ED can be read as evolutionary potential; preserving the evolutionary distinct species might preserve the future of life. This has been debated since Krajewski's (1991) 'twig-branch' argument (FIGURE 1.11): is it better to save the twigs or the branches of the tree of life in order to better preserve the potential of life? Mouquet et al. (2012) suggest that it would be best to preserve the twigs, as these represent the most recently radiated members of biota and are in a phase of radiation. However, others have pointed out that evolutionarily distinct species are more likely to feature unique

adaptations, allowing them to respond differently to environmental changes (Isaac et al. 2007; Collen et al. 2011), and by already surviving for a long period of time they may be better equipped to continue surviving.

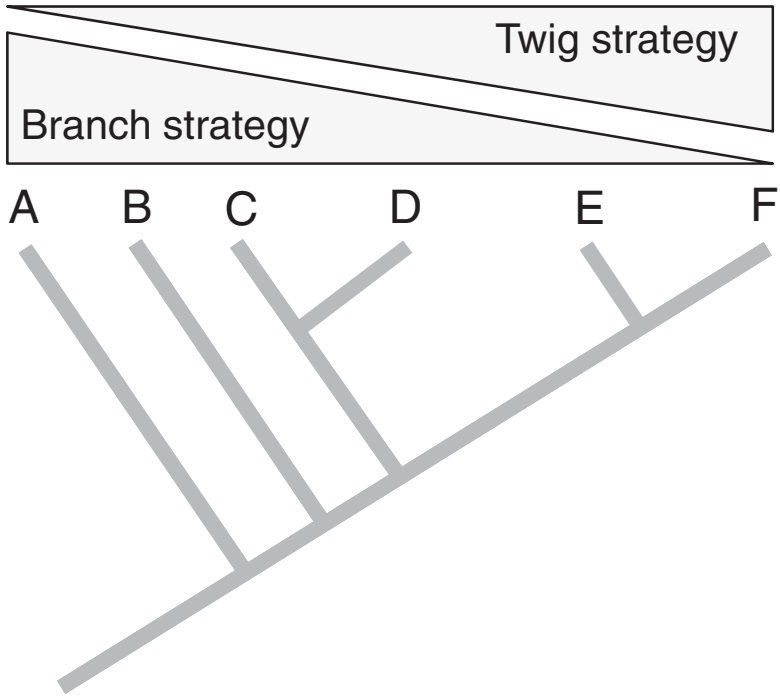


FIGURE 1.11 SAVE THE TWIGS OR BRANCHES?

How best to conserve evolutionary history. Is it better to save as many twigs as possible, or try to save a few branches? (Figure 6 from Mouquet et al. 2012.)

Palaeontological evidence may favour the branches side of the argument. The 'phylogenetic fuse', as coined by G.G. Simpson, is the idea that the largest radiations in Earth's history only occurred after a long period of 'tinkering' (Cooper and Fortey 1998). Examples include mammals, birds and land plants: in all of these cases the group first appeared long before it 'exploded' in diversity. It is speculated that during this time, these groups developed the unique adaptations necessary for their subsequent mass radiations (Cooper and Fortey 1998). For example, the ancestors of mammals are estimated to have split from the rest of the extant tetrapods in the Carboniferous c. 300MYA (Pyron 2010) yet they did not diversify into modern mammal groups until an estimated c. 170MYA

(Pyron 2010; Bininda-Emonds et al. 2007). During this tinkering phase, they might have been considered 'living fossils', being a small sister clade to the much larger tetrapod clades at the time. On the other hand, however, more recent studies exploring rates of diversification have consistently found no correlation between rates of extinction and lineage age (Van Valen 1973; Alroy 1996; Ezard et al. 2011); a fact that either makes the 'phylogenetic fuse' untenable or extremely rare.

As the twig/branch argument demonstrates, palaeontology and macroevolution have a role to play in informing conservation – particularly through research into the evolutionary potential and origins of the LF. Are they a unique form of life worthy of special protection or are they much like any other group and less in need for saving?

Thesis Outline

The LF is a concept with many issues, which relate to its exact definition; whether it is a useful concept under different models of evolution; and to what extent it may play a useful role in conservation biology. The aim of this thesis is to better elucidate what we mean when we say 'living fossil', and to demonstrate to what extent this term can be useful. In the next two chapters I will lay out methods for determining whether the LF is a useful or real concept for segregating organisms. In the fourth chapter, I suggest a new set of metrics for better clarifying what the term means. In the fifth chapter I use the metrics of chapter four to test which life-history traits, habitats and other factors are most often associated with the LF to determine if there are any common patterns that might explain the origin of the palaeobiotic condition. In the sixth, I detail the different computational tools I have developed for analysing the LF. Finally, in my concluding chapter I assemble the evidence to ask how useful a concept the LF is, both to those who study evolution and those who conserve biodiversity. In short, the aim of the thesis as a

whole is to answer the following question: is 'living fossil' a term we should wish to maintain?

2.

Testing the Living Fossil with a Tree-Growth Model

INTRODUCTION

Regardless of issues defining the concept of the 'living fossil' (chapter one), there is a greater concern over whether the concept corresponds to any meaningful delimitation of biodiversity. Even under a simple definition – 'a group that experiences low rates of speciation and extinction' – it is unclear whether or not LFs simply represent the tail of a distribution of evolutionary performances (Schopf 1984). To date, most work on determining living-fossil status has focussed on single taxonomic groups and has been primarily descriptive (e.g. Eldredge and Stanley 1984). This approach, however, does not address the generality of the LF concept and its reality as an evolutionary phenomenon. In this chapter, I test the concept from a phylogenetic tree perspective using an existing metric (evolutionary distinctness; ED) that has been suggested as a quantitative measure of LF-ness (Isaac et al. 2007; Cavin and Kemp 2011).

The uneven distribution of species among taxonomic groups – the 'hollow curve distribution' (HCD) – may intuitively point to evolutionary performances differing substantially between groups (Alfaro et al. 2009). Yule (1925) was the first to notice the HCD and related it to a property of phylogenetic tree shape, imbalance – the unequal number of descendants between sister groups. However, Yule demonstrated that this imbalance does not necessarily indicate unequal rates of diversification among species due to the phenomenon of preferential attachment: if speciation is random then clades with more species will speciate more and more because they increasingly represent a larger proportion of all species. Since Yule, however, the increase in the number and diversity of published modern-day phylogenetic trees has made it apparent that phylogenetic imbalance is found across all parts and scales of the tree of life at a level greater than expected for a random process (Blum and François 2006), indicating that evolutionary performance is not normally distributed (Mooers and Heard 1997). Indeed, various independent studies, which modelled the growth of phylogenetic trees through

time using different methods, have all found that strong positive 'age-dependent' speciation is able to generate the level of imbalance observed in empirical phylogenetic trees (Purvis et al. 2011; Hagen et al. 2015; Rabosky and Goldberg 2015). In other words, for phylogenetic trees to have the level of observed imbalance, species-rich groups containing young species must tend to have substantially higher rates of speciation than older species-poor groups.

Palaeobiologists will be more familiar with logistic models where speciation rates drop as clades reach predefined carrying capacities (Sepkoski 1978, 1979, 1984; Raup 1985). Although many spin-off models have been developed, such as hierarchical models (Brayard et al. 2009), models that incorporate mass extinctions (Courtillot and Gaudemer 1996), and coupled logistic models (Miller and Sepkoski 1988; Roy 1996; Sepkoski et al. 2000), these all share the central feature that speciation rates drop as clades grow in size. Although these logistic models and tree-growth models have been developed and applied towards different ends – the former mathematically for the description of clade taxonomic richness through time while the latter for the simulation of tree-growth with birth and death parameters – they both find that speciation rates change dynamically. Age-dependent tree-growth models that place higher speciation rates on younger tips and logistic models that place higher speciation rates on clades that have fewer species – both of which are not mutually exclusive – produce better fits, respectively, to observed phylogenetic tree shape and fossil record occurrences than alternative models.

This prevalence of age-dependent speciation rate bias would suggest that the LF – as an evolutionarily distinct lineage that experiences reduced rates of evolutionary change – could be a real phenomenon. Biases in extinction rates across the tree of life, however, have little effect on phylogenetic imbalance (Hagen et al. 2015), making it difficult, using imbalance alone, to determine what past levels of extinctions rates were. Yet determining whether extinction as well as speciation is differentially distributed is

important in understanding the nature and reality of LFs. If extinction rates also show a positive age-dependent bias, low-diversity groups – which may be identified as LFs – will experience reduced speciation and extinction rates, allowing them to persist for extended periods of time. I term this scenario the 'Panchronic LF hypothesis' (Pan). If extinction rates instead show a negative age-dependent bias, low-diversity groups will experience reduced speciation and increased extinction rates, in which case LF lineages are evolutionary relicts soon to go extinct. I term this scenario the 'Dead-End LF hypothesis' (DE). Alternatively, extinction rates may not show any age-dependent bias, in which case 'living fossil' as a label, when used generally to describe low-diversity groups, may be covering at least two distinct macroevolutionary phenomena – groups generated by both the Pan and DE phenomena, and potentially also by alternative phenomena – and would require splitting. I term this the 'Hydra LF hypothesis' (Hyd). Large-scale studies of the fossil record have consistently found extinction rates to be random with respect to lineage age for multiple groups (Van Valen 1973; Alroy 1996; Ezard et al. 2011), a signal that is even picked up during mass extinction events (Boyajian 1991). This would suggest that the Hyd scenario is the most likely, a finding that could discredit the use of ED as a proxy of living-fossil-ness as it implies there is no consistent process for generating evolutionarily distinct species.

One means of determining whether extinction rates show the same age-dependent bias as speciation rates using tree shape is to look at the temporal distribution of internal nodes – a property of tree shape that I term 'phylogenetic gravity' after centre of gravity or centre of mass as calculated for clade richness through time (Gould et al. 1987) (FIGURE 2.1.) Phylogenetic gravity is driven by temporal differences in births and deaths (Pybus and Harvey 2000). Low gravity or 'regressive' clades experience early bursts of diversification, causing most of their internal nodes in a phylogenetic tree to be distributed towards the root and the peak of their fossil occurrences to occur near their inception. High gravity or 'progressive' clades experience late bursts of diversification,

causing most of their internal nodes to distribute towards the tips and their historical diversity to peak near the present. Additionally, however, the phylogenetic location of speciation and extinction events will also have an impact on gravity (Hagen et al. 2015). For example, when birth and death rates are the same between evolutionary scenarios, if speciation and extinction rates are opposing on equivalent parts of the tree (as would be the case in the DE scenario), gravity is expected to be higher than if they were equal (such as in the Pan scenario) – because in the former scenario tips will be removed in places where there are few recently added tips, which increases the likelihood of loss of deep internal nodes. To date, however, most modellers of tree growth have focused on imbalance rather than gravity due to the relative scarcity of time-calibrated phylogenies (Purvis et al. 2011).

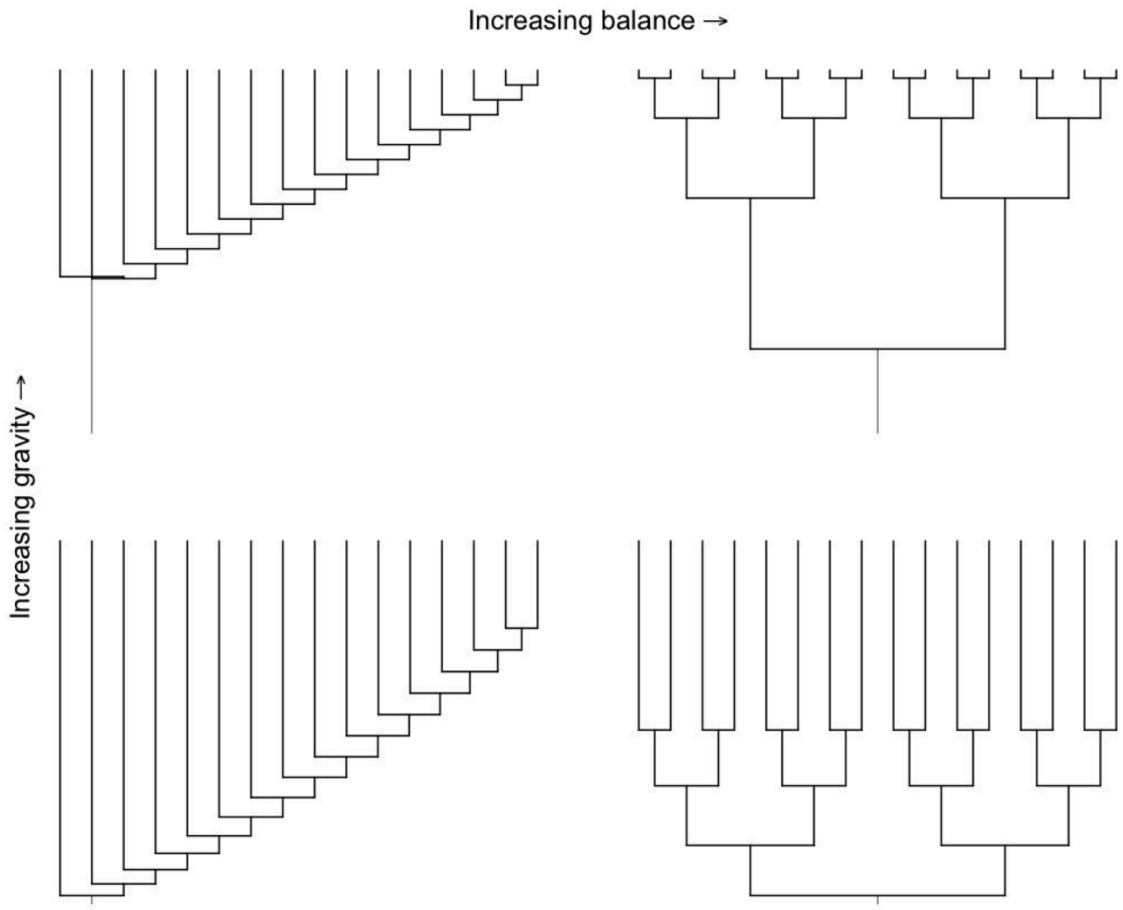


FIGURE 2.1 TREE DIMENSIONS

The two dimensions of phylogenetic tree shape for a 16 tipped tree: balance and gravity, a new term I coin after 'centre of gravity'. Trees with most nodes towards the tip of the tree would be more likely to fall over (high centre of gravity), whereas tips towards the root would be less likely to topple (low centre of gravity).

Here, I test how different models of evolution, each with different implications for the origin of LFs, affect the shape of the tree of life. As there is no agreed-upon definition of the LF, my aim is to take a particular phylogenetic concept for what LFs might represent, and test its self-consistency and utility for partitioning biodiversity. Because the majority of groups described as LFs are often distinct lineages with few close relatives (Eldredge and Stanley 1984), I used the Evolutionary Distinctness (ED) metric – the number of closely related species measured by phylogenetic isolation – as a simple proxy for 'living-fossil-ness'. For the LF concept to have general applicability, some or all of these low-diversity or evolutionarily distinct lineages must behave in similar ways over

macroevolutionary time, which will be shown by a strong signal for either a DE or Pan scenario. To this end, I developed a tree-growth model with age-dependent bias for speciation and extinction rates using a tip's ED value. An ED bias is equivalent to an age-dependent bias because time since last speciation correlates with a tip's ED value. With this model I simulated 10,000 trees under different scenarios by varying the bias in rates of speciation and extinction by the evolutionary distinctness of tips. To identify which hypothesis (DE, Pan, Hyd, or others) best recreates empirical evolutionary patterns, I compared simulated trees to over 2,000 empirical modern-day phylogenetic trees, representing sections of the tree of life, using both phylogenetic imbalance and a birth-death independent measure of phylogenetic gravity.

MATERIALS AND METHODS

Evolutionary Distinctness Biased Markov Model (EDBMM).

I developed a tree-growth model based on the Equal Rates Markov Model (ERMM) that biases speciation and extinction rates by the 'living-fossil-ness' of a species – the Evolutionary Distinctness Biased Markov Model (EDBMM). The ERMM starts with a seed tree, and adds and drops tips at random per unit branch length given birth (*b*) and death (*d*) parameters. The EDBMM biases selection for speciation or extinction by a tip's evolutionary distinctness value (ED). At every time step (*t*), the ED values of all the tips are calculated, and these are used to bias the random selection of tips for speciation and extinction. Tips are then added or dropped and the branch lengths of all extant tips are extended by $\frac{t}{n}$, where n is the number of tips in the tree, so that birth and death rates are constant in time. A simulation can be stopped after a set time is elapsed or the tree has reached a set number of tips. (FIGURE S2.1).

Because there is no fully agreed-upon definition of a LF, I use, as a proxy, 'a low diversity lineage over time'; this is applicable to most lineages described as LFs (Eldredge and Stanley 1984). This 'living-fossil-ness' proxy was calculated using ED as this is a measure of a species' level of phylogenetic isolation, making it an estimate for species' recent lineage diversification rate. I calculated ED using the metric of Isaac et al. (2007). This metric shares the total branch length of the tree between tips by equally apportioning branch lengths by the number of descendants. In this way it takes into account all branches and is conceptually intuitive (FIGURE 2.2).

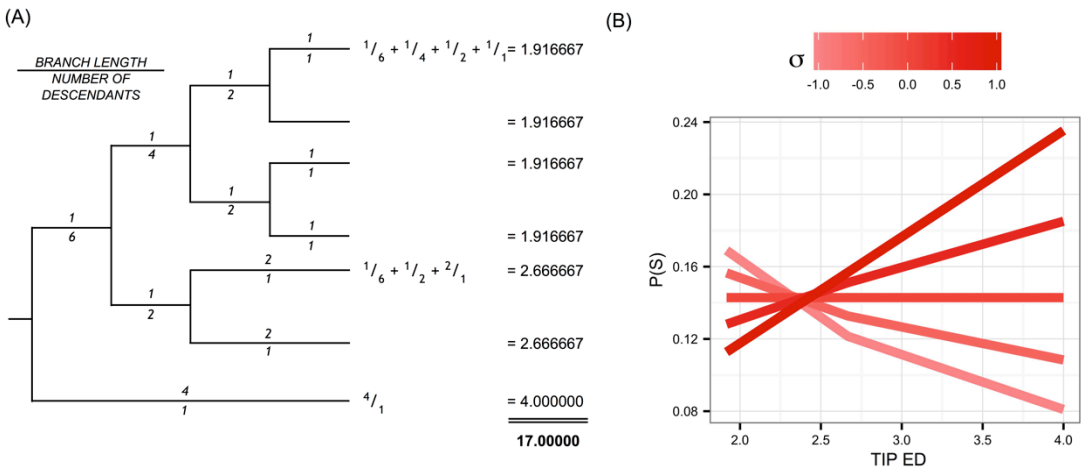


FIGURE 2.2 BIASING EVOLUTIONARY DISTINCTNESS

(A) How to calculate evolutionary distinctness from a phylogenetic tree: divide every branch length by the number of descendants, and sum these proportional branch lengths for every tip in the tree to calculate evolutionary distinctness scores. The total of these scores equals the total branch length of the tree. (B) How different ED values impact speciation probability, S, in the next time step given different σ values for tree in FIGURE (2.2A). Under an equal rates Markov model where rates of diversification are equal between tips (σ=0), all the tips have an equal chance of diversifying. This will maintain tree imbalance, as nodes with more descendent tips have a greater chance of diversifying, and gravity will remain at a mid-level provided birth and death parameters are equal. Modelling this same tree under an Evolutionary Distinctness Biased Markov model will lead to, depending upon the model's parameters, different outcomes. For example, under a Pan simulation (σ=-1) the most evolutionarily distinct tip (4.00000) will become ever more distinct as it will have lower rates of speciation and extinction, while the tips with lower scores will be more likely to speciate and go extinct, extenuating tree imbalance and increasing tree gravity.

Although speciation and extinction rates are held constant and determined by birth and death parameters, the relative probability of a tip being selected for speciation or extinction is determined by its ED value and two power parameters: σ ('sigma') and ε ('epsilon'). The relative probability of the ith tip (i) speciating (S) at each time step (where n is the number of tips in the tree and d is their ED values) is given by equation (1), while its probability of going extinct (E) is given by equation (2). Given these probabilities and global birth (λ) and death (μ) rates, the probability of diversifying (D) for the ith tip in the next time step can be determined by equation (3).

$$p_i(S) = \frac{d_i^{\sigma}}{\sum_{j=1}^{n} d_j^{\sigma}} \quad (1)$$

$$p_i(E) = \frac{d_i^{\varepsilon}}{\sum_{j=1}^{n} d_j^{\varepsilon}} \quad (2)$$

$$p_i'(D) = \lambda \cdot p_i(S) - \mu \cdot p_i(E) \quad (3)$$

In other words, species with higher ED (hereafter 'ED species') experience increased rates of speciation and extinction for positive values of σ and ε respectively, and reduced rates of speciation and extinction for negative values. Values of 0 for σ and ε provide no overall bias towards ED species, making speciation and extinction rates random for all tips in the tree. (See FIGURE 2.2 for visual representation of how different σ values influence probability of speciation for a 7-tipped tree.)

The σ-ε parameter space can be split into four quadrants, each with their own implications for the LF concept. I term each quadrant: 'Phylogenetic Fuse' (PF, +σ, -ε), 'Dead-End' (DE, -σ, +ε), 'Ephemeral' (Eph, +σ, +ε) and 'Panchronic' (Pan, -σ, -ε). Additionally, I identify five scenario points representing the extremes of each quadrant as well as the 'Hydra' (Hyd, σ=-1, ε=0) (see FIGURE 2.3A). In the PF scenario, ED species have low extinction rates and high speciation rates, such that species that could be described as LFs are 'fuses' that will experience future evolutionary radiations. In the DE scenario, ED species have low speciation and high extinction rates; LFs are evolutionary relicts doomed to disappear. In the Eph scenario, ED species have high speciation and extinction rates, making LFs transient phenomena. In the Pan scenario, ED species experience low speciation and extinction rates, meaning that LFs persist through time in low species numbers. In the Hyd scenario, ED species experience reduced speciation rates but extinction rates are random, making LFs potentially the products of both Pan and DE scenarios.

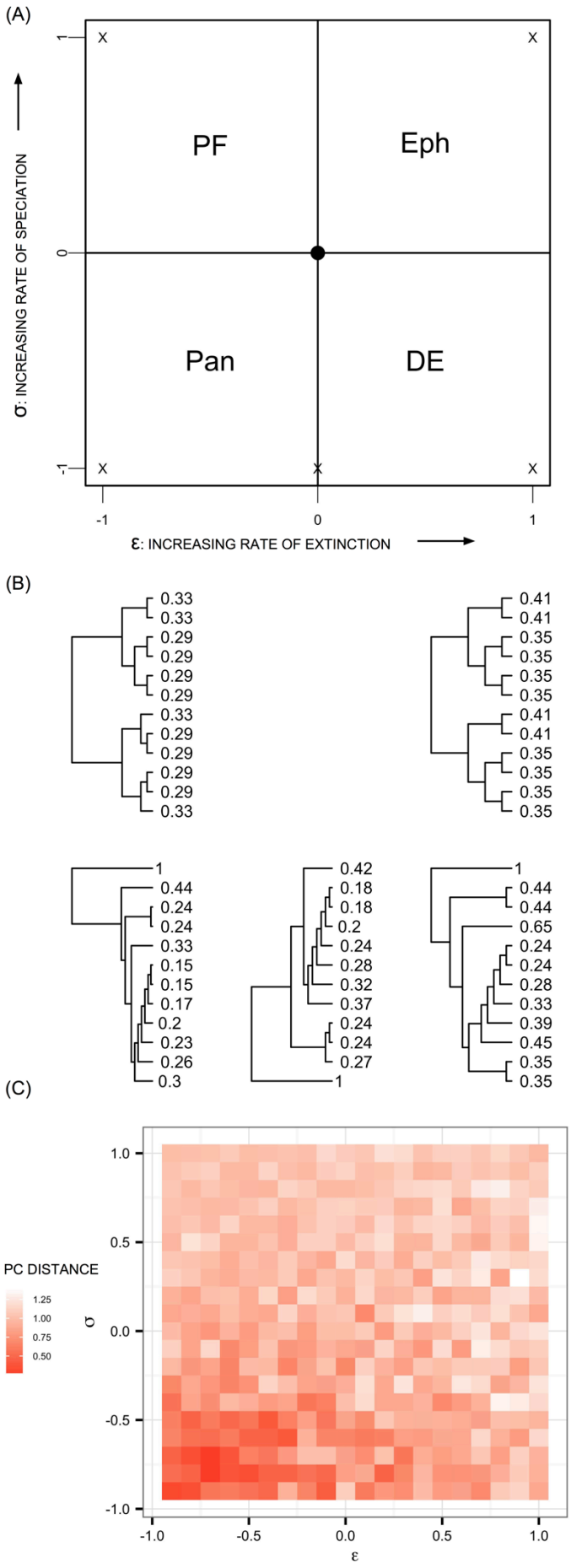


FIGURE 2.3
SIMULATIONS IN TREE SPACE

(A) σ-ε ("sigma"-"epsilon") parameter space indicating the four quadrants for LFs: Phylogenetic Fuse (PF), Dead-End (DE), Ephemeral (Eph) and Panchronic (Pan). The point at 0,0 indicates an Equal Rates Markov Model. The extreme values for each scenario are marked with "X"s. (B) Example12-tipped trees, their expected shapes and corresponding ED values for the five main simulation scenarios. PF and Eph produce balanced trees with moderate levels of gravity. Pan, Hyd and DE all produce unbalanced trees but with different values for gravity, as demonstrated in the more distributed ED values as gravity becomes higher. (C) Distances of 0.1x0.1 grid cells of mean PC1 and PC2 values in simulated space to empirical trees. The smaller the value, the closer to empirical tree shape. Values have been generated from a PCA of the Colless, Sackin and PSV metrics of simulated and empirical trees. Both PCs are weighted equally.

Tree Simulations.

To determine which part of the σ-ε parameter space best recreates empirical tree shape, I simulated 10,000 trees using randomly generated σ and ε values between -1 to +1. I used seed trees of two tips, birth and death parameters of 2 and 1 respectively, and halted tree growth once they had reached a set number of tips determined by randomly selecting a value between 50-500 for each of the simulations. I also used the same parameters to generate 1,000 trees for each of the 'extreme' scenarios PF, DE, Eph, Pan, and the additional Hyd in order to compare the differences between these scenarios with larger numbers of trees.

In addition to exploring phylogenetic tree shape I also explored how idealised fossil records differed between Hyd, DE and Pan. I took the simulated phylogenies generated from the 'extreme' scenarios of Hyd, DE and Pan and their simulations were continued for twice the starting age of the initial tree using new birth and death parameters of 1 and 1 to remove the influence of a higher birth rate on clade shape. In this stage extinct tips were not removed. One hundred equally spaced time samples were then extracted from the tree. Each sample recorded the number of descendants for each clade excluding all tips that were either extinct or not yet extant before and after the time point. A clade was defined in the strict monophyletic sense as a node and all its descendants. This produced a species richness time series for every clade in the tree that accounts for both extant and extinct lineages, unlike other time series based on present-day phylogenetic trees (e.g. Pybus and Harvey 2000). I calculated various clade statistics from these idealised fossil records, including the centre of gravity/mass, which measures the relative time point when a clade reaches its peak (Gould et al. 1987), and centre of gyration, which measures variance around that peak. Statistics were calculated for all clades with total sizes of more than 100 descendants after the 25th time point to reduce the influence of edge effects.

Empirical Tree Search, Manipulation and Comparison.

Empirical trees were gathered for comparison with the simulated trees. I first identified large species-level phylogenies with over 1,000 taxa from the literature (see TABLE S2.1). These trees were supplemented by downloading published phylogenies from TreeBase (Donoghue and Sanderson 2002) using the R package `treebase` (Boettiger and Temple Lang 2012). TreeBase searches were constrained to species-level phylogenies and to trees with more than 50 taxa. Trees that were polytomous were rendered fully bifurcating using the function `multi2di` (Paradis et al. 2004) because, upon inspection, the majority of these polytomies were due to inference error as they did not separate known taxonomic groups. Because this function randomly splits polytomies, I generated a distribution of 100 trees to account for any bias. Trees with branch lengths that were not ultrametric (i.e. branch lengths not relative to time) were converted by rate-smoothing. To account for differences between rate-smoothing algorithms I ran three different methods: D8 (Britton et al. 2007), MPL (Britton et al. 2002) and PL (Sanderson 2002). To make comparisons with the simulated trees, I ensured empirical trees had the same number of tips by reducing any tree with over 500 tips by extracting non-overlapping clades of between 50-500 tips. In total, I sourced 2,061 empirical trees with a mean of 101 (25th, 50th and 75th quantiles: 60.0, 78.0, 110.5) tips (FIGURE S2.2). The mean year of publication of the empirical trees was 2009 (SD 4). Of these trees, 1,432 (69%) were polytomous, 802 (39%) had given branch lengths, 64 (3%) were already ultrametric, and a mean 647 (31%), 492 (24%) and 370 (18%) were successfully made ultrametric using D8, PL and MPL respectively.

For all sourced empirical trees, the phylum, class and order represented by each tree were identified using the online Global Names Resolver taxonomic tool (Global Names Architecture 2015). The 215,752 unique names in all of the sourced trees represented 32 phyla, 87 classes and 260 orders. The number of trees represented by each

phylum was highly unequal; the top five phyla – Tracheophyta, Ascomycota, Chordata, Arthropoda and Basidiomycota – represented 85% of all trees (FIGURE S2.3). Sixty-one trees had names that could not be identified at the phylum, class or order rank.

For empirical and simulated trees I calculated 'shape metrics' for both imbalance and gravity. To avoid pseudoreplication, in cases where empirical tree data were available as distributions of trees rather than single trees – as produced from Bayesian analysis or by randomly removing polytomies – shape statistics were calculated for each tree in the distribution and the mean value was used in subsequent analysis.

To estimate imbalance I used both Colless (1982) and Sackin (1972) metrics. As imbalance metrics are affected by the number of tips in a tree, both measures were normalised with the expected value for a pure-birth tree of the same number of tips ('Yule normalisation') (Mooers and Heard 1997). Values greater than 0 indicate a tree that is more unbalanced than the Yule reference, and less than 0 indicate a tree that is more balanced. These metrics were calculated and normalised using the functions of the R package `apTreeShape` (Bortolussi et al. 2006).

The metric γ ('gamma') (Pybus and Harvey 2000) is used as a measure of gravity (termed 'tippiness' by Manceau et al. 2015). However, γ was originally devised to measure stable ($\gamma=0$), increasing ($\gamma>0$) or decreasing ($\gamma<0$) lineage generation through time, and is therefore sensitive to differences in birth-death parameters between trees – a factor I cannot control when sourcing empirical trees. Therefore, in addition to γ, I also calculated phylogenetic species variability (PSV) (Helmus et al. 2007) as a measure of phylogenetic gravity. As a measure of the relative distance between tips, PSV negatively covaries with gravity. Star trees where all tips are equally distant have low gravity and PSV values of 1. 'Dot' trees or 'line' trees where tips are separated from a single distant tip and internal nodes clustering have high gravity and PSV values tending towards 0 (FIGURE S2.4). Although originally developed as a measure of community phylogenetic diversity, I show in supplementary analysis (see FIGURE S2.5-6) that PSV is less

sensitive to the birth-death parameter (Pearson's Rs: -0.27 and 0.78, for PSV and γ) and more sensitive to ε (Pearson's Rs: 0.20 and -0.13, for PSV and γ) when birth-death varies randomly.

Normalisation was not required for gravity, as the metrics I use are independent of age and number of tips of the tree. Gravity metrics, however, require trees to be ultrametric, and as such only the ultrametric empirical trees (18-31% of sourced trees for the different rate-smoothing methods) were compared to simulated trees using gravity metrics. Because gravity and imbalance metrics partly correlate, I performed PCA using Sackin, Colless and PSV metrics to map the distances of empirical and simulated trees in in 'tree shape space'.

All of the methods described above were run in the R environment (v 3.2.3) and can be reproduced using online R scripts and functions (Bennett et al. 2016).

RESULTS

Simulated Trees

I found strong differences between the 10,000 simulated trees in each of the four quadrants of σ-ε parameter space. There was a strong negative correlation between σ and imbalance for both Colless and Sackin metrics (Pearson's Rs: -0.80 and -0.78 respectively; FIGURE S2.7). As such, the scenarios that represent positive σ in σ-ε space, PF and Eph, both produced trees more balanced than the Yule equivalent (TABLE 2.1). Because all empirical trees are less balanced than the equivalent Yule tree, I consider these scenarios to be implausible. Pan, Hyd and DE scenarios, however, represent negative σ in σ-ε space and produced trees more unbalanced than the equivalent Yule tree, consistent with empirical trees (TABLE 2.1).

TABLE 2.1 TREE SHAPE STATISTICS

Mean (and standard deviation) tree shape statistics for empirical and simulated trees in different quadrants and points of σ-ε ("sigma"-"epsilon") parameter space as indicated by FIGURE 2.3A.

	N	*Colless*	*Sackin*	*γ ('gamma')*	*PSV*	*Tree age*
Empirical trees	2061	4.04 (2.61)	3.54 (2.37)	3.54 (6.43)D8 2.75 (7.51)PL 3.68 (6.62)MPL	0.48 (0.20)D8 0.50 (0.23)PL 0.51 (0.20)MPL	--
Pan-extreme (σ = -1 ε = -1)	1000	4.30 (2.18)	3.80 (1.99)	3.32 (0.96)	0.52 (0.12)	4.04 (0.97)
Hyd-extreme (σ = +1 ε = 0)	1000	5.43 (2.71)	4.86 (2.51)	2.30 (0.83)	0.53 (0.12)	2.88 (0.77)
DE-extreme (σ = -1 ε = +1)	1000	3.96 (2.26)	3.46 (2.05)	0.33 (0.68)	0.61 (0.11)	2.03 (0.44)
PF-extreme (σ = +1 ε = -1)	1000	-1.65 (0.46)	-1.22 (0.29)	4.10 (1.05)	0.75 (0.06)	4.42 (0.87)
Eph-extreme (σ = +1 ε = +1)	1000	-1.17 (0.53)	-0.91 (0.36)	3.91 (1.01)	0.76 (0.07)	3.73 (0.87)
Pan-quadrant (σ- ε-)	2402	1.77 (2.11)	1.54 (1.84)	3.47 (1.01)	0.64 (0.13)	3.47 (0.99)
DE-quadrant (σ- ε+)	2542	1.82 (1.95)	1.57 (1.72)	2.35 (1.12)	0.67 (0.12)	2.35 (0.83)
PF-quadrant (σ+ ε-)	2514	-1.10 (0.70)	-0.82 (0.46)	4.03 (1.01)	0.74 (0.07)	4.03 (0.91)
Eph-quadrant (σ+ ε+)	2542	-0.71 (0.75)	-0.56 (0.56)	3.73 (1.05)	0.74 (0.08)	3.73 (0.91)

I found no significant differences between each of the four quadrants in terms of gravity, except for the DE scenario which produced trees with significantly lower γ values than all other simulated trees (37% lower, t-test: $p < 0.001$) and empirical trees (34% lower using D8, t-test: $p < 0.001$). Because in the DE scenario ED species have negative speciation and extinction rates, the most isolated branches of the tree are more likely to be pruned. This leads to the constant shrinking of the tree from the base, and causes the γ value to be low despite a birth-death parameter of 2-1. I term this the 'shrinking tree problem' (see FIGURE 2.4). This explanation was corroborated by the average age of trees simulated in the DE quadrant being significantly lower than all other simulated trees (26% lower, t-test: $p < 0.001$).

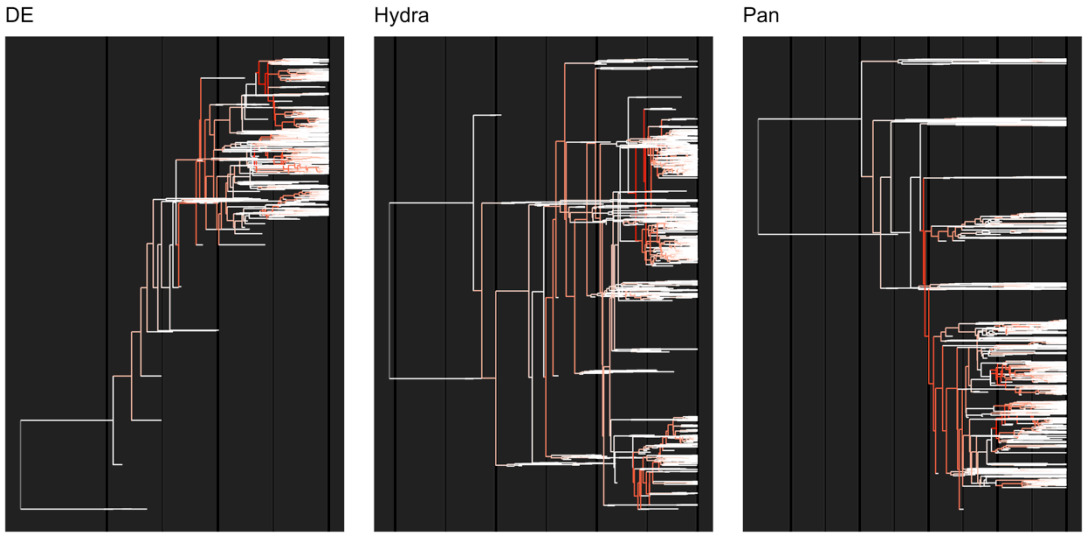


FIGURE 2.4 CHROMATOPHYLA OF SIMULATION SCENARIOS

Chromatophyla of trees with fossil taxa produced under the three most likely scenarios of ED-biased evolution: DE, Hyd, and Pan. Darker branches indicate greater numbers of descendants; darker areas of the tree indicate points of diversification. Under the DE scenario, the resulting tree without fossils is younger than the equivalents under both Pan and Hyd due to the pruning of ED tips – 'the shrinking tree problem'. The DE tree's most distinct branches are extinct, the Pan tree's ED branches are all extant and the tree is as old as the simulation time span.

Across all estimates of PSV, differences between each of the quadrants were not great, although Pan had the lowest PSV values, indicating higher gravity. In particular, PSV values were lowest for simulations with negative σ and mid-ranging or negative ε

(FIGURE S2.8). When excluding trees simulated with positive σ values, I found weak negative correlations between gravity and ε (Pearson's Rs: 0.18 (PSV) and -0.53 (γ); FIGURE S2.9).

The clade dynamics show different shapes between the three extreme scenarios DE, Hyd and Pan. Although Pan produced trees with higher phylogenetic gravity than both DE and Hyd, the mean clade centre of gravity was lowest (TABLE 2.2). Pan also produced the most clades, which on average lasted less time than clades of other scenarios, indicating a high turnover. Furthermore, Pan had the highest gyration, indicating that although its clades tended to peak closer to the mid-point of their time span, this peak was more plateaued than in DE and Hyd. DE produced the fewest number of clades and had the highest gravity, indicating relatively fast clade extinction. With random extinction, clades produced with Hyd contained on average more species at peak (TABLE 2.2, FIGURE 2.5).

TABLE 2.2 CLADE SHAPE STATISTICS

Mean (and standard deviation) clade shape statistics 3,000 simulated trees in three points of σ-ε ("sigma"-"epsilon") parameter space as indicated by FIGURE 2.3A. All statistics are calculated for clades representing more than 100 species between the 25th and 75th time intervals. N. clades – total number of clades, Gravity – centre of gravity/mass, Gyration – centre of gyration, t – clade time span, Peak Spp. – peak diversity, Total Spp. – total number of species throughout time span

	N. clades	*Gravity*	*Gyration**	*t*	*Peak Spp.*	*Total Spp.*
Pan-extreme (σ = -1 ε = -1)	3,815,188	0.525 (0.08)	7.30 (1.10)	24.5 (8.42)	15.1 (8.31)	185 (119)
Hyd-extreme (σ = +1 ε = 0)	3,225,536	0.528 (0.09)	6.72 (1.05)	22.9 (8.32)	29.9 (44.9)	372 (682)
DE-extreme (σ = -1 ε = +1)	2,603,544	0.534 (0.08)	6.61 (9.81)	23.7 (7.31)	26.3 (36.6)	347 (546)

**Gyration E-2*

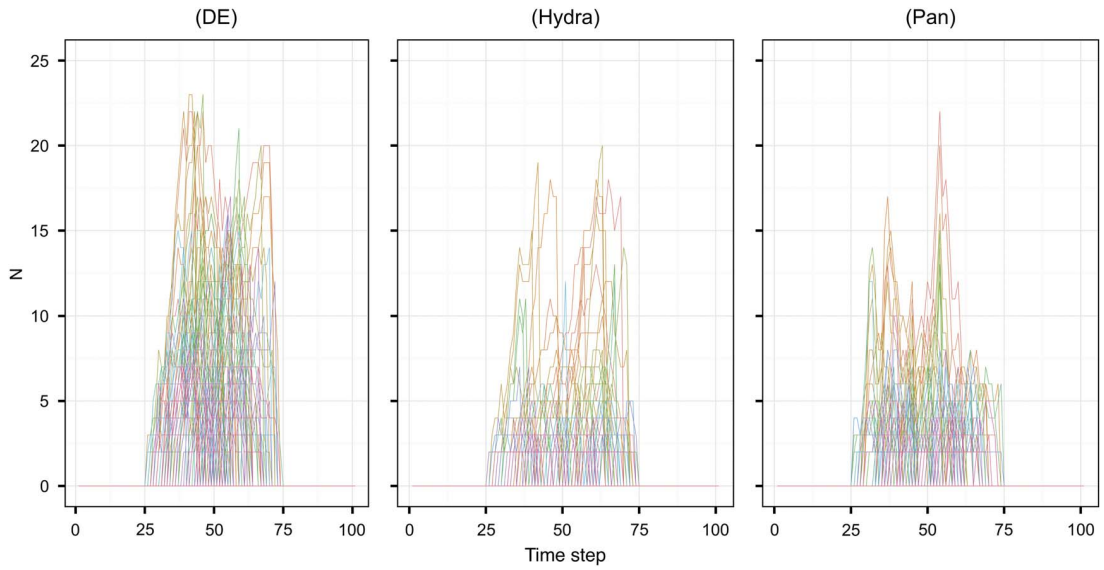


FIGURE 2.5 CLADE DYNAMICS OF SIMULATION SCENARIOS

Clade dynamics between the 25th and 75th time intervals for the 100 largest clades produced by exemplary simulations of the DE, Hydra and Pan scenarios. Each clade trajectory through time has a different colour.

Empirical Trees.

Empirical trees were more unbalanced than the Yule equivalent for both Colless and Sackin metrics, 4.0 (SD 2.6) and 3.5 (SD 2.4) respectively. I did not find significant differences between γ generated with each of the three rate-smoothing methods (t-test$_{D8\sim PLl}$: $p = 0.062$, t-test$_{D8\sim MPL}$: $p = 0.750$; referred to later as γ_{D8}, γ_{PL} and γ_{MPL}). The mean γ for empirical trees was greater than expected for a simulated tree where birth and death parameters are equal, implying increasing lineage generation for most trees (TABLE 2.1). I also found no significant differences between PSV values generated from each of the three methods (t-test$_{D8\sim PL}$: $p = 0.59$, t-test$_{D8\sim MPL}$: $p = 0.34$; referred to later as PSV_{D8}, PSV_{PL} and PSV_{MPL}). The PSV values were mid-ranging, indicating a mid-level gravity for most trees (TABLE 2.1). Although I found significant correlation between gravity and imbalance (Pearson's product moment correlation, Colless~PSV_{D8}: $R = -0.21$, $p < 0.001$), I deem this to be due to the physical impossibility of a tree with low gravity and high imbalance. I found significant differences between taxonomic groups at

all three taxonomic ranks for both imbalance metrics but not for either of the gravity metrics (TABLE S2.2). The variance of shape metrics was larger between orders than between either classes or phyla, which may indicate that lower-ranked groups have diverging macroevolutionary dynamics compared to higher ranks (FIGURE S2.10-12). For example, the variance of the means between taxonomic groups of Sackin and PSV_{D8} metrics increased 111% and 494%, respectively, from phyla to orders. However, I found that this increase in variance, or for any of the other metrics, was not significant (permutation tests of variances of the means from phyla to orders: $p = 0.89$ and $p = 0.76$ for Sackin and PSV_{D8} respectively) (TABLE S2.2).

Empirical and simulated compared.

In terms of imbalance, Pan and DE quadrants were both equally near to the empirical trees. However, for gravity as measured by PSV_{D8}, the Pan quadrant was closer to the empirical than DE (TABLE 2.1). To explore intra-quadrant differences, I plotted the distances of each 0.1x0.1 grid cell in σ-ε space to empirical trees for Colless, Sackin and PSV_{D8} metrics (FIGURE S2.13). Visually, this shows that simulated trees with extremely low values of σ and mid or negative levels of ε produce trees most similar to empirical trees in terms of imbalance and gravity. With a PCA of Sackin, Colless and PSV_{D8} for the simulated and empirical trees, I found the grid cells closest to empirical trees mostly fell within the Pan quadrant (FIGURE 2.3C).

This was further corroborated by a PCA of empirical trees and the simulated trees of each extreme scenario, which shows Pan to be closer to empirical trees in PC space than both of the other -σ scenarios Hyd and DE (FIGURE 2.6). However, the level of variance in empirical tree shape was not matched by any of the simulated scenarios, particularly in gravity metrics (see TABLE 2.1). This higher variance in gravity could be

the result of a greater diversity of birth-death rates; repeating the PCA above with empirical trees partitioned into $\gamma > 0$ and $\gamma < 0$ showed that empirical trees which are likely to have experienced reduced or negative diversification rates are closer in PC-space to DE and Hyd scenarios (FIGURE S2.14). Additionally, I found a strong difference between the trees sourced from the literature, which had lower gravity compared to all simulated trees, than those originating from TreeBase (FIGURE S2.15).

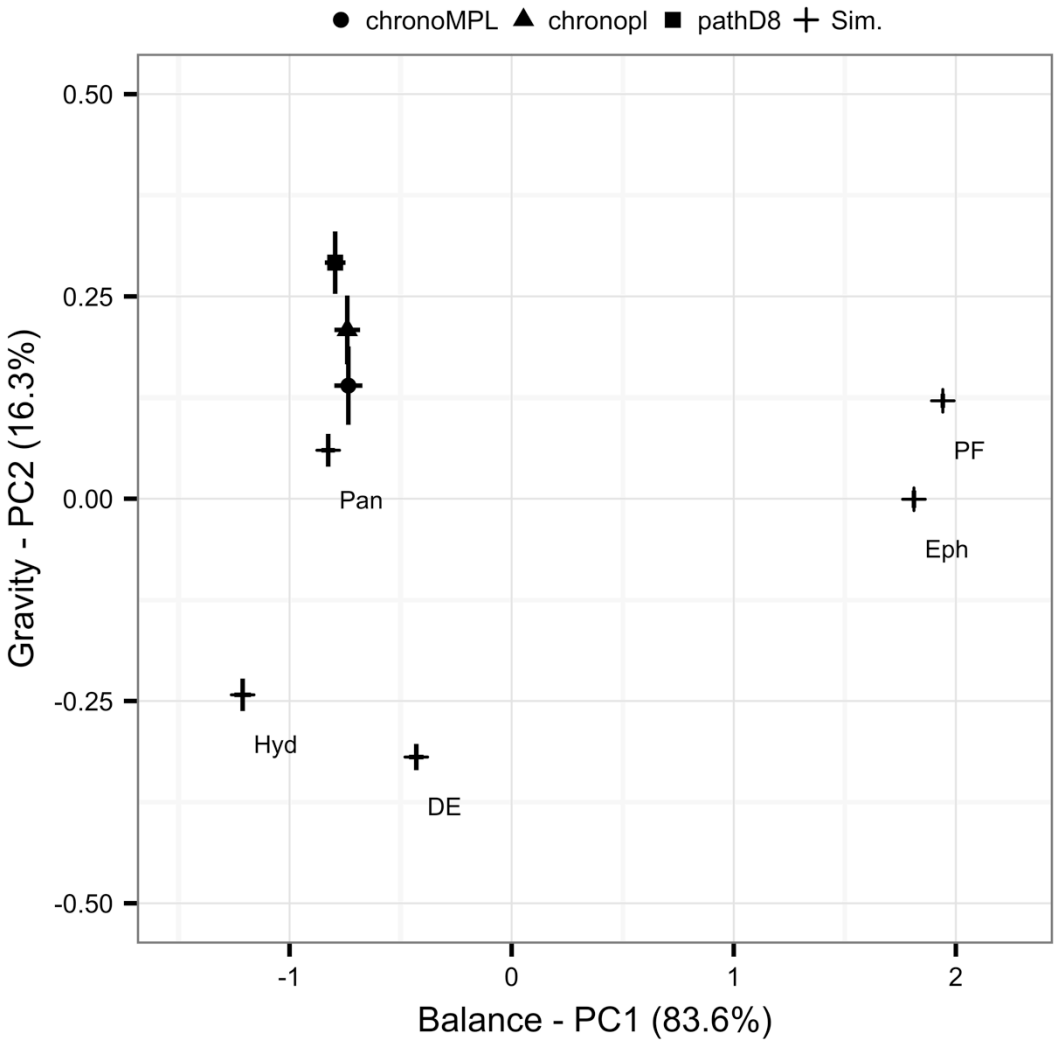


FIGURE 2.6 SIMULATED AND EMPIRICAL TREES IN TREE SPACE

PCA of trees simulated using the extreme scenarios and empirical trees using Yule-normalised Colless and Sackin and PSV. Error bars are standard errors. The three points for empirical trees represent the trees produced using different rate-smoothing methods. PC1 correlates with Sackin and Colless and PC2 correlates most with PSV, making the two PCs equivalent to imbalance and gravity dimensions of tree shape.

DISCUSSION

My phenomenological model of tree growth uses a tip's evolutionary distinctness value to bias speciation and extinction rates in order to test the reality of the LF. With this model, I confirm the results of previous studies where younger or more diverse clades have higher rates of net speciation (Purvis et al. 2011; Hagen et al. 2015; Rabosky and Goldberg 2015) by showing that scenarios where ED species have increased rates of speciation lead to trees with greater phylogenetic balance than in empirical trees. Scenarios where ED species have reduced rates of speciation (DE, Hyd and Pan) all produced levels of imbalance similar to empirical trees. Of these three scenarios, however, I found that the panchronic scenario (Pan), where speciation and extinction rates were reduced for ED species, produced trees closest in both gravity and imbalance to empirical trees. By eliminating the Hyd hypothesis I therefore show that the 'living fossil' label represents a true grouping and, by eliminating the DE hypothesis, that it does not merely represent 'evolutionary relicts' of once-large radiations that are soon to go extinct (Nagalingum et al. 2011). Instead, ED species are generally the product of the same macroevolutionary phenomenon and can therefore be grouped as a "real category" of biodiversity, and are able to persist without speciating or going extinct for long periods of time.

Potential Sources of Error

Although this approach was only able to work with a purely phylogenetic interpretation of the LF based on ED, I argue that these results reveal this proxy more closely recalls the original LF concept proposed by Darwin (1859) as a "window into the past" than more recent concepts of LFs as evolutionary dead-ends.

The results differentiate between the fit of each of my three main hypotheses (Pan, Hyd and DE) to the shape of the tree of life in terms of phylogenetic gravity. While these results might theoretically be contingent on methodological particulars (metrics of gravity

and rate-smoothing methods employed, and empirical tree sampling), I have good reasons to infer that this is not the case. Firstly, although PSV has never been used in this capacity before, it is still a useful measure of the shape of a tree, as it indicates whether the tree is more like a star or a line and is therefore equivalent to a metric of gravity. In supplementary analysis where I simulated trees with varying birth and death parameters, I demonstrated that PSV, although still strongly correlating with the birth parameter, also correlates more with ε than γ does. For this reason, I favoured PSV over γ for comparisons of gravity because the differences in γ between and among empirical trees were much greater than that produced by my simulations, obscuring any signal. Secondly, several rate-smoothing methods were employed, and all produced similar results. Additionally, I recovered the same tendency for empirical trees to have high levels of gravity ($\gamma > 0$ and PSV ~ 0.5) in trees that did not require rate-smoothing (i.e. were already ultrametric). I did, however, find significant differences between trees sourced from the literature and those from TreeBase. I was unable to break up the empirical trees based on the methods used to create them due to the lack of readily available data. Doing this, I may have found further sources of bias (e.g. taxonomic differences, tree estimation methods) that can impact tree shape, and therefore concede that this may be a weakness in my estimation of mean gravity of empirical trees. Thirdly, the higher levels in gravity for empirical trees might reflect a bias in the sourced data towards groups in the process of radiating. I think it unlikely that this is the case, however, as I sampled from a broad range of taxonomic groups. Furthermore, results indicate that the range of PSV values for radiating and non-radiating clades (i.e. the PSV values for those trees with positive and negative γ) are still greater than those produced by the Hyd or DE scenarios; higher births to deaths alone is unable to generate the higher gravity seen in empirical trees.

The Fossil Record

Although the results indicate that extinction rates show negative age dependence, there is little evidence for this from the fossil record. Most notably, Van Valen's Red Queen hypothesis (Van Valen 1973) was proposed on the discovery that extinction rates appeared to be independent of lineage age for a range of taxonomic groups. Boyajian (1991) corroborated this by showing that extinction rates were independent of lineage age even during periods of mass extinction for fossil marine families. More recently, Ezard et al. (2011) showed that extinction rates were more influenced by environmental factors in foraminiferans, rather than biotic factors such as relative clade size. Such evidence would point to a Hyd scenario of evolution instead of a Pan scenario. However, in some instances extinction rates have been found to demonstrate positive age dependence. Quental and Marshall (2013) demonstrated that an island biogeography model of clade rise and fall could explain the trajectories of recently extinct mammalian orders, a process akin to the DE scenario. Additionally, Wilshire et al. (2014) developed a new method for measuring age dependence in extinction rates and found that foraminiferans all showed positive age dependence.

Given this fossil evidence, how can I claim that extinction rates show negative age dependence as per my results? I would suggest that there are multiple factors determining extinction rates, which are generally independent of lineage age and its correlate, evolutionary distinctness. A Pan scenario, however, can be recreated with only the most evolutionarily distinct lineages, which represent a small subset of total biodiversity, having reduced extinction rates. Indeed, as a Pan model continues to run, the relative skew of evolutionary distinctness continues to grow, making the less evolutionarily distinct tips of the tree have random extinction with respect to one another. Statistically detecting such a non-linear relationship for a small subset of the most evolutionarily distinct species from the fossil record would be difficult. However, by taking a tree-growth modelling approach, I demonstrate how Hyd and DE scenarios are

implausible general models of evolution. Both Hyd and DE scenarios lead to the continual reduction in tree age due to the loss of the most evolutionarily distinct branches – the 'shrinking tree problem.' Only a Pan model of evolution can recreate highly evolutionarily distinct lineages such as horseshoe crabs, ginkgos and tuataras while maintaining tree imbalance. Trees produced with the Hyd and DE scenarios had respectively a third and a half less the age of Pan trees, which would make the persistence of such isolated lineages highly unlikely through geological time. Slowly evolving, species-poor lineages are required to maintain the age of a tree.

Alternative Palaeobiotic Models

In Raup's (1985) terminology, my modelling framework fits into a time-inhomogeneous category. This is because although the global parameters of speciation and extinction remained constant, the relative rates differed between clades as a function of the proportion of evolutionarily distinct species each clade represented. By placing differential rates of diversification on potentially competing clades, my modelling approach is related to the density-dependence logistic models of Raup (1985), Sepkoski (1978, 1979, 1984) and others. Furthermore, different scenarios resemble the simulations of previous modellers. The DE scenario that causes the sharp rise and fall of clades as a result of increasing and decreasing extinction and speciation rates respectively for older clades is most related to the island biogeography model of clade rise and fall of Quental and Marshall (2013). The Hyd scenario that modifies speciation rates and keeps extinction rates constant is akin to the coupled logistic models of directly competing clades (Miller and Sepkoski 1988; Roy 1996; Sepkoski et al. 2000). Despite these similarities, the differences in process from my approach should be highlighted: these models generally attempt to explain taxonomic diversity against time whereas my simulations modelled phylogenetic trees, and I make no explicit assumptions about the roles of biotic or abiotic forces shaping diversity nor attempt to capture potential competition (see review by Voje et al. 2015).

Clade Shape

Gould et al. (1987) found clades tended to be bottom-heavy or 'regressive' for a wide range of taxonomic groups. All of the clades in my simulations were top-heavy, although Pan produced the least top-heavy clades. My results, however, are not directly comparable to those generated from the fossil record. This is due to differences in how a clade is defined between my analyses and in the fossil record. I used a strict monophyletic definition because I was working with phylogenetic trees rather than simulating morphological shifts; those working on the fossil record do not have access to full phylogenetic information and have hence preferred a morphological paraclade/clade definition. In Raup's (1980) terminology I used a phylogeneticist approach, while Gould et al. (1987) used an evolutionary taxonomist approach. I would therefore expect to calculate higher estimates of centre of gravity in my simulated clades with respect to the results of Gould et al. (1987) because the phylogeneticist approach counts all descendants from a node, while the evolutionary taxonomist approach would only count those descendants that are deemed to still be part of the higher clade. Furthermore, the phylogeneticist approach should always lead to top-heavy estimates of gravity due to double counting as a result of the hierarchical nature of a tree: for every diversifying node there is a parent node, yet for every node soon to go extinct there are no child nodes. For these reasons I argue that the clade trajectories of the Pan scenario most closely match those found in the fossil record.

A Turnover Process

It should be stressed, however, that I have not set out to develop a 'general model of evolution'. I set out to determine what biases in diversification with respect to evolutionary distinctness best reproduce observed phylogenetic trees. Factors other than evolutionary distinctness and its correlates may prove to be more important in shaping other macroevolutionary patterns. For example, the high variance in γ in empirical trees – a factor none of my simulations were able to recreate – may reflect the importance of

adaptive radiations (Simpson 1944). Indeed, unexpectedly high levels of imbalance have previously been used to identify adaptive radiations (Purvis et al. 2011). Excluding the signal generated by radiations, however, indicates that turnover – speciation and extinction without net gain or loss in species number – is the secondary key process. Alfaro et al. (2009) found that up to 85% of present-day vertebrate species could be the result of adaptive radiations, with the remaining 15% the result of turnover. Turnover has been noted in the fossil records of many major clades, e.g. Carnivora (Liow and Finarelli 2014). The Pan scenario is essentially a turnover process as tips have the same rate of speciating or going extinct, with these rates being determined by ED values. Turnover alone is able to explain the phenomenon of high values of γ despite trees as a whole not experiencing higher births to deaths (Boettiger and Temple 2012), as typically seen in the fossil record (Alroy et al. 2008). Lineages with high turnover will continually add new nodes towards the tips, while lineages with low turnover will maintain tree age and provide few nodes towards the root.

Conclusions

Although in this chapter I have based the feasibility of 'living fossil' through the use of the ED metric, I do not suggest that evolutionarily distinct species equate to LFs under all possible definitions nor do these results rely on the use of the ED metric; alternative measures of phylogenetic isolation would be likely to produce the same results. By demonstrating that positive feedback in diversification rates is required for producing the observed shape of the tree of life, I show that evolutionary performance is unequally distributed and that 'living fossil' as a label can have more meaning than just the tail end of a normal distribution. It can be used to describe a true grouping of lineages with substantially lower rates of diversification than seen in other parts of the tree of life, rates that are reflective of the hollow curve distribution itself, and representing an emergent feature of a turnover-based model of evolution. The aim was not to determine what the processes are that allow LFs to arise, but to validate or invalidate potential definitions of

the LF. By demonstrating the validity of a panchronic definition, I hope to have opened the door for future research that seeks to determine what these processes may be.

3.

Testing the Living Fossil with the Fossil Record

INTRODUCTION

In the first line of Anna Karenina (1877), Tolstoy asserts that happy families are happy for much the same reason, while unhappy families are unhappy each in their own way. This 'Karenina principle' can be alternatively phrased to consider the evolutionarily distinct: Are all evolutionarily distinct species distinct for much the same reasons, or is each evolutionarily distinct species distinct in its own unique way? In the last chapter, I demonstrated how we might consider evolutionary distinct species to be happy families as they are by-and-large generated by a single scenario, the panchronic. Answering the Karenina question was not the main aim of the last chapter and a happy family conclusion depends on just phylogenetic data of the Recent. Instead, in this chapter I use fossil data to more explicitly ask whether evolutionary distinct species are happy families or not.

For evolutionary distinct species to be 'happy families' they must originate through a shared process. There are two commonly held models for this process: an undying lineage that remains at low species number for extended geological time ('panchronic') or the surviving remnants of a once diverse clade ('evolutionary dead-end'). For example, obligate river dolphins are relatively evolutionary distinct and are thought to have persisted for long periods of time (Mooers et al., 2009). Sloths (Folivora), on the other hand, are evolutionary distinct today but had much greater diversity in the recent past (Kurten and Anderson, 1980; Mooers et al., 2009). The evolutionary distinctness of river dolphins is therefore closer to a panchronic scenario while the evolutionary distinctness of sloths is closer to an evolutionary-relict scenario. The 'river dolphin' model evokes the initial concept of the LF as proposed by Darwin (1859) and the concept of tachytely proposed by Simpson (1944). The 'sloth' model fits better with more recent concepts of the LF, such as that of Nagalingum (2011), or the popular conception of the living-fossil status of coelacanths, which were diverse in the deep past

(Friedman and Coates, 2006). These two models are not strictly mutually exclusive, but are extreme ends of a continuum (FIGURE 3.1).

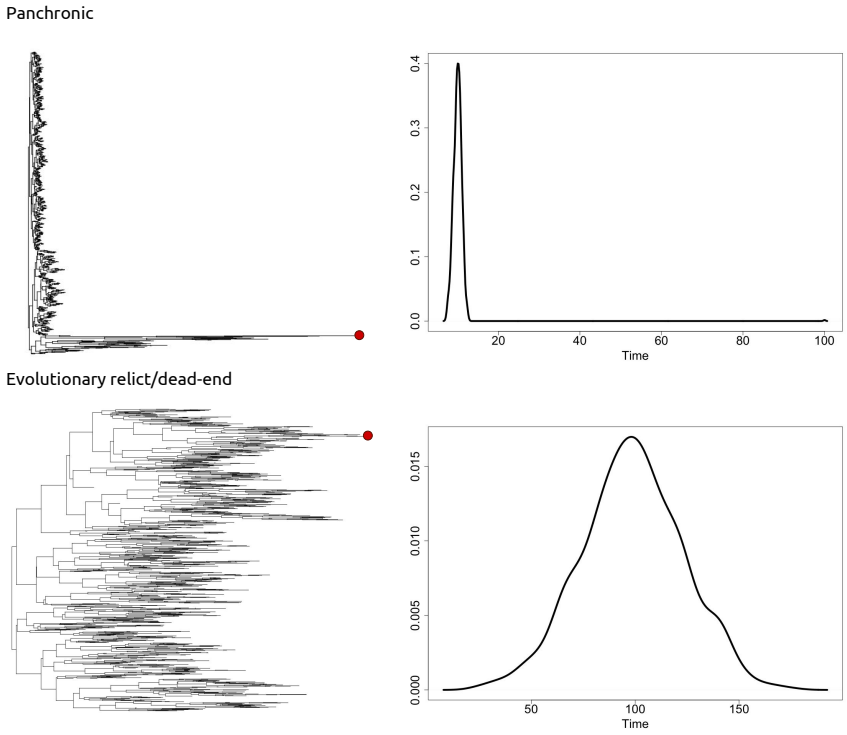


FIGURE 3.1 PANCHRONIC AND DEAD-END EXTREMES

Two opposing extremes for the origin of evolutionarily distinct species represented by phylogenetic tree (left) and clade diversity through time (right). Top, a lineage or species that may have been rich in the deep past but has for extended periods of time persisted without diversifying or going extinct; 'panchronic'. Bottom, a species that is distinct today as a survivor of a once diverse clade.

If evolutionary distinct species are more generally the result of the river dolphins model, then we should expect them to have a lower rates or risk of extinction than if they were the result of the sloths model. Evidence for low rates and risk of extinction for evolutionary distinct species is mixed. For example, it is well recognised that species at risk of extinction today are non-randomly distributed across the tree of life (Nee and May, 1997; von Euler, 2001; Purvis et al. 2000). If species at risk of extinction were to be lost, more evolutionary history will be lost than if the same number of species were lost at random. This is because at risk species tend to be more related – in which case whole clades are lost – or they tend to be members of smaller clades – in which case they are more evolutionary distinct (Nee and May, 1997). Equally, Mooers et al. (2009) found a

similar pattern in Holocene extinctions. Despite these findings of non-random extinction, Verde Arregoitia et al. (2013) found no relationship between the evolutionary distinctness of a species and its risk of extinction. Furthermore, it should be highlighted the above studies are based on recent rates and risks of extinction, yet we know that species and clades at risk of extinction can differ strongly between 'normal' times and mass extinctions (Jablonski, 1995).

Understanding the origins of the evolutionary distinct is important, as it may also provide insight into their 'evolutionary potential': are these species more likely to diversify, become extinct or simply persist? This is a question of great significance to conservation biologists (Krajewski, 1991; Winter et al. 2013) who do not wish to invest resources into species that are 'doomed'. History, however, does not always guide future. Cooper and Fortey (1999) argued that many large radiations in the past have their origins in species-poor clades, a process they called the 'phylogenetic fuse'. Understanding the nature of evolutionary distinct species through the two models above is therefore insufficient. A better way of assessing the nature of evolutionary distinct species is to test for a relationship between past evolutionary distinctness and future evolutionary distinctness. This can be achieved by assessing the relationship between the ED of clades at a given time point (ED_{t0}) and their subsequent EDs in a following time point (ED_{t1}). Once controlling for differences in the ages of trees between different time points and the number of species in a tree, we should expect a linear relationship between the ED values of the two time points if ED does not influence diversification; we should expect species to have on average the same ED value in the next time step. A linear relationship would be akin to an 'unhappy family' explanation for evolutionary distinctness: these species are the result of different processes. If, on the other hand, there are any differences in the future evolutionary potential of species of different evolutionary distinctnesses, we should expect a divergence from this linear relationship. For example, under a strict panchronic scenario we would expect that low ED species are more likely to speciate; at

the next time point they should have, on average, a lower ED value. High ED species would be less likely to speciate and as such should represent a disproportionately greater share of the phylogenetic tree in the next time step. The mean of their ED values should then be greater than expected under random speciation and extinction. Under an evolutionary relict scenario, mid-level ED species would become increasingly more evolutionarily distinct up to the point at which they become extinct. These species should then show increasingly higher than expected ED values in the next time point, while high ED values should tend towards expectation (i.e. to no change). The advantage of approaching the question in this manner allows us to re-visualise these two scenarios but also generate more complex multi-hypothesis scenarios (see FIGURE 3.2)

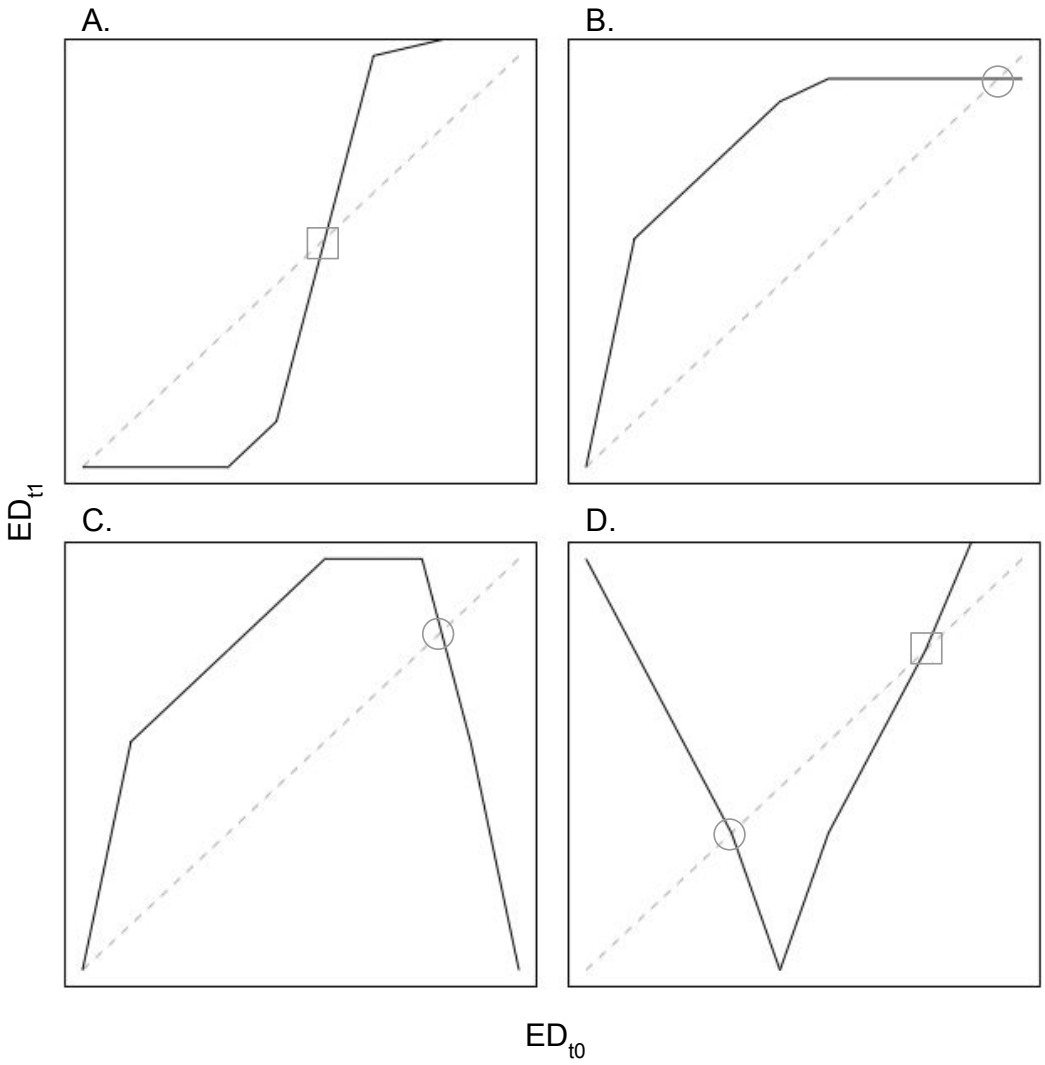


FIGURE 3.2 RICKER CURVE PREDICTIONS

Four Ricker Curve scenarios for the general relationship between ED at t0 and t1. A. Low values of ED leads to lower values in the next time frame, high values of ED lead to even higher values in the next time frame. This scenario is equivalent to the 'panchronic' hypothesis. B. Low to mid values of ED lead to increasing ED up to a maximum value. This scenario is equivalent to the 'evolutionary relict' or 'dead-end' scenario. C. Low ED values lead to higher ED values, high ED values lead to low ones. This is equivalent to a 'phylogenetic fuse' scenario. D. Low to mid values lead to low to mid values, very low or very high values lead to very high ED values. This scenario is a combination of a 'panchronic' and an 'evolutionary relict' scenario. Stable points – where ED values of species converge – are indicated with circles. Unstable points – which mark threshold points – are indicated with squares.

In this chapter, I compare the ED values between mammalian clades at different time points to investigate how evolutionary distinctness changes through time. I use mammals due to their well-established phylogenetic tree (Bininda-Edmonds et al. 2007) and the

availability of a relatively well-sampled fossil data (Liow et al. 2008). I generate a large molecular-fossil inframetric tree by taking a large mammalian supertree and adding fossil tips using a taxonomically constrained stochastic process. I then use this large tree to take time slices at comparable time points in the tree. I track the evolutionary distinctness of identifiable clades/species across these time points to generate an ED_{t0} and ED_{t1} dataset. With this dataset I then test whether the $ED_{t1} \sim ED_{t0}$ relationship is non-linear and whether it corresponds to any of the suggested scenarios (FIGURE 3.2).

METHODS AND MATERIALS

Data Acquisition

Mammalian fossil records were downloaded from the Paleobiological Database (PBDB) (paleobioDB, 2017) using the R package paleobioDB (Valera et al. 2015) on 30/01/2017. All records were constrained to 'Mammalia'. In total 104,071 fossil occurrence records were downloaded. These records were converted to species records by merging any record with shared species names through selecting the minimum and maximum age extremes in order to determine the maximum possible temporal extent. Taxonomic lineages for every species record were determined and any record without lineage information below the family level was dropped. Finally, any records with a temporal extent greater than the age of the mammalian supertree (166.2 MY) were also dropped. 123 species records were older than the mammalian tree and 427 species records had too few lineage ranks. In total 18,097 species records were assembled representing 130 families and 6,396 genera, and a temporal distribution skewed towards the recent (0.0059 – 0%, 7.0 – 25%, 17.3 – 50%, 39.7 – 75%, 164.8 – 100%).

The fossil species were added to a time-calibrated ultrametric phylogenetic supertree of 4,510 extant mammal species (Bininda-Edmonds et al. 2007). Taxonomic information was added to the tree by identifying the most likely taxonomic group for every node by matching descendants to named entries in the NCBI taxonomic database (Federhen, 2012) via the Global Names Resolver (Global Names Architecture,2015). For every node, the lowest shared taxonomic group was selected from all lineages of matching named entries. Taxonomic groups were identified for 6,603 of the 6,618 nodes in the tree (>99%).

Stochastic Fossil Adding

Tips representing fossil species were added to the molecular phylogeny using the `pinTips`() function of the `treeman` R package (Bennett et al. 2017). For every fossil to be added, the lineage was matched to the lineages present in the tree. The points at which to add a fossil was determined as any edge directly parent to or descending from the node(s) with the lowest ranked matching taxonomic label. Additionally, any edge or part of an edge that was younger than the minimum age range of the fossil was excluded. Depending on the number of ranks in the matched lineages and the number of edges under the lowest matched rank, there may be a range of equally likely edges to which the fossil tip can attach. Where this was the case, the fossil tip was added randomly. The age at which the fossil becomes extinct is determined as a random point within its estimated age range after splitting from the tree (see FIGURE 3.3 for outline of the process, see FIGURE S3.1 for example results). In this way, fossils added to the tree will all become extinct within the estimated age range, and their appearance would either occur before or during their estimated age range.

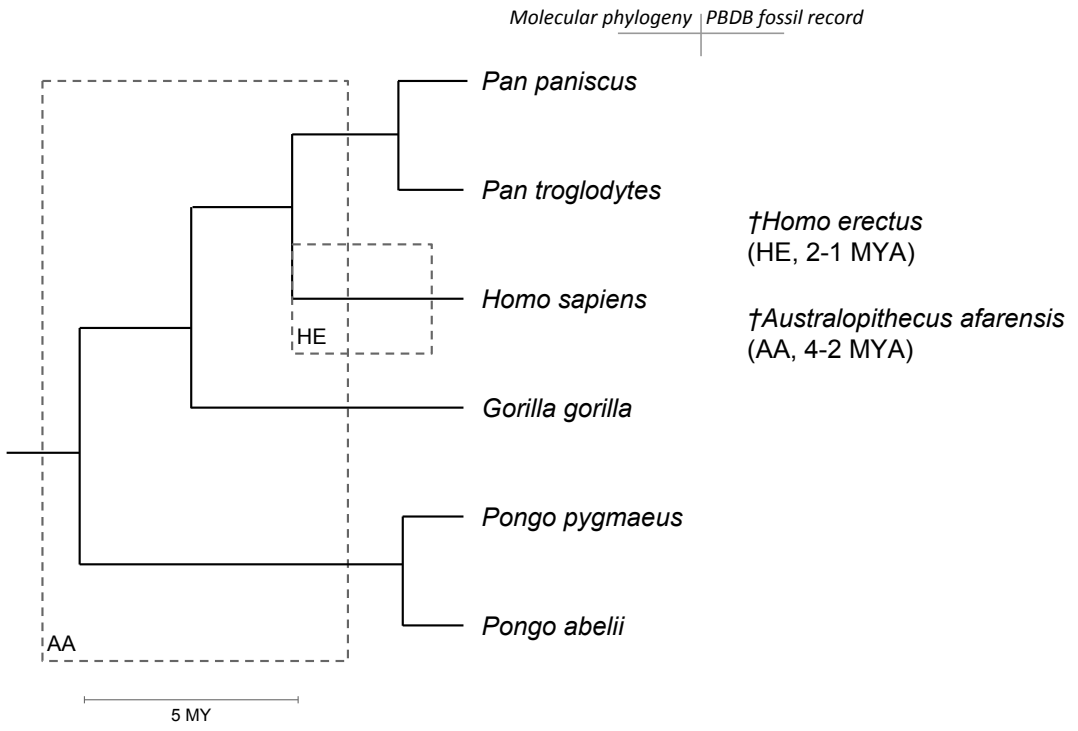


FIGURE 3.3 STOCHASTIC FOSSIL ADDING

Fossil placement using `treeman`'s `pinTips()`. This example uses a phylogenetic tree of extant apes, and two fossil ape species. Comparing the taxonomy of the tree and the fossil records we can identify branches the fossils could potentially branch from; these windows of placement are then limited by the expected occurrence of the fossil. In the case of *Homo erectus*, its genus is *Homo* and there is only one extant member of the Homo genus in the tree. This means its branch can be placed anywhere along this branch within its 1-2 MY time frame, hatched box 'HE'. The taxonomy for *Australopithecus*, however, is broader (Australopithecus, Hominoidea, Primates …), and all tips of the tree share the lowest matching rank (Hominoidea or Apes). Given this imprecision of the taxonomy, *Australopithecus afarensis* has a much wider range of possible branches to which it can attach, hatched box 'AA'. Although we know *Australopithecus afarensis* should occur along the branch towards modern humans, the PBDB taxonomy does not provide this information. As such, the fossil-adding process will not provide accurate estimates of changes in evolutionary distinctness towards the tips. Estimates, however, of deeper nodes should be more accurate. For example, *Australopithecus afarensis* will always be placed within the Hominoidea, and its lineage evolutionary distinctness estimate will be less impacted by uncertainty of *Australopithecis afarensis* placement below the family level.

To account for the range of possible random outcomes, the stochastic fossil-pinning process was iterated 100 times to generate a distribution of possible molecular-based and fossil 'inframetric' trees. 100 times was deemed sufficient as initial analyses demonstrated that trees generated through this process have similar distributions of ED values. Finally, to assess whether the inclusion of fossil data using this stochastic process was providing a detectable signal that was not just random, the fossil-pinning process was repeated with the fossil lineages and age ranges randomly shuffled. In this repeated analysis, the same dataset was used except before iterating, the lineages were randomly shuffled so that for each fossil their lineage did not match with their expected age range. As a result, I generated two sets of 100 hundred trees, *real* and *random*. Before analysing changes in ED between time points, I first tested whether these two sets of trees differed significantly.

Determining Change in ED

For each inframetric tree within the *real* and *random* distributions, a 'slice' was taken to generate an ultrametric tree for a given time point (see FIGURE S3.1.2 for an example slice). For each of these slices, EDs were calculated for all tips and internal nodes using the 'fair proportion' method of Isaac et al. (2007). The ED of internal nodes was calculated as the mean value of the ED of all descendants. In order to generate ultrametric trees that were as equally sampled as possible, time slices were taken at the midpoints of each epoch (Upper Jurassic to Recent) (see TABLE 3.1). ED_{t0} and ED_{t1} datasets were generated from the two distributions by matching ED values from one epoch to the next for all species/clades that spanned two or more epochs. For species/clades that occurred more than once across the tree distributions, 'shared nodes', mean ED values were calculated. To replace the skewed distribution of ED with a more statistically convenient normal distribution, all values were converted to their natural

logarithm before modelling. These datasets of species/clades were supplemented with taxonomic information (genus and order), the number of species present in the tree at t0, and the time (in MY) between t0 and t1.

TABLE 3.1 EPOCH-EPOCH MIDPOINTS

Epoch-to-epoch mid-point estimates taken from the International Chronostratigraphic Chart (2013) used for estimating changes in evolutionary distinctnesses from the inferred molecular-fossil mammalian phylogenetic tree

Period	Code	Span (MYA)			Time
Pleistocene - Recent	Pe-Re	1.30	-	0.00	1.30
Pliocene - Pleistocene	Pi-Pe	3.96	-	1.30	2.66
Miocene - Plicoene	Mi-Pi	14.18	-	3.96	10.22
Oligocene - Miocene	Ol-Mi	28.47	-	14.18	14.28
Eocene - Oligocene	Eo-Ol	44.95	-	28.47	16.49
Paleocene - Eocene	Pa-Eo	61.00	-	44.95	16.05
Cretaceous Upper - Paleocene	CU-Pa	83.25	-	61.00	22.25
Cretaceous Lower - Cretaceous Upper	CL-CU	122.75	-	83.25	39.50
Jurrasic Upper - Cretaceous Lower	JU-CL	154.25	-	122.75	31.50

Modelling

Comparing the Real and the Random

Before modelling the relationship of ED_{t0} and ED_{t1}, I first tested whether the *real* distribution of trees and their ED values differed significantly from the *random* distribution. Initially, I determined whether the estimates of ED of the *real* and *random* had different distributions by comparing the mean and variance of the estimated values using the t-test and F-test, respectively. Secondly, I tested whether ED estimates of shared nodes was greater or smaller for the *real* and *random* distributions. 'Shared nodes' are the nodes which appear multiple times across the iterations. Because fossil placement in the *random* is not informed by taxonomy, shared nodes in the *random* are either due to their existing already within the tree or chance. If taxonomic information informs the

stochastic adding process, we should expect ED estimates of shared nodes across the iterations of the *real* to vary less than those in the *random*. To test this, I calculated the standard deviation of ED values estimated for shared nodes across the iterations, and tested whether the *real* had a lower variance using a t-test.

Mixed Effects Models

For the *real* ED dataset, I then investigated how clade ED in an epoch (ED_{t0}) changed with respect to the ED in the subsequent epoch (ED_{t1}). ED values of epoch-to-epoch transitions are however not comparable. For example, the ED values of t0 to t1 in one epoch transition will not necessarily be independent of the t0 to t1 in another epoch transition because many of same species/clades are present across epoch-to-epoch transitions. Additionally, each epoch transition is different because they each have different starting number of tips in the t0 tree, the t0 trees have different ages and the time that separates them differs (TABLE 3.1). Both age and number of tips affect the ED of species in a tree. One solution would be to model each of the epoch transitions separately, this, however, would not allow the modelling of the overall relationship – independent of time – of how past ED impacts future ED. As such, I used linear mixed-effects models (LMEMs, Fisher, 1919; Bates et al. 2015) to control for differences between epochs. LMEMs are an extension of generalised linear models but can also take into account non-independence by estimating parameters of the 'fixed effects' for subsets of the data determined by a 'random-effects structure'. In this case, the random-effects structure is determined by the epoch transitions. Model formulae can be used to specify independent intercepts for epoch transitions (e.g. *t1~t0+(1|epoch)*, where *t1* and *t0* are fixed effects indicating ED_{t1} and ED_{t0}) or independent slopes, e.g. *t1~t0+(t0|epoch)*. In addition to controlling for epoch transitions, I also controlled for non-independence of taxonomic groups as we expect closely related species to have similar evolutionary distinctnesses, e.g. rodents have low values while afrotherians have high values. The

additional taxonomic information can be specified in the random effects structure by sub-setting by orders, e.g. *t1~t0+(1|epoch)+(1|order)*, or species/clade IDs, e.g. *t1~t0+(1|epoch)+(1|id)*. The random effects structure can also allow hierarchical categories with which taxonomic ranks can be specified, e.g. *t1~t0+(1|epoch)+(1|order/genus/id)*. For each extra level in the hierarchy, however, there is a non-linear gain in computational time, limiting the maximum number of levels that can be run.

With LMEMS, I used a model simplification approach where I started with a basic model and added additional terms to the random effect structures until there was no longer any significant gain in the explained variance. I compared models using ANOVA and the Akaike Information Criterion (AIC, Akaike, 1974). AIC is a measure of a model's likelihood weighted by the number of estimated parameters, the lower its value the better the model fit.

The Three Models

With this modelling framework I tested how past ED affects future ED. I used the approach to generate three models: a linear observed (*obs1*), a non-linear observed (*obs2*) and a linear expected (*exp*). I generated *obs1* using the modelling framework as described above to generate the best fitting linear model of ED_{t1}~ED_{t0} given different random-effects structures. I then took the same random-effect structure to generate *obs2* using orthogonal polynomials of different degrees.

In the event that the relationship between ED_{t1} and ED_{t0} is non-linear, a linear expected (*exp*) model was built for comparison with the observed. This model was a function of ED_{t1} and the set of factors that influence ED between epochs: the number of tips in the tree at t0 (*n*), the time difference between epochs (*tm*) and an ED_{t0} dummy variable (*t0_dummy*). The dummy variable was a linear representation of ED_{t0} calculated by rounding ED_{t0} to the nearest integer and dividing values by the maximum in order to

generate values between 0 and 1.

With these models I first tested whether ED_{t1}~ED_{t0} is non-linear by comparing the goodness of fit of *obs1* and *obs2*. Secondly I tested which scenario (see FIGURE 3.2) best describes ED_{t1}~ED_{t0} by visually comparing the best observed (*obs1* or *obs2*) to *exp*.

All of the methods were performed in the R environment (v3.2) and the scripts for reproducing these results can be found on the author's GitHub account (Bennett, 2017a).

RESULTS

Comparing the *Real* and the *Random*

The *real* dataset consisted of 129,089 species/clade ED values recorded across the nine epoch-to-epoch transitions. The distribution of shared nodes across the iterated trees was bimodal with the majority of species/clades occurring 10 or fewer times (1 - 0%, 3 - 25%, 10 - 50%, 26 - 75%, 100 - 100%). The *random* dataset was much bigger (225,151) and the distribution of shared nodes was more even (1 - 0%, 5 - 25%, 18 - 50%, 38 - 75%, 100 - 100%). Both the *real* and *random* distributions showed positive non-linear relationships between ED_{t0} and ED_{t1} and differences between the epoch-to-epoch transitions were great, particularly at low ED values. Two epoch-to-epoch transitions (JU-CL, CL-CU) showed a different relationship from the others for both the *random* and *real* (FIGURES 3.4-5). This difference is likely due to fewer data points for these epochs (7,825 and 3,209 for CL-CU and JU-CL respectively versus a mean 16,865 for all other transitions in the *real*) and the much longer time separating them (39.5 and 31.5 MY for CL-CU and JU-CL respectively versus a mean 11.9 for all other transitions). I removed JU-CL and CL-CU transitions from all subsequent analysis.

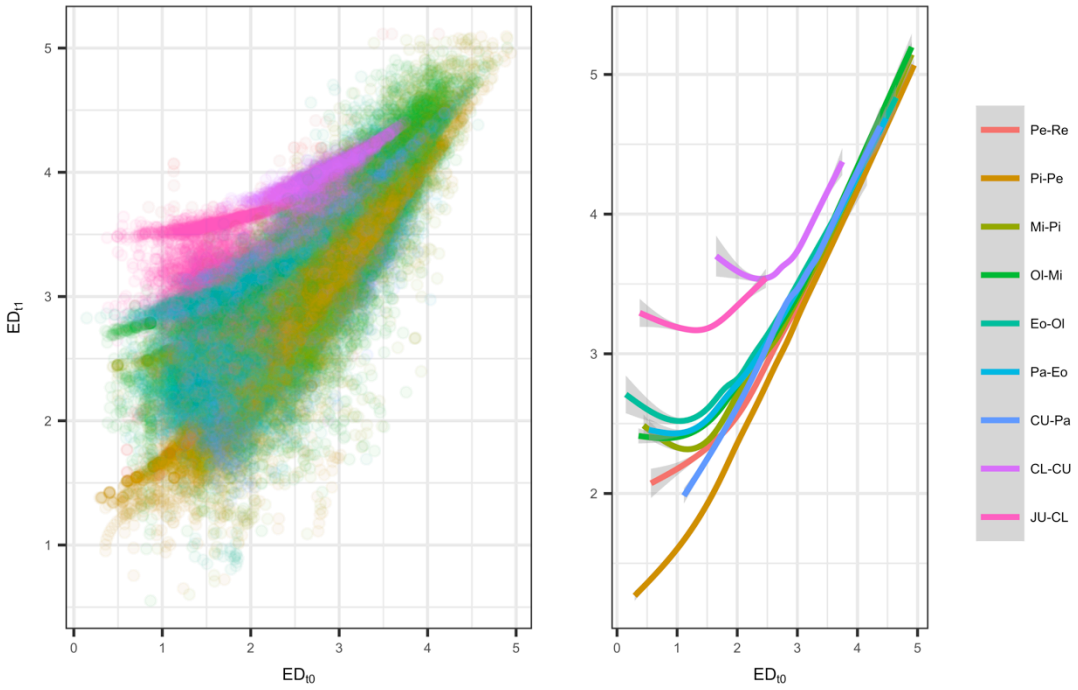


FIGURE 3.4 ED$_{t1}$~ED$_{t0}$ FROM THE REAL DATASET

ED values of clades in an epoch (ED$_{t0}$) against the following epoch (ED$_{t1}$) for estimates generated from the real distribution of inframetric trees. Left, points of all clade/species coloured by specific epoch-epoch transition. Right, for visual purposes, estimated General Additive Models by epoch.

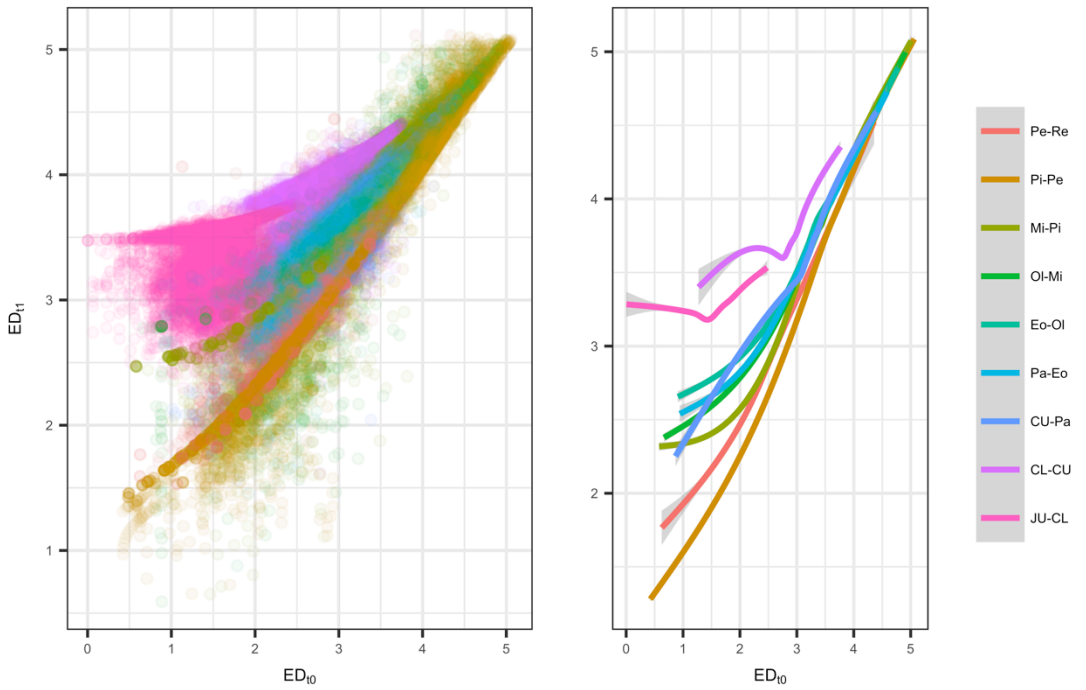


FIGURE 3.5 ED$_{t1}$~ED$_{t0}$ FROM THE RANDOM DATASET

ED values of clades in an epoch (ED$_{t0}$) against the following epoch (ED$_{t1}$) for estimates generated from the random distribution of inframetric trees. Left, points of all clade/species coloured by specific epoch-epoch transition. Right, for visual purposes, estimated General Additive Models by epoch.

The estimated ED values of t1 and t0 (calculated as ED$_{t0}$-ED$_{t1}$) for all unique nodes across all iterations differed significantly between the *real* and *random*. The mean ED for the *real* was lower (-0.56) than that of the *random* (-0.42) (t-test, $t = 128.43$, $p < 0.001$). The spread also differed significantly. The variance of the *real* distribution (1.226) was significantly greater than the *random* (0.033) (F-test, $F= 0.2676$,variance ratio = 0.268, $p < 0.001$). Additionally, the standard deviation of the mean ED for shared nodes across the iterations was much greater for the *random* (0.484) than the *real* (0.360) (t-test, $t=185.41$, $p < 0.001$). (See FIGURE 3.6.)

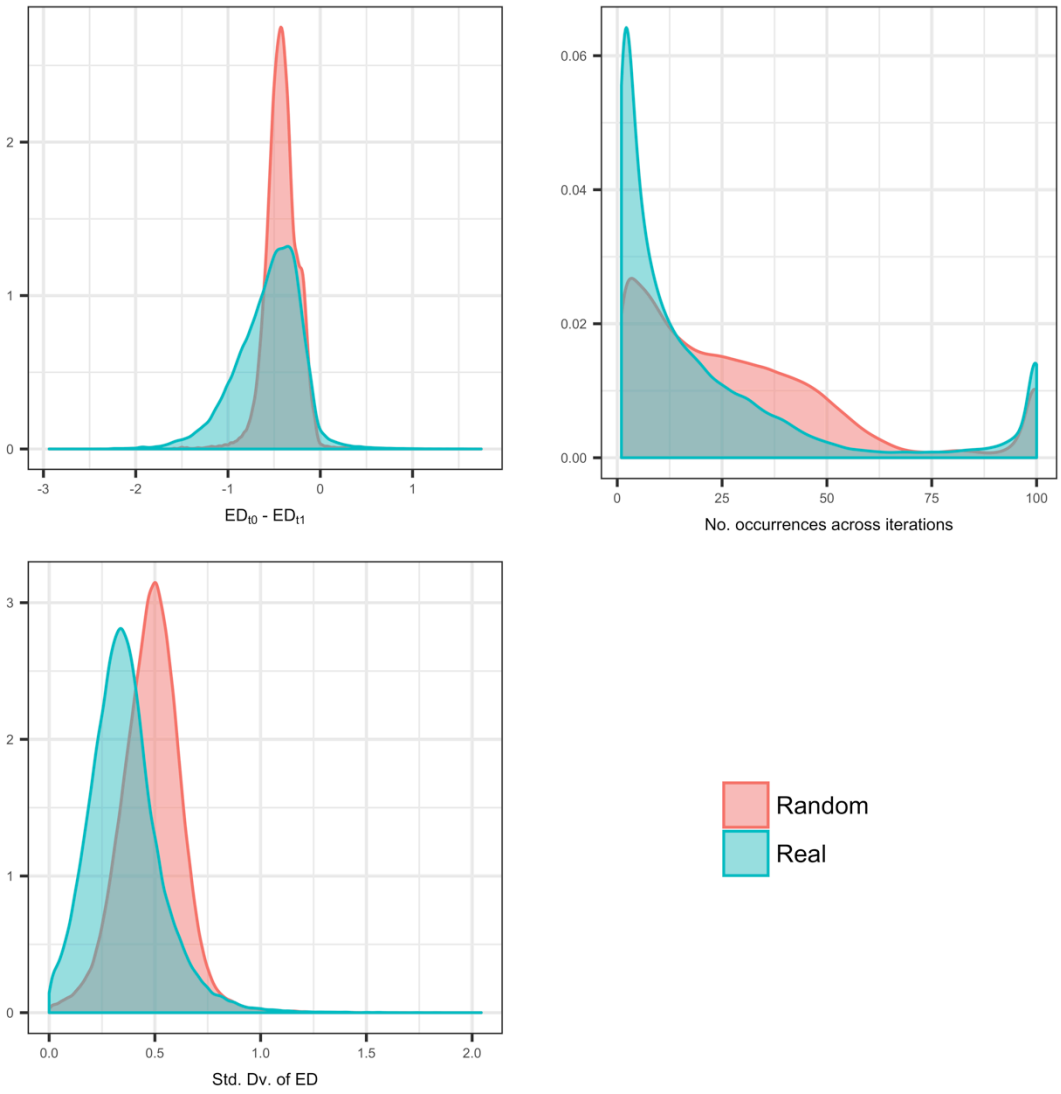


FIGURE 3.6 DIFFERENCES BETWEEN REAL AND RANDOM

Comparing the real and random tree distributions. Top-left, the *real* inframetric tree distribution has a greater range of mean ED values calculated across iterations than the *random* distribution. Top-right, proportion of times each node is shared across iterations for both *real* and *random*. Bottom-left, the real distribution shows less variance of ED (ED_{t0}-ED_{t1}) for identifiable clades across the distribution than does the random.

Estimating the Linear Model

I determined the best linear model of ED_{t1}~ED_{t0} to be *m2g* (TABLE 3.1). This model incorporated a random-effects structure that consisted of independent slopes for both epochs and genera. Although I found a marginal gain in terms of explained variance

using a hierarchical taxonomic random effect (order/genus) with random intercepts, the computation of such a model with independent slopes was not achievable in reasonable time. Furthermore, I did not deem model choice to be crucial in interpretation of results as estimated slopes across models were similar (0.65-0.70).

TABLE 3.2 LINEAR MODELS OF ED_{t1}~ED_{t0}

Formulae (Formula), intercepts (Int), slopes (Slp), degrees of freedom (DF), Akaike Information Criterion (AIC), and significance (P) are indicated. P-values were calculated by performing an ANOVA χ^2 test of the current row's model to the last significant model (*** p < 0.001, ** p < 0.01, * p < 0.05). Each new row features a model with greater complexity than the last row. The selected linear model (*m2g*) for comparison with polynomial models is highlighted in bold.

Id.	Formula	Int	Slp	DF	AIC	P
m0	t1~t0	1.4323	0.6509	1	42,953	
m1a	t1~t0+(1|epoch)	1.3462	0.6872	4	28,690	
m1b	t1~t0+(t0|epoch)	1.2938	0.7017	6	24,594	***
m2a	t1~t0+(t0|epoch)+(1|order)	1.3005	0.6983	7	23,407	***
m2b	t1~t0+(t0|epoch)+(1|genus)	1.409	0.6639	7	18,810	***
m2c	t1~t0+(t0|epoch)+(1|order/genus)	1.4116	0.6622	8	18,402	***
m2d	t1~t0+(t0|epoch)+(1|id)	1.3806	0.6736	7	19,794	
m2e	t1~t0+(t0|epoch)+(1|order/id)	1.4001	0.6656	8	18,502	
m2f	t1~t0+(t0|epoch)+(t0|order)	1.3344	0.6873	9	23,035	
m2g	**t1~t0+(t0|epoch)+(t0|genus)**	1.3919	0.6683	9	**16,862**	***
m2h	t1~t0+(t0|epoch)+(t0|id)	1.3757	0.6751	9	18,374	

Estimating the Non-Linear Model

I compared the best linear model (*m2g*) with a range of non-linear models, using the same random effects structure, based on orthogonal polynomials generated from ED_{t0} for different exponents. Using ANOVA and AIC values, I found all the polynomial models to have significantly better fits than the linear model, indicating that ED species do have differences in diversification potential (TABLE 3.2). Of the non-linear models, I determined *m3c*, the 4th order polynomial model, to be the best. The model had one of the lowest AICs and showed goodness of fit (FIGURE 3.7). Although there was a minor improvement in fit with a 5th order polynomial, this was not sufficiently great to warrant the greater complexity of model. The AIC decreased by only two and, upon visual

inspection, the models' predicted values produced the same relationship, indicating that any conclusions made would be the same.

TABLE 3.3 NON-LINEAR MODELS OF ED_{t1}~ED_{t0}

Formulae (Formula), order of polynomial (Poly), degrees of freedom (DF), Akaike Information Criterion (AIC), and significance (P) are indicated. P-values were calculated by performing an ANOVA χ^2 test of the current row's model to the last significant model (*** p < 0.001, ** p < 0.01, * p < 0.05). Each new row features a model with greater complexity than the last row. The best polynomial model (*m3c*) is highlighted in bold.

Id.	Poly	DF	AIC	P
m2g	1	9	16,862	
m3a	2	10	15,322	***
m3b	3	11	15,152	***
m3c	**4**	**12**	**15,108**	***
m3d	5	13	15,106	*
m3e	6	14	15,108	

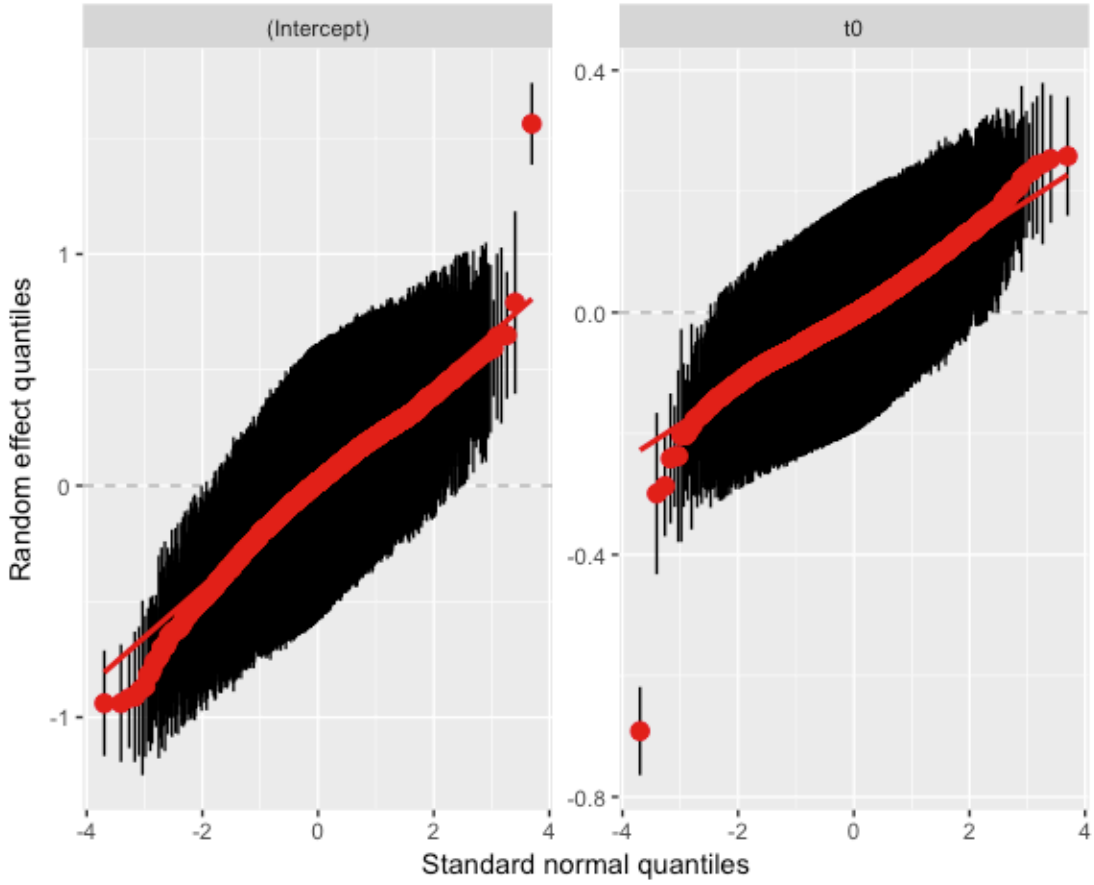


FIGURE 3.7 QQ-PLOTS OF BEST OBSERVED MODEL

Quantile-quantile plots of the random effects for best fitting model of ED_{t1}~ED_{t0}, *m3c*. Adherence of the points to the straight line indicates good fit of the model.

Estimating the Linear Expected Model

There was significant improvement in the expected linear model candidates when incorporating the ED_{t0} dummy variable (*t0_dummy*), difference in time between epochs (*tm*) and the starting number of species in a tree (*n*) (TABLE 3.3). I found the best-expected linear model to be *n1g*, a model that incorporates all three of these fixed effects as well as a genus random effect with random slopes for *t0_dummy* and *tm*. Although it is likely that a model with random slopes for all three fixed effects would have been a better fit, this model was not able to converge. As with the linear model, I found marginal improvement in the model with the use of a hierarchical taxonomic random effect structure (order/genus); however, this again was not computable in reasonable time.

TABLE 3.4 EXPECTED LINEAR MODELS OF ED_{t1}

Expected linear models of ED_{t1} against difference of time between epochs (*tm*), number of species in the tree at t0 (*n*) and an ED_{t0} dummy variable generated from the rounded figures of ED_{t0}. Formulae (Formula), degrees of freedom (DF), Akaike Information Criterion (AIC), and significance (P) are indicated. P-values were calculated by performing an ANOVA χ^2 test of the current row's model to the last significant model (*** p < 0.001, ** p < 0.01, * p < 0.05). The '-' indicates the separation of linear and linear-mixed-effects models. The selected expected model (*n1g*) for plotting with the observed polynomial model is highlighted in bold. This model was selected over *n1f*, which had a lower AIC, because *n1f* failed to converge.

Id.	Formula	DF	AIC	P
n0a	t1~tm	3	118,470	
n0b	t1~n	3	118,307	
n0c	t1~tm+n	4	118,133	***
n0d	t1~t0_dummy	3	64,344	
n0e	t1~t0_dummy+tm+n	5	56,295	***
n1a	t1~t0_dummy+tm+n+(1|order)	6	54,639	-
n1b	t1~t0_dummy+tm+n+(1|genus)	6	47,744	***
n1c	t1~t0_dummy+tm+n+(1|id)	6	52,221	
n1d	t1~t0_dummy+tm+n+(t0_dummy|genus)	8	46,270	***
n1e	t1~t0_dummy+tm+n+(tm|genus)	8	45,926	
n1f *x*	t1~t0_dummy+tm+n+(n|genus)	8	44,351	
n1g	**t1~t0_dummy+tm+n+(t0_dummy|genus)+(tm|genus)**	11	**44,742**	***
n1h	t1~t0_dummy+tm+n+(1|order/genus)	7	47,199	

Comparing the Expected and the Observed

I compared the expected model (*exp*, *n1g*) with the best-observed model (*obs2*, *m3c*) by plotting the parameterised lines to create an analogous figure to FIGURE 3.2. Both models, however, use random-effects structure based on epoch transitions and genera. Plotting the outputs of these models using the whole dataset would lead to a figure containing 6,396 x 7 x 2 individual lines – making visual interpretation of trend difficult. As such I generated predicted values from a representative dataset including one hundred different genera for all epoch transitions. I plotted these predicted values by taking the median across the different epoch transitions and genera (FIGURE 3.8). I found that, on average, high ED_{t0} values (> 20 MY) tend to lead to higher than expected ED_{t1} values, while low-to-mid ED_{t0} values (2.7-20 MY) tend to lead to lower than expected ED_{t0}

values; a pattern consistent with the 'panchronic' scenario. For very low ED_{t0} values (< 2.7 MY), however, ED_{t1} is much greater than expected. This pattern was consistent across the different epochs (see FIGURE 3.9).

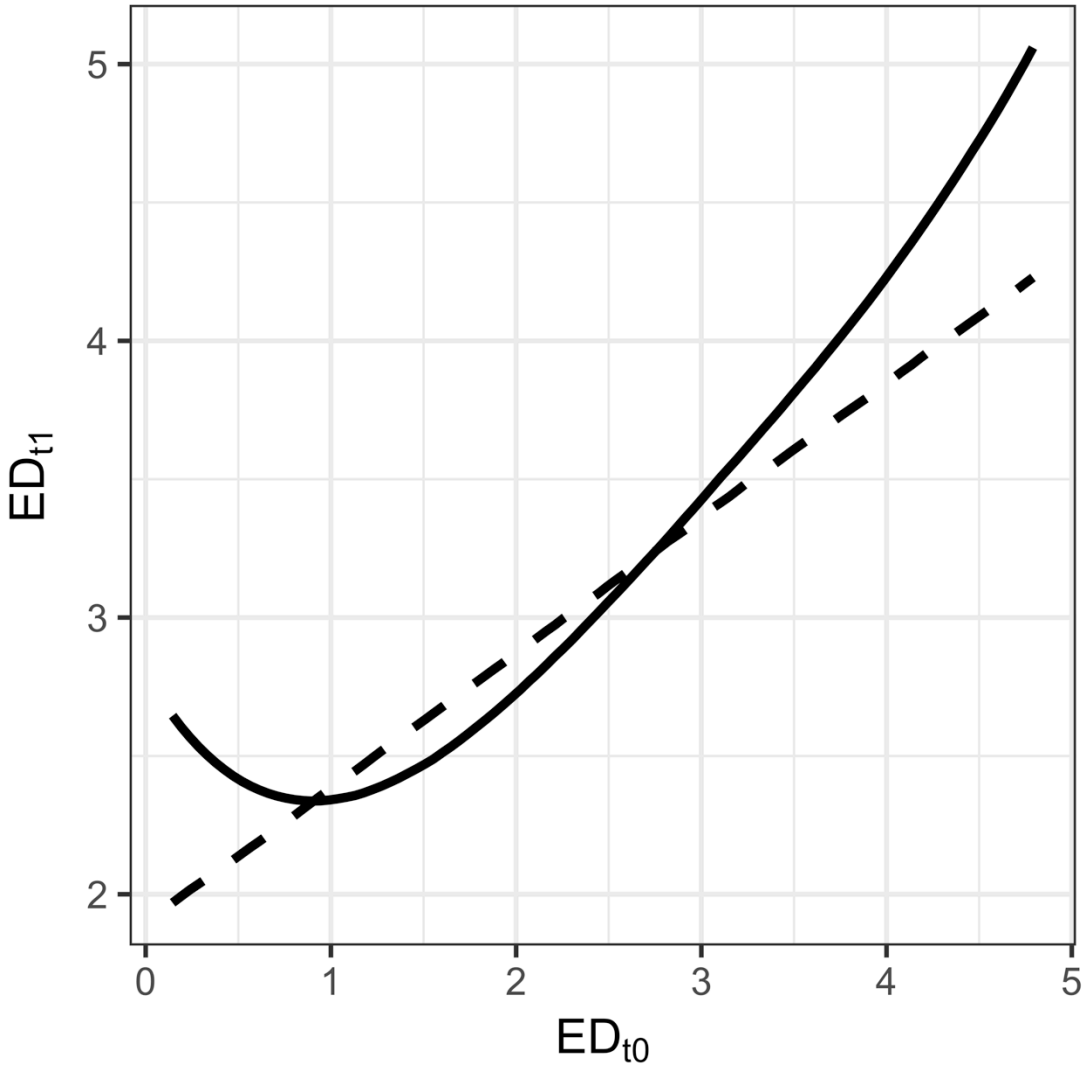


FIGURE 3.8 ESTIMATED RICKER CURVE

Predicted ED_{t1} values generated from the observed model (m3c, solid line) and the expected linear model (n1g, dashed line) for a representative dataset of a range of ED_{t0} values, a random subset of one hundred genera and all the epoch-to-epoch transitions. For plotting, ED_{t1} estimates across the different genera and epoch-to-epoch transitions were median averaged. Note, the values here are natural logged (1 = 2.7, 2 = 7.4, 3 = 20.1, 4 = 54.6, 5 = 148.4). Compare to FIGURE 3.2.

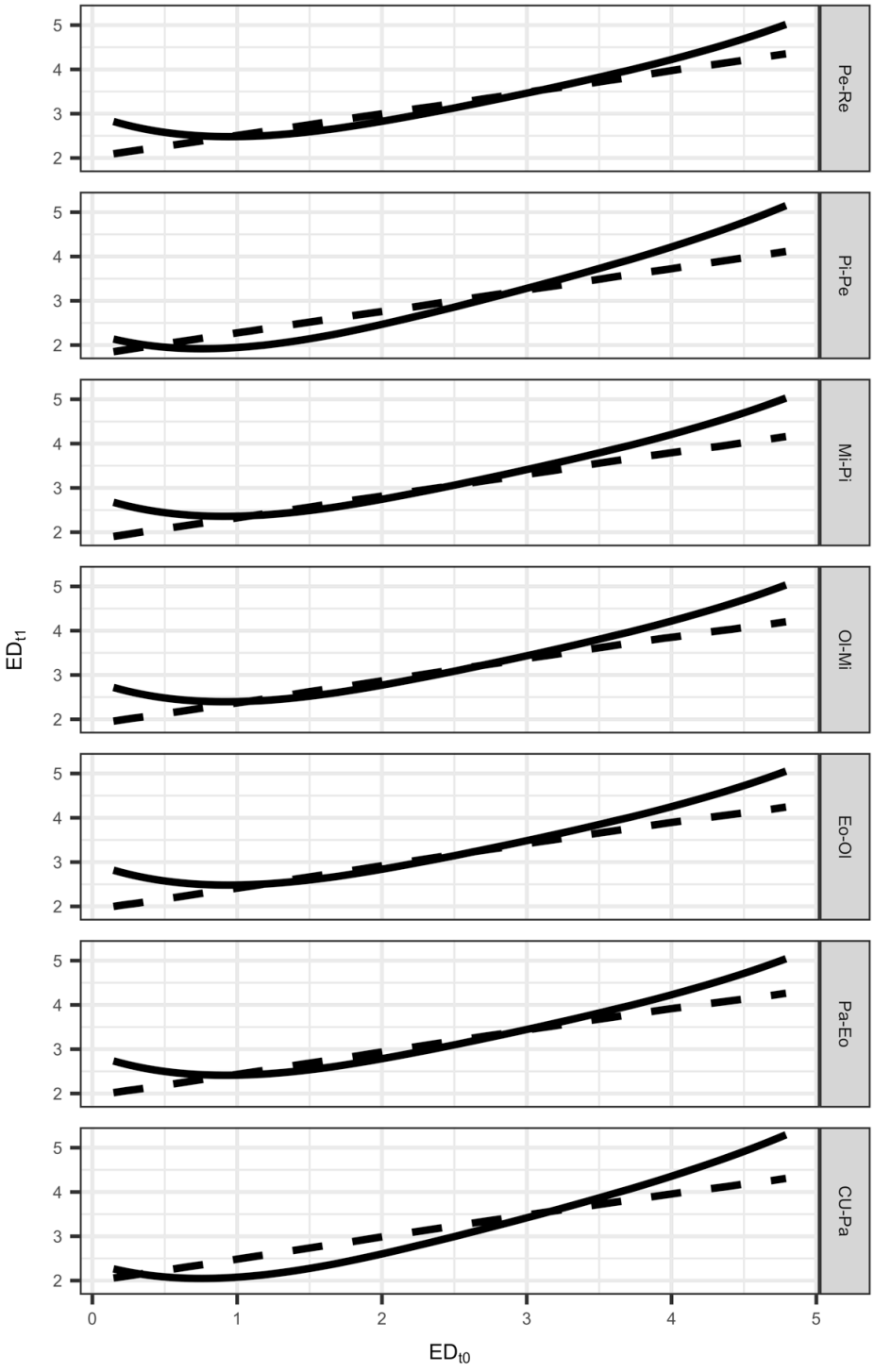


FIGURE 3.9 ESTIMATED RICKER CURVES ACROSS EPOCHS

Predicted ED_{t1} values generated from the observed non-linear model (*m3c*, solid line) and the expected linear model (*n1g*, dashed line) for a representative dataset of a range of ED_{t0} values and a random subset of one hundred genera across the different epoch-to-epoch transitions. For plotting, ED_{t1} estimates across the different genera were median averaged.

Testing Potential for Fossil Placement Bias

Finally, there is a possibility the non-linear observed results are a consequence of the random placement of fossils in the iterated trees of the *real*. Although I found a similar relationship for the *random* iterated tree distribution using an equivalent model to *obs2*, I found the predicted values for ED_{t1} at high ED_{t0} were not as extreme (FIGURE 3.10). Additionally, I compared the trend between ED_{t0} and ED_{t1} using a General Additive Model for species/clades that were shared 50 or fewer times across the *real* iterations to those shared more than 50 times. The species/clades shared more than 50 times showed a stronger non-linear relationship (FIGURE 3.11). Therefore the points of which we can be more confident indicate a stronger non-linear relationship indicating that any error in the stochastic fossil-adding process is likely to have *underestimated* the non-linear nature of the relationship rather than caused it.

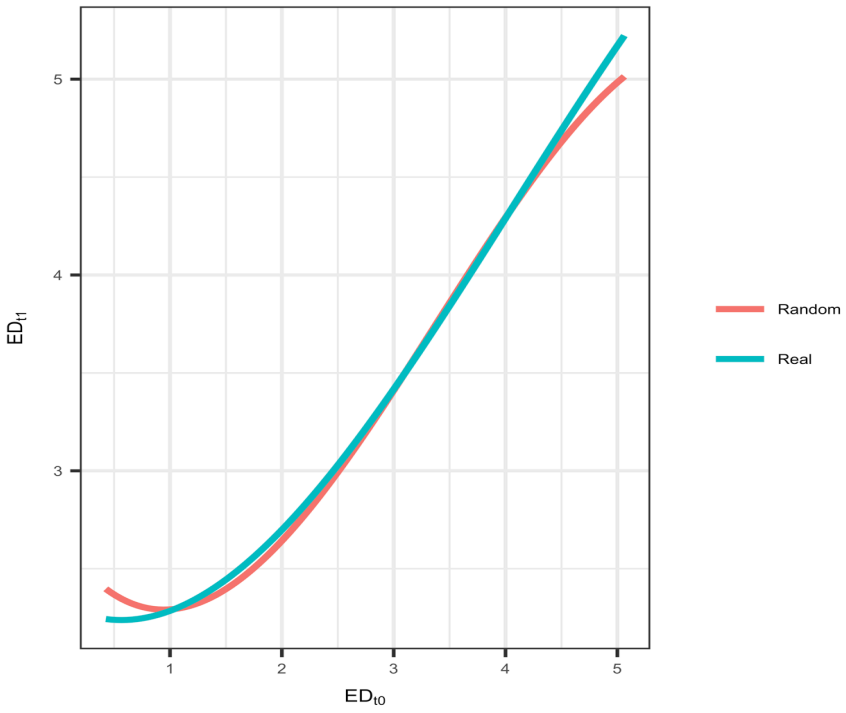


FIGURE 3.10 ED_{t1}~ED_{t0} MODELLED FOR REAL AND RANDOM

Predicted ED_{t1} values generated from quatrinomial models with random effect structure of (1|genus), a comparable model to *m3c* but which can be generated for both real and random inframetric distributions. Although both lines are similar, the *real* line has higher estimates for high ED_{t0} and lower estimates for low ED_{t0}, indicating that the observed ED_{t1}~ED_{t0} relationship may be more conservative than reality due to errors in the stochastic fossil-adding process. Values are generated for a representative dataset of a range of ED_{t0} values, a random subset of one hundred genera and all the epoch-to-epoch transitions. For plotting, ED_{t1} estimates across the different genera and epoch-to-epoch transitions were median averaged.

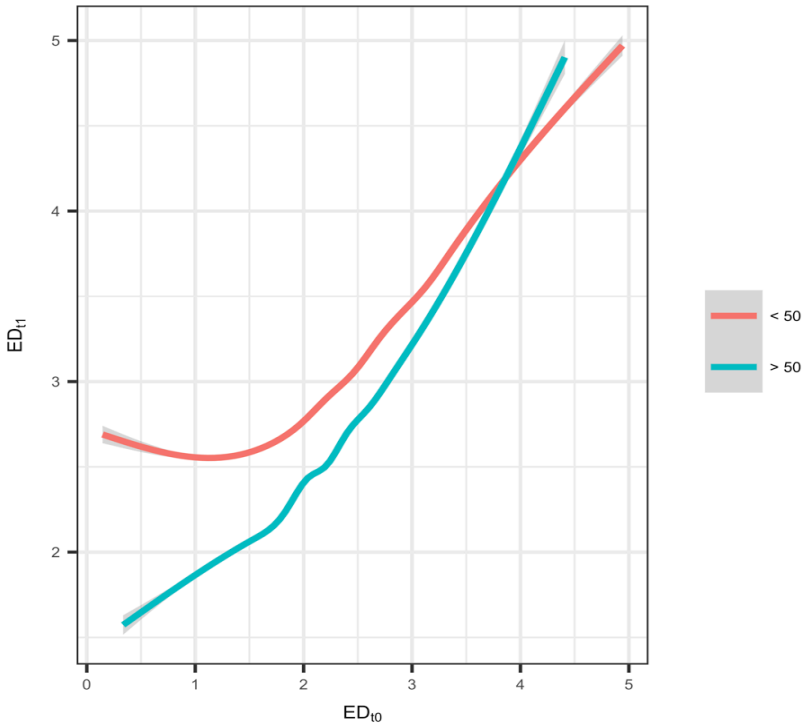


FIGURE 3.11 CONFIDENT VS. UNCONFIDENT

Generative Additive Models to explore the ED_{t0} and ED_{t1} relationship using subsets of the *real* dataset. Blue/green line indicates clades/species points that were shared over 50 times across the iterations of the *real*. Red/orange line indicates clades/species points that were shared 50 or fewer times across the iterations of the *real*.

DISCUSSION

Evolutionarily distinct mammal species are like happy families; they are evolutionarily distinct for much the same reason. Species that are or have been evolutionary distinct are more likely to become more evolutionary distinct in the future. This fits closest to a panchronic scenario for the evolution of the evolutionarily distinct.

Fossil Placement Biases

The observed non-linear relationship could be due to fossil placement biases. As FIGURE 3.3 shows, the level of detail of the fossil taxonomy limits the placement of fossils. This, however, is more likely to affect the ED estimates of lower-level clades, e.g. at the genus or subfamily level, than higher-level clades because although fossils may be added to the wrong subfamily due to lack of taxonomic resolution they will not be added to the wrong family. Misplacing of fossils will impact estimates of ED. Despite this source for error, there is evidence to suggest that phylogenetic trees that are not wholly accurate can still produce metrics more similar to true values than would be expected (Rodrigues et al. 2011). Another source of error in the placement of fossils is the age of speciation and extinction of the fossils. The ages of appearance of fossils are likely to be underestimates due to the Signor-Lipps effect (Signor and Lipps, 1982). Although, this effect is not directly accounted for using any statistical or mechanistic model, the effect's impact should not have been great because in the fossil adding process the speciation events of all the fossils added were set to occur before or during the estimated age ranges.

Regardless of the potential for biases produced by the fossil adding process, results indicate that the randomness of the process, if anything, reduced the non-linearity of the observed result rather than generating it. Firstly, comparing models fitted to the *real* and *random* showed that the pattern for high ED values in t0 to become even higher ED values in t1 was greater in the *real* than the *random*. Secondly, the observed non-linearity was greatest in the data points for in which we have the greatest confidence.

High ED values in t0 led to even higher ED values in t1 for the species/clades that were shared more than fifty times across the *real* iterations.

Comparing the Results of the *Real* and *Random*

I found a similar relationship between the distributions of trees generated using the *real* and *random* taxonomic information, although as stated above the *real* predicted higher ED_{t1} values for high ED_{t0} values. The similarity between these two distributions may be attributable to their both sharing the same base tree and the same ranges of fossil taxonomies and age ranges. This latter reason may have constrained the *random* process to placing tips in similar positions to the *real*. Furthermore, despite the taxonomic constraint there were large numbers of species/clades that were effectively placed in the *real* distribution randomly, as their positions were unique (i.e. not repeated in any other iteration). This finding applies particularly to early fossils, which had few branches on which to be added, due to the paucity of closely related species in the Recent. Where there are few branches and many fossil records, new tree sections, unique to each iteration, are generated as fossil records combine in novel ways. This difference in the spread of shared nodes may be due to the taxonomic constraint causing the consistent placement of uncertain nodes in unique points while also mapping certain nodes to the same locations. Additionally, the finding that so large a proportion of the *real* dataset was essentially added randomly indicates that increasing the number of iterations beyond one hundred is unlikely to have changed these results.

Improving the Fossil Adding Process

Other workers who have attempted similar processes for adding tips to trees have incorporated tree-modelling expectations to better inform placement, e.g. an equal-rates Markov model (Jetz et al. 2012). As discussed in chapter two, an equal-rates model is not an accurate model of tree-growth and multiple alternatives exist (e.g. the EDBMM of Bennett et al. 2017a). A practical algorithm for fossil pinning that would not require a tree-growth algorithm might involve constraining position by expected tree metrics (e.g.

balance and gravity) as well as taxonomy, using an Approximate Bayesian Computation (Rubin, 1984) framework (see FIGURE S3.2). The full implementation of such an approach is, however, beyond the scope of the initial study undertaken here.

Fossil Record Biases

The strong non-linear relationship I detect between ED_{t1} and ED_{t0} may theoretically be ascribed to fossil record biases, which may vary in a consistent manner between epoch transitions. However, this would require a bias whereby high ED species were sampled less frequently than low ED species. One mechanism that could achieve a fossil bias might be smaller body sizes and/or population sizes (Cooper et al. 2006) for evolutionarily distinct species. No studies have found significant correlations between evolutionary distinctness and population size. Many studies, however, have found evidence that evolutionary distinct species have greater body mass for a range of taxonomic groups (Ricklefs, 2005; Latiolais et al. 2006; Magnuson-Ford et al. 2009; Redding et al. 2010). Across all mammals, however, body mass is only weakly or not at all correlated with ED (Collen et al. 2011). Fossil record biases are, also, likely to be primarily taxonomic, as species that are less likely to be fossilised are also likely to be related, e.g. through occupying the same habitat (Foote and Raup, 1996). The non-linear relationship, however, is detected even when using a random-effects structure that controls for inter-relatedness.

Stable and Unstable Points

The fit of the data to a panchronic scenario, however, is not perfect. Instead of there being only a single unstable saddle point (at ~20 MY) as expected, there is also a stable point (at ~2.7 MY). Species below this stable point should be expected to increase in ED, while species above it should be expected to decrease in ED. At face value this might imply that today's mammals should have converged to an average ED of ~2.7 MY, but in fact the average is much greater (13.0 MY). This lack of convergence may reflect variation in this stable point through time; while the unstable saddle point remained

relatively fixed between different epoch transitions, the stable point did not, varying between 0 and 2.7 MY; it may thus not have acted as a consistent 'attractor'. More importantly, however, it should be noted that there are very few mammal species alive today with an ED value less than 2.7 (~10 species). This lack of sampling makes any conclusions on the shape of the relationship for this end of the data unreliable.

Conclusions

The fossil-record based modelling undertaken in this chapter reinforces the conclusions of chapter two; evolutionarily distinct species tend to become even more distinct through time. The relationship between ED_{t0} and ED_{t1} is non-linear, and the closest scenario for the origin of evolutionary distinctness is the panchronic. This provides independent corroboration of the conclusions of chapter two.

These observed results differ strongly from what would be expected from an "evolutionarily relict" or "dead-end" scenario, which posits that species become evolutionarily distinct by being the sole survivors of a once recently large clade (compare FIGURE 3.2A and FIGURE 3.8). Although not impossible, such a circumstance would be rare to occur given the results of the expected model, as it only shows a slow gain in ED for species that are *already* evolutionarily distinct. As I outline in FIGURE 3.2, for the 'evolutionary relict' scenario to regularly occur, species with mid- to low-ED values at t0 would have to become high-ED at t1. Instead, the results show these species with mid- to low-ED values have lower ED values than expected at t1.

Across the deep-time evolutionary history of mammals, evolutionarily distinct species (with an ED greater than ~20 MY) became more evolutionarily distinct as time progressed. Around 980 extant mammal species (~20%) have an evolutionary distinctness in this range (after Bininda-Edmonds et al. 2007). We should expect this set of species to be more likely to become either extinct or more evolutionarily distinct, but not to diversify in the future. If we consider evolutionarily distinct species as LFs, then

the conclusion that the LF phenomenon is the result of slow rates of diversification appears secure.

4.

Developing New Metrics for the Living Fossil

INTRODUCTION

As outlined in the chapter one, the term 'living fossil' is controversial and viewed unfavourably by many evolutionary biologists and palaeontologists. At the core of its contention are divergences over its definition and concerns whether the concept can exist. In chapters two and three I tested the latter of these issues through a tree growth model and the fossil record using the Evolutionary Distinctness metric (Isaac et al. 2007) as a proxy. These chapters demonstrate that, in principle, the concept of a lineage that experiences reduced rates of diversification for extended periods of Earth's history is feasible. The next issue, therefore, concerns the precise definition of 'living fossil': what are the precise limits of what we mean when we say LF? Which organisms alive today do we class as LFs?

Most work on the LF concept has hitherto been restricted to single groups, and has focused on describing the nature and magnitude of evolutionary changes seen in these groups within the fossil record (e.g. see Eldredge 1984). An alternative approach is a pan-group analysis, which could highlight and resolve inconsistencies around current usage of the term 'living fossil', with a view to producing a more precise and usable definition that maintains some congruence with common usage. A key element of such an analysis would be a quantitative scale of "living-fossil-ness", combining different aspects of the concept into a single index that could be used to determine which groups may be considered LFs in a more objective manner. The availability of such a metric would enable a more rigorous analysis of the degree to which the LFs are a phenomenon worthy of study. Such a measure would have to be applicable across the tree of life, be applicable to clades as well as species; be readily measurable; and allow fair inter-group comparisons. No explicit metric of living-fossil-ness has yet been proposed, although evolutionary distinctness (or ED) has been suggested as a proxy (Isaac et al. 2007; Cavin and Kemp 2011). ED is applicable across the tree of life, allows inter-group comparisons, and captures the element of phylogenetic isolation and lineage antiquity that is common to most interpretations of the LF concept. ED, however, is not measurable for clades above the species-

level, meaning that many candidate LF groups (e.g. coelacanths, monotremes) can only be considered on the basis of their individual species. It also requires a fully resolved, time-calibrated phylogenetic tree of the entire taxonomic group of interest, a substantial impracticality for any metric intending to identify LFs across all life. Finally, it does not take into account other elements frequently incorporated into the LF concept such as morphological or ecological change (Fisher 1990).

In this chapter, I propose a new metric to address these shortcomings, and demonstrate its fit to qualitative concepts of LF-ness in a range of groups. I surveyed the literature to find the most common ways that the term 'living fossil' is used, and broke these down into measurable elements: age of the clade (in MY), relative success in terms of numbers of species, and relative number of changes since the clade first appeared. I combined these three measurable elements into a single equation to produce an evolutionary performance index (EPI), and calculated EPI values across all metazoans and plants to generate a ranked list of the most LF-like clades. Clades that score low on this index are considered LF-like, while those that score high are not (i.e. have likely experienced recent adaptive radiations). I also introduce pEPI, a proxy for EPI for use where number of changes cannot be calculated. These indices provide not simply a measure of living-fossil-ness for any given clade, but a breakdown of which elements of the concept (age, relative success, number of changes) are responsible for a particularly low or high value.

MATERIALS AND METHODS

Literature Survey

Using 'living fossil' as a keyword, I searched for all literature, scientific articles and books that proposed a definition of the LF. In cases where a definition statement could not be found, a definition was inferred from the text as a whole. In total I discovered 56 sources from which a definition could be determined (see appendix 'literature survey' for a list of references and TABLE S4.1 for example of interpretation of text). I identified eight recurring themes from the survey: existing for a long time (1), morphologically conserved (2), some alternative form of conservatism (3), having 'primitive' features (4), phylogenetically/evolutionarily distinct (5), a survivor of a once large clade (6), geographically isolated (7) and having a generalist niche (8).

I disregarded themes 7 and 8 as I considered these to be potential explanatory or emergent characteristics of LFs, rather than defining features. For example, there are too many examples of clades, considered LFs, that do not exhibit these features (e.g. crocodiles, sharks, moss, horseshoe crabs); equally there are many clades which do exhibit these features that are not considered LFs (recently diverged island species, highly successful generalist clades like rodents and grasses). Additionally, there is disagreement between authors on whether a LF should solely be an unchanging species (e.g. Schopf 1984) or can be a higher-level taxon that may experience low rates of speciation (e.g. Yoshida 2002). For the purposes of this study, I opted to assess any monophyletic group (species or not) as a candidate for a LF.

I combined the remaining six themes into three quantifiable variables: 'success', 'time' and 'change'. These were in turn combined into an 'Evolutionary Performance Index', based on current success and levels of change relative to a clade's sister (see below for more details).

The Three Variables

Success

This variable captures themes 5 and 6. It is calculated simply as the number of extant species. I used the NCBI taxonomy (Federhen, 2012) to estimate success and other variables across all metazoans and plants. NCBI taxonomy was selected over other online taxonomies (e.g. ITIS, 2016) because, as a repository for sequence data, I deemed its names to be the most likely to have associated time-estimates. Furthermore, all clades within the database aim to be monophyletic and classifications are regularly updated according to the latest studies in the systematic literature (Federhen, 2012). To aid interpretation of results, all recovered clades were assigned to one of nine large well-known groupings: Aves, Mammalia, Teleostei, Lepidosauria, Amphibia, other vertebrates, Arthropoda, other metazoans, and Embryophyta. I downloaded all relevant taxonomies from NCBI and counted the number of descendent species for every clade. Many names for biological entities in the taxonomy are not necessarily recognised extant species or groups. We, therefore, excluded all 'unclassified', 'unassigned', 'unvouchered', 'extinct' and 'environmental' entries as well as next-generation sequencer identified species. I also identified each sister clade, and calculated the contrasted number of descendants by dividing the clade's number of descendants by its sister's. In cases where there was more than one possible sister, i.e. a polytomous node, I selected the sister with the greatest number of descendants in order to tend towards the lowest possible EPI scores.

Time

This variable captures themes one and five and is calculated as the time in millions of years since the lineage diverged. I used two approaches for estimating time. First, I sourced large-scale time-calibrated phylogenetic trees for two major vertebrate groups, mammals (Bininda-Emonds et al. 2007) and birds (Jetz et al. 2012; Birdtree, 2016). Because the source data for the bird tree is composed of two posterior distributions generated from different avian taxonomies, I selected a random set of one hundred trees from the distribution based on the Hackett (2008) taxonomy.

For every clade in these phylogenetic trees I estimated the clade's age, determined as the time since the lineage diverged from the tree of life, using the R package `treeman` (Bennett 2017c; Bennett et al. 2017b; see chapter six). Common clades between those of NCBI and those found in the phylogenetic trees were identified using name matching of descendants. The second approach used timetree (Hedges et al. 2006; Hedges et al. 2015), an online database of divergence times. This database hosts a list of estimated divergence times across all life as reported in the scientific literature, including all published estimates for the divergence date for two given taxa, and a weighted average measure of divergence based on these estimates. I sought the age of a given clade by performing timetree searches of the clade against all possible sister clades. In order to estimate the lowest possible EPI values I then selected the highest reported divergence date among all sisters. To reduce computational time and to limit the dependence of the results on averaged estimates of divergence, I conservatively restricted the timetree searches only to clades I considered more likely to be LFs, identified as clades whose parent clade contained more than 500 species and which had a contrasted numbers of descendants of less than 0.1. This restriction only prevents the unnecessary searching for high EPI clades because parental clades containing fewer than 500 species are likely to have only existed for relatively short periods of time. Additionally, ten times fewer descendants than your sister is a common score even for clades not considered LFs, e.g. rabbits (e.g. *Oryctolagus* [1 sp.] vs. *Lepus* [32 spp.]: 0.03125).

Change

This variable captures themes 2, 3 and 4 and is calculated as the number of changes (morphological and ecological) that have occurred in the clade's lineage since its initial divergence. I estimated change from datasets of morphological, ecological and life-history traits using ancestral character reconstruction. This required phylogenetic trees, and therefore I only sourced datasets for the major taxonomic groups for which I had trees (mammals and birds). For all other clades I calculated only pEPI values, which do not require a measure of change (see

below). For both mammals and birds, I sourced one morphological character dataset (O'Leary et al. 2013; Livezey and Zusi, 2007) and one ecological/life-history dataset (Jones et al. 2009; Lislevand et al. 2007). The names found within the datasets were matched to tips in the phylogenetic trees using character matching. The combined datasets represented 4,572 characters and 4,510 species for mammals and 2,988 characters and 3,509 species for birds. For character reconstruction I used the R package `ape` (Paradis et al. 2004) to implement maximum parsimony reconstruction (Hanazawa et al. 1995). I chose not to perform reconstruction with any model based approach (i.e. Brownian motion or Ornstein-Uhlenbeck); doubts exist (Thomas et al. 2014) over their accuracy, there is evidence that they may be prone to undetectable trends (Webster and Purvis, 2002) and they do not always outperform parsimony (Royer-Carenzi et al. 2013). Continuous traits were made discrete by binning into ten equally spaced categories. I used the mammalian supertree (Bininda-Edmonds, 2007) and a consensus bird tree (Birdtree, 2016) to perform ancestral character reconstruction for all characters. Change scores were calculated for every branch in a tree based on differences between the ranges of possible states of the nodes of the tree. Score values of one indicate no possibility of change, less than two the possibility of change, and more than two that multiple changes must have occurred. Mean change scores per character were calculated for each clade in a tree using the scores of all descendent branches. Sister contrast values were then calculated for each character by dividing the clade mean by the sister mean. An overall change score from these sister contrasted means per character was then determined using a weighted mean to control for non-independence and number of states per character. No score was calculated for any clade that had fewer than four characters represented. (See appendix 'estimating change' for more details.)

Derivation of EPI and pEPI

EPI (Evolutionary Performance Index) combines the three variables of success, time and change into a scalar. Because performance is relative, EPI uses values of success and change measured as 'sister contrasts', i.e. relative to the equivalent values of the sister clade, rather than raw values. Sister clades are appropriate benchmarks of performance as a clade and its sister share the same evolutionary trajectory until the point of divergence – as originally suggested by Vbra (1984). The use of sister contrasts allows EPI comparisons across taxonomic groups, and also has the benefit of forcing the values of success and change onto the same scale (> 0, skewed distribution). The EPI of a clade can therefore be thought of as the amount of evolutionary 'activity', measured as the number of species and changes relative to its sister, over the amount of time a clade has existed. Because it can be difficult to source the data required to measure change, I present two indices, EPI (Eq. 1) where all three variables can be estimated, and 'proximate' EPI or pEPI (Eq. 2) where only time and success can be estimated:

$$EPI = log(\frac{\frac{S_i}{S_j} + \frac{C_i}{C_j}}{T_i}) \quad (1)$$

$$pEPI = log(\frac{\frac{S_i}{S_j}}{T_i}) \quad (2)$$

These define the performance for any clade of interest (*i*) relative to its sister (*j*). *S*, *C* and *T* refer to the estimated values for success, change and time respectively (see FIGURE 4.1). Because the resulting division of success and change over time produces a heavily skewed distribution, the logarithm of this ratio is used to generate a normal distribution.

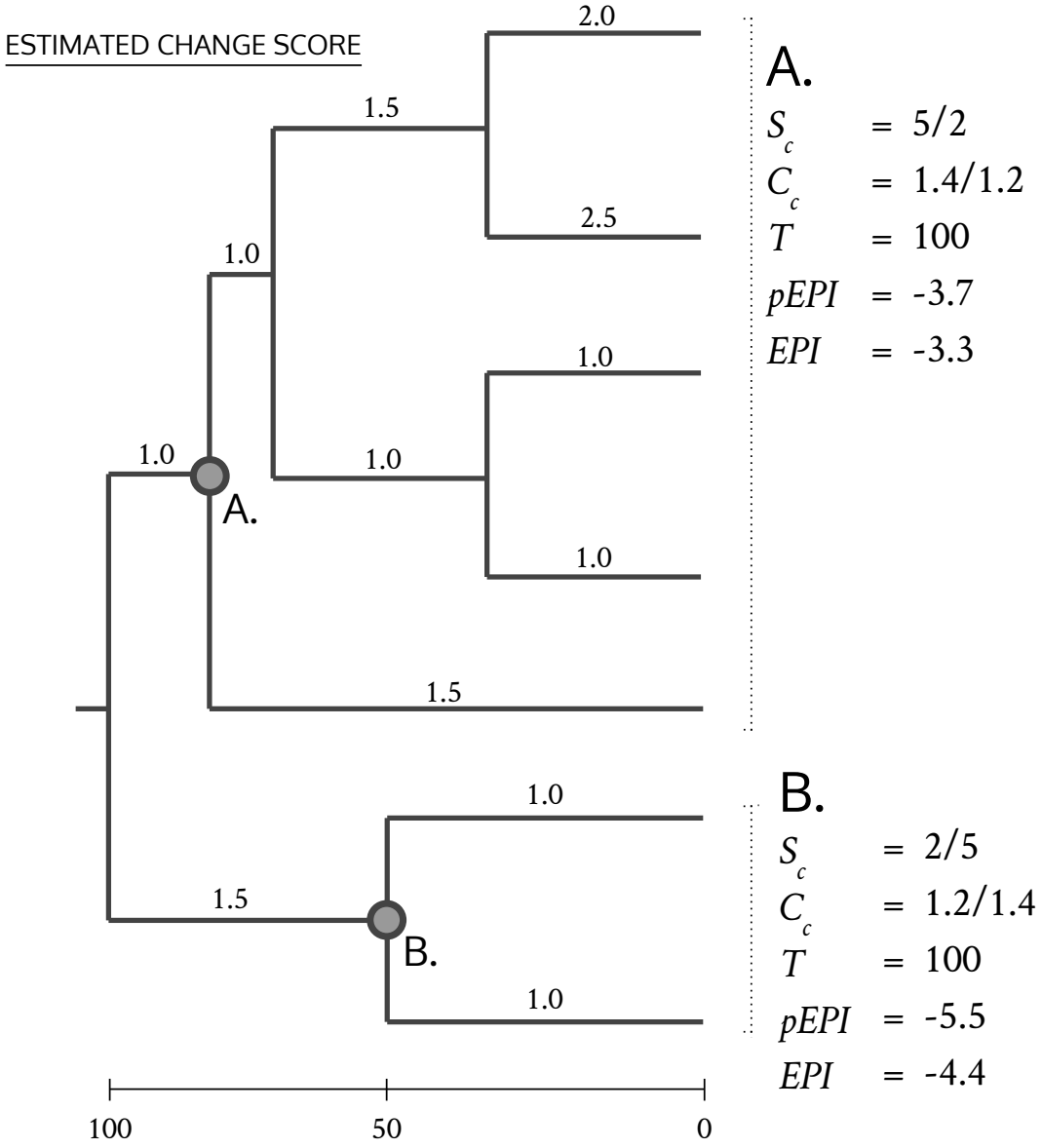


FIGURE 4.1 CALCULATING EVOLUTIONARY PERFORMANCE

How to calculate pEPI and EPI from a phylogenetic tree. Numbers above the branches are estimated change scores. Clades A and B are sisters, their performance scores depend on each other. Contrasted change (C_c) is calculated as the mean change score for a clade divided by its sister's. Contrasted success (S_c) is calculated as the number of species in a clade divided by its sister's. Time (T) is the amount of time in MY since the clade first appeared. EPI and pEPI are then calculated using equations 1 and 2.

Analysis

Comparing the Fit of Metrics to Qualitative Assessments of LF-ness

All three metrics were calculated for every clade where data permitted. For all clades where an index was calculated, I then searched for the clade's corresponding Wikipedia article (Wikipedia, 2016) and generated presence/absence data on whether the phrase 'Living Fossil(s)' appeared. While this measure is crude, it represents a practical and relatively unbiased assessment of the popular applicability of the term to a clade. I ran generalised linear models with binomial error distributions (Hastie and Pregibon, 1992) to determine whether the three indices correspond to this measure of popular usage.

R (3.2.4) was used to run all analyses. The pipeline used to generate all results is available via the main author's GitHub account (Bennett, 2016).

RESULTS

In total I calculated pEPI scores for 21,740 clades, with the majority of values being calculated for mammals and birds through the use of time-calibrated phylogenetic trees (10,136 Aves, 5,774 Mammalia, 280 Teleosti, 103 Lepidosauria, 93 Amphibia, 18 other vertebrates, 2,049 Arthropoda, 296 other metazoans, and 2,992 Embryophyta). The majority of values were negative (pEPI quantiles: -16.3–0%, -6.5–25%, -4.7–50%, -3.3–75%, 3.5–100%). Many of the lowest scoring clades have been considered LFs by previous authors, for example *Trichoplax* (-16.2), coelacanths (-15.6), lancelets (-14.9) and limulids (-13.6). For EPI, scores were calculated for 2,433 and 3,012 clades for mammals and birds respectively. The resulting range of values was less negative compared to pEPI (EPI quantiles: -5.1–0%, -2.8–25%, -2.0–50%, -1.2–75%, 3.7–100%). Again, taxa regularly considered to represent LFs had the lowest scores: monotremes (-5.4), hoatzin (-4.6), marsupials (-4.9) and palaeognaths (-4.7). The pEPI of *Homo sapiens* (-2.2) was near to the median value, but for apes overall the score was relatively low for both pEPI (-5.1) and EPI (-3.4). (See TABLE 4.1 and 4.2, see appendix "full results" TABLE S4.2 for statistics calculated for all 21,740 clades and TABLE S4.3 for estimates of contrasted change.)

TABLE 4.1 TOP pEPI LIVING FOSSILS

TABLE 4.1. Top five LFs according to *pEPI* and their associated statistics for Amphibia, Arthropoda, Aves, Embryophyta, Lepidosauria, Mammalia, Metazoa, Vertebrata and Teleostei. '-' indicates no data is available.

Common name	Scientific name	Change	Success	Time	ED	*EPI*	*pEPI*	%
Amphibians	**Amphibia**	-	3.24E-01	351.80	-	-	-6.99	20%-21%
Tailed frog	*Ascaphus*	-	3.47E-04	183.50	-	-	-13.18	0%-1%
New Zealand primitive frogs	*Leiopelma*	-	6.95E-04	183.50	-	-	-12.48	0%-1%
Gastric-brooding frog	*Rheobatrachus silus*	-	1.49E-03	123.90	-	-	-11.33	1%-2%
Fire-bellied toads	Bombinatoridae	-	1.91E-03	156.90	-	-	-11.32	1%-2%
The Seychelles frogs	Sooglossoidea	-	1.80E-03	135.30	-	-	-11.23	1%-2%

Arthropods	**Arthropoda**	-	4.10E+02	680.50	-	-	-0.51	98%-99%
Kauri moth	*Agathiphaga queenslandensis*	-	2.64E-05	224.00	-	-	-15.95	0%-1%
Southern beech moth	*Heterobathmia pseuderiocrania*	-	2.64E-05	224.00	-	-	-15.95	0%-1%
-	*Andesiana lamellata*	-	2.70E-05	194.00	-	-	-15.79	0%-1%
Archaic bell moths	Neopseustidae	-	5.29E-05	194.00	-	-	-15.12	0%-1%
-	*Prionodiaptomus*	-	2.18E-04	507.35	-	-	-14.66	0%-1%
Birds	**Aves**	-	3.41E+02	236.50	-	-	0.37	99%-100%
Hoatzin	*Opisthocomus hoazin*	0.74	1.13E-04	72.45	72.69	-4.56	-13.37	0%-1%
New Zealand wrens	Acanthisittidae	0.80	3.72E-04	73.10	41.68	-4.55	-12.19	0%-1%
Hoopoes, wood-hoopoes, scimitarbills	Upupiformes	0.96	3.41E-04	59.58	20.68	-4.13	-12.07	0%-1%
Grey hypocolius	*Hypocolius ampelinus*	-	1.86E-04	29.02	32.41	-	-11.96	0%-1%
Mousebirds	Coliidae	0.83	6.81E-04	81.59	38.97	-4.54	-11.69	0%-1%
Land Plants	**Embryophyta**	-	1.99E+02	919.80	-	-	-1.53	94%-95%
Coontails or hornworts	Ceratophyllum	-	7.91E-05	139.00	-	-	-14.38	0%-1%
-	Berberidopsidales	-	1.19E-04	113.70	-	-	-13.77	0%-1%
-	*Andreaeobryum macrosporum*	-	3.11E-04	220.00	-	-	-13.47	0%-1%
Griffith's oedipodium moss	*Oedipodium griffithianum*	-	3.24E-04	214.10	-	-	-13.40	0%-1%
Soap bark tree	*Quillaja saponaria*	-	1.13E-04	65.40	-	-	-13.27	0%-1%
Lepidosaurs	**Lepidosauria**	-	7.06E-01	279.70	-	-	-5.98	30%-31%
Tuatara	*Sphenodon*	-	3.06E-04	251.80	-	-	-13.62	0%-1%
Cat gecko	*Aeluroscalabotes felinus*	-	1.19E-03	116.00	-	-	-11.49	0%-1%
-	Dibamidae	-	2.15E-03	201.00	-	-	-11.45	0%-1%
Straight-fingered geckos	*Altiphylax*	-	2.37E-03	110.67	-	-	-10.75	1%-2%
-	Microgecko	-	2.37E-03	108.00	-	-	-10.73	1%-2%

Mammals	**Mammalia**	-	3.54E-01	311.90	-	-	-6.78	22%-23%
Egg-laying mammals	Monotremata	0.97	5.37E-04	166.20	80.13	-5.15	-12.64	0%-1%
Mountain beaver	*Aplodontia rufa*	-	5.34E-04	58.70	58.82	-	-11.61	0%-1%
Springhare	*Pedetes capensis*	0.85	5.34E-04	56.90	59.83	-4.20	-11.58	0%-1%
Flying lemurs	Cynocephalidae	-	1.10E-03	91.30	52.18	-	-11.33	1%-2%
Beavers	*Castor*	0.91	1.07E-03	71.60	41.88	-4.37	-11.11	1%-2%
Multicelled animals	**Metazoa**	-	3.39E+00	998.10	-	-	-5.68	34%-35%
-	*Limnognathia maerski*	-	5.77E-05	662.00	-	-	-16.26	0%-1%
Placozoans	*Trichoplax*	-	8.74E-05	951.80	-	-	-16.20	0%-1%
Mesozoans	Mesozoa	-	1.10E-04	951.80	-	-	-15.97	0%-1%
Jaw worms	Gnathostomulida	-	1.36E-04	745.60	-	-	-15.51	0%-1%
Comb jellies	Ctenophora	-	2.18E-04	824.00	-	-	-15.15	0%-1%
Vertebrates	**Vertebrata**	-	1.27E+02	680.20	-	-	-1.68	93%-94%
Coelacanth	*Latimeria*	-	7.06E-05	413.00	-	-	-15.58	0%-1%
Lungfishes	Ceratodontimorpha	-	4.23E-04	413.00	-	-	-13.79	0%-1%
Bichirs	Polypteridae	-	7.66E-04	386.30	-	-	-13.13	0%-1%
Jawless vertebrates	Cyclostomata	-	2.20E-03	615.00	-	-	-12.54	0%-1%
Crocodiles	Crocodylia	-	2.93E-03	236.50	-	-	-11.30	1%-2%
Bony fish	**Teleostei**	-	2.32E+03	314.70	-	-	2.00	99%-100%
Dwarf pencilfish	*Lepidogalaxias salamandroides*	-	8.32E-05	209.40	-	-	-14.74	0%-1%
-	Holostei	-	4.32E-04	314.70	-	-	-13.50	0%-1%
Beardfish	Polymixia	-	5.31E-04	148.00	-	-	-12.54	0%-1%
Convict/engineer blenny/goby	*Pholidichthys leucotaenia*	-	4.49E-04	93.00	-	-	-12.24	0%-1%
Jellynose fish	Ateleopodidae	-	8.33E-04	166.90	-	-	-12.21	0%-1%

TABLE 4.2 TOP EPI LIVING FOSSILS

Top twenty-five LFs according to *EPI* and their associated statistics for birds (B) and mammals (M) for which the change variable could be calculated.

Common name	Scientific name	B/M	Change	Success	Time	ED	*EPI*	*pEPI*
Egg-laying mammals	Monotremata	M	0.97	5.37E-04	166.20	80.13	-5.15	-12.64
Marsupials	Metatheria	M	1.00	6.68E-02	147.70	18.28	-4.93	-7.70
Ratites and Tinamous	Palaeognathae	B	1.01	6.24E-03	116.75	25.58	-4.74	-9.84
Anteaters, sloths and armadillos	Xenarthra	M	0.99	6.92E-03	101.10	25.48	-4.62	-9.59
Afrotherians	Afrotheria	M	0.98	2.08E-02	101.30	30.44	-4.62	-8.49
Fowl	Galloanserae	B	0.97	5.52E-02	103.54	7.23	-4.61	-7.54
Aardvark	*Orycteropus afer*	M	0.92	9.43E-03	93.20	93.34	-4.61	-9.20
Odd-toed ungulates	Perissodactyla	M	0.86	1.20E-02	87.30	29.38	-4.61	-8.89
Hoatzin	*Opisthocomus hoazin*	B	0.76	1.13E-04	72.45	72.69	-4.56	-13.37
New Zealand wrens	Acanthisittidae	B	0.77	3.72E-04	73.10	41.68	-4.55	-12.19
Trogons	Trogonidae	B	0.82	4.45E-03	77.23	12.35	-4.54	-9.76
Oilbird	*Steatornis caripensis*	B	0.83	1.10E-02	79.12	79.17	-4.54	-8.88
Mouse birds	Coliidae	B	0.87	6.81E-04	81.59	38.97	-4.54	-11.69
Pangolins	*Manis*	M	0.91	6.40E-03	84.90	25.73	-4.53	-9.49
Seriemas	Cariamidae	B	0.89	1.26E-02	82.20	48.49	-4.51	-8.78
Solenodons	*Solenodon*	M	0.94	4.72E-03	84.20	62.54	-4.49	-9.79
Rabbits and hares	Lagomorpha	M	0.98	4.64E-02	91.80	12.99	-4.49	-7.59
Dormice	Gliridae	M	0.85	9.16E-03	76.30	24.13	-4.49	-9.03
Tree shrews	Tupaiidae	M	1.05	9.95E-03	94.30	28.62	-4.49	-9.16
Divers/Loons	*Gavia*	B	0.75	5.68E-04	64.98	30.23	-4.46	-11.65
Shorebirds	Charadriiformes	B	0.97	3.56E-02	85.18	12.14	-4.45	-7.78

							4.44	
Mesites	Mesitornithidae	B	0.93	1.26E-02	77.54	36.09	-4.41	-8.73
Owls	Strigiformes	B	0.97	1.78E-02	80.44	10.57	-4.40	-8.42
Falcons and caracaras	Falconiformes	B	0.96	3.33E-02	80.68	11.15	-4.40	-7.79
Parrots	Psittaciformes	B	0.96	4.00E-02	80.48	8.76	-4.39	-7.61

It is likely, however, that these scores are impacted by differences in sampling effort between taxonomic groups. Although I found amphibians, birds, lepidosaurs, mammals, vertebrates and teleosts all had total number species counts in the NCBI taxonomic database I would expect; embryophytes, metazoans and particularly arthropods were clearly under-sampled when compared to estimated numbers of species for these groups (see TABLE 4.3 for sampled and expected species counts). Furthermore, sampling effort differed within these under-sampled groups. For example, although it is well known that Coleoptera is the most species-rich insect order, in the NCBI database it is less sampled (26,240 spp.) than Lepidoptera (37,934 spp.).

For clades that had both EPI and pEPI scores, there was a strong correlation between the two indices (Pearson's R = 0.77, Spearman's R = 0.75; see FIGURE 4.1), indicating either the over-dominance of a single variable or correlations between the variables success and change against the time variable. I confirmed the latter by finding a positive correlation between the change variable and pEPI (Spearman's R = 0.45). Both indices also showed relatively strong correlations with ED (Pearson's R = -0.41 and -0.72 for pEPI and EPI respectively against log ED; FIGURE 4.2 and 4.3).

TABLE 4.3 SPECIES COUNTS BY GROUP

Number of species counted from NCBI taxonomy and number of expected for each taxonomic grouping. Metazoan estimate is based on the sum of descendent clades

Group	Spp. Count	Expected Count		%	Reference
Amphibia	6,936	7,571		92	(AmphibiaWeb, 2016)
Arthropoda	166,900	7,500,000	*	2	(Ødegaard, 2000)
Aves	8,867	10,050		88	(Gill and Wright, 2006)
Embryophyta	131,987	350,669		38	(The Plant List, 2006)
Lepidosauria	6,539	9,000	*	73	(Uetz, 2010)
Mammalia	5,593	5,416		103	(Wilson and Reeder, 2005)
Metazoa	265,332	7,972,706		3	-
Vertebrata	50,498	64,000		79	(Baillie et al. 2004)
Telostei	20,847	26,000	*	80	(Froese and Pauly, 2016)
		Mean %		62	

*Author estimates based on description

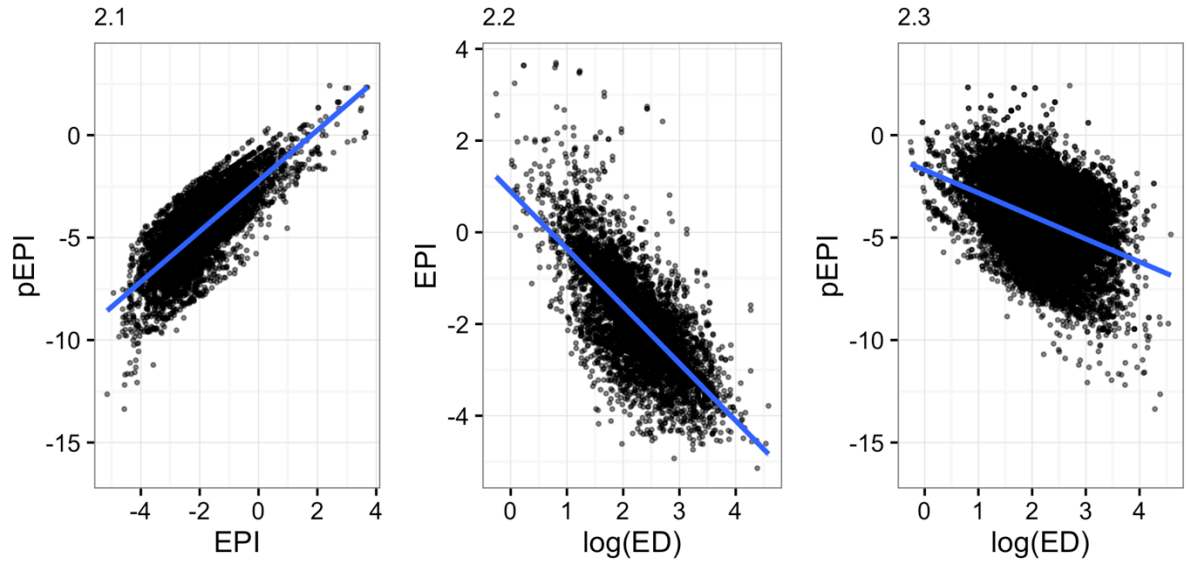


FIGURE 4.2 pEPI, EPI AND ED CORRELATIONS

(2.1) pEPI and EPI show a strong correlation, evolutionary distinctness (ED) when logged shows strong correlations with both EPI (2.2) and pEPI (2.3).

Each variable experienced different levels of dispersion, with success having the greatest coefficients of variation (39.5, 1.2 and 0.2 for success, time and change respectively).

Despite these great differences, all of the variables had an impact on the overall scores

(Spearman's R 0.78, and -0.68 for success and time against pEPI and Spearman's R 0.21, 0.25 and -0.95 for success, change and time respectively for EPI). In the case of EPI, however, the score was largely determined by the time variable causing fewer single species clades to rank at the bottom. This was in part due to the stabilising impact of the change variable when combined with the success variable. For example, the Palaeognathae that ranked among the bottom ten birds for pEPI, ranked as having the lowest EPI score. Although its success (0.06) and change (1.0) variables were not as low as for other bird groups (e.g. 0.0006 and 0.9 for Menuridae, 0.0003 and 1.0 for Upupiformes), when added they were closer to the values of other groups – making the time variable the deciding factor. As a consequence, fewer single species clades, which tend to be younger, ranked among the most likely LFs according to EPI than was the case for pEPI. Both indices, also, showed an increasing range of variables towards higher values of time (FIGURE 4.3).

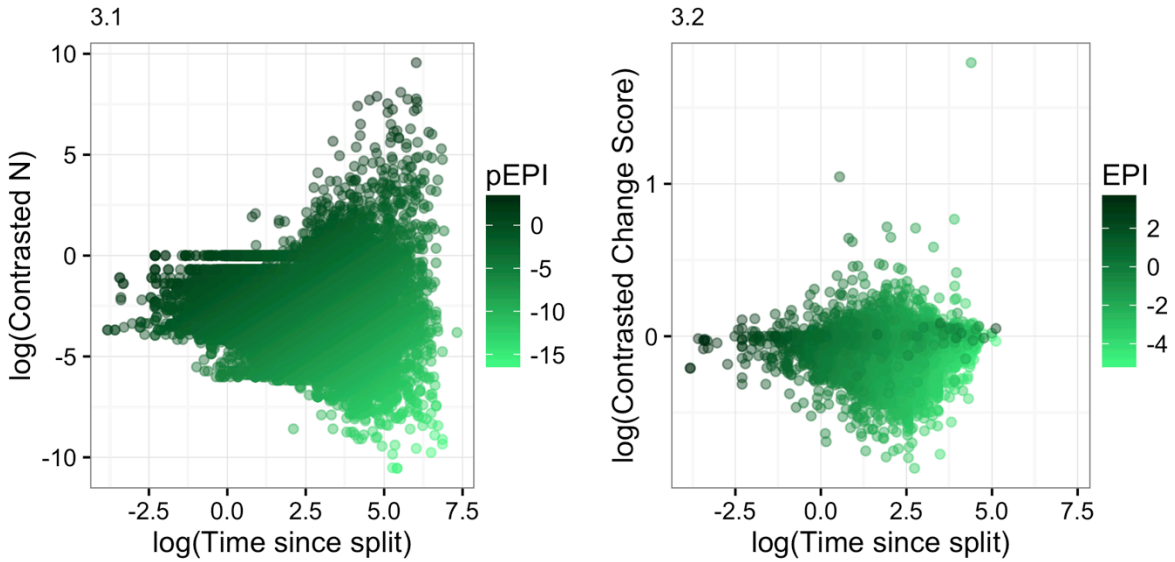


FIGURE 4.3 DISPERSAL OF pEPI AND EPI

Dispersion increases as a clade's time since splitting increases for both success (3.1) and change (3.2). LF clades are found towards the bottom-right, while clades that have likely experienced an adaptive radiation aggregate in the top-right.

In total I sourced 13,898 Wikipedia articles for all clades where I had calculated indices. Of these articles, 62 contained the term 'living fossil'. EPI and pEPI both showed that the lower the value,

the more likely a clade's article is to include the term. Within the bottom 0-25% quantile of EPI and pEPI clades, 23 and 40 included 'living fossil' in their articles, whereas there were only 2 and 19 respectively in total in the subsequent three quantiles (25-100%). For ED the pattern was as expected given that higher values indicate greater distinctness: 31 mentions in the 75-100%, and 4 in the 0-75%. I did not identify any of these indices to be significantly better; none of the binomial models for each of the indices differed significantly when explaining the 62 instances of the phrase (residual deviances of each metric modelled against 'living fossil' mentions: -1 – 168.4, ED – 115.4, pEPI – 120.8, EPI – 102.9)(see FIGURE 4.4).

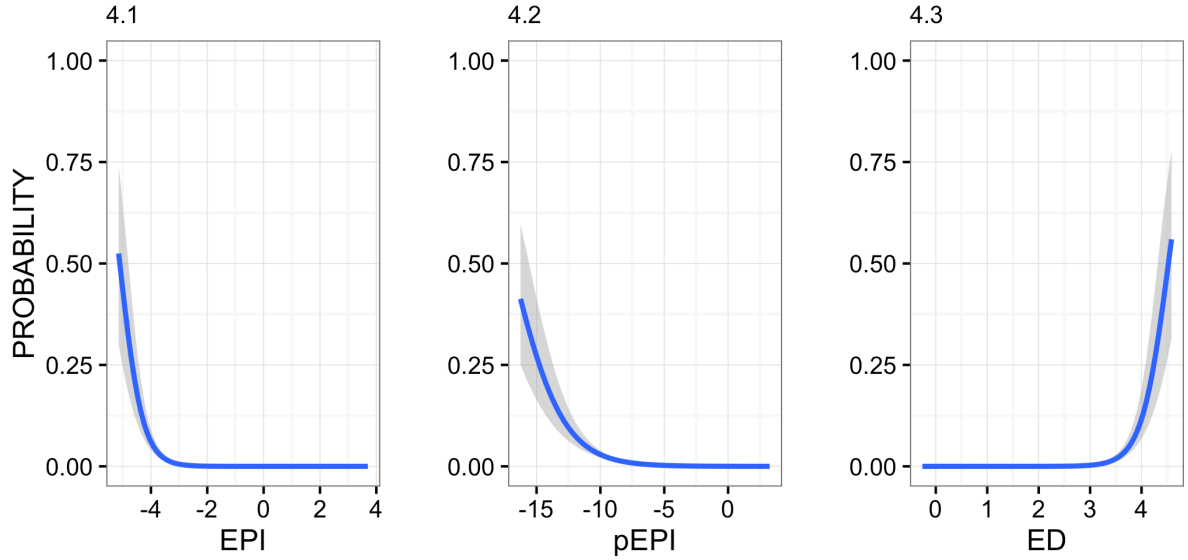


FIGURE 4.4 METRICS AND MENTIONS

Probability of the Wikipedia article for a clade containing the phrase 'living fossil(s)' against different scores for *EPI* (4.1), *pEPI* (4.2) and *ED* (4.3).

DISCUSSION

I have developed practical metrics for determining the extent to which clades can be associated with the LF concept. I have demonstrated that these indices, EPI and pEPI, correspond well to the popular conception of the LF by showing that low-scoring clades are much more likely to be described as LFs in their Wikipedia articles. Furthermore, I have demonstrated that the more easily calculated pEPI correlates well with EPI. Many well-known LF clades appeared in the top rankings for pEPI, including, among others, coelacanths (Cavin and Guinot, 2014), tadpole shrimps (Mathers et al. 2013), lancelets (Garcia-Fernàndez and Benito-Gutiérrez, 2009), lungfishes (Cavin and Kemp, 2011), limulids (Kin and Błazejowski, 2014), tuataras (Hay et al. 2008), crocodiles (Buckley et al. 2000) and the ginkgo (Royer, 2003). By better describing what constitutes a LF it will be easier to resolve conflicts over its meaning, and investigate any underlying causes that may lead to the condition.

Overlooked Living Fossils

I identified many clades that have not commonly been considered LFs, but which have low scores in these indices. These clades are mainly microscopic metazoans that split early in the evolution of eumetazoa. The top three LFs according to pEPI were the recently discovered *Limnognathia maerski*, whose likely closest relatives are the rotifers (Kristensen, 2002) from which it split 662 MYA; the amoeboid placozoa (*Trichoplax*), which is possibly an early diverging sister to all diploblasts (Syed and Schierwater, 2002; Voigt et al. 2004; Schierwater et al. 2009); and the worm-like mesozoans, which are potentially sister to all Lophotrochozoa (Suzuki et al. 2010). Although their exact phylogenetic positions and species counts have not yet been fully resolved, their ages are in the 100s of MY and their species counts are below 100; they fit most people's conception of a LF.

I also identified low scoring clades among larger bodied metazoans that have been overlooked for LF status. For example, the mountain beaver (*Aplondontia rufa*) is sister to all

rodents and had one of the lowest scores among all mammals, the hoatzin (*Opisthocomus hoazin*) is an early diverging neognath had the lowest score among birds, and the dwarf pencil fish (*Lepidogalaxias salamandroides*) was the lowest scoring teleost. These animals are not regularly cited as LFs in the most well known literature that discuss the phenomenon (e.g. Schopf, 1984; Eldredge and Stanely, 1984; Fisher, 1990), yet this analysis shows they are just as good candidates for the term as many oft-cited examples. By including previously overlooked groups such as these, future work on the common causes of the LF phenomenon will be able to sample a broader set of clades.

Taxonomic Hierarchy

A few clades that have been described as LFs that did not have low scores belonged to larger clades that did. For example, although the term is commonly applied to the cycads, I found this group to have a mid-ranging pEPI score. Nagalingum et al. (2011) argued against cycads being LFs due to recent radiations within the group, but in this analysis their score is modest as they perform comparably to their sister taxa, which include other "low performing" gymnosperm clades such as conifers, gnetophytes, and *Ginkgo*. However, the *parent* clade of these gymnosperm clades, Acrogymnospermae, has a very low pEPI score (in the lowest 2% of plants and metazoans). I hence contend that acrogymnosperms as a whole, rather than the cycads in particular, are good candidates to receive the LF label. Equally, although 'sharks' (Elasmobranchii, a grouping that includes rays) are commonly described as LFs, they had a high pEPI score due to their relatively better performance compared to their sister lineage (Holocephali - Chimaeriformes). Again the LF label for 'sharks' is not so much inappropriate as misplaced in the hierarchy; I found the parent group of both these groups, Chondrichthyes, to have a particularly low pEPI score due to it being sister to all bony fish and tetrapods (Euteleostomi). The Hula painted frog (*Latonia nigriventer*) provides another example; it was labelled a LF when first described (Biton et al. 2013), but, as a species, I did not find it among the top LFs within Amphibia. Instead, the family (Alytidae, painted frogs) *was* among the top ten.

These examples demonstrate the benefit of this larger scale approach based around monophyletic clades as it allows us to better pinpoint the full extent of the region of the tree of life that has experienced evolutionary stagnation. Indeed, this transfer of LF status to higher-level clades may explain some inconsistencies in the Wikipedia analysis. For example, Anispotera, Macroscelididae and *Solenodon paradoxus* are all described as LFs in their articles but have unexceptional EPI/pEPI scores, while their parents (Palaeoptera, Afrotheria and *Solenondon*) all have low scores, without necessarily being described as LFs themselves in Wikipedia.

Data Limits

I argue that pEPI and EPI are less prone to sampling bias compared to ED because they only require the clade and its sister to have equal levels of sampling rather than a complete species-level phylogenetic tree. Despite this, it was evident that the relative sampling of the different clades impacted the scores. Arthropods had less than three per cent of their expected number of species accounted for in the NCBI taxonomy. According to the pEPI ranking, among the top ten clades there were three lepidopteran species: *Agathiphaga queenslandensis*, *Heterobathmia pseuderiocrania* and *Andesiana lamellata*. I would not expect these Lepidoptera to have lower scores than other low diversity, early-branching ('basal') arthropod clades – such as the Diplura, Protura, Zygentoma or Limulidae. Instead, it is likely that the unexpected low scores for these species are due to the order of magnitude more DNA barcode records for Lepidoptera than for other arthropods (BOLD, 2016). Low sampling within a group is more likely to affect larger clades than smaller ones because researchers tend to sample a range of diverse groups, e.g. for phylogenetic analysis. With the amelioration of taxonomic sampling of DNA databases, I would therefore expect low-scoring clades within the under-sampled plants and arthropods to have still lower scores.

Conclusions

The performance of ED, pEPI and EPI metrics (measured against the Wikipedia-sourced measure of popular usage) is similar, but the metrics (EPI and pEPI) have many practical

advantages. By covering all six identified aspects of the LF, pEPI and EPI capture more elements of the LF concept. Additionally, they are calculable above the species-level and are easier to gather data for. pEPI does not require a fully resolved time-calibrated phylogenetic tree because estimates of success can be determined from online taxonomies, and large available datasets of 'time since split' are also now available online. EPI is more demanding of information in that it requires a sufficient number of character states and a phylogenetic tree in order to infer ancestral states. This tree, however, need not be time-calibrated nor fully resolved. Additionally, sister contrasts result in there being less need to ensure that characters selected are representative of a group as a whole; instead, they need only be comparable with the sister.

In summary, EPI and pEPI can be readily calculated from taxonomy, time since divergence and, optionally, character matrices; they provide the most reliable means available of quantifying the degree to which a clade fits the 'living fossil' concept. These metrics enable the LF concept to be investigated in more quantitative manner, and hence to provide a better grounding for investigations of the reality, significance, and potential causes of the phenomenon.

5.

Identifying Shared Ecological Characteristics of the Living Fossil

INTRODUCTION

In this penultimate chapter I tackle the question: what are the shared ecological features of these LFs that may allow them to persist?

What might the factors be that could promote evolutionary persistence? Palaeontologists have demonstrated that extinction is selective, it cannot simply be explained by 'bad luck' (Raup, 1988; Raup, 1991; Jablonski, 1995). This selectivity implies that certain groups share features that make them more resilient to extinction, a process termed 'species selection' or 'species sorting' as such features must occur at the species-level or above (Jablonski, 2008). In previous macroevolutionary and macroecological, palaeontological and neontological studies, a suite of ecological and life-history traits that occur above the level of the individual (termed 'emergent traits' [Jablonski, 2008], such as population size, geographical extent and environmental context) have been cited for influencing extinction rates and selectivity (TABLE 5.1). Previous authors often cite the unchanging habitat and a non-specialist autecology as common to LFs. No species can persist if its environment is lost (Fortey, 1981) and a generalist species will be less dependent on specific energy sources that may be lost (Eldredge, 1979; Stanley, 1998). If, however, we consider LFs as unchanging and species-poor lineages (as the metrics pEPI and EPI attempt to measure, see chapter four), we should equally consider the factors that promote speciation (TABLE 5.2) or any processes that may bring about adaptive radiations that would lead to large scale evolutionary change (Schluter, 2000). Neontologists have demonstrated that we can consider factors that influence speciation through understanding what generates and impedes gene flow and reproductive isolation. For example, assuming speciation is primarily allopatric, lineages that readily disperse and are not impeded by geographical barriers should experience lower rates of speciation than lineages that do the opposite (Kisel and Barraclough, 2010).

It can be difficult, however, to ascertain which traits and/or factors are responsible for the evolutionary success or not of a given clade. Species' shared ancestries, non-independence of traits and non-conformity of processes over different temporal and geographic scales lead to complexity that can be intractable in datasets of few species. For example, one of the best examples of documented evidence of species sorting is of the relative evolutionary success of self-incompatibility in the nightshade family (Solanaceace, Goldberg et al. 2010). Goldberg et al. (2010) discovered that although self-incompatibility led to lower speciation rates than self-compatibility, over larger temporal timescales self-incompatible species had lower extinction rates and therefore outperformed self-compatible species. This example demonstrates that temporal scale is important: what may confer an advantage over small timescales may be a disadvantage over larger timescales. Equally, it demonstrates the requirement of large datasets; such a conclusion would not be possible for a family that contained fewer species (Solanaceae consist of 1,710 species).

TABLE 5.1 FACTORS AFFECTING EXTINCTION

A non-exhaustive list of commonly proposed biological and ecological factors that influence extinction rates and risk. A suggested mechanism for how each factor can affect extinction is provided. See references for either an empirical demonstration or theoretical suggestion of the factor's role in affecting extinction.

Factor	Suggested mechanism	Reference
Population size	Species with small population sizes are more likely to go extinct than larger ones, as fewer individuals will need to die for the species to disappear.	Pimm et al. (1988), Lande (1993)
Reproductive rate	Species with slow reproductive rates are more vulnerable to extinction as their populations will take longer to rebound after catastrophic events.	Lande (1993), Bennet and Owens (1997)
Body size	Greater body size of a species will lead to greater risk of extinction as it is correlated with smaller populations sizes and slower reproductive rates.	Cardillo et al. (2005), Barnosky (2008)
Geographic range	Greater range reduces the risk of all populations becoming extinct.	Jablonski and Raup (1995), Cardillo et al. (2005)
Specialist/Generalist	Highly ecologically specialised species are more likely to become extinct, as they have more restrictive dietary or habitat requirements, and so are more vulnerable to loss of key food resources or habitats.	Eldredge (1979), Stanley (1998), Cieslak et al. (2014)
Sociality	Social species are more likely to go extinct, as they tend to require multiple individuals in a group: positive correlation between population size and average individual fitness. Populations with too few individuals are said to be 'undercrowded' and are more likely to become die-off, this is termed the Allee effect.	Courchamp et al. (1999), Muñoz-Durán (2002)
Metabolic rate	Species with low metabolic rates require less energy, meaning fewer resources to maintain larger populations.	Liow et al. (2009)
Environmental stability	A species will be less likely to go extinct if its environment remains stable through time	Fortey (1980)
Greater dispersal ability	Species that are able to move far and fast will be less likely to go extinct as they will be able to move away from damaged or threatened environments.	Marzluff and Dial (2002)

TABLE 5.2 FACTORS AFFECTING SPECIATION

A non-exhaustive list of commonly proposed biological and ecological factors that influence speciation rates. A suggested mechanism for how each factor can affect speciation is provided. See references for either empirical demonstration or theoretical suggestion of the factor's role in impacting speciation.

Factor	Description	Reference
Population size	Smaller population sizes will allow novel mutations to become fixed more rapidly in the population, potentially speeding reproductive isolation from other populations.	Templeton (1980), Stanley (1986)
Reproductive rate	Faster reproduction cycles will allow species to respond sooner to changes in their environment or niche, potentially speeding the evolution of adaptations and accelerating differences between populations.	Marzluff and Dial (2002)
Geographic range	Broader range, particularly over complex landscapes, will increase the likelihood of populations diverging through allopatric speciation.	Jackson (1974), Marzluff and Dial (2002)
Sexual selection	Mate choice can lead to increased rates of speciation as minor changes in the process of mate selection, e.g. variations in birdsong, can exclude members of a population, increasing the probability of reproductive isolation.	Darwin (1871), West-Eberhard (1983), Barraclough (1998)
Environmental heterogeneity	Complex environments will increase the likelihood of physical separation of populations, and will expose different populations to differing selection pressures.	Gavrilets and Losos (2009)
Dispersal ability	Low dispersal ability will lead to species becoming reproductively isolated at smaller distances, increasing speciation rates.	Vrba (1987); Marzluff and Dial (2002); Kisel and Barraclough (2010)

Many of the factors mooted as promoting speciation have also been suggested to decrease extinction. For example, a geographically widespread species that has multiple populations might be less likely to become extinct as all the populations would need to become extinct simultaneously (Hanski, 1998). Equally, however, such a species might be more likely to experience a speciation event as populations living in different habitats or environmental gradients experience differing selection pressures (Jackson, 1974; Stanley, 1986). Factors more commonly associated with the LF must then lower the risks of extinction without increasing the rate of speciation. For a lineage whose species are geographically widespread lower speciation rates can be achieved by high gene flow across species' populations (Barraclough 1998). Increased gene flow is produced by species whose individuals disperse widely (Kisel and Barraclough, 2010), do not perform sexual selection (Barraclough, 1998) and do not inhabit complex environments, which contain geographical barriers (Gavrilets and Losos, 2009). Any restriction in gene flow

for a widely distributed lineage, however, may turn such a species into a source for adaptive radiations, as its wide geographic range would improve its chances of encountering novel ecological opportunities (Yoder et al., 2010). Lineages that are highly specialised and confined to a geologically stable environment (Fortey, 1981) will be better placed to avoid ecological opportunities and not radiate. In this context, speciation rates could remain low through reduced dispersal ability. Equally, however, we could argue for a Goldilocks scenario for maintaining low speciation and extinction rates: not too big, not too widely distributed, etc. In short, there may be multiple ways that LFs are generated and there could in fact be no defining set of features.

In this chapter, I use the Evolutionary Performance Index (EPI) and the proximate Evolutionary Performance Index (pEPI, see chapter four) to test, which, if any, biological or ecological factors are statistically associated with LFs. I source data concerning the key life-history, ecological, habitat variables and extinction risks across all Metazoa. In my first analysis I look for factors commonly associated with low-scoring EPI and pEPI clades with datasets that cover as many taxonomic groups as possible. In my second analysis, I focus on analyses of mammals and birds as more life-history and ecological data are available. I run linear regressions between variables of the assembled mammal and bird datasets and the LF metrics using models that control for taxonomic and phylogenetic pseudo-replication.

MATERIALS AND METHODS

Life-History and Ecological Datasets

IUCN Red List

No detailed and consistent, pan-metazoan life-history and ecological datasets exist. To test what characteristics are common to LFs across the groups for which I estimated EPI and pEPI, I used the IUCN Red List (RedList, 2017). This data source not only provides the Red List categories of risk of extinction for each species, but also the number of countries in which the species occurs; a coded set of suitable habitats (RedList, 2017); and a textual description of habitat and ecology. I used the Red List API (ver. 3 IUCN, 2016) to download these data for all species for which I had EPI scores. Red List categories were quantified by setting the lowest risk of extinction as 0 and adding 1 for each ranked increase in category (e.g. "Least Concern" = 0, "Near Threatened" = 1 …), following e.g. Cardillo et al. (2005). All species that were categorised as "Data-Deficient" (DD) according to the IUCN were excluded.

Clade-Specific Datasets

More detailed life-history and ecology datasets were sourced for mammalian (Jones et al. 2009) and avian (Lislevand et al. 2007) species. The data for mammals consisted of 45 measured variables for 4,510 species covering body size measurements; reproductive and diet characteristics; habitat type, habitat extent, and habitat breadth. The data for birds consisted of 8 measured parameters for 3,801 species covering body shape and size, and reproductive behaviour. I supplemented these data by taking estimates of species' ranges from the IUCN website using an HTML 'webscraper'. The webscraper was an in-house script that downloaded the webpage of the bird species of interest, and extracted the number associated with the tag 'estimated extent of occurrence (EOO) - km^2'. In addition to these two separate datasets, I also sourced avian and mammalian categorical values

relating to lifestyle: volancy (i.e. ability to fly), fossoriality, etc. from (Healy et al. 2014).

See TABLE 5.3 for a list of all sourced data variables.

TABLE 5.3 ASSEMBLED VARIABLES

All assembled variables, groups represented, source and number of observations.

Variable	Group	Description	Source	No. obs
cate	All	Numericisation of category of extinction	RedList, 2017	11,093
nhbbts_log	All	Log of number of suitable habitats	RedList, 2017	11,018
ncntrs_log	All	Log of number of countries	RedList, 2017	11,087
iucn_range_log	Aves	Log of estimated home range	RedList, 2017	7,290
unsexed_mass_log	Aves	Log of body mass	Lislevand et al. 2007	689
Unsexed_tarsus_log	Aves	Log of length of tarsus	Lislevand et al. 2007	109
Unsexed_bill	Aves	Log of bill length	Lislevand et al. 2007	100
Unsexed_tail	Aves	Log of tail length	Lislevand et al. 2007	104
Clutch_size	Aves	Number of eggs per clutch	Lislevand et al. 2007	1,912
Egg_mass_log	Aves	Log of egg mass	Lislevand et al. 2007	2,054
Mating_System	Aves	Mating system code	Lislevand et al. 2007	1,019
Display	Aves	Display code	Lislevand et al. 2007	1,007
X1.1_ActivityCycle	Mammalia	1 (nocturnal) to 3 (diurnal)	Jones et al. 2009	1,350
X5.1_AdultBodyMass_g_log	Mammalia	Log of adult body mass	Jones et al. 2009	2,711
X8.1_AdultForearmLen_mm_log	Mammalia	Log of adult forearm length	Jones et al. 2009	608
X13.1_AdultHeadBodyLen_mm_log	Mammalia	Log of adult head body length	Jones et al. 2009	1,524
X2.1_AgeatEyeOpening_d_log	Mammalia	Log of age at first eye opening	Jones et al. 2009	315
X3.1_AgeatFirstBirth_d_log	Mammalia	Log of age of first young	Jones et al. 2009	399
X18.1_BasalMetRate_mLO2hr_log	Mammalia	Log of basal metabolic rate	Jones et al. 2009	502
X5.2_BasalMetRateMass_g_log	Mammalia	Log of basal metabolic rate by mass	Jones et al. 2009	502
X6.1_DietBreadth	Mammalia	Number of diet categories	Jones et al. 2009	1,752
X7.1_DispersalAge_d_log	Mammalia	Log of age of first dispersal	Jones et al. 2009	135
X9.1_GestationLen_d_log	Mammalia	Log of gestation length	Jones et al. 2009	1,190
X12.1_HabitatBreadth	Mammalia	Habitat breadth code	Jones et al. 2009	2,081
X22.1_HomeRange_km2_log	Mammalia	Log of home range	Jones et al. 2009	624
X22.2_HomeRange_Indiv_km2_log	Mammalia	Log of individual home range	Jones et al. 2009	558
X14.1_InterBirthInterval_d_log	Mammalia	Log of number of days between births	Jones et al. 2009	620
X15.1_LitterSize	Mammalia	Litter size	Jones et al. 2009	2,055
X16.1_LittersPerYear	Mammalia	Number of litters per year	Jones et al. 2009	777
X17.1_MaxLongevity_m_log	Mammalia	Log of maximum longevity	Jones et al. 2009	916
X5.3_NeonateBodyMass_g_log	Mammalia	Log of neonate mass	Jones et al. 2009	966

X13.2_NeonateHeadBodyLen_mm_log	Mammalia	Log of neonate head body length	Jones et al. 2009	208
X21.1_PopulationDensity_n.km2_log	Mammalia	Log of population density	Jones et al. 2009	814
X10.1_PopulationGrpSize_log	Mammalia	Log of population group size	Jones et al. 2009	336
X23.1_SexualMaturityAge_d_log	Mammalia	Log of sexual maturity	Jones et al. 2009	933
X10.2_SocialGrpSize_log	Mammalia	Log of social group size	Jones et al. 2009	580
X24.1_TeatNumber	Mammalia	Number of teats	Jones et al. 2009	512
X12.2_Terrestriality	Mammalia	Terrestriality code	Jones et al. 2009	2,000
X6.2_TrophicLevel	Mammalia	Trophic level (herbivore to carnivore)	Jones et al. 2009	1,752
X25.1_WeaningAge_d_log	Mammalia	Log of weaning age	Jones et al. 2009	1,013
X5.4_WeaningBodyMass_g_log	Mammalia	Log of body mass	Jones et al. 2009	434
X13.3_WeaningHeadBodyLen_mm	Mammalia	Log of weaning head body length	Jones et al. 2009	41
X26.1_GR_Area_km2_log	Mammalia	Geographic range (area)	Jones et al. 2009	3,046
X26.2_GR_MaxLat_dd	Mammalia	Geographic range (max. latitude)	Jones et al. 2009	3,046
X26.3_GR_MinLat_dd	Mammalia	Geographic range (min. latitude)	Jones et al. 2009	3,046
X26.4_GR_MRLat_dd	Mammalia	Geographic range (mid-range latitude)	Jones et al. 2009	3,046
X26.5_GR_MaxLong_dd	Mammalia	Geographic range (max. longitude)	Jones et al. 2009	3,046
X26.6_GR_MinLong_dd	Mammalia	Geographic range (min. longitude)	Jones et al. 2009	3,046
X26.7_GR_MRLong_dd	Mammalia	Geographic range (mid-range longitude)	Jones et al. 2009	3,046
X27.1_HuPopDen_Min_n.km2_log	Mammalia	Log of minimum human population denisty	Jones et al. 2009	1,605
X27.2_HuPopDen_Mean_n.km2_log	Mammalia	Log of mean human population density	Jones et al. 2009	3,018
X27.3_HuPopDen_5p_n.km2_log	Mammalia	Log of 5th percentile human population density	Jones et al. 2009	2,106
X27.4_HuPopDen_Change	Mammalia	Human population change	Jones et al. 2009	3,019
X28.1_Precip_Mean_mm_log	Mammalia	Log mean precipitation	Jones et al. 2009	2,993
X28.2_Temp_Mean_01degC	Mammalia	Mean temperature	Jones et al. 2009	2,993
X30.1_AET_Mean_mm	Mammalia	Mean monthly Actual Evapotranspiration Rate	Jones et al. 2009	2,907
X30.2_PET_Mean_mm	Mammalia	Mean monthly Potential Evapotranspiration Rate	Jones et al. 2009	2,907
maximum_lifespan_yr_log	Aves and Mammalia	Log of maximum longevity	Healy et al. 2014	424
mass_g_log	Aves and Mammalia	Log of mass	Healy et al. 2014	424
BMR_log	Aves and Mammalia	Log of body mass ratio	Healy et al. 2014	424
volancy	Aves and Mammalia	Volant or non-volant	Healy et al. 2014	424
fossoriallity	Aves and Mammalia	Fossorial or non-fossorial	Healy et al. 2014	424
foraging_environment	Aves and Mammalia	Code for habitat type	Healy et al. 2014	424
daily_activity	Aves and Mammalia	Code for time of activity	Healy et al. 2014	424
hermit	All	Synonym score of hermitic lifestyle	RedList, 2017	449
lethary	All	Synonym score of lethargic lifestyle	RedList, 2017	5
refugium	All	Synonym score of refugia habitat	RedList, 2017	149
specialist	All	Synonym score of specialist lifestyle	RedList, 2017	52

primitive	All	Synonym score of primitive features	RedList, 2017	10
oddness	All	Synonym score of odd organism	RedList, 2017	2

Synonyms

Given the availability of the RedList habitat and ecology descriptions for so many species I used these textual descriptions to test for possible ecological attributes that have been associated with causes for the LF condition. I constructed lists of synonyms and antonyms for each of these attributes and searched all habitat and ecology texts for their presence and absence. For example, it has been argued whether LFs are generalists or specialists. For this attribute, words relating to specialist ('specialist', 'distinct'… etc.) were searched as synonyms and words relating to generalist ('generalist', 'unspecialised' … etc.) were searched as antonyms using online dictionaries. I then created a score for each species based on whether synonyms (1), antonyms (-1) or both (0) were present. Although it is possible that these words may be used in a negative context (e.g. "the species is *not* a specialist"), I have assumed that such occurrences are relatively rare as the RedList descriptions are short. All species lacking either synonyms or antonyms were excluded from this analysis (TABLE 5.4).

TABLE 5.4 SYNONYMS AND ANTONYMS

Searching for specific meanings within the RedList habitat and ecology descriptions (RedList, 2017) with synonyms and antonyms. Synonyms and antonyms are listed with their regular expression flags (POSIX 1003.2). These flags are used to match either the beginnings and/or endings of words to prevent discovering negative word forms e.g. the pattern 'sociable' without flags would also match to words like unsociable.

Meaning	Synonyms	Antonyms	Description	Reference
Hermit	^sole, ^alone$, ^lone, ^single$, ^solitary$, ^unsociable, ^hermit, ^individual$	^group, ^pack, ^altruistic, ^convivial, ^gather, ^herd, ^flock, ^aggregate, ^swarm, ^horde, ^sociable	The taxon lives solitarily, is not social and rarely forms groups.	Courchamp et al. (1999), Muñoz-Durán (2002)
Lethargy	^lethargic$, ^inactive$, '^torpid$, ^inactivity$, ^passive$, ^languid$, $unresponsive$, ^sedate$, ^slow-moving$, ^slow-going$	^energetic, ^dynamic, ^vigou?r, ^aggressive$, ^nimble$, ^lively$, ^swift, ^fast-moving$	The taxon has a 'slow', ecology: low metabolic rate, low reproductive rates, low mobility	Vrba (1984), Liow et al. (2009)
Refugium	^refug, ^stable$, ^fossorial, ^underground$, ^static, ^dig	^dynamic, ^changing$, ^unstable$	The taxon inhabits stable, static habitats.	Alexander (1977), Fortey (1980) Norris (1992)
Specialist	^specialist$, ^unique$, ^specialised$, ^specialized$, ^idiosyncratic, ^adapted$, ^distinctive$, ^distinct, ^uncommon	^generalist, ^generic, ^unspecialised, ^unspecialized, ^opportunist$, ^opportunistic, ^omnivore$	The taxon is an ecological specialist.	Eldredge (1979), Stanley (1998)
Primitive	^primitive, ^undeveloped, ^basic, ^simple, ^rudimentary$	^developed$, ^specialised$, ^specialized$, ^adapted$, ^advanced$, ^evolved$	The taxon features 'primitive' traits.	Schopf (1984)
Odd	^odd, ^unusual, ^bizarre, ^aberrant, ^unconventional, ^peculiar, ^strange, ^atypical, ^unexpected, ^untypical, ^anomalous, ^remarkable	^standard, ^conventional, ^typical$, ^unremarkable$	The taxon has unique features not shared by other taxa.	Liow (2004, 2006), Collen et al. (2011)

Testing for Shared Ecologies of Living Fossils

Text Analyses

I used the Red List 'habitat and ecology' descriptions to test for differences between LF clades and other clades. Mean textual distances were calculated for LFs and compared to the mean text distances of null distributions of randomly selected species. Textual/string distances or similarities are numerical values generated by comparing texts to determine how similar/distant they are. Because there is no single method, I used three textual distances metrics – Levenshtein (Levenshtein, 1966), cosine (Singhal, 2001) and Jaro-Winkler (Winkler, 1990) – calculated using the `stringdist` R package (van der Loo, 2014). To calculate the mean textual distance of the LFs I selected up to 250 of the lowest scoring clades according to pEPI. With this selection, I then calculated the textual distance for each of the three distance metrics by selecting two clades at random. For clades consisting of multiple species, all the habitat and ecology descriptions were merged into a single text. Because there are too many possible pair combinations for 250 texts, I iterated the random selection of two pairs 999 times. The LF textual distance was then calculated as the mean of these 999 textual distances. The significance of these LF distance values for the three distance metrics was then determined by comparing to a null distribution of textual distances. A null distribution was generated from a random selection of the same number of species repeated 999 times (the control non-LF sample).

Secondly, I tested whether specific words occurred more often in the top LFs. For clades, word frequencies were determined using a weighting method across all descendants to account for differences in word presence. This weighting method worked by dividing the number of occurrences of a word across all descendent species' descriptions by the number of descendent species. Words of fewer than five letters or which only appeared five times across the descriptions were ignored. To determine which of these words occurred more often than expected, I compared their frequencies to

frequencies generated from a null distribution of the same number of randomly selected species repeated 999 times.

I ran these textual analyses for different taxonomic subsets (Amphibia, Aves, Lepidosauria, Mammalia, Vertebrata and Teleosteii) and null distributions were generated from all available IUCN data, not just those with pEPI scores.

Linear Regressions

Linear models were evaluated for either pEPI or EPI with each of the collated variables. Taxonomic and phylogenetic non-independence must be taken into account with models that test for associations between species' features as any statistical signal detected could be the result of shared ancestry rather than common cause (Gittleman and Purvis, 1998). For non-mammalian variables, Linear Mixed-Effects Models (LMEMs, Bates et al. 2015) were used to account for the taxonomic non-independence between species using, as random-effects, genus, family or order groupings ascertained with NCBI taxonomy (Federhen, 2012). LMEMs, as explained in chapter three, are an extension of generalised linear models but which can also take into account non-independence by estimating parameters – intercepts, slopes or both – for subsets of the data determined by a 'random-effects structure'. This random-effects structure can be used to account for taxonomic independence (Stone et al. 2011). As such, even if the values of pEPI for different taxonomic groups respond differently to a given variable, overall parameters across a dataset can be estimated. For example, birds have radiated more recently than mammals and as a consequence have higher pEPI values. A LMEM would allow for the better control of this difference in evolutionary history.

For each variable, a modelling dataset was generated by excluding all species that did not have a value for either the variable and/or the performance metric (pEPI/EPI). A series of null models of EPI or pEPI was estimated from these modelling datasets using different sets of random-effects (e.g. *pEPI~1(1|genus)*, *pEPI~1(1|family)* …) that

would model changing intercepts or slopes by taxonomy. Because the inter-relatedness of species is tiered (species of a family are closer to one another than species of an order), hierarchical taxonomic random-effects structures (e.g. *order/family/genus*) were also used. These, however, were limited to a maximum of two levels as more would not have been computable in reasonable time. The model with the lowest Akaike Information Criterion (AIC, Aikaike, 1974) was selected as the null model as this was deemed the model that explained the most variance given the evolutionary performance metric and the inter-relatedness of the species within the dataset. The variable parameters were estimated from a model using the same random-effects structure as the null and the significance of these parameters were determined by running a χ^2 test of the null and variable model. In other words, if the model with the observed variable could explain more of the variance in pEPI or EPI than the null model of just the taxonomic random-effects, then the association was determined as significant. All observed variables that were deemed non-normal upon visual inspection were logged before modelling. For habitat types and subtypes and all binomial variables, the above process was repeated but because these data are binomial and do not fit a normal error distribution the variable was instead modelled against EPI or pEPI using a generalised LMEM (Bates et al. 2015) with a binomial error structure. Because many mammal and bird species are exclusively terrestrial or marine and therefore cannot move between these habitats, I modelled terrestrial and marine habitat types separately.

Because a mammalian supertree is available, Generalised Least Squares (GLS, Pinheiro et al. 2017) regression with phylogenetic autocorrelation was used instead for mammalian variables. GLS with a correlation matrix determined using a phylogenetic tree is better at controlling for non-independence than an LMEM with taxonomic random-error structure (Symonds and Bloomberg, 2014) because the correlation matrix can take into account the closeness of species at all levels of the tree of life whereas the taxonomic random-effects structure is limited to only the taxonomic ranks specified. The

process of selecting a null model was similar to above. For every variable, a null model of pEPI or EPI with a correlation matrix based on a non-fixed Pagel's λ (Pagel, 1999) was generated; this model was compared to a model estimating parameters for a variable using the χ^2 test.

R (3.2.4) was used to run all analyses. The pipeline used to generate all results is available on GitHub (Bennett, 2017b).

RESULTS

Textual analysis

I found significantly greater textual distances for at least one of the text distance metrics between LFs and non-LFs within Aves, Teleosteii, Mammalia and Vertebrata. For the Levenshtein distance of vertebrates, however, I found LFs were significantly closer than expected (TABLE 5.5). Sampling may have impacted significance as the groups with the fewest number of available habitat and ecology description texts (Amphibia [87 texts] and Lepidosaurs [86 texts]) showed no significance (TABLE 5.5). Words with significantly greater frequencies for LFs were discovered for all taxonomic groups with the exception of Amphibia (TABLE 5.6). Although many words with significantly different frequencies were meaningless outside of the context of their usage (e.g. 'which', 'primarily', 'restricted'), others could be interpreted independently. For example, among bony fish words like 'benthopelagic' and 'demersal' occurred more frequently within LF descriptions; and 'nocturnal' occurred more frequently in the descriptions of LFs independently for lepidosaurs, mammals and vertebrates. Other words that occurred more frequently for LFs included descriptors for habitats (*inter alia* 'rocky', 'arboreal', 'grass') and life-history traits ('solitary', 'burrow'). Additionally, we may interpret significance from 'aquarium' occurring 9 times more often for LF bony fish, and 'savannah' and 'range' respectively occurring 5.5 and 1.5 times more often for LF birds (see FIGURE 5.1).

TABLE 5.5 TEXTUAL DIFFERENCES

Testing the textual differences between LFs and non-LFs. Means for textual distances for three different metrics (cosine = cs, Leveshtein = lv, Jaro-Winkler = jw) within LF (Obs.) and non-LF (Null) RedList 'habitat and ecology' descriptions for different taxonomic groups.

		Obs. mean	Null mean	Z score	P
Amphibia (87 texts)	cs	0.0368	0.0366	0.13	0.56
	lv	252.0805	241.3333	0.57	0.70
	jw	0.3125	0.3073	0.81	0.80
Aves (98 texts)	cs	0.0229	0.0278	-2.83	**0.00**
	lv	646.8776	636.3571	0.13	0.56
	jw	0.3463	0.3591	-1.35	0.09
Teleosteii (204 texts)	cs	0.0450	0.0526	-2.04	**0.01**
	lv	294.7279	340.2328	-1.72	**0.03**
	jw	0.3658	0.3848	-3.15	**0.00**
Lepidosauria (86 texts)	cs	0.0406	0.0418	-0.46	0.31
	lv	248.8605	261.9767	-0.60	0.28
	jw	0.3194	0.3290	-1.31	0.08
Mammalia (235 texts)	cs	0.0345	0.0339	0.25	0.58
	lv	353.2851	477.6596	-3.35	**0.00**
	jw	0.3487	0.3660	-2.94	**0.00**
Vertebrata (185 texts)	cs	0.0337	0.0413	-3.14	**0.00**
	lv	440.1811	369.4865	1.98	0.97
	jw	0.3619	0.3636	-0.24	0.42

TABLE 5.6 WORD FREQUENCIES

Top 25 words most commonly associated with LFs across different taxonomic groups. Word morphologies (and Z-scores) are indicated. All words that did not have a frequency greater than two among LF texts and did not have a significantly greater frequency in the LF texts were excluded. Amphibia are excluded in this table as none of the LF words met these criteria. Note that words have been manipulated to avoid plurals (e.g. "tree" or "trees" appears as "tre")

Aves	Teleosteii	Lepidosauria	Mammalia	Vertebrata
savanna (2.8)	aquarium (7.3)	under (2.2)	flannery (4.5)	nocturnal (4.5)
range (2.3)	known (5.1)	nocturnal (2.2)	speci (3.4)	which (4.5)
below (2.3)	mal (3.7)	recorded (1.6)	stream (3.4)	nest (3.8)
africa (2)	rock (3.5)	-	collected (3.2)	young (3.3)
primary (1.9)	breeding (2.8)	-	moist (3.1)	tre (3.2)
including (1.9)	level (2.8)	-	terrestrial (2.9)	insect (3.1)
year (1.8)	habitat (2.8)	-	nocturnal (2.5)	speci (3)
which (1.6)	water (2.7)	-	redford (2.5)	moist (2.7)
record (1.5)	region (2.7)	-	water (2.1)	present (2.7)
-	observed (2.7)	-	restricted (2)	forest (2.5)
-	benthopelagic (2.6)	-	specimen (1.9)	burrow (2.4)
-	prefer (2.5)	-	arboreal (1.8)	roost (2.4)
-	caught (2.1)	-	known (1.8)	large (2.3)
-	invertebrat (2)	-	modified (1.8)	probably (2.3)
-	demersal (1.9)	-	animal (1.7)	gestation (2.2)
-	occur (1.9)	-	limestone (1.6)	animal (2.2)
-	between (1.8)	-	largely (1.4)	close (2.1)
-	liv (1.8)	-	-	collected (2.1)
-	-	-	-	between (2.1)
-	-	-	-	shrub (2.1)



FIGURE 5.1 LIVING FOSSIL WORD CLOUD

Cloud of words more often associated with 'living fossil' habitat and ecology descriptions among vertebrates. Word size is determined from the log of the Z-score calculated by comparing word frequencies of LFs to non-LFs. Note that words have been manipulated to avoid plurals (e.g. "tree" or "trees" appears as "tre"). Colours are random.

Linear regressions

Risk of Extinction

I found significant but very weak positive relationships between measures of extinction risk and EPI and pEPI indicating LF species are slightly less likely to be at risk of extinction (TABLE 5.7). Although across all taxonomic groups the relationship between pEPI and extinction risk was not significant, it was significant for the separate datasets of birds and mammals. The relationship between EPI and extinction risk was also positive

and significant for datasets of mammals and birds and birds. Additionally when considering extinction risk as a binomial distribution (Numericised Category of Extinction Value > 0), low EPI species are slightly less likely to be at risk for the datasets of birds and mammals and birds. This was not the case, however, for any of the other EPI or pEPI datasets.

TABLE 5.7 EXTINCTION RISK OF LIVING FOSSILS

Category of extinction risk modelled against EPI and pEPI across different datasets as both a continuous variable (*cate*) and a binomial (*cate_bn*). Significant results are emboldened. Negative slopes (Slp) indicate greater association with the LF condition.

Group	N	X	Y	Type	Formula	Int	Slp	NULL	AIC	P
All	11062	cate	pEPI	LMEM	y ~ x + (1 | order/genus)	-5.55	0.01	31,837	31,836	.
EPI dataset	3523	cate	pEPI	LMEM	y ~ x + (1 | family/genus)	-4.54	0.03	10,564	10,563	.
EPI dataset	3523	cate	**EPI**	LMEM	y ~ x + (1 | order/genus)	-1.94	**0.03**	8,629	8,626	*
Aves	7329	cate	**pEPI**	LMEM	y ~ x + (1 | family/genus)	-4.04	**0.02**	22,089	22,087	*
Aves	1945	cate	**EPI**	LMEM	y ~ x + (1 | family/genus)	-1.49	**0.05**	5,230	5,227	*
Mammalia	2874	cate	**pEPI**	GLS	y ~ x	-4.54	**0.00**	8,650	8,654	**
Mammalia	1577	cate	EPI	GLS	y ~ x	-2.73	0.00	2,737	2,735	
All	11062	pepi	cate_bn	GLMEM	y ~ x + (1 | order/family)	-1.34	-0.01	11,919	11,919	
EPI dataset	3523	pepi	cate_bn	GLMEM	y ~ x + (1 | order/genus)	-1.35	0.04	3,704	3,704	
EPI dataset	3523	epi	**cate_bn**	GLMEM	y ~ x + (1 | order/genus)	-1.35	**0.09**	3,704	3,701	*
Aves	7329	pepi	cate_bn	GLMEM	y ~ x + (1 | order/family)	-1.53	0.00	7,215	7,216	
Aves	1945	epi	**cate_bn**	GLMEM	y ~ x + (1 | order/family)	-1.46	**0.12**	1,908	1,904	*
Mammalia	3644	pepi	cate_bn	GLMEM	y ~ x + (1 | order/genus)	-1.16	-0.01	4,549	4,551	
Mammalia	1578	epi	cate_bn	GLMEM	y ~ x + (1 | order/genus)	-1.22	0.06	1,791	1,792	

The Lonely Living Fossil

Synonym scores could be determined for relatively few species due to the absence of many synonyms or antonyms in the RedList habitat and ecology descriptions. Of the six meanings searched, only three were able to include more than 50 species with either synonyms or antonyms. For the 'hermitic' meaning I found a strong and significant relationship with pEPI for a dataset across all taxonomic groups ($m = -0.26$, $p < 0.05$),

and also for a dataset of just mammals ($m = -0.79$, $p < 0.01$), indicating that LFs – particularly among mammals – are more solitary.

Far-Ranging, Terrestrial Palaeobiotic Birds

Among the birds I found higher EPI and pEPI values were slightly associated with smaller ranges. Conversely, I also found that living fossil birds were associated with a smaller number of habitat types. Additionally, there were indications that high EPI and pEPI species were associated with greater clutch size and greater body size. Greater body size, however, was for a small dataset of individuals (105) that cannot represent the full range of LF and non-living-fossil birds. In terms of habitat, I found LF birds were less associated with arable and marine, coastal and related habitat types, while they were more likely to be found in savannah (TABLE 5.8).

TABLE 5.8 TRAITS OF LIVING FOSSILS BIRDS

Selected significant relationships between EPI and pEPI and the assembled variables and habitat types for birds. Negative slopes (Slp) indicate greater association with the LF condition.

N	X	Y	Type	Formula	Int	Slp	NULL	AIC	P
7288	iucn_range_log	pEPI	LMEM	y ~ x + (x | family/genus)	-3.81	-0.02	21,959	21,954	**
1936	iucn_range_log	EPI	LMEM	y ~ x + (1 | family/genus)	-1.02	-0.03	5,207	5,191	***
7328	nhbbts_log	pEPI	LMEM	y ~ x + (x | family/genus)	-4.09	0.05	22,072	22,069	*
1912	Clutch_size	pEPI	LMEM	y ~ x + (1 | order/genus)	-3.92	0.05	6,276	6,274	*
105	mass_g_log	pEPI	LMEM	y ~ x + (1 | order)	-4.91	0.22	415	413	*
7350	pEPI	marine	GLMEM	y ~ x + (1 | order/family)	-3.31	0.05	2,766	2,763	*
1948	EPI	marine	GLMEM	y ~ x + (1 | order/family)	-3.16	0.14	781	779	*
7211	pEPI	Sea Cliffs and Rocky Offshore Islands	GLMEM	y ~ x + (1 | family/genus)	-9.17	0.41	632	623	**
7211	pEPI	Savanna	GLMEM	y ~ x + (1 | family/genus)	-2.10	-0.04	6,941	6,939	*
7211	pEPI	Rocky Shoreline	GLMEM	y ~ x + (1 | genus)	-8.68	0.27	910	905	*
7211	pEPI	Marine Intertidal	GLMEM	y ~ x + (1 | order/family)	-3.93	0.10	1,789	1,784	**
7211	pEPI	Marine Coastal/ Supratidal	GLMEM	y ~ x + (1 | order/family)	-3.92	0.08	1,675	1,673	*
1915	EPI	Sea Cliffs and Rocky Offshore Islands	GLMEM	y ~ x + (1 | genus)	-9.33	0.63	195	192	*
1915	EPI	Marine Coastal/Supratidal	GLMEM	y ~ x + (1 | order/family)	-3.67	0.29	444	437	**
1915	EPI	Arable Land	GLMEM	y ~ x + (1 | family/genus)	-1.94	0.17	1,781	1,774	**

Nocturnal, Long-Lived Palaeobiotic Mammals

Although through the use of GLS models I found no significant relationships between any of the assembled variables and pEPI, there were many significant relationships with EPI, which unlike pEPI takes into account 'change' as well as the other aspects of the LF (see chapter four). LF mammals tended to have longer lifespans, greater diet breadth and were more likely to be nocturnal (low activity cycle values indicate greater activity at night time) (TABLE 5.9). Although through the synonyms analysis I found LFs tended to be more solitary and that in particular mammalian LFs tended to be more hermitic, the relationship between EPI and social group size was not significant. Finally, in terms of habitat, the only habitat type that showed significance was the 'Subtropical/Tropical Dry Shrubland'. This was a weak positive relationship, indicating LF mammals are only slightly less likely to occur there.

TABLE 5.9 TRAITS OF LIVING FOSSILS MAMMALS

Selected significant relationships between EPI and pEPI and the assembled variables and habitat types for mammals. Negative slopes (Slp) indicate greater association with the LF condition.

N	X	Y	Type	Formula	Int	Slp	NULL	AIC	P
619	X22.1_HomeRange_km2_log	pEPI	GLS	y~x	-4.11	0.02	2,073	2,078	**
2068	X12.1_HabitatBreadth	pEPI	GLS	y~x	-4.47	0.01	6,379	6,389	***
1681	X27.4_HuPopDen_Change	EPI	GLS	y~x	-2.72	0.40	2,862	2,864	*
410	X17.1_MaxLongevity_m_log	EPI	GLS	y~x	-2.28	-0.08	969	978	***
395	X16.1_LittersPerYear	EPI	GLS	y~x	-2.74	0.09	843	863	***
812	X6.1_DietBreadth	EPI	GLS	y~x	-2.60	-0.04	1,712	1,726	***
711	X1.1_ActivityCycle	EPI	GLS	y~x	-2.84	0.11	1,402	1,404	*
274	X10.2_SocialGrpSize_log	EPI	GLS	y~x	-2.50	-0.13	709	711	.
3741	pEPI	Subtropical/Tropical Dry Shrubland	GLMEM	y ~ x + (1 | family/genus)	-2.03	0.06	3,012	3,010	*

DISCUSSION

The analyses presented here demonstrate that several ecological factors are associated with the LF. Many of these factors, however, were not strongly linked to EPI or pEPI. Across different taxonomic groups and species grouped by common environments (marine and terrestrial), different ecologies, biologies and habitats were identified as either positively or negatively associated with the LF. No *single* set of factors could be identified to explain the 'secret' across all LFs. I therefore argue that the ways in which LFs become panchronic are different for each lineage, as they are not shared across different groups and within groups they are only weakly associated with either EPI or pEPI. For some factors, however, I argue that a lack of signal is an indication of a tendency towards the average, the Goldilocks principle. For example, large-bodied vertebrates have a much greater risk of extinction due to their smaller population sizes (Bennett and Owens, 1997; Cardillo et al. 2005; Barnosky 2008) and small-bodied animals may have a higher chance of speciation due to their tendency to have higher rates of reproduction and an increased likelihood of becoming reproductively isolated over smaller geographical distances (Gavrilets and Losos, 2009). Factors, such as body mass, which have positively co-varying impacts on speciation and extinction, should tend towards the mean for LFs. We should therefore expect no linear association between body size and living-fossil-ness: this was indeed found to be the case for mammals, and while birds did show such an association, the number of observations was small.

Across All Groups

For many taxonomic groups there were significant differences between the habitat and ecology preferences of LFs and non-living-fossils. To perform an analysis that was as taxonomically representative as possible, I was limited to performing a textual analysis

based on IUCN data. With such an analysis, it is difficult to gauge the level and the exact factors responsible for any differences detected. Furthermore, the methods employed do not take into account any phylogenetic non-independence. Despite this, certain words that occurred at different frequencies in LF and non-living-fossil descriptions stood out. The high frequency of 'benthopelagic' and 'demersal' in Teleosteii indicates that there is an aspect of the seabed environment that permits the evolution of LFs. Coelacanths (*Latimeria*), which are not members of the Teleosteii, are also benthopelagic. Equally, words like 'burrow' and 'nocturnal' occurred more often for living-fossil vertebrates. Such words are in keeping with the thinking that species that burrow, hibernate and come out only at night ('sleep-or-hide' traits) have lower risks of extinction (Liow et al. 2009). Additionally, many of the words discovered to have greater frequency in LF descriptions are congruous with the results of the linear regression analyses: for example, 'solitary' implying that LFs are not social, or 'savannah' being more commonly associated with LF birds.

Specialist/Generalist

Factors that would be expected to have strong relationships with EPI and/or pEPI turned out not to have statistically detectable relationships. For example, there is an argument over whether LFs should be generalists or specialists (Eldredge, 1979; Stanley, 1998; Cieslack et al. 2012). Results from the synonym/antonym analysis for 'specialist/generalist' were inconclusive, however, due to the low frequency of synonyms/antonyms in the habitat and ecology descriptions. If LFs were highly specialist or generalist species, we would also predict that they would show positive or negative relationships with the 'number of habitats' variable. This was not the case; no significant relationships between the number of suitable habitats for a species and its pEPI or EPI scores were found for any taxonomic group, with the exception of a weak positive relationship in birds. Equally, although I found an association between diet

breadth and EPI – indicating that LF mammals may be more generalist – this association was very weak.

Additionally, we may consider habitat type, as different habitats may be better suited to generalists or specialists. Non-living-fossil birds and mammals were, respectively, more often associated with arable environments and areas where human density population has increased. These human dominated landscapes may favour the generalist species that are able to expand their ranges readily and exploit a range of food sources (McKinsey et al. 1999; Fried et al. 2010; Clavel et al. 2011). Although we might, then, consider non-living-fossils to be generalists, this does not imply LFs are specialist.

Taking these results together, it cannot be said that LFs fit well into either of the specialist or generalist categories. It should, however, be noted that the generalist/specialist division is not strict. For example, Vrba (1984) described how the aardvark (*Orycteropus afer*) could persist for extended periods of geological time even though it is a specialist in terms of its food source – termites. Vrba (1984) argued that it was in fact a generalist in the sense of its environment as termites are available across multiple environment types, a phenomenon which she described as 'persistent-patch-specialisation'.

Environments for the Living Fossil

For LF mammals and birds there were negative associations, respectively, with subtropical/tropical dry shrubland and marine or coastal habitats. Both habitats are interface habitats connected with other habitats, e.g. land/sea or arboreal/grassland. In this way, the two habitats may act as a source of future speciation events. This may be particularly the case for coastal habitats that, through their linearity, have a higher probability of separating populations thereby increasing the likelihood of speciation (Gavrilets and Losos, 2009). Savannah was the only habitat type to be significantly more associated with LFs, in this case LF birds. Savannah is also an interface habitat between

grasslands and forest. Unlike marine or coastal habitats however savannah is not a linear habitat and if species forage throughout the environment then reproductive isolation should be less likely to occur (Gavrilets and Losos, 2009). Why savannah as a non-linear habitat should be particularly more associated with LF birds remains to be explored. This result appears contradictory with work that compared the diversification success of lineages based their habitat done on mammals. For example, Vrba (1984) suggested that the bovid tribe Alcelaphini (blesbuck-hartebeest-wildebeest group) comprises more species than the sister group the Aepycerotini (impalas) because the latter inhabits grasslands with greater wood coverage. Vrba suggested the greater number of species for the Alcelaphini is due to the spread and contraction of the grasslands of Africa over recent evolutionarily time, while the Aepycerotini maintained large populations in the relatively more stable wooded habitats which reduced their chances of geographic separation and promoted stabilising selection. For birds, however, the growth and contractions in the savannah/grassland habitat has clearly not led to an increase in diversification rates, perhaps this is due to their greater ability than mammals to maintain large contiguous populations over fragmented habitats thanks to their greater dispersal ability.

Are Living Fossils Threatened with Extinction?

LFs are less likely to be at risk of extinction (see 'Risk of Extinction' above). This is compatible with previous analyses of linkages between evolutionary distinctness and extinction (Verde Arregoitia et al. 2013), as well as reports of the resilience of specific LFs in the face of severe threats (Pereira et al. 2006; Franco et al. 2011; Turvey et al. *in press*). Nonetheless, the association between extinction risk and living-fossil-ness in the current study is weak, and its strength differed between taxonomic groups – it was strongest for birds. There are also many issues with the extinction-risk metric. It derives from a coarse qualitative measure (Cardillo et al. 2005); the increase in extinction risk

from 'Least Concern' to 'Critically Endangered' is unlikely to be consistent across species, and even less likely to be linear (Cardillo et al. 2005). Extinction risk also acts at the local level; species may have ecologies and/or biologies that lead to a greater risk of extinction (e.g. large body size, slow reproductive rates), but not be at risk in their local or regional environment due to absence of usually human-induced threats (Davies et al. 2008), which should therefore only be interpreted as the potential risk of extinction at a global level for today's current distribution of threats. These may not be typical of either 'background' extinction across evolutionary time or of non-anthropogenic mass extinction events, where causes of extinction and correlates of survival may change (Jablonski and Raup, 1995).

Living Fossil Mammals

The results suggest that generally the shared features of the LF mammal are (1) being solitary, (2) having a slow reproductive rate, (3) living a long time, and (4) being active mostly at night. Taking a few examples of LF mammals, we can see the majority of these features exemplified. Mountain beavers (*Aplodontia rufa*, pEPI = -11.6) have low rates of reproduction; they are more active at night, are usually solitary and can live up to five or six years (Carraway and Verts, 1993; Epple et al. 1993) or even up to ten years (Macdonald, 1985). (The median maximum lifespan of rodents is approximately 5.1 years [Jones et al. 2009].) Springhares (*Pedetes capensis*, pEPI = -11.6) are also nocturnal but often forage in groups of two to six (RedList, 2017). Aardvarks (*Orycteropus afer*, pEPI = -9.2) dig burrows, are generally nocturnal and are solitary (RedList, 2017).

Species that are able to survive as solitary individuals are less prone to extinction due to their ability to persist as smaller populations (Muñoz-Durán, 2002). I found no significant relationship between living-fossil-ness and the number of individuals in a social group. The only indication that solitariness is an important factor for the LF was through the 'hermit' synonym analysis. The lack of correlation with social group size

may be due to species often living in groups but also being able to survive as individuals, i.e. they are not obligate socialists. For example, lesser short-tailed bats (*Mystacina tuberculata*, pEPI = -11.0) usually roost in communal groups, but they can also roost as individuals (Sedgeley, 2006).

As it is well known that slow rates of an individual's senescence is a consequence of a species' low extrinsic mortality (Williams 1957; Healy et al. 2014), the long lifespans of LFs indicate that they experience reduced mortality rates compared to non-living-fossils. Provided there is not a proportionate drop in reproductive rate, as indicated by the association of LF mammals with smaller litter sizes, the longer lifespans of LFs may be associated with a lower risk of extinction, as it would provide more opportunity for an individual to procreate. Furthermore, lower mortality rates may indicate that the environment and niche in which they inhabit are more stable over not just short but also long timescales.

Finally, LF mammals tend to be nocturnal. This corroborates the finding by Liow et al. (2009) that 'sleep-or-hide' mammals tend to have lower risks of extinction. Nocturnal species may have lower risks of extinction because they are able to move more readily through fragmented landscapes than diurnal animals (Daily and Ehrlich, 1996). It should be noted, however, that the majority of terrestrial animals are nocturnal, including, it is suggested, many dinosaur species (Schmitz and Motani et al. 2011). In what way then could nocturnality be suggested as a uniquely LF trait? Healy et al. (2014) suggested that there are multiple reasons for species to become nocturnal, for example heat avoidance in tropical areas, better access to nocturnal prey or better avoidance of predators. The longer lifespans – and hence lower extrinsic mortality rates – of LF mammals suggests that the latter reason may apply here.

Living Fossil Birds

LF birds were typified by their slightly wider ranges, low reproductive rates as measured by the number of eggs in a clutch and their disassociation with the sea. Birds are able to fly and disperse widely, so a wide range may correlate with broader breeding areas, reducing speciation (Kisel and Barraclough, 2009). Lower reproductive rates, as for mammals, are associated with lower speciation rates (Marzluff and Dial, 2002) but also higher risk of extinction (Bennett and Owens, 1997). Although lower reproductive rates may increase extinction risk, lineages that a geographically widespread with higher reproductive rates would be much more likely to speciate and/or undergo an adaptive radiation (Schluter, 2000). We should, however, expect the strength and types of associations between EPI and pEPI to be different for birds and mammals. Aside from experiencing different ecological pressures and life-history traits, both clades have different evolutionary performances; pEPI values for Aves and Mammalia are respectively 0.37 and -6.8. LF birds will not be as low-scoring as LF mammals, and as such direct comparisons between the two cannot be made due to this non-equivalence.

Detecting Traits that Drive Speciation and Extinction

As discussed above, the processes that generate low evolutionary performance are patterned by taxonomy and ecology, and to investigate further correlates of living-fossil-ness these factors need to be controlled for. In this analysis I separated the analyses of birds and mammals, providing a crude form of control. A future study could separate analyses further, by such factors as body size, terrestriality or range size. For example, Healy et al. (2014) explored the life-history traits that influence vertebrate lifespan. Given the large number of studies that demonstrate volancy is the single most important factor, however, these authors separated their analyses between volant and non-volant taxa. Cardillo et al. (2005) found that the most important factor in determining species risk to extinction for mammals was body size. For this reason they similarly modelled large-

and small-bodied mammals separately, and identified different factors contributed to extinction risk for the two sets. The different pathways of extinction risk was also demonstrated by Davidson et al. (2009). These authors used a machine-learning approach that revealed how different combinations of life-history and habitat produced different predictions of extinction risk in mammals. 'Living-fossil-ness' conflates speciation and extinction rates, while the studies discussed above only considered extinction; a future analysis, however, could use a similar approach to see if there are any common factor combinations for LFs. More simply, a future analysis could test for non-linear interactions between any observed variable and EPI/pEPI, although power of analysis, unit of observation and non-independence of observable variables, will constrain all such analyses. In this study I have controlled for ancestral differences, but this control necessarily reduces the statistical power of analysis. For example, I found (as expected) that volant species have much higher EPI and pEPI values, yet this difference was not significant. This was due to the control for taxonomic non-independence for the shared ancestry of volant species; the presence of two volant groups – birds and bats – greatly reduced statistical power. Additionally, in the linear regressions, analyses were limited to the species level. This reduces the size of the dataset and thus the ability to detect signal. One solution would be to include clades as well as species. Doing so, however, would require the approximation of values of variables for clades from species-level values. This could be achieved through direct fossil observation, ancestral character estimation, or simply calculating the average from descendant species' values; any such method, however, would be contentious. Finally, it is well established that many observed variables co-vary, e.g. body mass and geographic range (Davies et al. 2008). Any correlation between EPI or pEPI with a trait may thus in fact be due to a causal link with an unmeasured correlate. Any future analysis must therefore also establish independent factors to test for association with the LF. This inevitably requires *a priori* knowledge that may preclude the discovery of unexpected results.

Conclusions

Despite the limitations in the results presented in this chapter and analyses of this kind, some broad conclusions about the shared ecological characteristics of LFs can be drawn. Although I highlight that different groups and subgroups can arrive at a LF-ness through different pathways, there is a common element to these pathways i.e. a tendency for low speciation-rates to be more important than low extinction-rates. For both LF mammals and birds there has been a tendency towards lower rates of reproduction. Lower reproductive rates reduce the probability of a species speciating while not reducing its extinction risk. When considering the reasons why particular species or clades are 'living fossils', we should hence at least be as aware of their avoidance of ecological opportunity as we are of their ability to evade extinction.

6.

Coding the Living Fossil

OVERVIEW

All the analyses presented in this thesis have been programmed in the R statistical environment (R Core Team, 2016). R allows for users to create, modify and run various statistics on phylogenies, however, it does lack in some important respects: manipulation of the phylogeny (such as adding new tips as I did in chapters two and three) and ready calculation of many statistical, phylogenetic metrics (such as evolutionary distinctness as I did in chapter two). As a result, as part of my PhD I generated many new R functions and classes with which I have created two packages: `MoreTreeTools` and `treeman`. The former is a repository available via the website GitHub (Bennett, 2016c) containing a series of useful functions that I have informally developed during the course of my PhD and upon which much of my work depends. The second is a formal R package available via CRAN (Bennett, 2017c) and published in BMC Research Notes (Bennett et al. 2017b). The two packages, however, are not independent. Many of the ideas I developed during the development of `MoreTreeTools` I incorporated into `treeman`. In this chapter I will outline the purpose, functioning and basis of the `treeman` package as presented in Bennett et al. 2017. I will then describe how the development of `MoreTreeTools` led to `treeman`, as well as highlighting additional functions unique to `MoreTreeTools`. All usage examples and code snippets were produced with `treeman` (v1.0.0) and `MoreTreeTools` (edbmm-branch).

TREEMAN

Introduction

Phylogenetic trees have been a mainstay of the R statistical software environment since the release of Emmanuel Paradis' `ape` package in 2002 (Paradis et al. 2016; Paradis et al. 2002). This package introduced the `phylo` object, an S3 class for the presentation and manipulation of phylogenetic tree data in the R environment. In its most basic implementation, the `phylo` object contains a list of three elements: an edge matrix, a vector of tip labels and an integer of the number of internal nodes. The use of an edge matrix facilitates phylogenetically structured statistical analyses because of its convenience for generating distance, cophenetic or covariance matrices. For this reason the `ape` package's `phylo` is the dominant class for phylogenetic tree representation in R and is used by many well-known phylogenetic R packages e.g. `phangorn` (Schliep, 2011), `phytools` (Revell, 2012). Since `phylo`'s first incarnation the number of available functions in the `ape` package has risen from 28 to 171 (versions 0.1 to 3.4), and to date there are 147 reverse dependencies, i.e. packages on CRAN that depend on the Alternatives to `phylo` class. More recently, the `phylo` class has been updated to S4 as part of the `phylobase` package (Michonneau, 2016).

An edge matrix, however, leads to a dependence on index referencing, leading to certain computational scenarios in which the `phylo` object performs poorly: in particular, analyses that require the manipulation of the tree itself (i.e. tip and node addition/deletion). Such analyses include simulating, comparing, pruning, and merging trees, and calculating phylogenetic statistics such as measures of phylogenetic richness (Faith, 1992) and evolutionary distinctness (Isaac et al. 2007). These have become the preserve of software solutions external to R (e.g. Smith and Dunn, 2008; Bogdanowicz et al. 2012), hindering their integration with the many packages in biomolecular,

evolutionary and ecological packages already available for R. Although there are alternatives to the `phylo` class for phylogenetics or more generally 'networks' available in R (e.g. Csardi, 2016), these packages and classes are rarely used for phylogenetics and may lack the intuitive functional framework for manipulating evolutionary trees.

Here I present the new phylogenetic tree manipulation class '`TreeMan`' (see FIGURE 6.1 for an overview); this is presented as the R package '`treeman`' (N.B. the package name is all lowercase). This class is built around a list of named nodes rather than an index-based edge matrix as is the case for the `phylo` class. Using an edge matrix, whenever a node is added or removed the new positions of all nodes in the matrix must be determined and the tree must be re-computed. With a node list, however, order does not matter; nodes can be added and removed without altering the entire tree structure. Manipulations are also less dependent on tree size because all that is required is to update the local nodes: those that directly descend or ascend from the new node, converting that scale of computation time from $O(N^2)$ to $O(N)$ (see FIGURE 6.2 for a comparison of growing a tree with the `phylo` and `TreeMan` classes). Furthermore, with a node list the nodes in the tree can have unique IDs, which persist after insertions or deletions, allowing elements in a tree (such as node labels) to be more easily tracked during analysis. Below, I describe the overall structure of the new class, I describe `treeman`'s naming convention, and I provide examples of tree manipulations that use the new package. The aims of `treeman` are to be conceptually intuitive for tree manipulation and as computationally efficient as possible within the R environment.

Implementation

The `TreeMan` object in R is an S4 formal class whose main data slot is a list – which in R is a vector whose elements can be named. All nodes in a `TreeMan` object are named

elements in this list (ndlst). Each node usually contains the following data slots: the node ID (id), the length of the preceding edge (spn, for 'span'), the IDs of all connecting ascending/ancestral nodes to root (pre-node IDs, prid), the IDs of the immediately descending nodes (post-node IDs, ptid), and the IDs of all descending tips (kids). Additionally, if all nodes in a tree contain the spn slot, then each node will also contain: the total edge length of all descending nodes (phylogenetic diversity, pd), total edge length of all connected pre-nodes (prdst; in a rooted tree this is the root-to-tip distance), and the relative distance of the node in the tree (age, for a time-calibrated rooted tree). All nodes must have either a prid and/or ptid data slots: tip nodes have only prid slots, root nodes have only ptid slots, and internal nodes have both. These slots must contain IDs that are found within the ndlst; if they do not, an error is raised. These core slots are supplemented by optional slots, a non-unique taxonomic name that can be used to generate lineages (txnym) and user-defined slots that can contain any kind of information. In addition to the ndlst, the `TreeMan` object contains informative slots that are generated upon reading or generating the tree, and are updated whenever modified. Basic tree information can be seen by printing the tree to console.

The `treeman` package implements an intuitive naming convention in which each tree feature has a specific name that all methods and objects must use (see FIGURE 6.1 and TABLE 6.1). Specific tree or node information can be accessed using R indexing by character-string. For example, entering tree["tips"] will output data on a tree's tips. Double square brackets are used for pulling out information on individual nodes, e.g. tree[['t1']] will return node information on tip/node t1. The majority of methods in the `treeman` package are grouped into four main classes: get, set, calc and manip (see TABLE 6.1). The get methods return node or tree specific information, set methods change the tree's or its nodes' parameters, calc methods generate tree statistics, and manip methods alter the tree, usually by adding or removing tips and nodes. Methods that act across nodes are indicated with 'nd' or 'nds' in their function name, e.g.

`getNdAge`() for a single node and `getNdsAge`() for multiple nodes. These core methods are designed to be fast and modular, allowing them to be readily combined into more complex functions. For example, evolutionary distinctness using the Fair Proportion metric (Isaac et al. 2007) can be calculated by using the method `calcFrPrp`(). In implementation, this method uses `getNdPrid`() to get all pre-node IDs, runs `getNdsKids`() on these IDs to find the number of descendants per pre-node, and then sums the division of the numbers of descendants over the pre-nodes' spans.

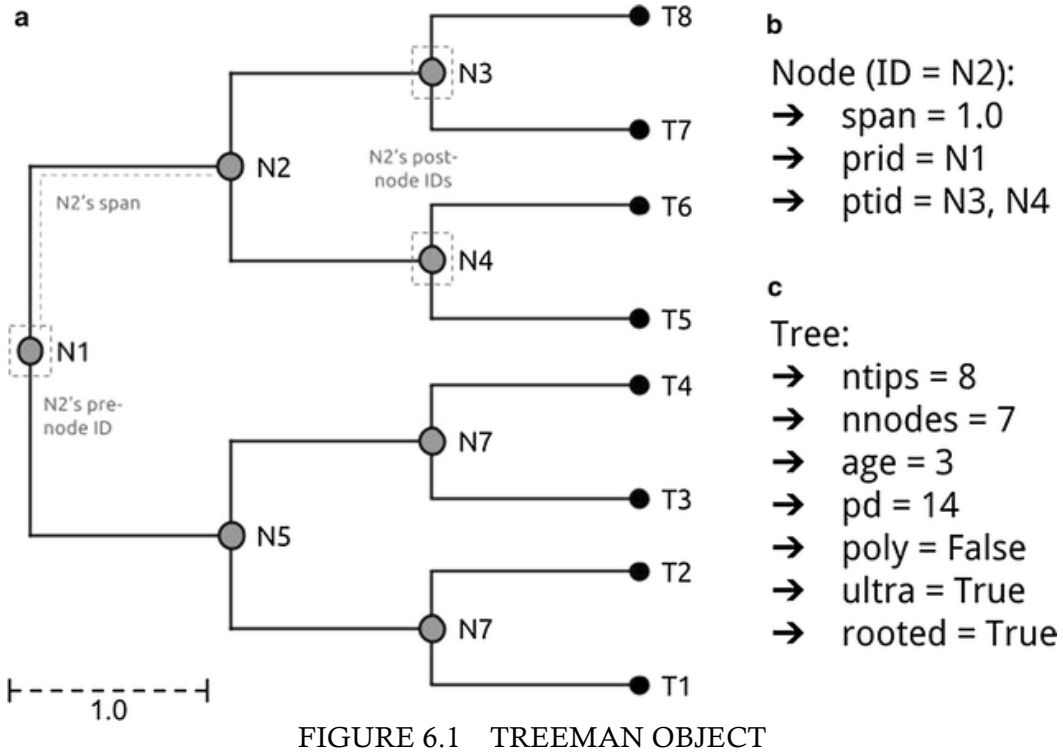


FIGURE 6.1 TREEMAN OBJECT

Representation of simple tree as `TreeMan` object: A. eight-tipped tree with node and tip IDs annotated and N2's key slots identified, B. representation of printed Node information for N2, C. representation of printed `TreeMan` information.

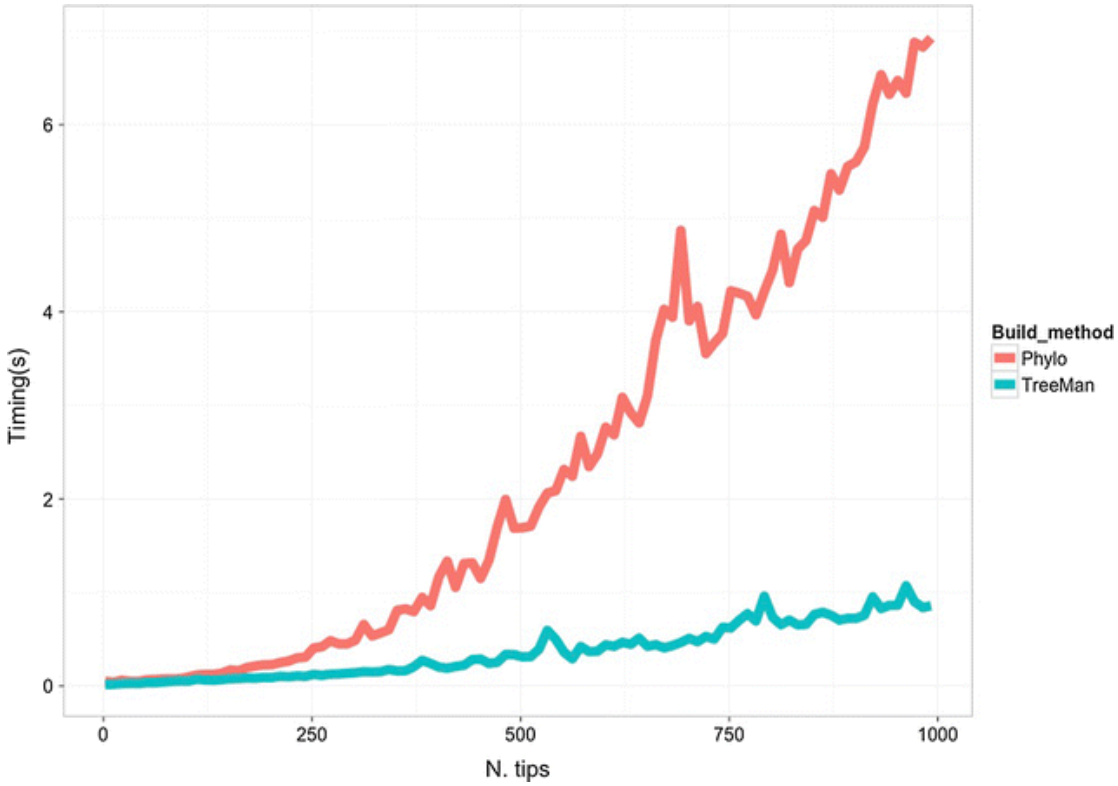


FIGURE 6.2 PHYLO AND TREEMAN COMPARED

Comparison of tree building using `ape`'s `phylo` and `treeman`'s `TreeMan` classes. Starting with trees of two tips, 1000 new tips were added to the trees and the time taken to run the process was recorded every ten tips. The rate of increase in time taken for larger trees increases faster for the `phylo` class than `TreeMan`.

Because the `TreeMan` class depends on the ndlst, all functions that run over this list are vectorised. All `treeman` methods that can be vectorised are done so using `plyr` vectorisation (Wickham, 2011), providing substantial performance benefits, as computation is no longer taking place at the scripting level. Through the use of `plyr` these functions can also be parallelised using the '.parallel' argument that is passed onto `plyr` functions, which work in conjunction with parallel R packages such as `DoMC` (Calaway and Weston, 2015) and `doSNOW` (Calaway and Weston, 2015).

Usage Examples

To demonstrate the `TreeMan` class and how its methods can be combined to complete complex tasks, I demonstrate three use-cases: pinning missing taxa using online taxonomic databases to a molecular phylogenetic tree; simulating phylogenetic trees through time using different models of evolution; and testing for significant phylogenetic turnover between ecological communities.

Tip Pinning: Adding Missing Taxa to a Tree using Online Taxonomies

Trees often have missing tips due to a lack of data for phylogenetic construction. One approach to placing these tips is to use the taxonomy of the missing taxa and constraining placement with a model of evolution, as implemented by the `PASTIS` R package (Thomas et al. 2013). Similar methods can be implemented in R using `treeman`. `TreeMan` provides an `addTip`() function that takes as arguments the incipient edge and an age range. Furthermore, `TreeMan` objects can be taxonomically informed: taxonomic names (txnyms) can be assigned to every node in a tree, allowing a user to constrain random placement of nodes. To demonstrate this functionality I present the mammalian supertree (Bininda-Edmonds et al. 2007) that has been taxonomically informed using NCBI's taxonomy (Federhen, 2012). I retrieved all the species names listed in NCBI but not present in the supertree, and pinned an example set of 100 new tips to the supertree. New tips were added to the supertree at any point in the branches that shared the lowest matching taxonomic rank with the taxonomy of the new tip (FIGURE 6.3). This was implemented with `pinTips`(), a function of 49 lines. The equivalent function using a `phylo` object is approximately 500 lines (see 'pinning-with-phylo.R' in 'chapter_6.zip').

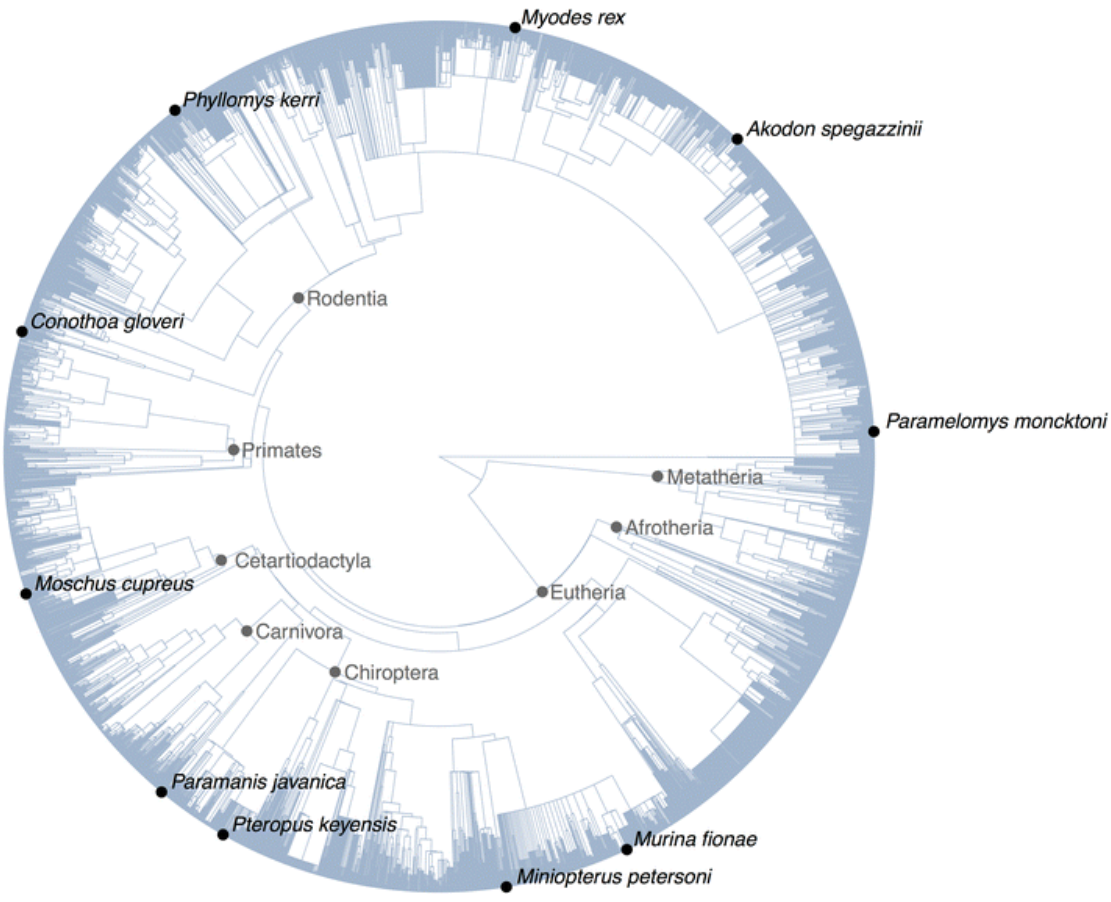
FIGURE 6.3 PINNING TIPS TO A TREE

```
# DATA
data(mammals)  # example mammal tree is 'taxonomically informed', all nodes have taxonyms
# rslvd names (i.e. with lineages) not in mammals tree generated with
# MoreTreeTools::taxaResolve load pre-generated dataset from github
load(url("https://github.com/DomBennett/treeman/raw/master/other/1_pinning.RData"))

# PARAMETERS
n <- 100  # number of missing mammal species to pin

# PIN
rnds <- sample(1:nrow(rslvd_mammals), n)
rslvd_mammals <- rslvd_mammals[rnds, ]
lngs <- plyr::mlply(rslvd_mammals, function(lineage, ...) strsplit(lineage, '\\|')[[1]])
tids <- gsub("\\s+", "_", rslvd_mammals$search_name)  # always replace spaces with _
ends <- rep(0, length(tids))  # all tips end in the present
pinned_tree <- pinTips(tree=mammals, lngs=lngs, tids=tids, ends=ends)
p_added <- sum(tids %in% pinned_tree['tips'])*100/n
cat('[', p_added, '%] of n pinned to mammals\n', sep='')
```

A. Code snippet used to generated "pinned" tree: load mammal supertree that comes with the `treeman` package, load resolved names of missing taxa pre-generated with `MoreTreeTools`, select 100 names at random, pin using `pinTips()`.



B. Mammalian supertree showing ten tips that have been "pinned" using taxonomically constrained random placement.

Tree Simulation: Generating Trees Using Different Models of Evolution

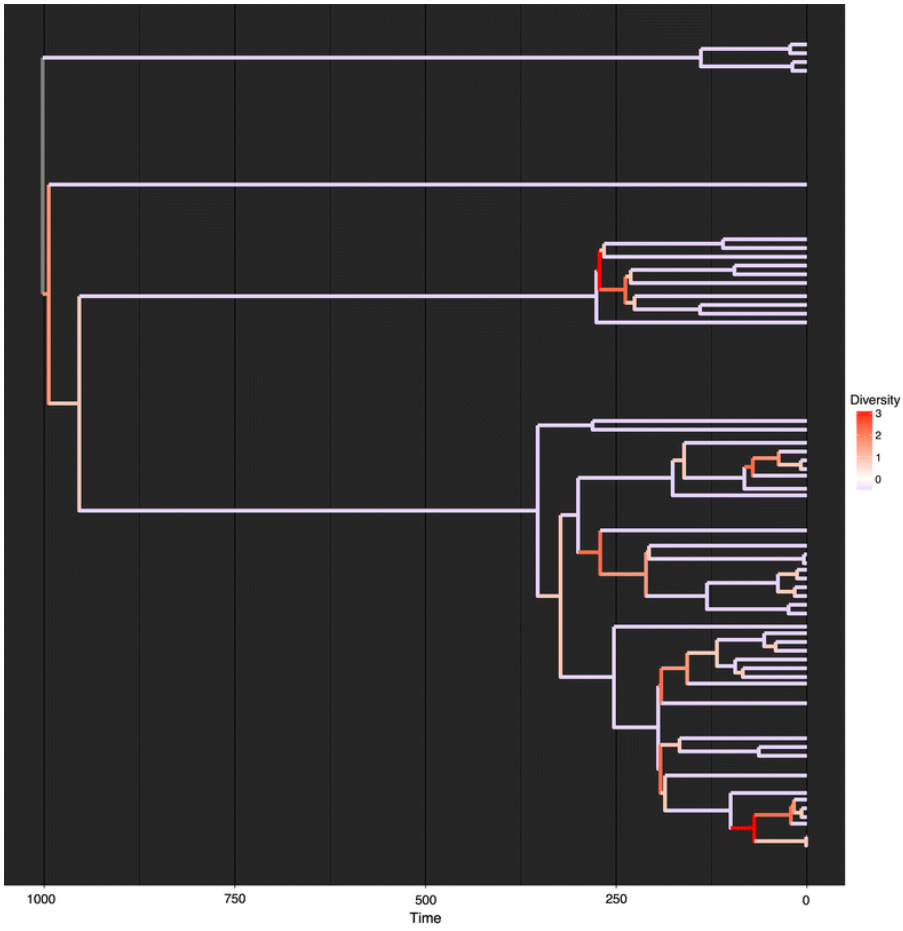
Tree simulation is an important tool for exploring the processes that may have generated biodiversity. A common tool for simulating trees is the birth-death simulation or equals-rates Markov model (ERMM) that randomly removes and adds tips on a tree (Mooers and Heard, 1997). Many publications – and as I did in chapter two – have independently explored alterations of the ERMM, such as modifying the rates of speciation for different tips in the tree (Purvis et al. 2011; Hagen et al. 2015; Rabosky and Goldberg, 2015; Bennett et al. 2017). All such studies, however, have developed their own software tools for tree simulation. The `treeman` object is easily modified using the `addTip`() and `rmTip`() functions and its speed of processing and implementation makes `TreeMan` an ideal software tool for tree simulation. Furthermore, as part of the R environment a user has access to the wide range of eco-evolutionary R packages already available to expand on these earlier tree simulations. To demonstrate this functionality, I produced an R script for simulating a tree using an Evolutionary Distinctness Biased Markov Model (EDBMM) with fewer than 40 lines of R code. The simulation randomly adds and removes tips but with a bias towards evolutionary distinctness: more evolutionarily distinct tips have a lower rate of speciation and extinction (FIGURE 6.4). The equivalent script for running a vectorised EDBMM with a `phylo` object takes 188 lines (see 'edbmm-with-phylo.R' in 'chapter_6.zip').

FIGURE 6.4 SIMULATING TREE-GROWTH

```
# PARAMETERS
# balanced tree to start
tree_string <- "((A:1.0,B:1.0):1.0,(C:1.0,D:1.0):1.0);"
tree <- readTree(text=tree_string)
iterations <- 200
burnin <- 10
d <- 1
b <- 2  # birth for burnin
b_true <- 1  # birth after burnin
ext <- tree["tips"]

# LOOP
for(i in 1:iterations) {
  if(length(ext) < 3) {
    stop('Too few tips remaining!')
  }
  if(i > burnin) {
    b <- b_true
  }
  cat('.... i=[', i, ']\n', sep='')
  # calculate fair proportion
  fps <- calcFrPrp(tree, ext)
  # add/remove based on b and d
  to_add <- sample(c(TRUE, FALSE), size=1, prob=c(b,d))
  if(to_add) {
    sid <- sample(ext, prob=1/fps, size=1)  # sister ID of the new tip
    tid <- paste0('t', i)  # new tip ID
    tree <- treeman::addTip(tree, tid=tid, sid=sid, start=0, end=0)
  } else {
    tid <- sample(ext, prob=1/fps, size=1)
    tree <- rmTip(tree, tid=tid)
  }
  # grow tree
  ext <- tree['tips']
  spns <- getNdsSlt(tree, slt_nm="spn", ids=ext)
  tree <- setNdsSpn(tree, ids=ext, vals=spns+1)
}
```

A. Code snippet for running EDBMM with `treeman`: read in balanced tree and set parameters for simulation; in loop, calculate fair proportion measure of evolutionary distinctness and add or remove tips based on these values; finally, extend the tip edges by adding 1.



B. Phylogenetic tree simulated using an Evolutionary Distinctness Biased Markov Model (EDBMM), where tips with greater ED have lower rates of speciation and extinction. Branch colours indicate the local number of descending branches, in this case the number of descendants within 20 branch length units.

Testing for Significant Phylogenetic Turnover

A common need in biological analysis is the detection of phylogenetic signal. Such analyses are often executed using model-based statistical analyses for which `ape` has been primarily designed (Paradis et al. 2016). A model-based approach, however, is not always applicable to every question related to phylogenetic signal. One such question is whether the changes in species between habitat types are due to phylogenetic signal in species' gains and losses, e.g. as a result of human-caused habitat loss (Frishkoff et al. 2015). A useful metric for calculating such a difference is the UniFrac measure (Lozupone and Knight, 2014), which measures the unique and shared fractions of

branches represented between communities. The `treeman` object is well suited to calculating this metric as all nodes in a tree have IDs; even if nodes are added or removed, IDs are constant. To demonstrate this I randomly generated community data with different intensities of overlap. I then ran permutation tests that detect whether there has been significant phylogenetic turnover between these communities using `TreeMan`'s `calcOvrlp()` (FIGURE 6.5).

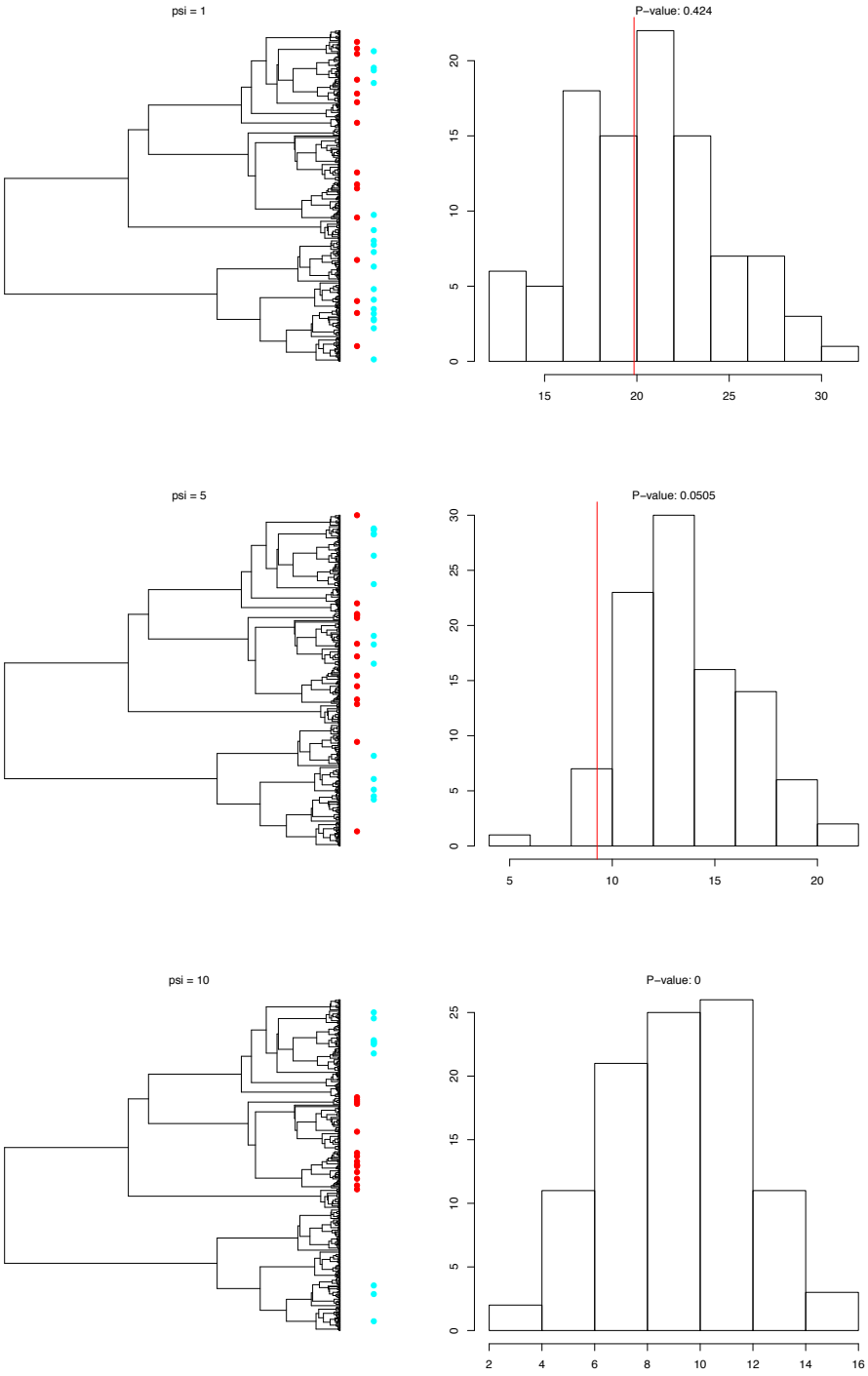
FIGURE 6.5 DETECTING PHYLOGENETIC TURNOVER

```
# PARAMETERS
ntips <- 500
tree <- rtree(ntips)
dpsi <- 10  # power difference

# GENERATE DATA
psi_1 <- dpsi
psi_2 <- -dpsi
focal <- round(ntips*0.5)
mean.incid <- ntips*0.05
c1 <- genCommData(tree=tree, psi=psi_1, mean.incid=mean.incid,
                  mean.abun=mean.incid, nsites=1, focal=focal)
c2 <- genCommData(tree=tree, psi=psi_2, mean.incid=mean.incid,
                  mean.abun=mean.incid, nsites=1, focal=focal)

# PERMUTATION TEST WITH TREEMAN
tree_tm <- as(tree, 'TreeMan')
c1_ids <- colnames(c1)[c1[1, ] > 0]
c2_ids <- colnames(c2)[c2[1, ] > 0]
# determine the proportion of shared branch length
obs_ovrlp <- calcOvrlp(tree_tm, c1_ids, c2_ids)
iterations <- 99
null <- rep(NA, iterations)
for(i in 1:iterations) {
  null_tips <- sample(tree_tm['tips'], length(c1_ids))  # generate null distributions
  null[i] <- calcOvrlp(tree_tm, c1_ids, null_tips)
}
p_value <- sum(obs_ovrlp >= null)/iterations
```

A. Code snippet for calculating overlap between two different communities using `treeman` and `MoreTreeTools`: generate random communities using parameters of community overlap for a random tree, convert trees from `phylo` to `TreeMan` using MoreTreeTool's `as()`, generate null communities to test whether the two communities have significant overlap.



B. Using `calcOvrlp()` to test for phylogenetic turnover. Left, phylogenetic trees with distributions of taxa found in two non-overlapping communities (red and blue) randomly generated with phylogenetic distance bias. The strength of this bias is determined by psi; greater psi leads to reduced overlap between communities. Right, distribution of 99 iterations for null communities' overlap in phylogenetic diversity with red community. Red line indicates observed overlap between red and blue communities.

Conclusions

`TreeMan` is an S4 class that encodes a phylogenetic tree using a node list. The advantage of a node list is the faster computational processing, and the ready capacity to track nodes between manipulations and vectorise or parallelise large-scale tree manipulations. The `treeman` package introduces new terminology to describe different elements of a tree and uses a naming convention to combine these new terms to make a more intuitive set of methods for tree manipulation.

MORETREETOOLS

Introduction

Before `treeman`, there was `MoreTreeTools`. This repository has acted as a testing bed for all the functions and classes that I have developed for `treeman`. For example, the utility functions for extracting node and branch information, calculating statistics, re-setting tree values, were are all first produced as part of the `MoreTreeTools` package. Not all the functions contained within `MoreTreeTools`, however, could be transferred into `treeman`. Many functions are fundamentally based on the `phylo` class on which `MoreTreeTools` depends. Furthermore, many functions remain in an early, experimental stage of development. In this section I will outline some of the functions I estimate to be of potential future value. I will provide usage examples, code snippets and figure outputs, where possible.

Usage Examples

Speed Improvements and Additional Utility Functions

`MoreTreeTools` functions depend on `ape`'s `phylo` class (Paradis et al. 2016). With this class, I have re-engineered functions to run faster. For example, a key measure of 'living-fossil-ness', is evolutionary distinctness. Prior to `MoreTreeTools`, there was only one function in R used for calculating this statistic, `evol.distinct()` (Kembel et al. 2016). With `calcED()`, I have produced a vectorised version of this function, so that it now runs much faster for large phylogenetic trees (FIGURE 6.6).

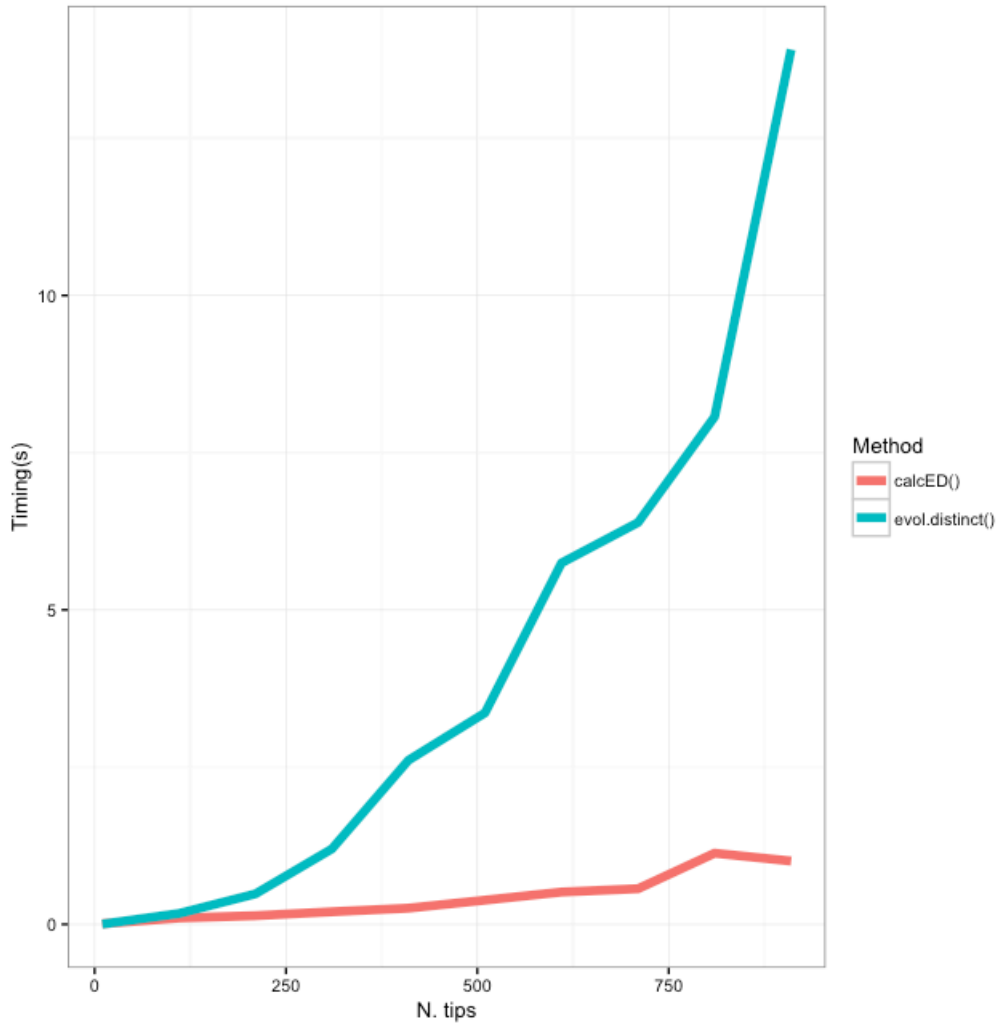


FIGURE 6.6 COMPARING ED FUNCTIONS

Timing the function run time for `calcED()` and `evol.distinct()` for trees of different numbers of tips when calculating the 'fair proportion' evolutionary distinctness metric (Isaac et al. 2007).

Additionally, `MoreTreeTools` contains many additional functions not currently available in other phylogenetic R packages. E.g. `calcDist()` measures the distance between trees of the same tips using four different metrics (Critchlow et al. 1996; Billera et al. 2001; Kuhner and Felsenstein, 1994; Nei and Kumar, 2000; Penny and Hendy, 1985; Rzhetsky and Nei, 1992), `commplot()` generates community/trait plots as demonstrated in FIGURE 6.5B, `getEdges()` efficiently extracts connected branches based on a given vector of tip labels. This latter function has recently been integrated into an R package for measuring phylogenetic signals in microbial communities (Washburne et al. 2017).

Re-visualising the Phylogenetic Tree

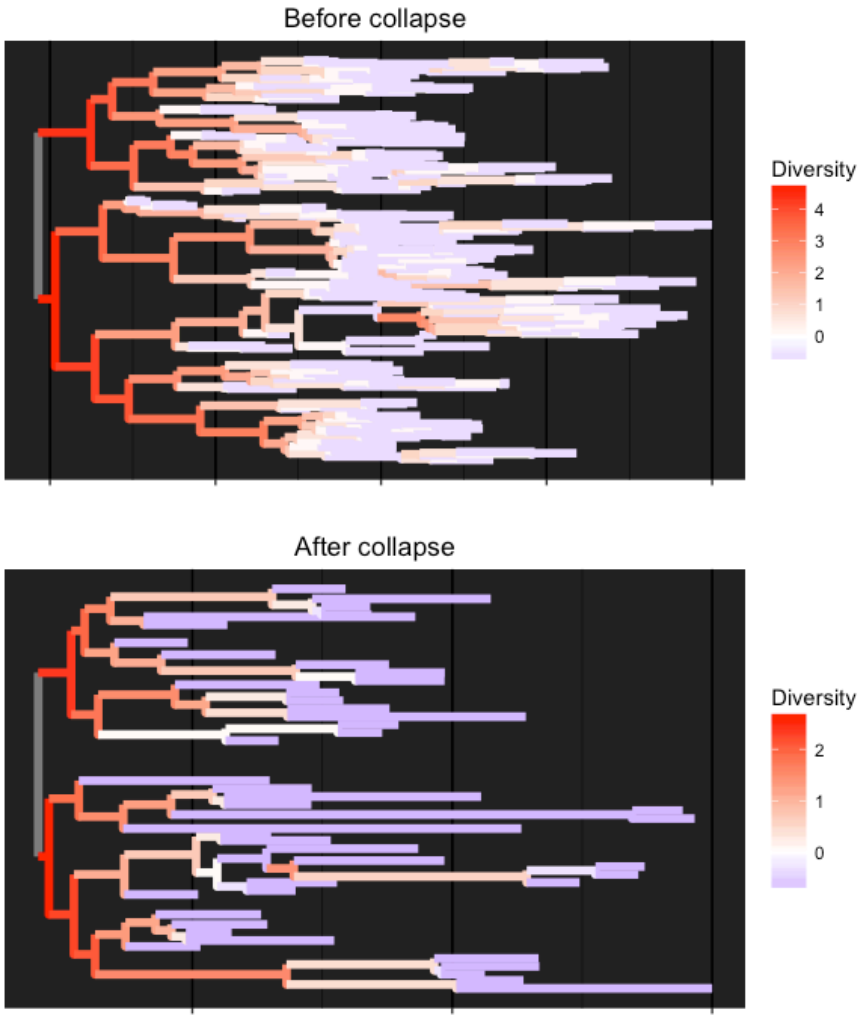
Phylogenetic trees were first introduced to evolutionary thought by Darwin (1859) and later popularised by Haeckel (1894). Since this time, however, the structure of the phylogenetic tree has remained relatively constant. With the growth of ever-larger trees containing upwards of thousands of tips, the ready visualisation of trees has become increasingly difficult. New ideas on how to tackle this problem often arise; a notable recent example is OneZoom (Rosindell and Harmon, 2012) that takes a 3D fractal interpretation. Given that I would be handling large phylogenies I attempted to create my own solution to the problem. I developed a series of functions that would take a large tree; identify the most significant branches based on their age, number of descendants and time span; drop minor branches and plot a re-configured 'skeleton' tree where branch colours indicate the relative diversity (see FIGURE 6.7).

I also attempted to reconsider the concept of the phylogeny as just a tree, for example when exploring phylogenetic signal in a trait. Visually plotting the distribution of traits of the tips of a tree can give an impression of how strong the signal can be, see results of `commplot()` in FIGURE 6.5(B). For larger trees with complex branching structures where proximity of tips provides no indication of phylogenetic distance, simply plotting trait values by tips will not be informative. An attempted solution I developed is `blockplot()`. This reconsiders branches as blocks coloured equally based on descending trait values. For a binary trait all blocks are composed of two colours. For balanced, ultrametric trees that are uniformly structured, perfect phylogenetic signal and maximum dispersion of trait values produce ordered patterns; random trait distribution disrupts these patterns (see FIGURE 6.8). Future development work would have to seek a way to plot realistic unbalanced trees. One method could be to look into ways of contrasting patterns of perfect and distributed signals against observed trait distributions.

FIGURE 6.7 PLOTTING CHROMATOPHYLA

```
# Example with collapseTips() + calcEdgeDiversity()
# generate random tree
tree <- rtree (250)
# add edge labels to track edges after collapse
tree$edge.label <- paste0 ('edge_', 1:nrow (tree$edge))
# generate edge diversity using two intervals
ed <- calcEdgeDiversity (tree, n.intervals=2)
# iteratively drop all tip edges with > 10% tree age
tree.age <- getSize (tree, 'rtt')
ctree <- collapseTips (tree, min.length=tree.age*0.1, iterative=TRUE)
# reconstruct edge diversity data frame without dropped edges
new.ed <- data.frame (edge.label=ctree$edge.label)
new.ed$edge <- 1:nrow (new.ed)
match.counts <- match (ctree$edge.label, ed$edge.label)
new.ed$count <- ed$count[match.counts]
# convert count to logged Z-score for maximum colour separation
new.ed$col <- (log (new.ed$count) - mean (log (new.ed$count))) /
  sd (log (new.ed$count))
ed$col <- (log (ed$count) - mean (log (ed$count))) /
  sd (log (ed$count))
# plot before
p1 <- chromatophylo (tree, edge.cols=ed, legend.title='Diversity') +
  ggtitle ('Before collapse')
# plot after
p2 <- chromatophylo (ctree, edge.cols=new.ed, legend.title='Diversity') +
  ggtitle ('After collapse')
```

A. Code snippet for collapsing larger phylogenetic tree into 'skeleton' tree: generate random tree of 250 tips, calculate branch diversity within a set time interval, collapse tree by dropping all branches representing less than 10% of tree age, generate colours for branches in collapsed tree, plot with chromatophylo.

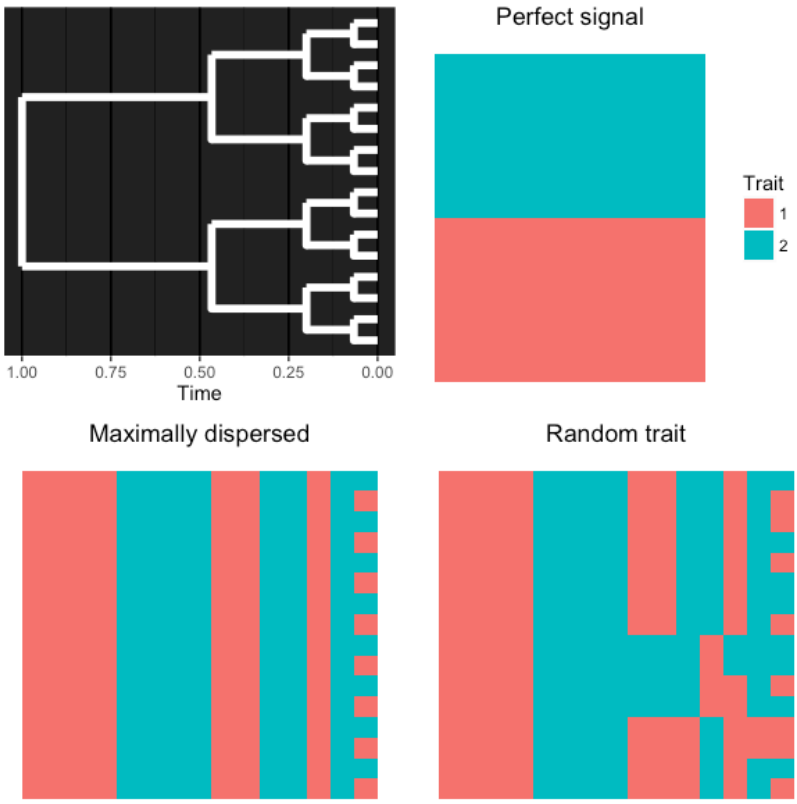


B. 'Chromatophyla' of large 250 tipped tree before and after collapsing.

FIGURE 6.8 VISUALISING PHYLOGENETIC SIGNAL

```
tree <- compute.brlen (stree (16, 'balanced'))
p0 <- chromatophylo(tree)
# Generate perfect phylogenetic signal traits
# (Note: trait vector should be named)
trait <- c (rep (1, 8), rep (0, 8))
names (trait) <- tree$tip.label
# Plot blocks ...
p1 <- blockplot (tree, factor(trait))
p1 <- p1 + ggtitle('Perfect signal')
# Repeat with evenly distributed traits ...
trait <- rep (c (0, 1), 8)
names (trait) <- tree$tip.label
p2 <- blockplot (tree, factor(trait))
p2 <- p2 + ggtitle('Maximally dispersed')
# The more evenly distributed, the more symetrical
# the pattern
# A random distribution of traits ...
trait <- randCommData (tree, nsites = 1, nspp = 8)[1, ]
p3 <- blockplot (tree, factor(trait))
p3 <- p3 + ggtitle('Random trait')
```

A. Explore phylogenetic signal with `blockplot()`: generate balanced and ultrametric tree and generate three sets of trait distributions.



B. Top-left, phylogenetic tree, top-right, block-plot if a binary trait were distributed with perfect phylogenetic signal, bottom-left, block-plot if a binary trait were distributed with maximum dispersion, bottom-right, block-plot for a random trait.

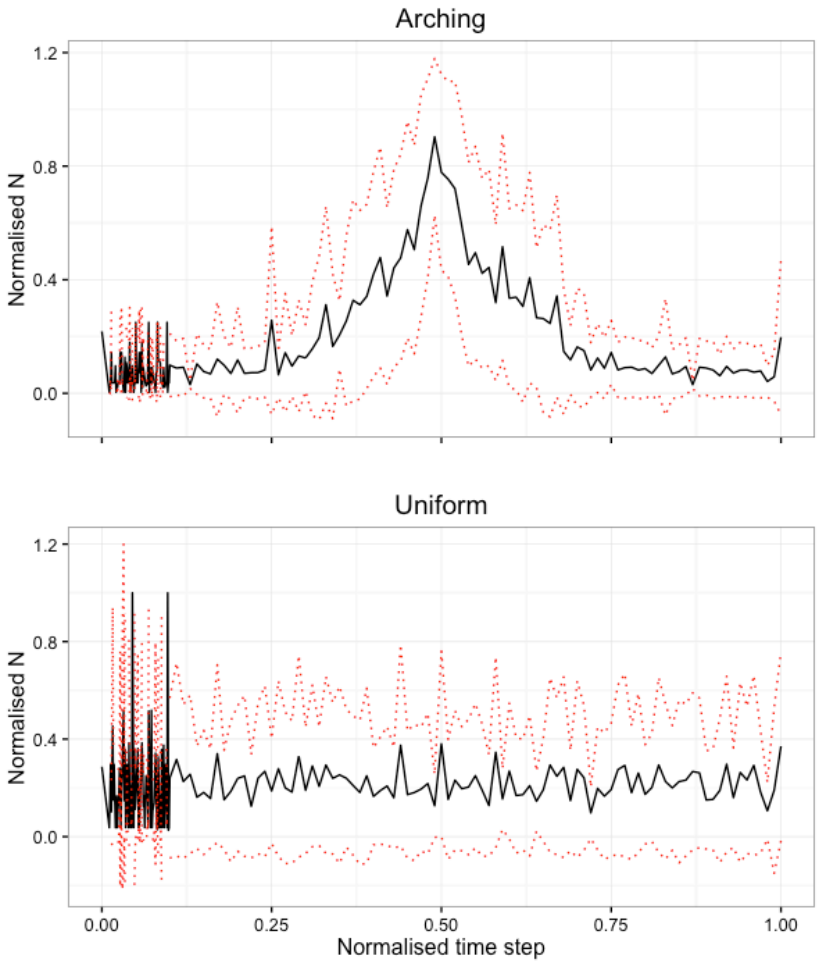
Analysing Clades

Few R packages allow the integration of analysis of phylogenetic trees and the clades contained within them. This was a requirement of my work in chapter two and as such I developed a series of functions for extracting clades from trees, `getCladeSuccess`(), calculating clade statistics, `calcCladeStats`(), such as centre of gravity and centre of mass, and visualising clades, `plotClades`(), see FIGURE 6.9. Future development with these functions could be to take a large tree with fossils and convert it into clade time series on which these functions depend. Currently, `getCladeSuccess`() works with output from an EDBMM model. Additionally, these functions could be readily incorporated into `treeman` in the future.

FIGURE 6.9 PLOTTING CLADES

```
# load test data
# arch.clades -- data on clades that peak and fall
# unif.clades -- data on clades that remain stagnant
data("testclades")
# calcCladeStats(arch.clades)  # view clade results:
# | cid | tot.size | max.size | time.span | start | end | cm | cg |
# merge to plot normalised clade trajectories
p1 <- plotClades(arch.clades, merge = TRUE) +
  ggtitle('Arching') + theme(axis.text.x=element_blank()) +
  xlab('')
p2 <- plotClades(unif.clades, merge = TRUE) +
  ggtitle('Uniform')
```

A. Plot normalised clade trajectories: load test clades from `MoreTreeTools` package data, use `calcCladeStats()` to view statistics on individual clades, plot clades with merge.



B. Normalised and merged clade trajectories for arching and uniform test clades. All clades extant species are counted for set time steps. These time series are normalised for each clade by maximum size and age. The mean (black line) and 95% confidence intervals are plotted (red dotted lines).

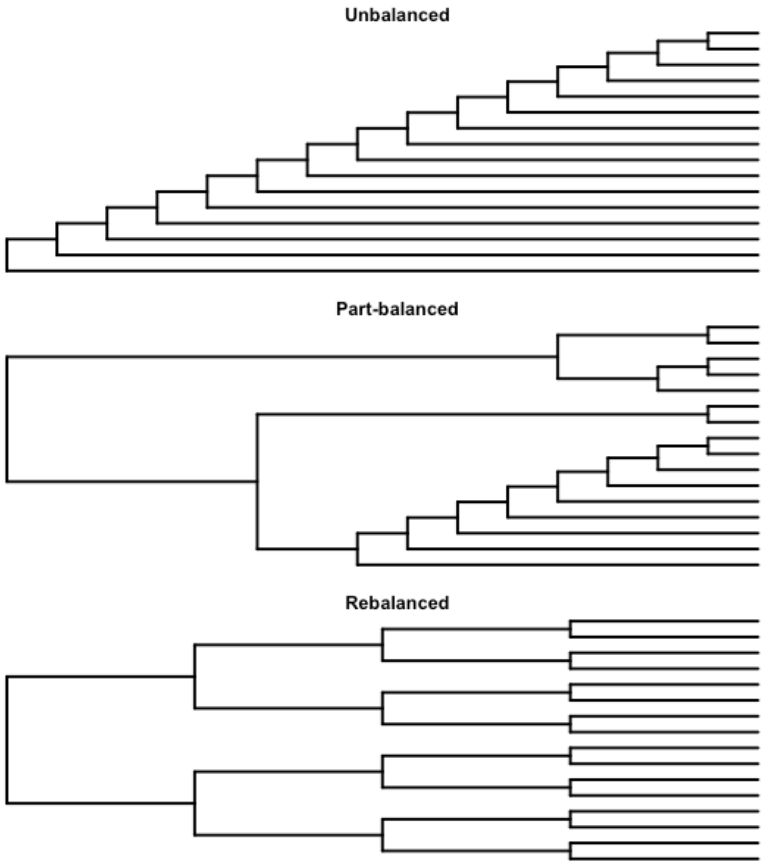
Manipulating Tree Shapes

As previously discussed earlier in this thesis, a tree has multiple dimensions to its shape. These principally are what I call 'balance' – the global difference in number of descendants between sister clades – and 'gravity' – the temporal distribution of internal nodes. `MoreTreeTools` has two functions that allow the user to manipulate each of these dimensions in a tree: `reBalance`() and `reGravitise`(). The former takes a tree and the argument 'steps', a positive or negative integer determining whether to increase or decrease the level of balance of the tree. Starting with the most unbalance nodes, the function iteratively moves tips between nodes 'steps' times to increase or decrease balance. The latter function, takes a tree and a 'factor' argument between -1 and +1. The function partitions branch lengths proportionately between parent and child branches to either increase or decrease the gravity of the tree. Through use of these functions, the two tree dimensions can be readily interpreted (see FIGURE 6.10 and FIGURE 6.11). Currently, these functions are limited to balanced and ultrametric trees. Future work could look at ways of making them work for any form of tree. Doing so may provide new ways of measuring balance and gravity. For example, a new metric of balance could be the number of steps to perfect balance. A new metric of gravity could be the global proportion of shared branch between parent and children.

FIGURE 6.10 RE-BALANCING A TREE

```
n <- 16
par (mfrow = c (3, 1), mar = c (1, 1, 1, 1))
tree <- compute.brlen (stree (n, 'left'))
plot (tree, show.tip.label=FALSE, edge.width=2, main='Unbalanced')
# +11 steps to make tree balanced
tree <- compute.brlen(reBalance (tree, 5))
plot (tree, show.tip.label=FALSE, edge.width=2, main='Part-balanced')
tree <- reBalance (tree, 6)
plot (tree, show.tip.label=FALSE, edge.width=2, main='Rebalanced')
```

A. Using `reBalance()`: generate a perfectly unbalanced tree of 16 tips, take 5 steps towards re-balancing it, re-compute its branch lengths to make it ultrametric, complete the rest of the steps towards re-balancing the tree.

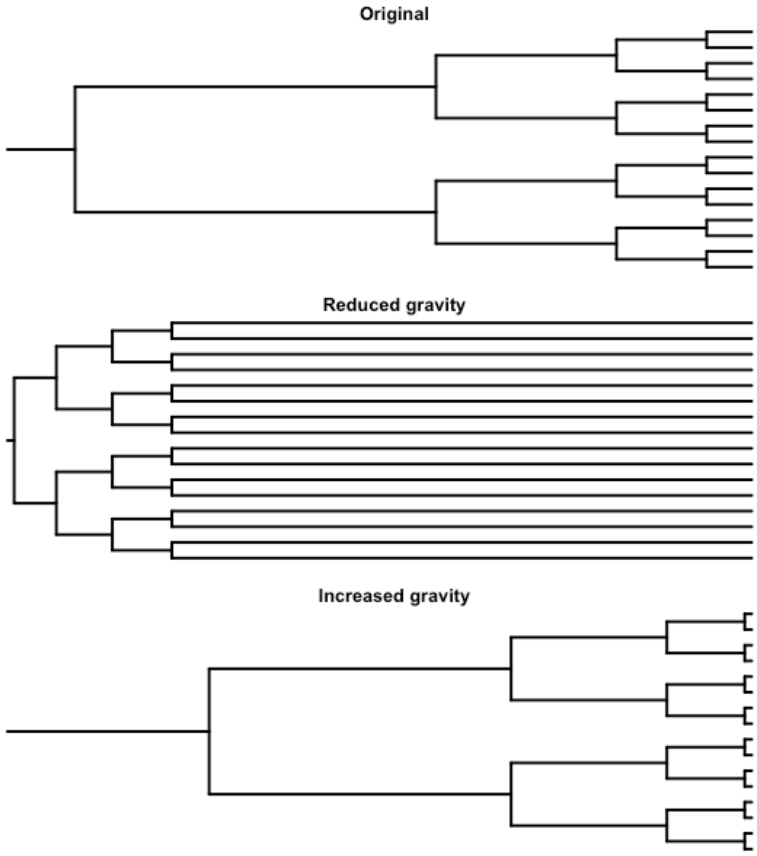


B. Re-balancing a tree from perfectly unbalanced.

FIGURE 6.11 RE-GRAVITISING A TREE

```
# Create balanced tree, plot positively and negatively reGravitised
n <- 16
par (mfrow = c (3, 1), mar = c (1, 1, 1, 1))
tree <- compute.brlen (stree (n, 'balanced'))
tree$root.edge <- 0.1  # add a root edge
plot (tree, show.tip.label=FALSE, edge.width=2, root.edge=TRUE,
      main='Original')
plot (reGravitise (tree, factor = -0.9), show.tip.label=FALSE,
      edge.width=2, root.edge=TRUE, main='Reduced gravity')
plot (reGravitise (tree, factor = 0.9), show.tip.label=FALSE,
      edge.width=2, root.edge=TRUE, main='Increased gravity')
```

A. Using `reGravitise()`: generate a balanced 16 tipped tree, add a small root branch in order for re-gravitised trees to be compared, plot original tree and two re-gravitised trees where 90% of the parent-child branches lengths have been re-distributed.



B. Re-gravitising a tree.

Summary

Currently, `MoreTreeTools` exists as a repository of semi-developed functions. Many of the functions that speed up common processes and analyses or add small additional utilities could be incorporated into `treeman` after conversion to work with the `TreeMan` class, or they could be incorporated into other `phylo` based packages. Other functions such as those highlighted in this section, however, are not ready for general release. Although I have outlined here how they may be useful in the near future, they require further work to ensure they work in a wider variety of circumstances; additionally the concepts underlying them need to be fully tested.

7.

Conclusions

A TERM TO KEEP?

The 'living fossil' concept is controversial, but much of this controversy stems from the absence of a clear definition, and its misapplication to disjointed sets of taxa. To resolve the issues surrounding the term I utilised two different approaches. The first was to test whether, under a simple definition, 'living fossil' could be said to refer to a particular set of taxa that experience a unique, different or separate evolutionarily history. In chapters two and three I demonstrated, using two different approaches, that LFs (defined as evolutionarily distinct species), typically belong to clades that persist for long periods of time without diversifying (*panchronic* in my terminological scheme). This sense of what an LF is bears more resemblance to Darwin's original concept of an 'unchanging form' than it does to more recent assumptions that living fossils are evolutionarily relicts. The second approach was to generate a quantitative metric for assessment of whether species and clades could be considered to be LFs. In chapter four I developed a set of metrics of 'living fossilness', that would be able to identify LFs across a wide range of taxonomic groups and which incorporated as broad an interpretation of the meaning as possible. Despite the term being applied without a consistent definition since Darwin coined it over 200 years ago, using these metrics I was able to identify many of the 'classic' LF species and clades as indeed LFs. Additionally, in chapter five I used these metrics of LF-ness to investigate which common factors are more often associated with the LF state.

With these two approaches I have demonstrated that 'living fossil' can be a useful term for evolutionary science. It can be used to describe a distinct subset of the diversity of life and it can be quantified to make its definition clearer and more tractable.

An Absence of 'Change'

The assertion that 'living fossil' is a useful term for a distinct category of life depends on the results of chapters two and three. Both these chapters equate evolutionary distinctness with LF-ness. Evolutionary distinctness, however, is only a proxy for the LF

as it cannot capture all elements of the LF concept, most notably "little change through time" (Eldredge and Stanley 1984). Equally, all results that depend on pEPI, which also does not take into account change (see chapter four), can also be questioned in the same way. Are there any reasons, however, to suspect that change and diversification are linked? There is tentative evidence as well as strong theoretical grounds for believing that reduced diversification rates leads to reduced morphological change.

The first line of evidence comes from correlations between trait distance and evolutionary distinctness. Under a Brownian motion model of character evolution we should expect ED species to be more ecologically and morphologically distinct as they have the greatest evolutionary distance from other species (Isaac et al. 2007). There are only two studies that have been conducted to test for this correlation, both on mammals. Redding et al. (2010) found that high ED primates were 'odder' across a range of traits compared to low ED primates. Collen et al. (2011) repeated the analysis but for all mammals and found that although for different sets of traits high ED species did have 'odder' characteristics, at the mammalian level as a whole high ED species were not 'odder'.

A second line of evidence comes from comparing morphological distance to a lineage's age. Under a Brownian motion model of character evolution, we should again, expect older lineages to be more morphologically distinct. Liow (2004) and (2006) compared the morphological similarity of clades by their lineage ages. Older lineages of fossil crinoids were found to be less morphologically distinct than younger ones (Liow 2004) and trachyleberidid ostracodes were found to be morphological equivalent between older and younger lineages (Liow 2006). From these studies we should conclude that reduced rates of diversification do not necessarily lead to decreased rates of trait or morphological change. Such a conclusion, however, requires the assumption that older lineages and evolutionary distinct species have experienced a comparably lower rate of diversification to younger lineages or evolutionary indistinct species at the time of

observation. Equally, this interpretation of the above studies' results is countered by the finding in chapter four of a positive correlation between pEPI values and the independently determined change variable. A better test for how reduced diversification rates impact morphological or trait evolution would be to directly track changes alongside speciation and extinction in the fossil record.

Wagner and Estabrook (2014) performed such a study by tracking the fate of primitive and derived states across a wide range of fossil groups and periods. They showed that traits showed 'hierarchical stratigraphic compatibility', i.e. character states show morphological progression through the loss of earlier states. From this result it could be argued that there is a positive link between diversification and change as species that may still retain these 'primitive' character states as they disappear globally are more likely to be evolutionarily distinct or have low pEPI and are less likely to produce future progeny.

On a theoretical basis there is an argument that there is a positive correlation between diversification and character trait evolution. Under a punctuated equilibrium model of evolution, where most character evolution occurs during speciation events (Gould and Eldredge 1993), only a scenario where diversification rates are low for extended periods of time, i.e. the panchronic scenario of reduced diversification rates for evolutionarily distinct species, would recreate the morphological primitiveness of LFs as it would generate high ED species through long periods of low diversification rates rather than recent rapid extinction. There is evidence that speciation events do indeed accelerate such change (Venditti and Pagel 2010). Ezard et al. (2013) demonstrated that the number of speciation events for foraminiferan linages correlated strongly with the rate of molecular evolution, a precursor for character trait evolution.

Finally, recent large-scale genomic studies are finding that commonly identified LFs have experienced reduced molecular change. Amemiya et al. (2013), in a large and comprehensive genomic study of protein coding regions, demonstrated that coelacanths

have experienced reduced substitution rates compared to other vertebrate lineages. Venkatesh et al. (2014) analysed the incidence of genomic rearrangements – a key process in driving evolutionary change – across different vertebrate lineages and found that coelacanths and the elephant shark (*Callorhincus milli*) had the least derived genomes.

Current empirical evidence suggests a positive relationship between character trait evolution and diversification rates. This evidence is corroborated by the expectation of the punctuated equilibrium model of evolution, which posits that most evolution occurs around speciation events (Gould and Eldredge, 1993). In this regard, the conclusion that low pEPI species and high ED species are likely to be LFs is strong. There is reason to believe that there is a distinct category of life, which can be identified using either pEPI or ED metrics, and that its members experience reduced rates of diversification as well as reduced rates of morphological evolution.

Implications for Conservation Biology

As indicated in the introduction, LF species are of interest to conservation biologists as a means to more fairly spread funds and efforts to neglected parts of the tree of life. The Evolutionarily Distinct and Globally Endangered (EDGE, 2017) programme is a much-publicised attempt to push for the conservation of evolutionarily distinct species, which might equate to LFs. There are three main reasons for conserving evolutionarily distinct species in particular. Firstly, it is argued that through preserving distinct parts of the tree of life, distinct traits and features of life will be preserved (Isaac et al. 2007; Redding et al. 2008; Collen et al. 2011), which could contribute to safeguarding ecosystem functions and services (Díaz et al. 2013). Secondly, it is deemed that these species and clades represent greater proportions of evolutionarily history (Mouquet et al. 2012; Winter et al. 2013). The extinction, for example, of all the monotreme species would lead to the loss of the 167 MY branch connecting them to the rest of the mammals. With this 'museum curator' argument, it can be said, that within the monotremes are the living traces (in

their DNA, ecologies and behaviours) of this 167 MY history. Thirdly, these species and clades may represent the seeds of future adaptive radiations — the phylogenetic fuse argument (Copper and Fortey, 1998). Being more distant from other species, these species may contain unique traits that would allow them to respond differently to any extreme environmental changes (Redding et al. 2010), allowing them to radiate into recently vacated ecological space (Schluter, 2000). To put it grandly, conserving these species would therefore be preserving the 'evolvability' of life on Earth (Mouquet et al. 2012; Winter et al. 2013). On the other hand, it has been argued that these evolutionary distinct species, in fact, represent relictual species that are more likely to become extinct (Collen et al. 2011). It is for this third dispute that the results of this thesis have consequence.

In chapter two I demonstrated that a tree-growth model where evolutionary distinct species had lower rates of speciation and extinction recreates phylogenetic trees similar in shape to real ones. This indicates that evolutionary distinct species are not doomed to extinction nor are they likely to radiate. Instead, evolutionarily distinct species fit best to the panchronic interpretation of LFs; they persist for extended periods of time without going extinct or radiating. The results of chapter two, however, require the assumption that speciation and extinction rates show a consistent bias through time. We know, however, that extinction biases can change through time. Lineages more prone to extinction can differ between 'normal' times and periods of mass extinction extinction (Jablonski, 1995). The biological traits associated with extinction can simply change between different geological periods (Bromham et al. 2012). Furthermore, the results of chapter two also depend solely on comparing phylogenetic trees through their shape. In chapter three, I took a different approach by using the fossil record of the mammals to test how past ED affected future ED. The results were consistent with the findings of chapter two: highly evolutionarily distinct species were more likely to become more evolutionarily distinct.

Evolutionary distinct species are like LFs, they are panchronic taxa that persist and diversify little. Though protecting them would not be preserving the future 'evolvability' of life on Earth, it would not be a wasted effort either. They are merely the slow evolving ends of the tree of life and have just as much right of survival through the Anthropocene as any other clade. Unlike other clades, however, they are unique. They represent a greater part of evolutionary history and they may feature distinctive traits not seen in any other lineages.

It Means Just What I say it Means

Finally and briefly, I would like to talk about definitions of words and scientific progress. Although I have demonstrated that 'living fossil' can be a term of scientific value, this will not necessarily be a welcome conclusion for many in the palaeontological field. Upon beginning this research project, I was impressed by the level of hostility held towards the term, 'living fossil'. Upon simply hearing the phrase spoken, many would immediately dismiss it. As I outlined in chapter one, I believe this rather emotional reaction to the term has its roots in its origin and its association with creationism. Nevertheless, despite palaeontologists often arguing against the concept (e.g. Schopf, 1984; Casane and Laurenti, 2013), a list of 'classic' LFs has persisted and whenever these species or clades are mentioned, 'living fossil' is found. For example, articles written in a range of fields other than evolutionary biology (molecular biology, medical science, nutrition, etc.) will append the tag 'living fossil' if they concern one of these classic LFs (e.g. horseshoe crabs [Dai et al. 2008], coelacanths [Schwarze and Burmester 2013] or ginkgo [Mohanta et al. 2014]).

Such antipathy towards a term despite its common usage is paralleled by an argument within linguistics: should a word's meaning be based on its history or its usage? For example, the word 'decimate' originates from the Latin for tenth and was used in the Roman world to describe the punishment of a group of people where every tenth person

was killed. Today, however, we use the word with an even more extreme meaning, 'exterminate' or 'wipe out'. It would be ridiculous to police every use of the word to force it to conform to the original Roman meaning. Therefore, in compiling dictionaries, philologists define words based on their observed usage and not their etymology. Likewise, it could be argued that 'living fossil' should only ever be used in the same way that we believe Darwin intended. I think, however, that this would be a mistake for two broad reasons. Firstly, it would put palaeontologists in the same position as those etymologists who wish to police the usage of words. This policing of a phrase would also be missing the underlying problem of the phrase's usage: the perpetration of an erroneous version of evolutionary theory. Secondly, arguing for specific definitions of words and phrases echoes the failed philosophy of essentialism – where science depends on the precise definitions of elements of reality based on their 'essences' (Popper, 1945). After all, words are localised models of reality rooted in the context of their usage, not global indisputable facts. Put simply, words, terms and phrases, in and of themselves, cannot have locked-in, strict and precise meanings, they must be allowed to change as our scientific understanding of the world grows. (Or, as Popper (1968) put it "In so far as scientific statements refer to the world of experience, they must be refutable; and, in so far as they are irrefutable, they do not refer to the world of experience." Words with concrete meaning are not refutable.)

In this thesis, I have used a range of alternative words to 'living fossil', e.g. palaeobiotic and panchronic. Because of the controversy over 'living fossil', perhaps in time these and similar alternatives, though less evocative, will supplant Darwin's expression. Ultimately, however, the words do not matter so long as they do not prevent the empirical research. In exploring "What is a Living Fossil?" I have had to respond to some important macroevolutionary questions. For example, in chapters two and three I determined that the evolutionary past does impact evolutionary future. Lineages that experience low rates of diversification are likely to continue to experience low rates of

diversification. In chapter four, I showed how a simple set of metrics of evolutionary performance could be used to identify species and clades that have experienced distinct rates of evolutionary change. In chapter five, I used these metrics to identify common biologies that could explain these differences. As a result of these final chapters, I discovered that low evolutionarily performing or LF lineages tended to have lower reproductive rates, indicative of these clades experiencing reduced speciation rates. Low speciation rates are key to maintaining a low evolutionary performance. Although these results do not directly depend on the existence of 'living fossil' itself, they are the product of the inspiration that it invokes. In this sense, perhaps, we can be grateful to Darwin for coining the term.

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Appendix

CHAPTER 2

Figures

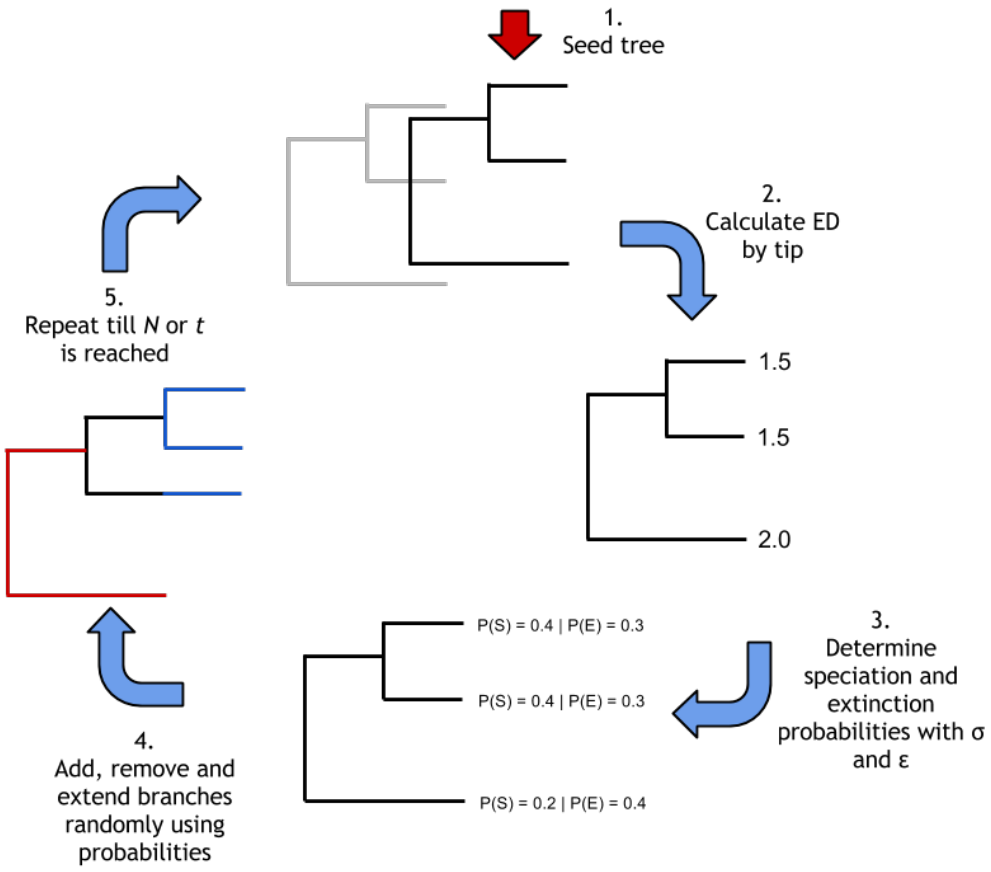

FIGURE S2.1

Concept map of the Evolutionary Distinctness Biased Markov Model (EDBMM). In this example, the tree is being simulated under a DE scenario (-σ, +ε), meaning speciation and extinction rates are lower for ED tips and as such the resulting tree (in grey) has effectively the same shape as the original tree (in black).

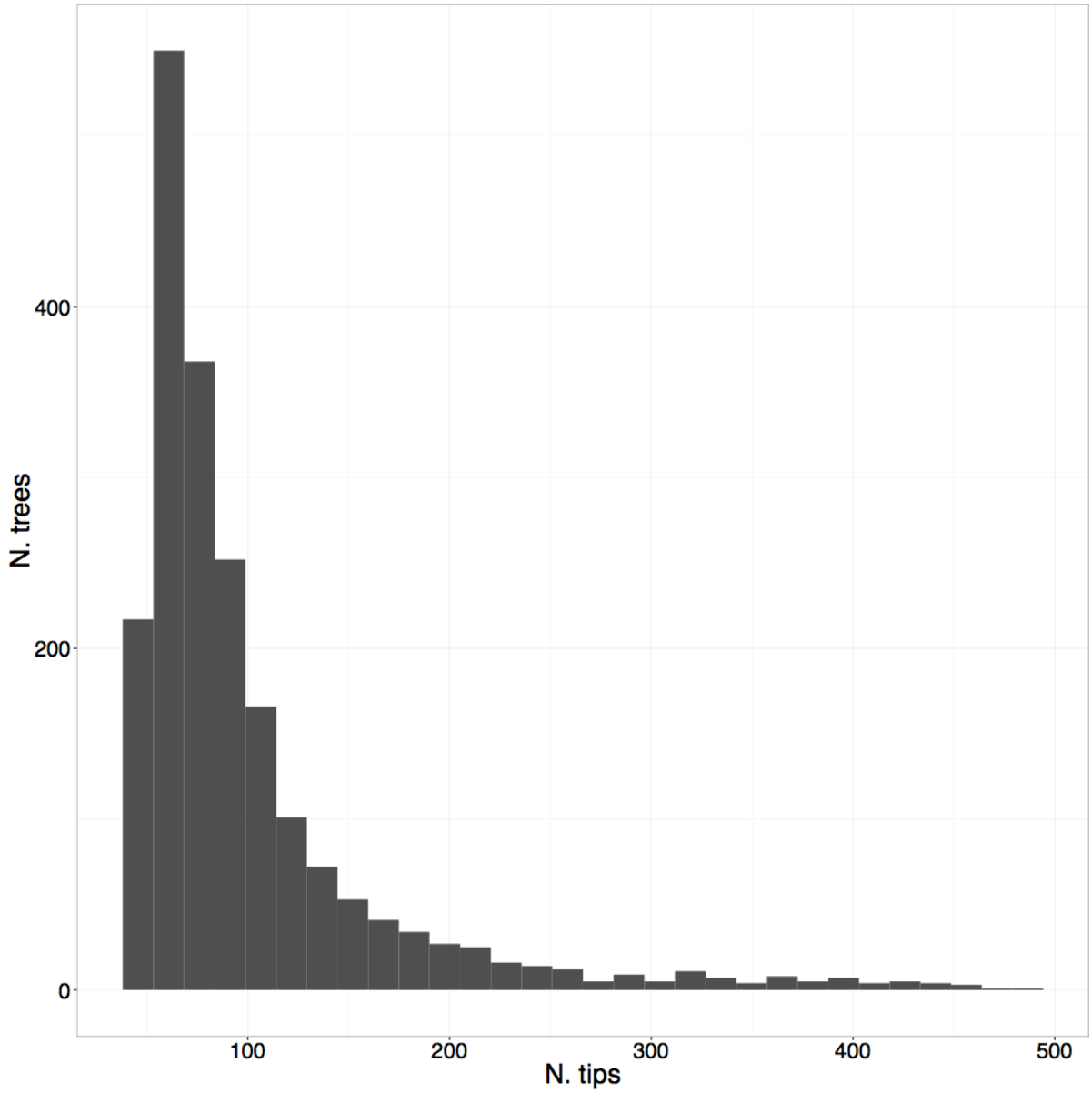

FIGURE S2.2

Number of tips represented by empirical sourced trees.

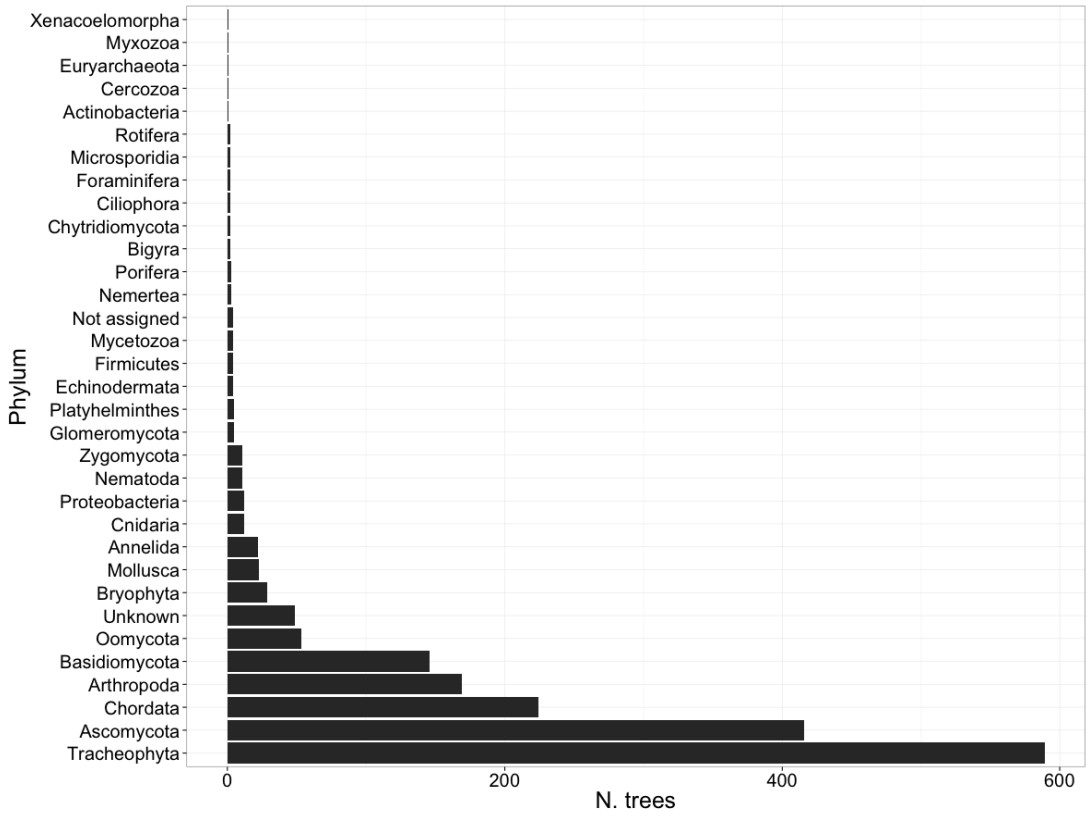

FIGURE S2.3

Number of sourced trees represented by each phylum.

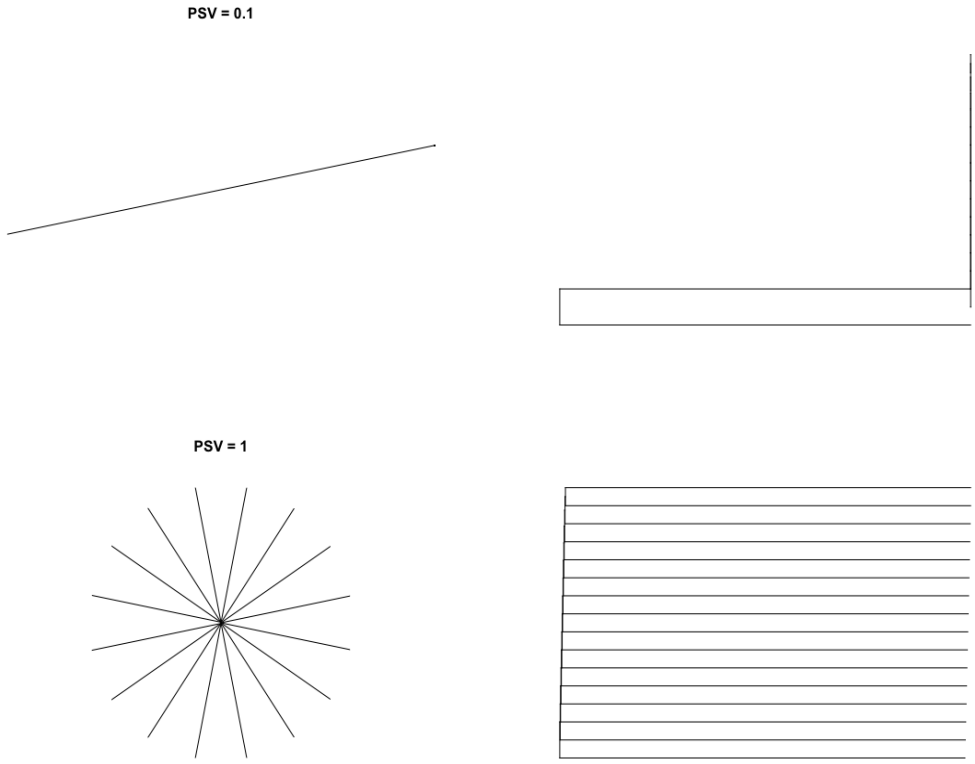

FIGURE S2.4

How PSV and phylogenetic gravity relate. (Top) A line/dot tree has a single cluster of highly related tips separated by a very distant tip, causing low phylogenetic species variability (PSV). When rooted on this long branch and keeping the tree ultrametric, internal nodes are clustered towards the tips. (Bottom) A star tree has all tips being equally distant and all internals nodes are distributed to the root.

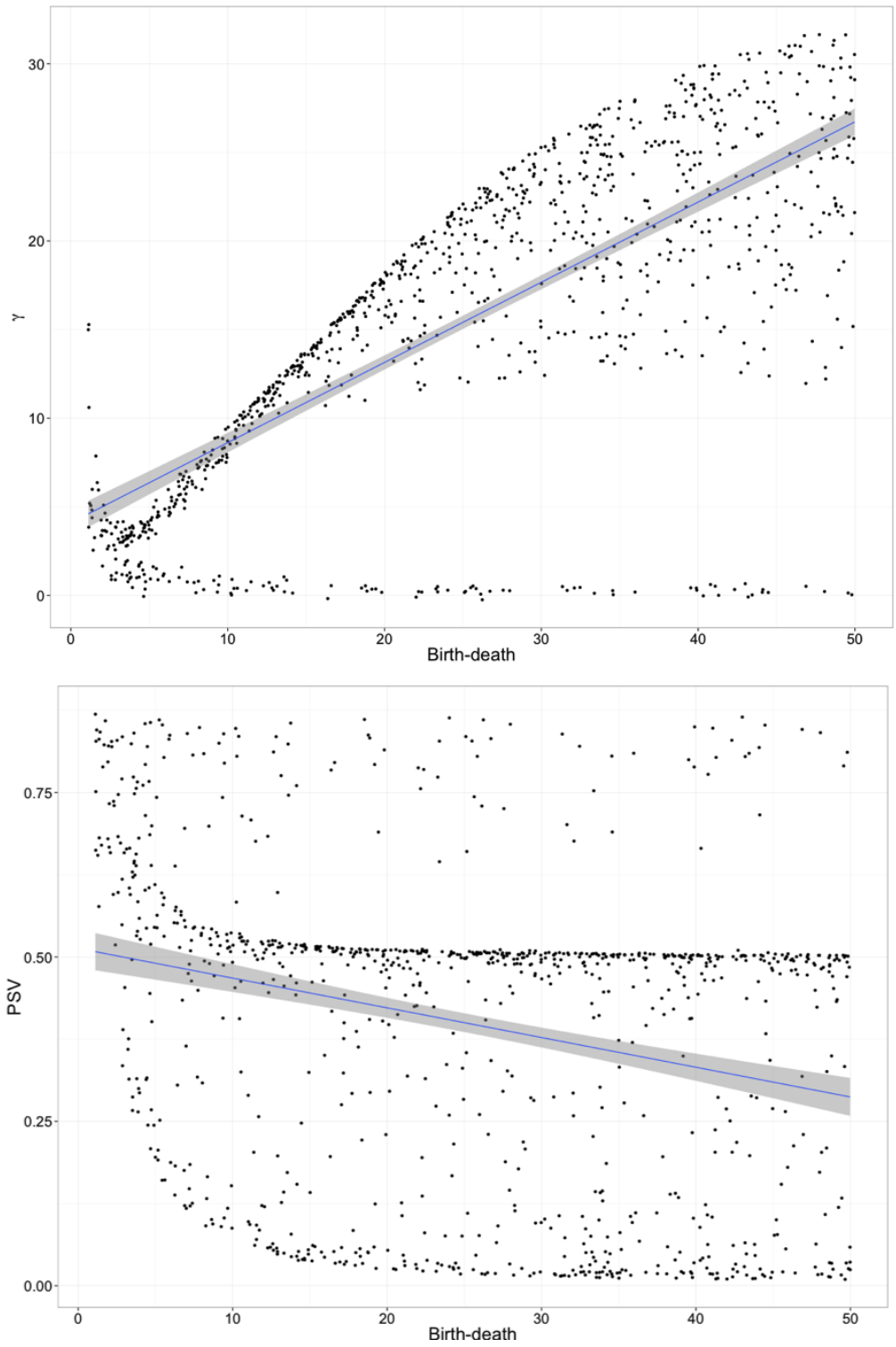

FIGURE S2.5

Correlations between birth-death parameters and γ (top) and PSV (bottom) for 1,000 simulated trees using random σ and ε parameters. PSV shows a weaker correlation to the birth-death parameter (Pearson's R -0.27) than γ (Pearson's R 0.78). The complex structuring of the points is caused by the interaction of birth-death, σ and ε with PSV and γ.

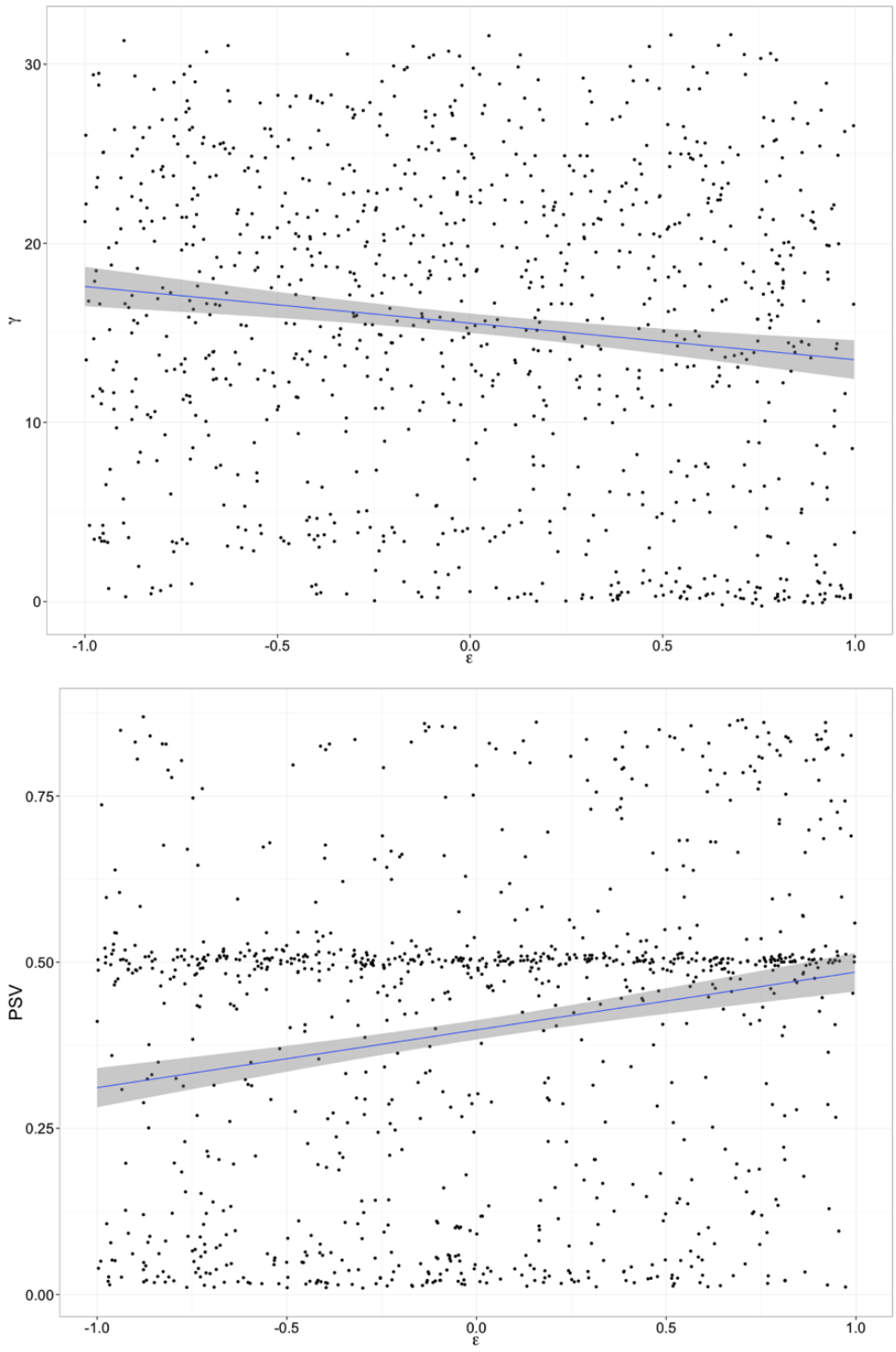

FIGURE S2.6

Correlations between ε parameters and γ (top) and PSV (bottom) for 1,000 simulated trees using random σ and ε parameters. Although weak, PSV shows a stronger correlation to the ε parameter (Pearson's R 0.20) than γ (Pearson's R -0.13). The structuring present in the PSV plot is indicative of a nonlinear interaction between σ and PSV.

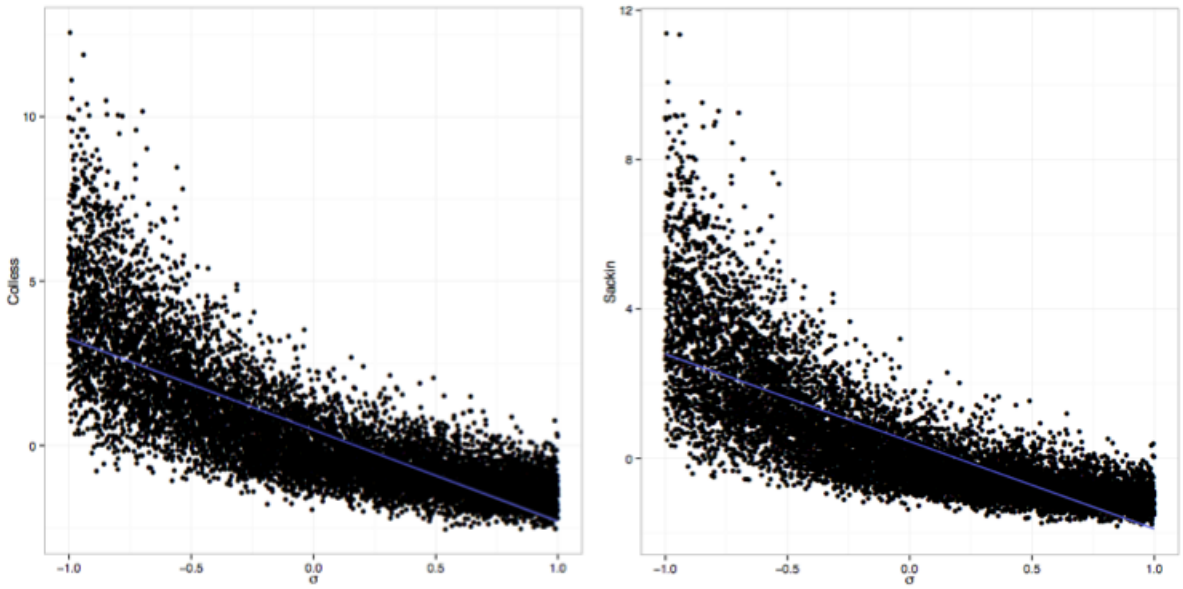

FIGURE S2.7

Yule normalised Colless (left) and Sackin (right) statistics against σ ("sigma").

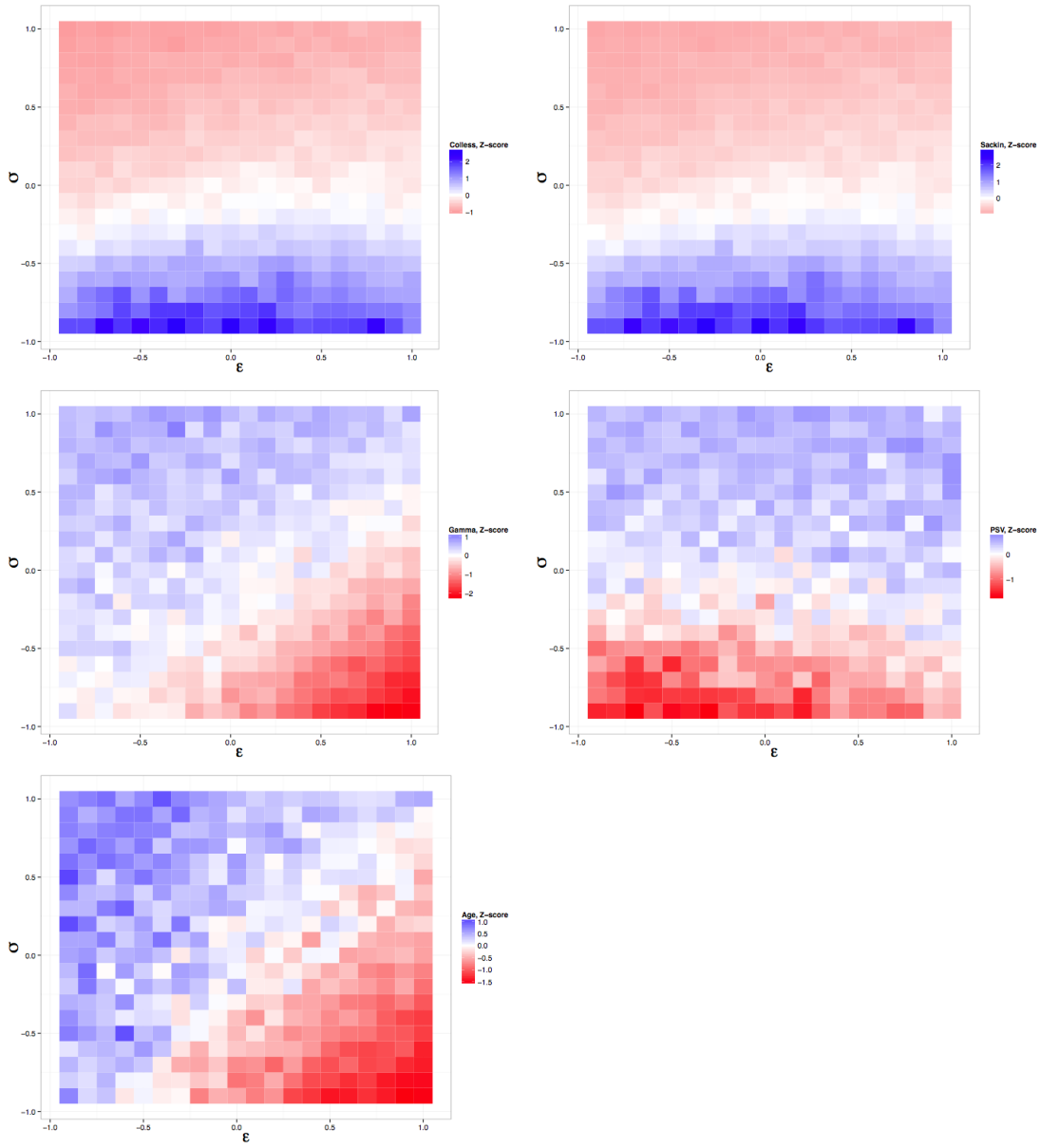

FIGURE S2.8

Z-scores for mean simulated tree statistics in σ-ε ("sigma"-"epsilon") parameter space: (top-left) Yule normalised Colless, (top-right) Yule normalised Sackin, (middle-left) γ ("gamma"), (middle-right) phylogenetic species variability (PSV) and (bottom-left) age (root to tip distance).

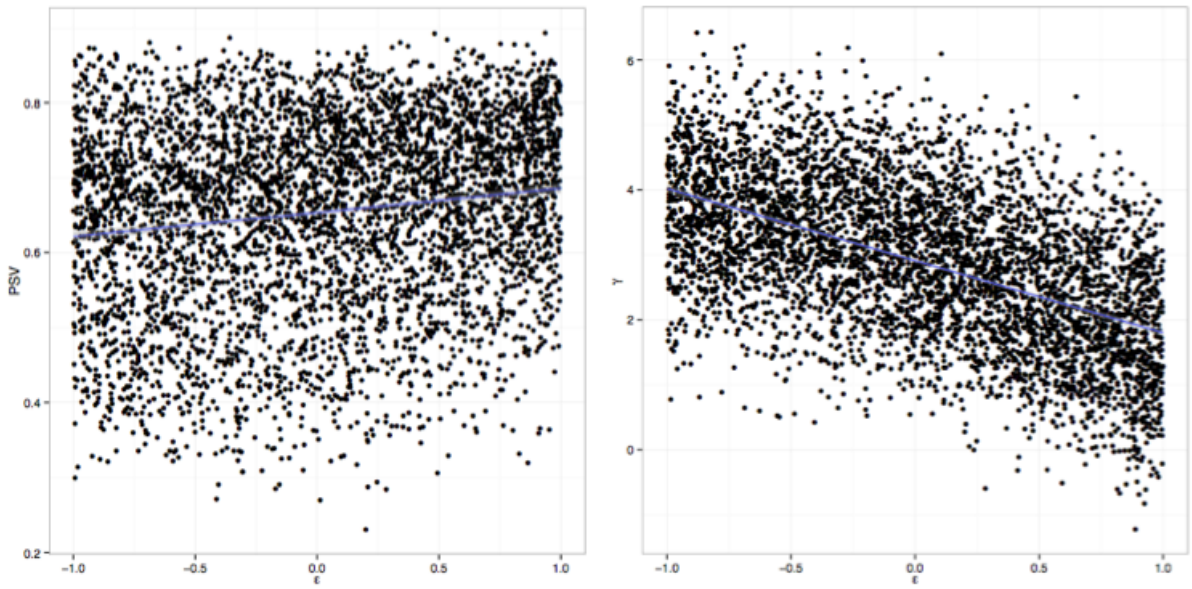

FIGURE S2.9

PSV (left) and ɣ ("gamma") against ε ("epsilon") for simulations with σ ("sigma") < 0.

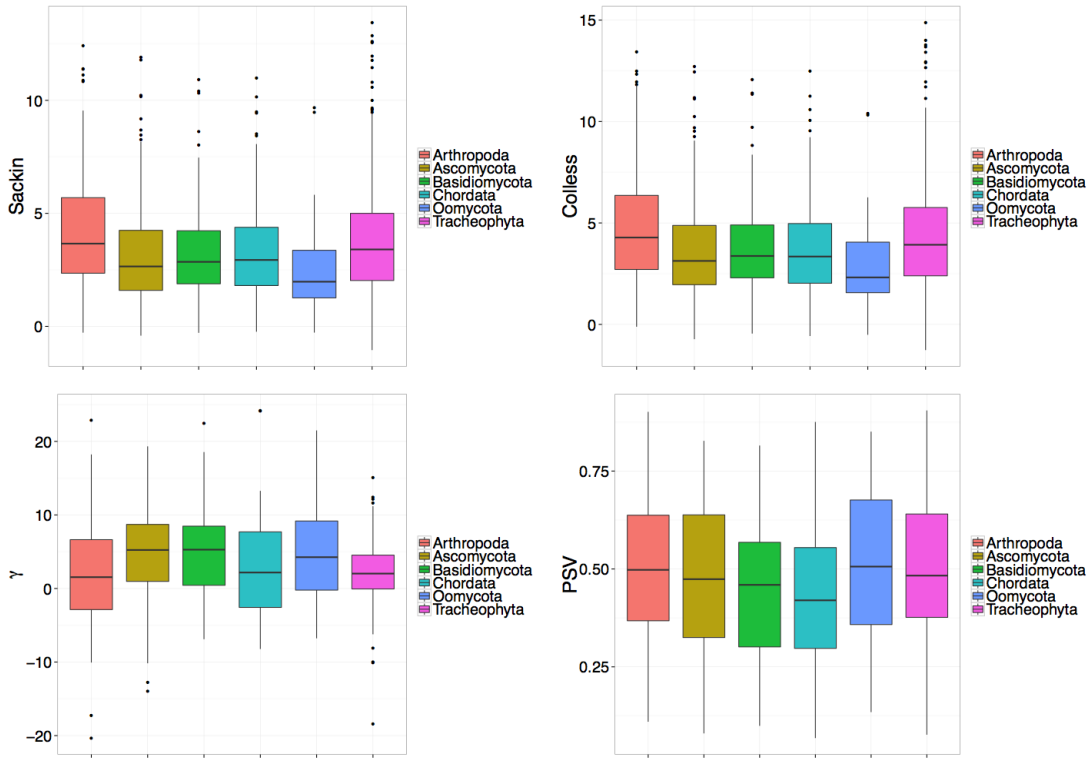

FIGURE S2.10

Shape metric ranges for different Phyla: Sackin (top-left), Colless (top-right), γ_{D8} (bottom-left) and PSV (bottom-right)

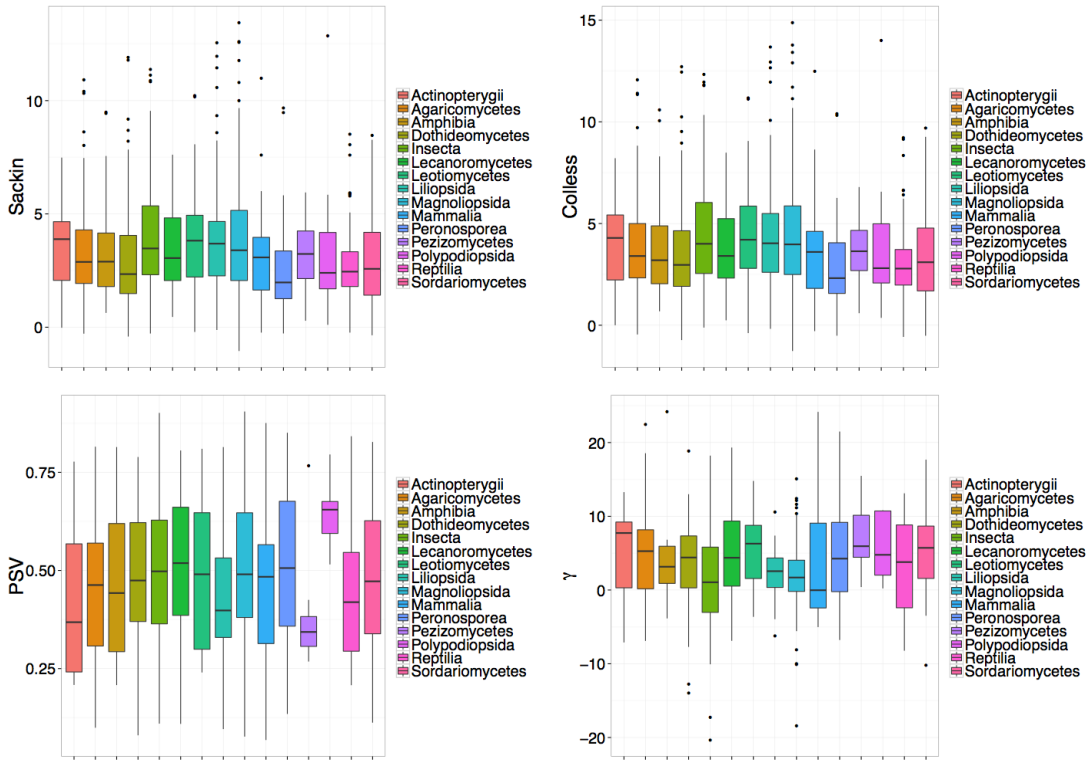

FIGURE S2.11

Shape metric ranges for different Classes: Sackin (top-left), Colless (top-right), γ_{D8} (bottom-left) and PSV (bottom-right)

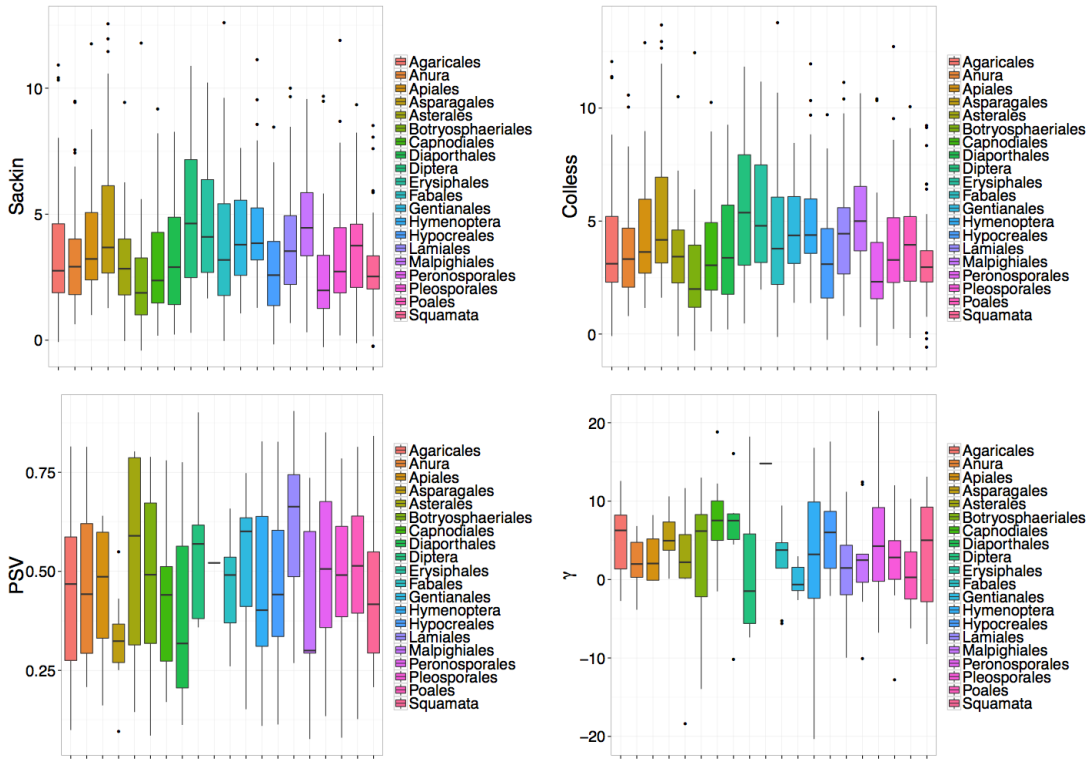

FIGURE S2.12

Shape metric ranges for top-ten most sourced Orders: Sackin (top-left), Colless (top-right), γ_{D8} (bottom-left) and PSV (bottom-right)

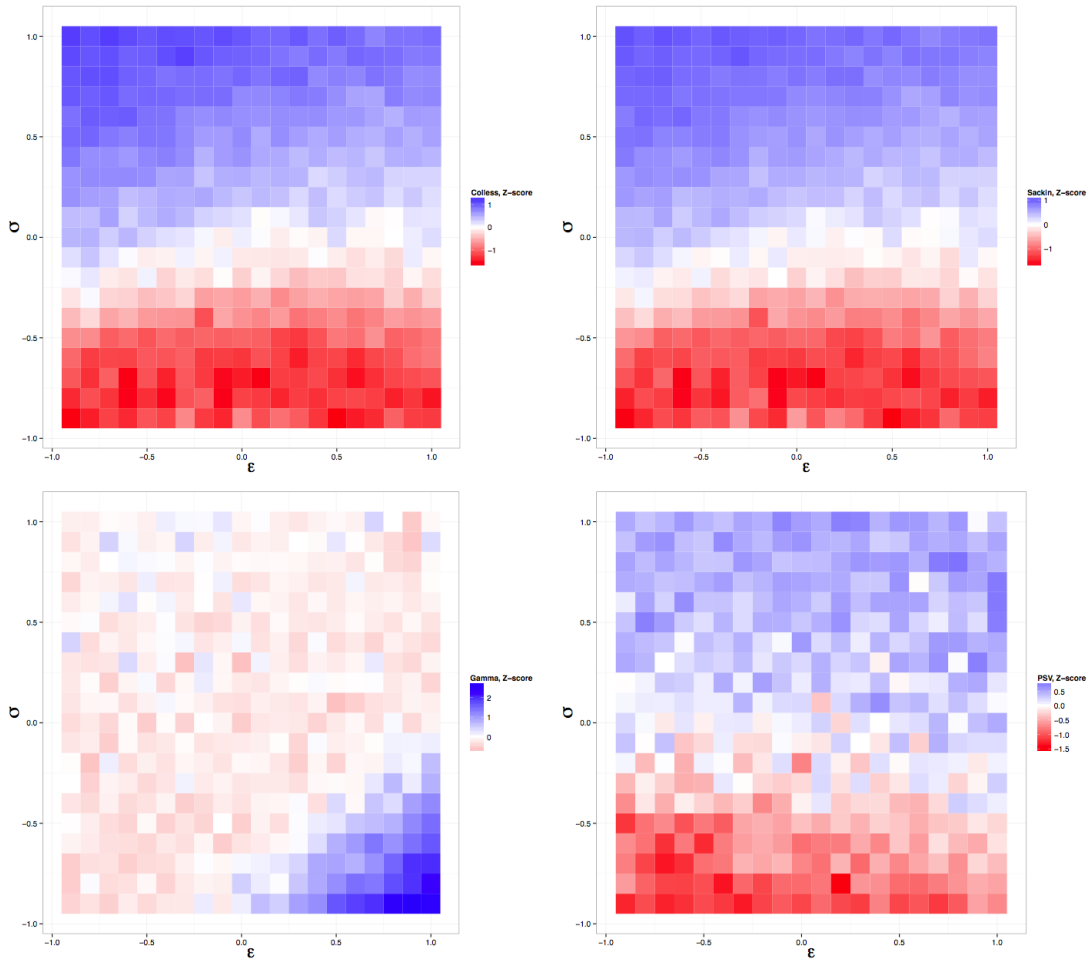

FIGURE S2.13

Z-scores for distance to empirical trees for mean simulated tree statistics in σ-ε ("sigma"-"epsilon") parameter space, the lower the value the nearer the simulated is to the empirical: (top-right) Yule normalised Colless, (top-left) Yule normalised Sackin, (bottom-left) phylogenetic species variability (PSVD8) and (bottom-right) ϒD8 ("gamma").

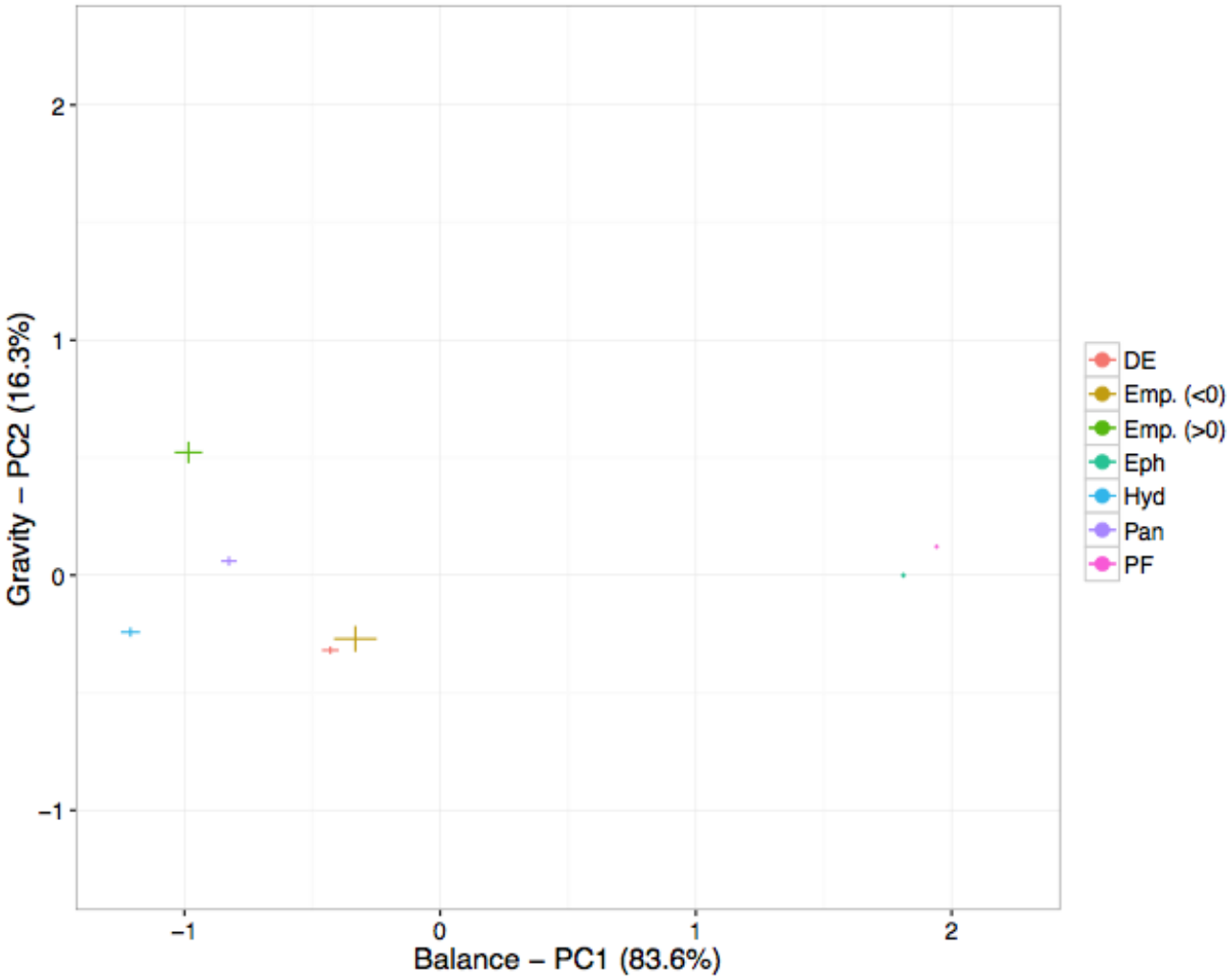

FIGURE S2.14

PCA of the extreme scenarios using Yule normalised Sackin, Colless and PSV. Colours indicate simulation scenario and whether tree is empirical with $\gamma > 0$ (high) or $\gamma < 0$ (low). 'Low' empirical tree shape is closer to the DE scenario, while 'high' has greater gravity than all simulated trees indicating that although Pan is closest in terms of gravity than any other simulation a diversity in birth-death rate is required to create the range of empirical gravity.

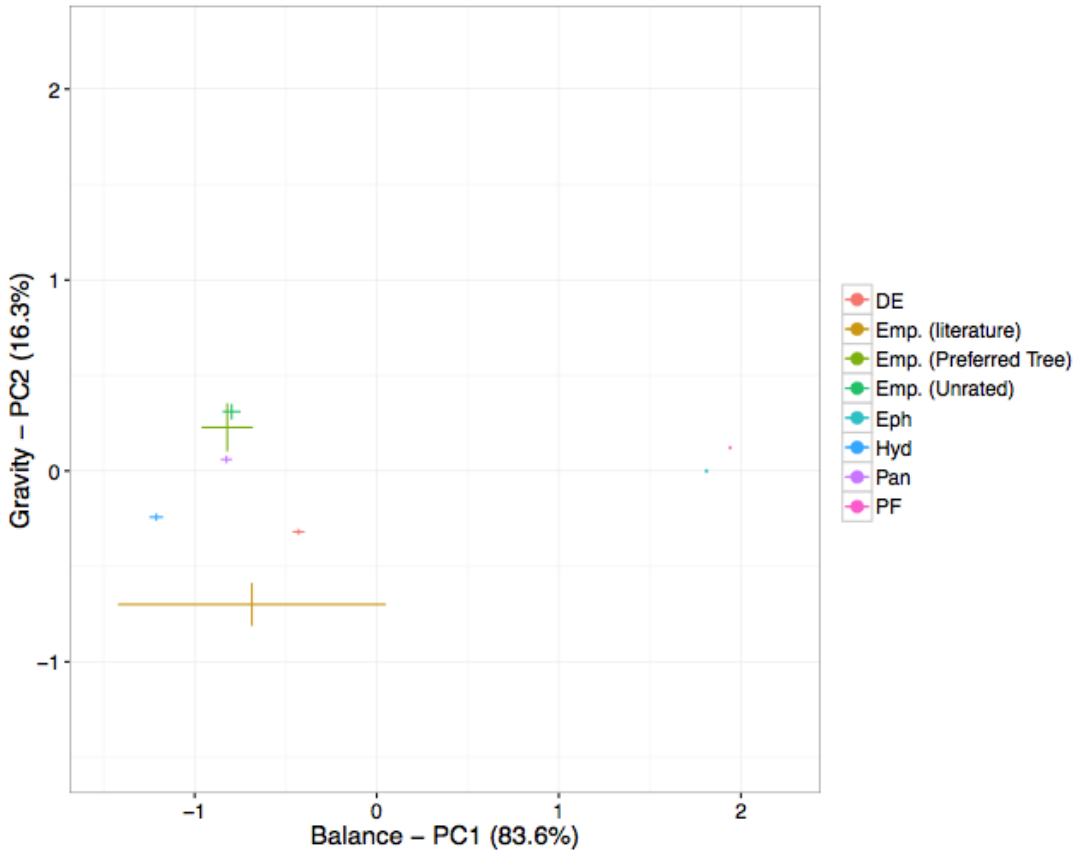

FIGURE S2.15

PCA of the extreme scenarios using Yule normalised Sackin, Colless and PSV. Colours indicate simulation scenario and empirical trees split between source/quality. 'Literature' refers to all the trees originating from Table S1, rate-smoothed with pathD8 if not already ultrametric. 'Preferred Tree' and 'Unrated' refer to quality tags for trees originating from TreeBase.

Tables

TABLE S2.1

Published trees taken from the literature

Group	*No. tips*	*Ultrametric*
Mammalia [1]	4510	Yes
Hymenoptera [2]	1376	No
Trachaeophyta [3]	5122	Yes
Aves* [4]	9993	Yes
Squamata [5]	4162	No

**Hackett backbone, 100 tree sample from 9,000-10,000 posterior distribution*

TABLE S2.2

Variance of means of taxonomic groups at Phylum, Class and Order for each shape metric (σ^2). Proportional change between variance of means between ranks (±). Significant differences between observed variance of means or change in variance of means generated using a permutation test are indicated with asterisks (* < 0.05, ** < 0.01, *** < 0.001).

	Colless	Sackin	PSV^{D8}	γ^{D8}
Phylum (σ^2)	8.60E-01 ***	6.00E-01 **	9.94E-05	2.36E+00
Class (σ^2)	9.80E-01 ***	7.70E-01 ***	6.09E-03	5.91E+00
Order (σ^2)	8.10E-01 ***	6.60E-01 ***	4.91E-03	9.85E+00
Phylum-Class (±)	1.13E+00	1.28E+00	6.12E+00	2.50E+00
Phylum-Order (±)	9.40E-01	1.11E+00	4.94E+00	4.17E+00

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CHAPTER 3

Figures

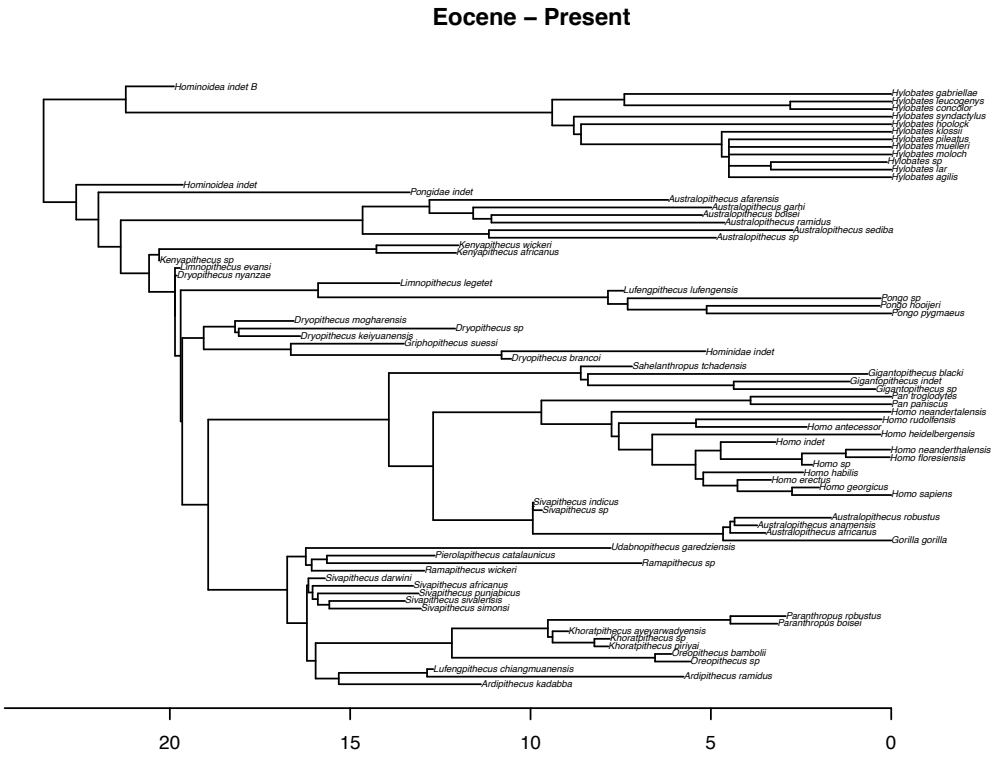

FIGURE S3.1.1

Example output of taxonomically constrained stochastic fossil placement: extinct and extant apes from the Eocene to the present.

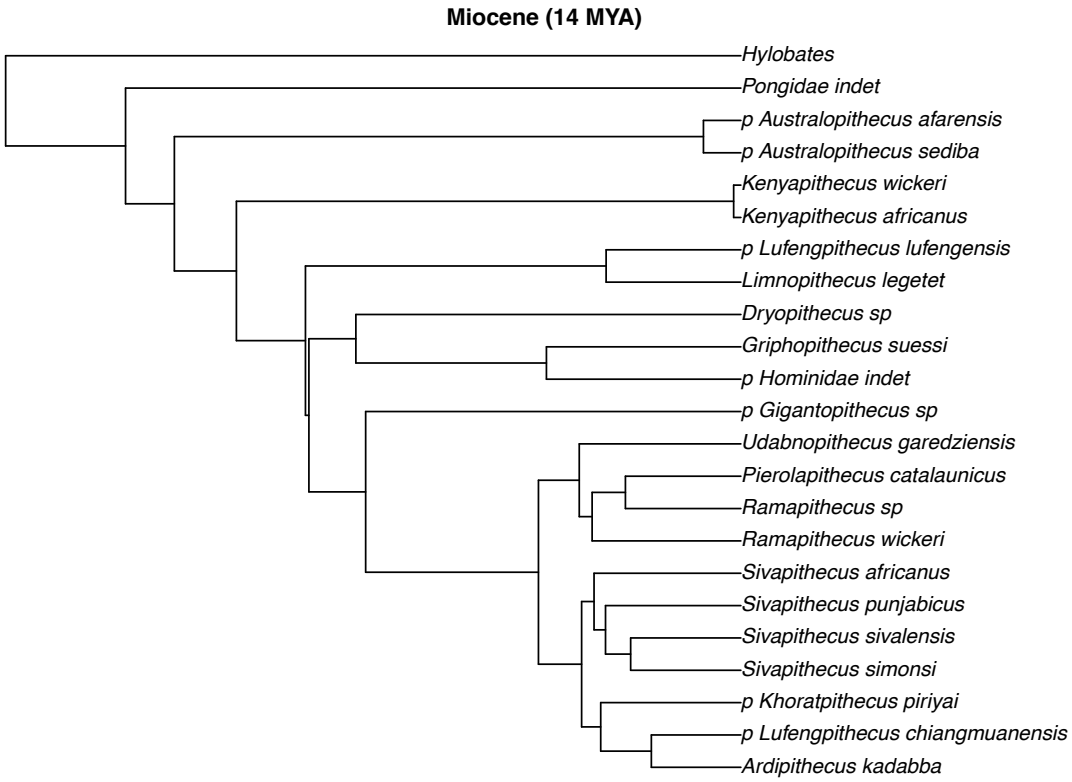

FIGURE S3.1.2

Example output of taxonomically constrained stochastic fossil placement: a reconstructed tree of extant apes during the Miocene.

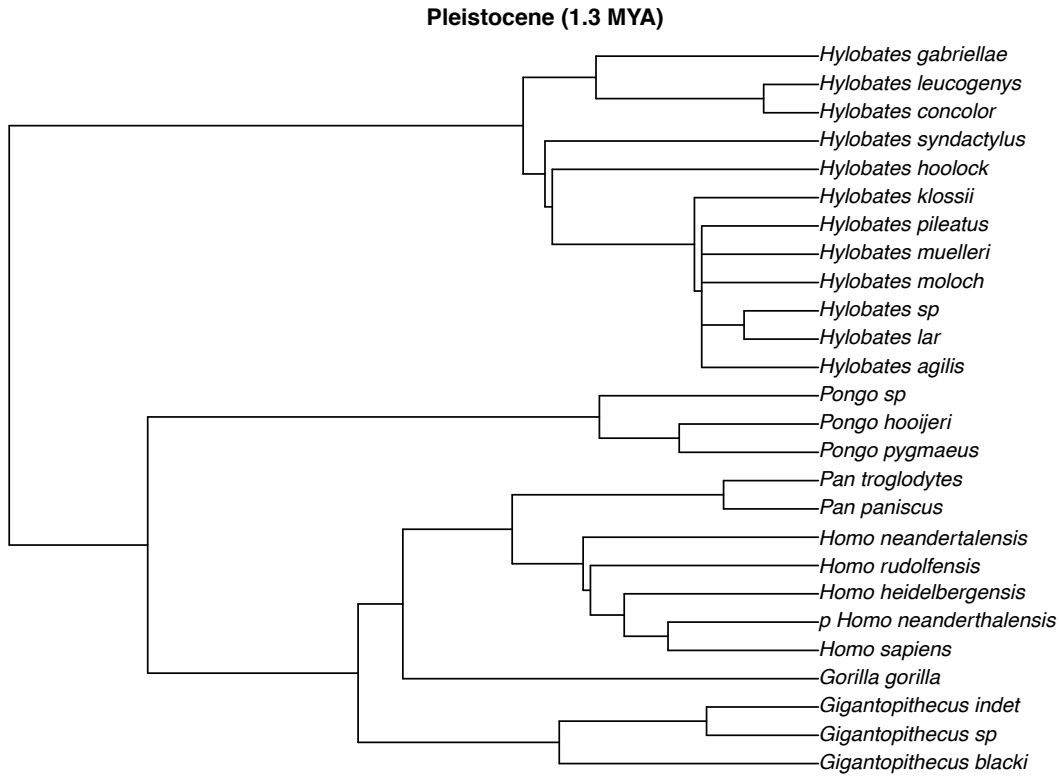

FIGURE S3.1.3

Example output of taxonomically constrained stochastic fossil placement: reconstructed tree of extant apes during the Pleistocene.

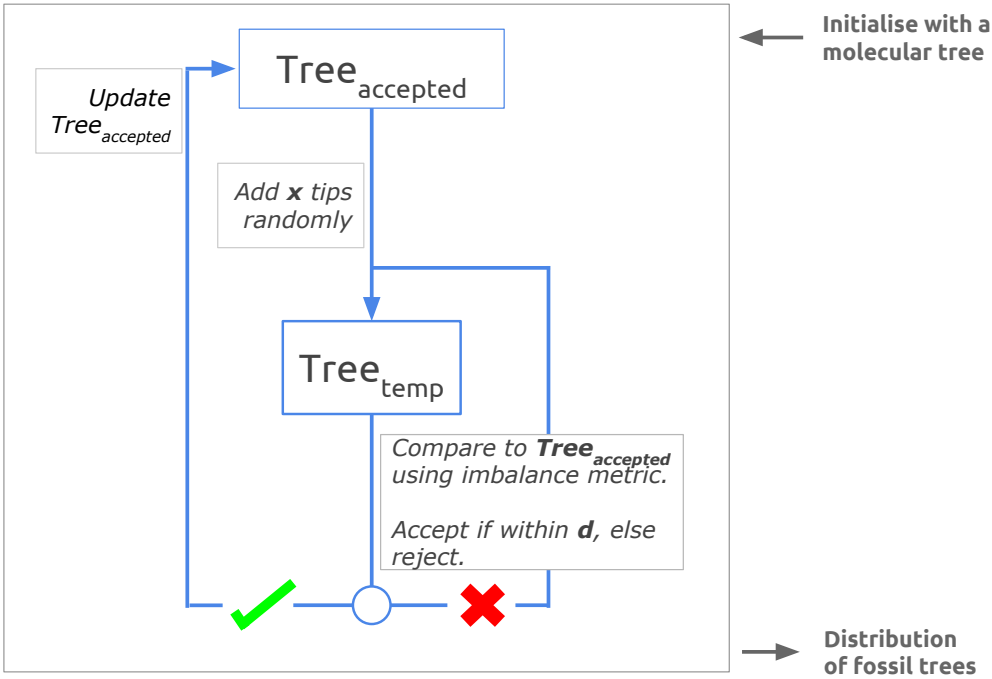

FIGURE S3.2

Suggested Approximate Bayesian Computation (ABC) process for improving pinning tips to tree. It is well established that phylogenetic trees are heavily unbalanced. This expectation can be used to improve pinning tips by ensuring that tip adding does not result in a tree more balanced than expected. Initiating this process with a molecular tree, x new tips can be added, the change in balance can be assessed, if the change is within d, then the tree is accepted and a new set of x tips are added. If the change is not within d, the tips are added at different locations. Repeat until all tips are added.

CHAPTER 4

Literature Survey

I conducted a literature survey by searching ISI Web of Knowledge and Google Scholar with the search phrase 'living fossil'. In total I read 56 papers, chapters and books. Below I list all the references from which I found an explicit or implied definition.

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TABLE S4.1

Identifying commonly cited aspects of the living fossil and the inconsistency of their use. Here I demonstrate how our nine aspects of the living fossil are represented by examples taken from the casebook of living fossils (Eldredge and Stanley 1984).

[Refer to chapter_4.zip]

Estimating Change

I used maximum parsimony reconstruction (MPR) (Narushima and Hanazawa, 1997) for estimating the states of internal nodes. This requires that all character traits provided are numeric integers. I, therefore, converted all continuous traits (such as body mass) by binning into ten equally spaced units. For traits that were non-numeric, I made them

numeric by randomly assigning numbers. Because trait states are not available for all parts of a tree, I reduce the tree by dropping absent tips and estimated ancestral states for this subset (FIGURE S4.1.1).

MPR estimates upper and lower internal node states (Farris, 1970). I used these numbers to estimate a change score (*score*) defined as one plus the absolute difference between the summed upper and lower estimates for the ascending and descending nodes that define a branch (eq. 1), see FIGURE S4.1.2.

$$score = 1 + |(upper_a + lower_a) - (upper_d + lower_d)| \quad \text{(Eq. 1)}$$

Values of one indicate no change has occurred, values between one and two indicate that change must have occurred for some of the most parsimonious trees, values above two indicate more than one change must have occurred. The score begins at one to prevent zero division errors when calculating contrasted change.

The change scores for each trait were then mapped to the full tree using name matching based on descendants. Scores per trait were equally shared between additional branches that are represented in the full tree (FIGURE S4.1.3). Finally, when calculating the contrasted change score by node, the mean score is calculated for all descendent branches from a node and its previous branch (FIGURE S4.1.4). The sister contrasted change ($score_c$) is then calculated from this data as the mean of contrasted mean changes for all (n) shared traits (t) between a clade (a) and its sister (b). Because traits are non-independent, and some show more possibility of variance than others, our mean was weighted based on the absolute mean of Spearman's R for shared traits between a clade and its sister (r), and the number of states represented by a trait (s) (eq. 2). $Score_c$ was only

calculated for clades with estimated change scores for more than four contrastable characters.

$$score_c = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{t_{a,i}}{t_{b,i}}\right).\frac{1}{s_i}.(1-r_i) \quad \text{(Eq. 2)}$$

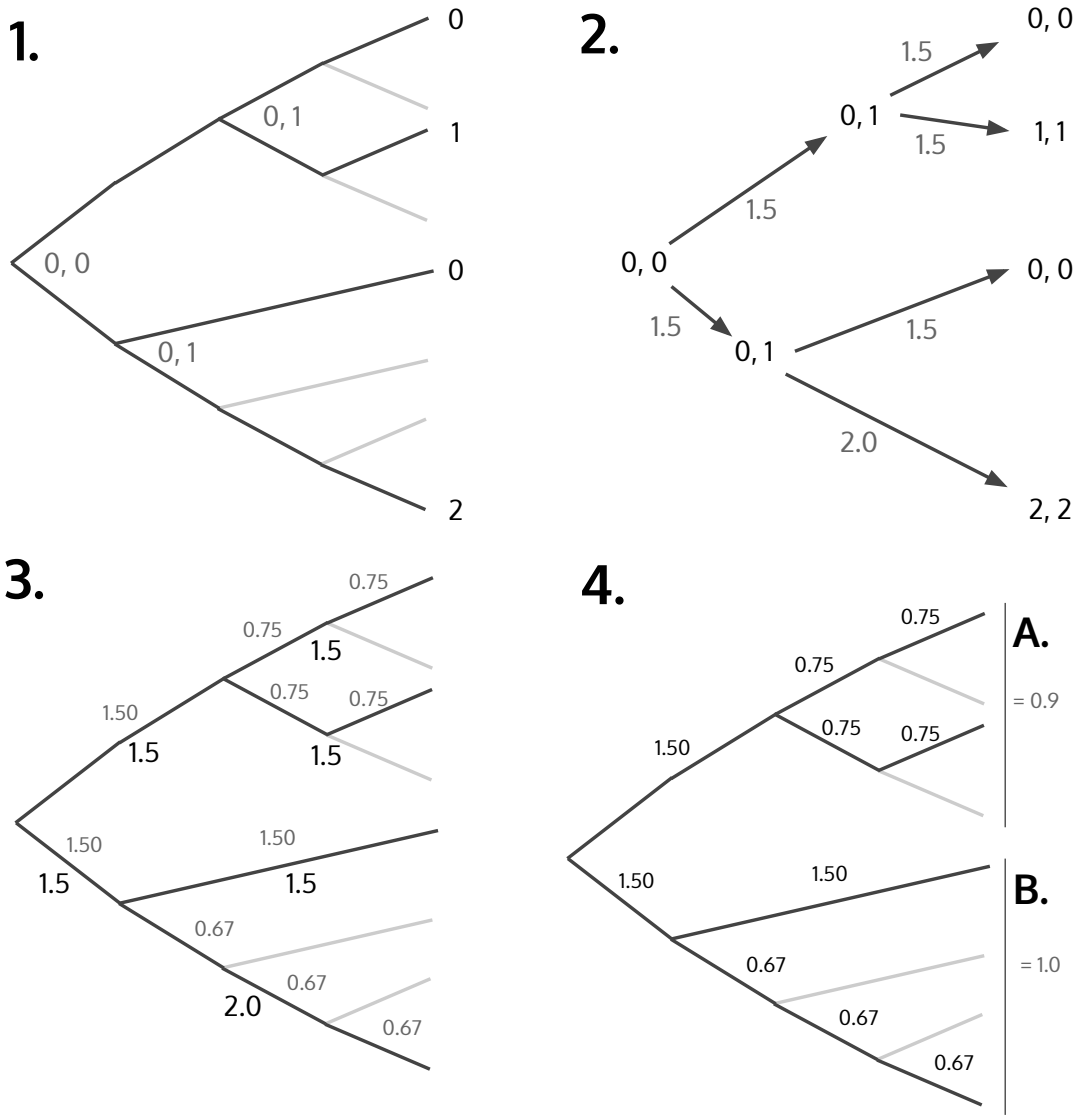

FIGURE S4.1

Calculating amount of change that has occurred for a single trait. (1) Use maximum parsimony reconstruction to estimate upper and lower states of trait at internal nodes. (2) Calculate change score based on the absolute difference of upper and lower states between previous and next node. (3) Map changes onto larger original tree by equally splitting scores for all branch parts. (4) Calculate score for every node and its sister by calculating mean scores from parental and descendant branches.

Estimating Change References.

Narushima, H. and Hanazawa, M. 1997. A more efficient algorithm for MPR problems in phylogeny. *Discrete Applied Mathematics*, 80, 231–238. DOI: 10.1016/S0166-218X(97)00088-7

Farris, J.S. 1970. Methods for Computing Wagner Trees. *Systematic Biology*, 19(1), 83–92. DOI: 10.1093/sysbio/19.1.83

Full Results

TABLE S4.2

Information and statistics for all clades for which pEPI, EPI and ED scores were calculated. Clades are ordered by pEPI. Common names are not available for all clades, these are automatically taken from NCBI. If you are looking for a specific clade, you may not find it because I only calculate values for clades that split. For example, lungfishes are represented by the clade "Ceratodontimorpha" even though they are more commonly known as "Dipnoi". This is because, according to NCBI taxonomy, the Dipnoi group only has a single child, Ceratodonitmorpha, which from the perspective of evolutionary performance make them the same clade. To find a clade, first search for it at the NCBI taxonomy website (www.ncbi.nlm.nih.gov/taxonomy) to ensure it is splitting. Additionally, many clades were ommitted if their "Success" was greater than 0.01 and/or their parent had fewer than 500 descendent species.

[Refer to 'chapter_4.zip' in archive]

TABLE S4.3

Contrasted change by character for bottom 250 clades most likely to be living fossils according to pEPI. Values below one indicate character has changed more in sister clade, above one indicate the inverse.

[Refer to 'chapter_4.zip' in archive]