Exploring the correlations between sequence evolution rate and phenotypic divergence across the Mammalian tree provides insights into adaptive evolution

JAN JANECKA*, BHANU CHOWDHARY and WILLIAM MURPHY

Veterinary Integrative Biosciences, Texas A and M University, College Station, Texas, USA

**Corresponding author (Fax, 979-845-9972; Email, jjanecka@cvm.tamu.edu)*

Sequence evolution behaves in a relatively consistent manner, leading to one of the fundamental paradigms in biology, the existence of a 'molecular clock'. The molecular clock can be distilled to the concept of accumulation of substitutions, through time yielding a stable rate from which we can estimate lineage divergence. Over the last 50 years, evolutionary biologists have obtained an in-depth understanding of this clock's nuances. It has been fine-tuned by taking into account the vast heterogeneity in rates across lineages and genes, leading to 'relaxed' molecular clock methods for timetree reconstruction. Sequence rate varies with life history traits including body size, generation time and metabolic rate, and we review recent studies on this topic. However, few studies have explicitly examined correlates between molecular evolution and morphological evolution. The patterns observed across diverse lineages suggest that rates of molecular and morphological evolution are largely decoupled. We discuss how identifying the molecular mechanisms behind rapid functional radiations are central to understanding evolution. The vast functional divergence within mammalian lineages that have relatively 'slow' sequence evolution refutes the hypotheses that pulses in diversification yielding major phenotypic change are the result of steady accumulation of substitutions. Patterns rather suggest phenotypic divergence is likely caused by regulatory alterations mediated through mechanisms such as insertions/deletions in functional regions. These can rapidly arise and sweep to fixation faster than predicted from a lineage's sequence neutral substitution rate, enabling species to leapfrog between phenotypic 'islands'. We suggest research directions that could illuminate mechanisms behind the functional diversity we see today.

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1. Introduction

The molecular clock proposed by Zuckerkandl and Pauling (1965) is one of the fundamental discoveries in evolutionary biology of the 20th century. It was first observed when these authors examined the level of divergence in globin genes and found that the estimated rate roughly behaved as a clock, with each substitution representing a 'tick' in time (Zuckerkandl and Pauling 1965). As mutations become fixed they accumulate, and if we know the rate and the number of mutational 'ticks' between any two species, we can estimate the time of their shared ancestry.

It was quickly noted that there are remarkable differences in rates of sequence evolution across lineages of mammals, and other organisms, leading to the development of relaxed molecular clocks that allow the rate to vary (Laird *et al.* 1969; Britten and Davidson 1971; Hart and Setlow 1974; Welch and Bromham 2005). These observations led to our understanding of radiations that occurred within life forms, allowed us to link diversification with paleoecological events, and identify cryptic diversity among taxa that are phenotypically conserved (Murphy *et al.* 2004, 2007; Janecka *et al.* 2007, 2008; Meredith *et al.* 2011). Hypotheses developed now explain how pivotal events including major asteroid impacts, continental drift or changes in atmospheric

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temperature have shaped life (Teeling *et al.* 2005; Murphy *et al.* 2007; Meredith *et al.* 2011).

Factors that influence the sequence evolution rate and are correlated with each other are important for several reasons. First, mutations and the subsequent fixation of these aberrations yield the initial heritable variation that leads to adaptation and subsequently speciation. By understanding factors that affect the substitution rate, i.e. the rate of fixed heritable genetic change, we gain insight into the fundamental processes that underpin the evolution of life forms. Second, these interactions are critical for applying sequence data to resolve both phylogenetic relationships and to estimate the timing of events that occurred millions of years ago (Welch and Bromham 2005; Bromham 2009). One of the missing parameters in our current methods of estimating timetrees is one that captures how rates vary in time; the majority of models that allow the rate to vary are yet to incorporate temporal variation along a branch. Finally, there is a remarkable diversity of organisms as a result of many well-described adaptive radiations. By studying molecular evolutionary rates, and how they are correlated with phenotypic change, we will better understand the fundamental processes that lead to adaptive radiations.

2. Molecular and phenotypic evolution

Evolution can be construed in two ways. The first is simply any change in a heritable character, even if it has no major phenotypic manifestations, which we refer to as 'static evolution'. However, another interpretation is change in a heritable character that yields morphological and functional alterations, in other words, continued change in the function of an organism, or 'dynamic evolution'. These are likely the consequences of two different processes.

Static evolution is largely the product of time. As time ticks by, populations eventually diverge into subpopulations, then into subspecies, then into species and later into higher-level lineages as mutations are fixed through drift, repeated bottlenecks and/or selection (Orr and Turelli 2001). Yet all these news species may appear and function roughly the same and thus be ecologically equivalent, and so in effect they have not 'evolved' in the sense of acquiring novel forms or adapting to new niches. In contrast, dynamic evolution is the acquisition of new traits and features that were not present in the common ancestor and are absent from related extant species. Therefore, the changes that accumulate during dynamic evolution have major functional repercussions.

It is fascinating that these two processes (i.e. the steady accumulation of mutations during static evolution versus the acquisition of new traits during dynamic evolution), and hence the two types of evolution described above, may be decoupled. Most mutations are nearly neutral and therefore become fixed through drift (Kimura 1983). In contrast, because phenotypic change can be adaptive, mutations that cause it could escape neutral processes through strong selection (Kimura 1983). Early on it was noted that larger animals evolved more quickly in terms of morphology (i.e. faster dynamic evolutionary rate) (Simpson 1953; Stanley 1973). Stanley (1973) suggested that lower population sizes and fecundity restrict gene flow, increasing opportunities for divergence. On the other hand, body size has been found to be negatively correlated with sequence evolution (i.e. static evolution evolutionary rate) (Nabholz *et al.* 2008; Welch *et al.* 2008; Galtier *et al.* 2009; Nabholz *et al.* 2009; Tsantes and Steiper 2009; Lartillot and Poujol 2011).

Interestingly, in cases where there is great functional change, one often sees very little molecular change and vice versa. This was noted early on in comparative genetics; one of the classical examples shocking many people at the time, particularly among the lay public, described high sequence similarity between humans and chimpanzees despite their drastic phenotypic divergence (Britten and Davidson 1971; King and Wilson 1975). Later, there was evidence that phenotypic evolution is not linked to species diversification (Adams *et al.* 2009). Therefore, the forces that have the greatest effects on dynamic versus static evolution must differ. The disassociations observed led to a synthesis of developmental and genetic studies and the establishment of evolutionary development biology (Carroll 2008). The resulting theory of morphological evolution attributes major phenotypic changes to mutations in *cis*-regulatory sequences that alter expression of regulatory loci and their targets, thus modifying fundamental gene networks (Carroll 2008).

The phenotypic diversity present among organism is not distributed equally across the tree of life. Understanding the relationship between molecular and phenotypic evolution is fundamental to grasping the diversification of organisms, such as the post-Cretaceous radiation of mammals. In the Cretaceous-Paleogene (KPg) boundary there is no evidence for a pulse in diversification rate (Meredith *et al.* 2011). However, the modern crown groups (i.e. orders) originated at that time and underwent functional diversification into novel ecomorphs (Meredith *et al.* 2011). This included the acquisition of many traits used to define the respective orders. Therefore, the evolutionary rate in terms of sequences and lineage diversification did not increase, but the level of phenotypic diversification did. Although there are numerous specific genomic alterations observed in select species or lineages found to explain phenotypic evolution (Fondon and Garner 2004), we are yet to understand the systematic genomic changes that yield rapid functional changes across entire lineages during periods with ecosystem-level community turnovers, such as those that transpired at the KPg boundary. Once we elucidate these mechanisms and establish a framework for understanding their complexity, we will better understand how phenotypic diversity evolves. We

may even be able to predict the potential for adaptation to changing environmental conditions and novel environments for particular lineages.

In this article we review literature that explores the correlation of molecular evolution, life history traits and phenotypic evolution, and discusses potential hypotheses on how these are linked. Recently we completed a family-level phylogenetic and timetree reconstruction of Mammalia (Meredith *et al.* 2011), giving us the opportunity to examine the distribution of rapid functional radiations relative to sequence evolution rate. We discuss the repercussions on understanding mechanisms behind adaptive radiations and propose research directions that may lead to advances in this field.

2.1 *Importance of taxon and gene sampling*

Molecular rate differences have been observed across and within lineages. Overall, the rate of nucleotide substitutions increases from fish to amphibians to birds to mammals (Adachi *et al.* 1993). Within these groups there are correlations with life history traits – for example, body size and generation time (table 1). However, many cases studies have been biased by selecting only a few representatives of a particular lineage or the use of only a few genes (table 1). Even within groups there are additional rate differences (Bleiweiss 1998; Lanfear *et al.* 2010; Meredith *et al.* 2011). For example, in rodents the rate varies greatly; yet, most studies only include mouse or rat, which happen to be among the fastest rodents (Bromham 2009, 2011). The observed effect when comparing different lineages may depend on which taxon was selected to represent that group. To date, the correlations among all major lineages of mammals have not been examined, nor have there been studies that have sampled genes or taxa with the explicit purpose of exploring these rate trends. This greatly hampers the ability to characterize relationships between molecular rate and life history traits.

2.2 *Components of molecular evolution*

It is important to consider the processes leading to variation in populations and species. The first step is the introduction of a mutation into the genome due to a replication error or DNA damage. The second is the fixation of this mutation over successive generations, thereby making the molecular change a feature of the entire phylogenetic unit (i.e. population, species or lineage). The molecular variation we typically use for comparing genes or species comes from these fixed mutations (usually in the form of nucleotide substitutions), and therefore the evolutionary rate we estimate is a combination of the mutation rate and the rate of fixation (table 1). Under the neutral model, the substitution rate is equal to the mutation rate (Kimura 1983); however, there are factors that disconnect these two values. When we compare the rate between species or genes, a portion of the difference comes from changes in the mutation rate and a portion from changes in the rate of fixation (Bromham 2009, 2011). The overall influence that a life history trait has on sequence evolution rate is then largely a result of the magnitude and directions of its effects on mutation and fixation rates. Because genetic drift will overcome selection to a greater extent in smaller populations, slightly deleterious mutations are more likely to become fixed in species with small effective population sizes (N_e) (Kimura 1983; Welch *et al.* 2008). This is supported by the greater rates of nonsynonymous substitutions in mtDNA of species with larger body sizes (a proxy for smaller N_e) (Popadin *et al.* 2007).

3. Correlations with life history

In many studies, sequence evolution scales with body size and other related life history traits within and among lineages of mammals, fish, birds and other organisms for both nuclear, mitochondrial and plastid DNA (Read and Harvey 1989; Martin and Palumbi 1993; Douzery *et al.* 1995; Bromham *et al.* 1996; Oli 2004; Welch and Bromham 2005; Davies and Savolainen 2006; Welch *et al.* 2008; Bromham 2009; Jeschke and Kokko 2009; Bromham 2011) (table 1). In addition, differences in substitution rates are also observed among genomic locations, genes and even nucleotide positions (Bromham *et al.* 1996; Rowe and Honeycutt 2002; Welch *et al.* 2008; Tsantes and Steiper 2009). The sequence rate is tied so closely with body size, generation time and population size that Bromham (2009) proposed it be considered a life history trait.

However, many life history traits are also correlated with each other (e.g. body size, metabolic rate, range size, population size and generation time), and therefore it is hard to determine which of these drives the relationship and if any of them are causal. To complicate matters, the metric used to capture particular life history traits are often not clearly defined in many studies. In addition, among groups there can be differences in the relationships between life history traits. For example, in mammals and birds, species with slower life histories (i.e. longer life span and generation time) also tend to have lower fecundity, and yet in fish the opposite pattern is more universal (Jeschke and Kokko 2009). These discrepancies add to the difficulties faced when attempting to resolve which specific factors contribute to rate heterogeneity. Nonetheless, there has been a wealth of research leading to some common patterns that have led to hypotheses explaining correlations between sequence evolution rate and life history traits. We highlight some of the most important studies below and in table 1.

Table 1. A list of studies examining correlations between molecular evolution and life history

Reference	Species/Lineages	Genes	Molecular evolution	Estimator	Phenotypic trait	Estimator	Correlation	Comments
Bromham *et al.* 1996	Mammalia	Nuclear (2), mitochondrial (2)	Substitution rate (T, Kn, Ks)	HKY85	Body size	Mean adult female mass	Negative	Pattern variable among gene segments
Lartillot and Poujol 2011	Carnivores and Therians (Nabholz 2008 data se)	Cytochrome b	Substitution rate	GTR + G	Body Size	Mass	Negative	Pattern in Ks; in both therians and carnivores
Lartillot and Poujol 2011	Carnivores and Therians (Nabholz 2008 data set)	Cytochrome b	dN/dS	dN/dS	Body Size	Mass	Positive	Pattern for therians only
Martin and Palumbi 1993	Various vertebrates	Nuclear, mitochondrial	Substitution rate (various studies)	Various	Body size	Mean adult mass	Negative	Pattern holds more within major vertebrate groups
Mooers and Harvey 1994	Birds	Genomic (Sibley and Ahlquist 1990)	Substitution rate	DNA-DNA hybridization	Body size	Mass	None	Tested across birds
Nabholz *et al.* 2008	Mammalia (1,696 species)	Cytochrome b	Substitution rate (Ks)	GTR + G	Body Size	Mass	Negative	Explained the lowest fraction of variance
Nabholz *et al.* 2009	Aves (1, 571 species)	Cytochrome b	Substitution rate (Ks)	GTR + G	Body Size	Mass	Negative	Substitution rate varies among bird lineages
Rowe and Honeycutt 2002	Rodents (Cavioidea)	Nuclear (2), mitochondria (1)	Substitution rate (T)	GTR + G	Body size	Adult body mass	Negative	Nuclear genes only
Tsantes and Steiper 2009	Strepsirrhine primates	Nuclear (17), mitochondrial (6)	Substitution rate	HKY + G, GTR + G + I	Body size	Mass	None	Pattern for introns, 3rd positions, and intergenic
Welch *et al.* 2008	Mammalia, all major lineages	Nuclear (6), mitochondrial (9)	Substitution rate	Codon-based model	Body size	Mass	Negative	Pattern for mtDNA Ks and nuclear Ks and Kn
Bromham *et al.* 1996	Mammalia	Nuclear (2), mitochondrial (2)	Substitution rate (T, Kn, Ks)	HKY85	Generation time	Age 1st repro. + gest.	Negative	For Ks in cyt b and beta globin exons
Laird *et al.* 1969	Rodents and Artiodactyls	Genomic	Substitution rate	DNA-DNA hybridization	Generation time	Generations per year	Negative	Correlation not directly tested
Lartillot and Poujol 2011	Carnivores and Therians (Nabholz 2008 data set)	Cytochrome b	Substitution rate	GTR + G	Generation time	Age of Fat maturity	None	No in correlation therians, marginal in carnivores
Lartillot and Poujol 2011	Carnivores and Therians (Nabholz 2008 data set)	Cytochrome b	dN/dS	dN/dS	Generation time	Age of F at maturity	None	Therians and carnivores
Martin and Palumbi 1993	Various vertebrates	Nuclear, mitochondrial	Substitution rate (various studies)	Various	Generation time	Age at first reproduction	Negative	Pattern holds more within major vertebrate groups
Mooers and Harvey 1994	Birds	Genomic (Sibley and Ahlquist 1990)	Substitution rate	DNA-DNA hybridization	Generation time	Age at first breeding	Negative	Highly significant correlation
Nabholz *et al.* 2008	Mammalia (1,696 species)	Cytochrome b	Substitution rate (Ks)	GTR + G	Generation time	Age of F at maturity	Negative	Explained the highest fraction of variance
Ohta 1993	Mammalia	Nuclear (17)	Substitution rate (Ks, Kn)	Li *et al.* (1985) method	Generation time	No formal test	Negative	Effect stronger for Ks
Rowe and Honeycutt 2002	Rodents (Cavioidea)	Nuclear (2), mitochondria (1)	Substitution rate (T)	GTR + G	Generation time	Gestation time	Negative	Nuclear genes only
Tsantes and Steiper 2009	Strepsirrhine primates	Nuclear (17), mitochondrial (6)	Substitution rate	HKY + G, GTR + G + I	Generation time	Age at first reproduction	Negative	Pattern for introns, 3rd positions, and intergenic
			Substitution rate				Negative	

Welch *et al.* 2008	Mammalia, all major lineages	Nuclear (6), mitochondrial (9)		Codon-based model	Generation time	Age at first reproduction		Pattern for nuclear Ks and Kn
Li *et al.* 1987	Mammalia	Nuclear, mitochondrial	Substitution rate (Ks, Kn)	Li *et al.* (1985) method	Generation time	No formal test	Negative	No formal test, stronger effect for Ks
Li *et al.* 1996	Primates and rodents	Nuclear (8, 1 pseudogene)	Substitution rate (Knc, Ks)	Data from other studies	Generation time	No formal test	Negative	No formal test, stronger effect for Ks
Lartillot and Poujol 2011	Carnivores and Therians (Nabholz 2008 data set)	Cytochrome b	Substitution rate	GTR + G	Longevity	Lifespan	Negative	Pattern in Ks; in both therians and carnivores
Lartillot and Poujol 2011	Carnivores and Therians (Nabholz 2008 data set)	Cytochrome b	dN/dS	dN/dS	Longevity	Lifespan	Positive	Therians only
Nabholz *et al.* 2008	Mammalia (1,696 species)	Cytochrome b	Substitution rate (Ks)	GTR + G	Longevity	Lifespan	Negative	Stronger effect in long-lived mammals
Nabholz *et al.* 2009	Aves (1, 571 species)	Cytochrome b	Substitution rate (Ks)	GTR + G	Longevity	Lifespan	Negative	Substitution rate varies among bird lineages
Welch *et al.* 2008	Mammalia, all major lineages	Nuclear (6), mitochondrial (9)	Substitution rate	Codon-based model	Longevity	Maximum lifespan	Negative	mtDNA Ks, life span strongest in mtDNA
Bromham *et al.* 1996	Mammalia	Nuclear (2), mitochondrial (2)	Substitution rate (T, Kn, Ks)	HKY85	Metabolic rate	Mass-specific	None	Same pattern in all segments
Gillooly *et al.* 2005	Major taxonomic groups (inverts. and verts).	Nuclear, mitochondrial	Substitution rate	Data from other studies	Metabolic rate	Mass-specific	Negative	Model included body size and temperature
Mooers and Harvey 1994	Birds	Genomic (Sibley and Ahlquist 1990)	Substitution rate	DNA-DNA hybridization	Metabolic rate	Mass-specific	None	Tested across birds
Rowe and Honeycutt 2002	Rodents (Cavioidea)	Nuclear (2), mitochondria (1)	Substitution rate (T)	GTR + G	Metabolic rate	Basal	None	No pattern observed across all genes
Welch *et al.* 2008	Mammalia, all major lineages	Nuclear (6), mitochondrial (9)	Substitution rate	Codon-based model	Fecundity	Litter size	Positive	Nuclear Ks and Kn
Li *et al.* 1996	Primates and rodents	Nuclear (8, 1 pseudogene)	Substitution rate (Knc, Ks)	Data from other studies	Germ cell divisions	No formal test	Negative	Compared differences for males and females
Mayrose and Otto 2011	*Daphnia*	Mitochondrial (12S, 16S rRNA	Substitution rate	GTR	Habitat effects	Salinity	Positive	Greater substitution rate in saline environment

This compilation is not exhaustive and serves as an overview of the patterns commonly tested and observation made that have become the basis for hypotheses explaining sequence evolution rate variation. The number in parenthesis in the "Genes" column refers to the number of loci examined in the respective dataset.

T = Substitution rate for total sites in a sequence.

Kn = Substitution rate at nonsynonymous sites.

Ks = Substitution rate at synonymous sites.

Knc = Substitution rate at non-coding sites.

GTR = General Time Reversible sequence evolution model.

HKY85 = Hasegawa, Kishino and Yano 1985 sequence evolution model.

G = Gamma.

I = Invariant sites.

3.1 *Body size and longevity*

Negative correlations with body size and longevity have been observed in both nuclear and mitochondrial genes (Bromham *et al.* 1996; Nabholz *et al.* 2008; Welch *et al.* 2008; Lartillot and Poujol 2011) (table 1). Body size and longevity are hypothesized to put selective pressure on processes that directly affect mutation rate including polymerase fidelity, proofreading, excision-repair and the SOS response (Kornberg 1980; Echols and Goodman 1991; Kunkel 1992; Bromham *et al.* 1996). Larger, longer-lived animals are theoretically better served with a more effective DNA copy and repair systems to minimize cellular abnormalities that may affect fitness. Therefore, selection should work to reduce mutation rates, particularly in mitochondria (Bromham *et al.* 1996; Nabholz *et al.* 2008, 2009). This body size and longevity effect is stronger in longer-lived mammals, further supporting this hypothesis, and also across Aves (Nabholz *et al.* 2008, 2009). Indeed, differences in the efficiency of repair mechanisms have been correlated with body size and longevity as would be expected if these were under selection (Hart and Setlow 1974). For example, unscheduled DNA synthesis in fibroblast lines is proportional to logarithm of life span (Hart and Setlow 1974). The mutation rate differences between primates compared to rodents, and apes and monkeys compared to more ancestral primates have been also explained by differences in repair efficiency (Hart and Setlow 1974, Britten 1986).

3.2 *Generation time*

Generation time has been correlated with rate both within and among diverse lineages (Li *et al.* 1987; Ohta 1993; Mooers and Harvey 1994; Li *et al.* 1996; Rowe and Honeycutt 2002; Bromham 2011) (table 1). One of the first papers that showed the rate is affected by generation time observed rodents had a tenfold faster rate than artiodactyls, but this diminished when scaled with generation time instead of years (Laird *et al.* 1969). Generation time is believed to affect rate in two ways. First, species with shorter generation times undergo more meiotic events in a given amount of time; therefore, there are more chances for mutations to occur. In addition, with more generations per unit time, there is a greater opportunity for any mutations to become fixed. Both these factors would increase the substitution rate.

The slope of the log of the generation time and log of the mutation rate has been estimated between −0.15 to −0.44, which is not as steep as predicted if there was a direct relationship (Nabholz *et al.* 2008; Welch *et al.* 2008). Therefore, the generation time effect is likely mitigated by other factors (Bromham 2011). One possibility is that variation in the number of germline divisions reduces the impact from generation time (Li *et al.* 1996). For example, humans have a greater number of germline cell divisions than mice, and therefore their substitution rate is not as low relative to mice as would be predicted from just generation time (Li *et al.* 1996; Bromham 2011). Males have more germline divisions than females, and this has been proposed to explain the higher mutation rates in males for both primates and rodents (Li *et al.* 1996). The number of divisions has also been used to explain greater mutation rates in mtDNA compared to nuclear loci (Bromham 2011).

3.3 *Metabolic rate*

It has been hypothesized that in species with higher metabolic activity, DNA is exposed to a greater number oxidative byproducts that cause damage (Martin and Palumbi 1993; Mooers and Harvey 1994; Bromham *et al.* 1996; Gillooly *et al.* 2001; Rowe and Honeycutt 2002, Lanfear *et al.* 2007). This has also been used to explain the higher rate in mitochondrial compared to nuclear DNA (Bromham *et al.* 1996). However, there is more inconsistency in the correlation between metabolic rate and sequence evolution than for the above life history traits; therefore, its effects are likely not as universal or strong (Mooers and Harvey 1994; Bromham *et al.* 1996; Rowe and Honeycutt 2002; Gillooly *et al.* 2005) (table 1). For example, birds have a higher mass-specific metabolic rate, and yet birds with small body mass (< 500 g) evolve fourfold slower than mammals (Nabholz *et al.* 2009). One potential explanation for the inconsistency is that the primary factors that contribute to evolutionary rate differ depending on the cellular environment of the locus (Bromham 2011). For instance, because mitochondria have greater numbers of oxidative by-products, rate differences for mtDNA genes may be driven more by metabolism (Brown 1983; Martin and Palumbi 1993; Bromham *et al.* 1996). In contrast, nuclear DNA is not exposed to as many oxidative byproducts, and so it may be more influenced by generation time (Bromham *et al.* 1996; Tsantes and Steiper 2009). Martin and Palumbi (1993) suggested that although metabolic rate may not be the primary factor for rate variation, it may potentially explain outliers that do not fit the sequence rate–generation time correlation.

3.4 *Modelling effects of multiple factors*

There have been several attempts to model life history variables together to test correlations with sequence rate (table 1). One approach took into account the influence of body size and temperature on metabolic rate, and the neutral theory, to explain variation in sequence evolution with mass-specific metabolic energy (Gillooly *et al.* 2001, 2002, 2005). Gillooly *et al.* (2005) proposed such models may resolve inconsistencies between molecular and fossil estimates of divergence

time. Indeed, they were able to obtain more accurate divergence dates by taking into account mass-specific metabolic energy. This approach was recently used to reconcile molecular and fossil estimates of crown primate origins by predicting the molecular rate based on morphological traits reconstructed from fossils assigned to specific time points on branches (Steiper and Seiffert 2012). If a pattern can be found that holds across diverse species and genes, this model could be also used to predict the correct sequence evolution rate for lineages that have very long branches and few good fossil calibrations, e.g. marsupial moles and aardvarks.

4. Correlations with phenotypic evolution

Diversification patterns for many major clades are very uneven across phylogenies, both in terms of species numbers and phenotypes (Adams *et al.* 2009; Meredith *et al.* 2011; Purvis *et al.* 2011) (table 2). Even sequence divergence appears to have punctuated episodes of acceleration and slowdown that lead to departures from the molecular clock (Soltis *et al.* 2002; Pagel *et al.* 2006; Venditti and Pagel 2010). Previous studies have detected rapid evolution and speciation as a result of selection on standing genetic variation over short time periods (Fondon and Garner 2004, Schluter and Conte 2009). However, at broader temporal and genomic scales few studies have identified process leading to large-scale functional diversification. Regulatory changes are more likely to contribute to phenotypic divergence during radiations than the accumulation of nucleotide substitutions (Britten and Davidson 1971; Beall *et al.* 1999; Carroll 2008). This is suggested in a transcriptome analysis of two cichlids (*Amphilophus astorquii* and *Amphilophus zaliosus*) that showed only 6 expressed sequence tag (EST) contigs were under strong diversifying selection out of 13,106 orthologous ESTs, despite their rapid adaptive divergence (Elmer *et al.* 2010).

Numerous natural history traits and environmental variables may impact both neutral drift and selection, including geographic range, ecological specialization, body size, mating preferences, breeding patterns and interspecific competition. Therefore, they would also affect morphological evolution (table 1). However, there has been controversy about which of these predominate (Bromham 2009). This is very difficult to resolve because the relative influence of each one depends on ecological traits, effective population size, functional diversity in the ecosystem and saturation of niches (Davies and Savolainen 2006; Cooper and Purvis 2010; Mahler *et al.* 2010). For example, a wide geographic range across diverse ecosystems would subject subpopulations to different selective pressures facilitating divergence. However, local selection can be overcome by gene flow if individuals disperse long distances and there is frequent migration (Stanley 1973). The dispersal ability of a species mitigates the correlation between geographic range and the rate of phenotypic divergence of subpopulations. From an evolutionary historical perspective these interactions will be even more complex because selection regimes, ranges and even species themselves change through time. Despite these challenges, are there patterns from which we can infer the primary factors that shape functional divergence and lead to adaptive radiations?

Attempts have been made to answer the above question by testing for correlations between phenotypic divergence and diversification rate, substitution rate or life history traits (table 2). Life history correlations with morphological evolution are in general the opposite of those observed for sequence evolution, or are not present at all (Omland 1997; Bromham *et al.* 2002; Davies and Savolainen 2006; Seligmann 2010; Collar *et al.* 2011; Goldie *et al.* 2011; Safi *et al.* 2011)(table 2). Cooper and Purvis (2009) examined the associations between phenotypic evolutionary rate with numerous traits including body mass, mass-specific metabolic rate, gestation length, maximum longevity, population density, geographic range size, mean annual temp across the range, competition and ecological speciation (Adams *et al.* 2009; Cooper and Purvis 2009, 2010). The fastest rates of morphological change occurred with larger body sizes (Cooper and Purvis 2009). However, the rate and patterns were not always consistent. Morphological evolution was faster in small- and large-bodied rodents than in rodents with moderate body sizes (Cooper and Purvis 2009). Other factors appeared to be more important in some lineages. Low mass-specific metabolic rate contributed the most to morphological change in marmotine squirrels, while high environmental temperatures did so in phyllostomid bats (Cooper and Purvis 2009). There are also likely environmental effects on phenotypic divergence; in a study of mammalian body size evolution (3473 species in dataset) this trait appeared to be influenced by a combination of geography, climate and history (Cooper and Purvis 2010).

Bromham *et al.* (2002) found no association between molecular and morphological rates of evolution, which was also supported in several other studies (Davies and Savolainen 2006). However, Seligmann (2010) reanalysed data of Bromham *et al.* (2002) and observed a positive correlation between the two, refuting the original work. The discrepancy observed was attributed to the loss of power in the Bromham *et al.* (2002) analyses caused by exclusion of tips. For *Lamellodiscus* gill parasites, morphology remained nearly constant even with rapid sequence evolutionary rates (Poisot *et al.* 2011). This was partly explained by very strong host-induced constraints on form and function (Poisot *et al.* 2011). In plants, Davies and Savolainen (2006) detected weak positive correlations, potentially a result of processes linked to size and growth. This relationship only held when data were combined and individual clades were analysed

Table 2. A list of studies examining correlations between phenotypic evolution and either molecular or ecological traits

Reference	Species/Lineages	Genes	Evolution parameters	Estimator	Phenotypic trait	Estimator	Correlation	Comments
Bromham *et al.* 2002	Mammals and reptiles	Nuclear, mitochondrial	Substitution rate	HKY + G	Morphological divergence	Numerous traits	None	Used methods to avoid phylogenetic bias
Davies and Savolainen 2006	Mammals, reptiles	Nuclear, mitochondrial	Substitution rate	HKY85	Morphological divergence	Numerous traits	None	Variable among groups
Davies and Savolainen 2006	Plants	Chloropast	Substitution rate	HKY85	Morphological divergence	Numerous traits	Positive	2–11 % morph. variation explained in plants
Goldie *et al.* 2011	Mammalia (1,696 species)	Nuclear, mitochondrial	Substitution rate (T, Kn, Ks, dN/dS)	GTR and MG94	Species diversification	Body size	Negative	mtDNA Ks, nuclear T, Kn, Ks
Goldie *et al.* 2011	Mammalia (1,696 species)	Nuclear, mitochondrial	Substitution rate (T, Kn, Ks, dN/dS)	GTR and MG94	Species diversification	Clade size	None	No relationship detected
Poisot *et al.* 2011	*Lamellodiscus* gill parasites	Nuclear, mitochondrial	Substitution rate	GTR	Morphological divergence	Numerous traits	None	Pattern explained by host-parasite constraints
Seligman 2010	Mammals and plants	Nuclear, mt., chloropast	Substitution rate	Not specified	Morphological divergence	Numerous traits	Positive	Re-analyzed data and showed weak correlation
Omland 1997	Plants, verts., inverts.	Mitochondrial, chloroplast	Sequence divergence	Maximum parsimony	Morphological divergence	Numerous traits	Positive	Positive in 7 of 8 groups, some variable
Collar *et al.* 2005	Centrarchid fish (black basses and sunfishes)	Nuclear (4) mitochondrial (3)	Divergence time	relaxed clock (r8s program)	Morphological divergence	6 skull traits	Negative	Greater phenotypic evolution in younger lineage
Safi *et al.* 2011	Mammalia, all major lineages	Beninda-Emonds *et al.* (2008)	Phylogenetic diversification	Sum of branch lengths in a group	Functional diversity	4 ecological traits	Positive	The slope varied with continents
Adams *et al.* 2009	Plethodontid salamanders	Nuclear, mitochondrial	Phylogenetic diversification	Method-of-moments estimator	Morphological divergence	7 morphometric variables	None	No correlation in 15 clades
Mahler *et al.* 2010	*Anolis* lizards	Mitochondrial (6)	Ecological opportunity	Lineage diversity and relative age	Morphological divergence	12 morphological traits	Negative	Traits examined were known to be adaptive
Cooper et al. 2009	Mammalia (4 groups)	n.a.	Morphological divergence	Combination of 11 skull traits	Body size	Adult mass	Positive	Pattern in all 4 groups tested.
Cooper et al. 2009	Phyllostomid bats	n.a.	Morphological divergence	Combination of 11 skull traits	Environmental temperature	Mean annual temperature	Positive	Did not hold in other 3 groups
Cooper et al. 2009	Marmotine squirrels	n.a.	Morphological divergence	Combination of 11 skull traits	Metabolic rate	Mass-specific metabolic rate	Negative	Did not hold in other 3 groups

This compilation is not exhaustive and serves as an overview. The number in parenthesis in the "Genes" column refers to the number of loci examined in the respective dataset.

T = Substitution rate for total sites in a sequence.

Kn = Substitution rate at nonsynonymous sites.

Ks = Substitution rate at synonymous sites.

Knc = Substitution rate at non-coding sites.

dN/DS=Nonsynonymous to synonymous substitution ration.

GTR=General Time Reversible sequence evolution model.

HKY85=Hasegawa, Kishino and Yano 1985 sequence evolution model.

MG95=Muse and Gaut model of codon substitution.

Mt=Mitochondrial.

G=Gamma.

I=Invariant sites.

together. The authors speculated that the lack of correlations in mammals may have resulted from fewer sampling points (Davies and Savolainen 2006). The absence of evidence for significant patterns in other studies may have also been due to insufficient samples sizes (Bromham *et al.* 2002; Davies and Savolainen 2006; Goldie *et al.* 2011). However, it is also possible that the difference among lineages has biological significance and may reflect fundamental dissimilarities in molecular mechanisms and selection coefficients. The discrepancies suggest that there is no strong relationship between phenotypic evolution and substitution or diversification rates.

5. Patterns of phenotypic divergence across the mammalian phylogeny

A recent family-level phylogeny of Mammalia provides an opportunity to develop a framework for understanding genomic mechanisms that are responsible for the extensive phenotypic diversification that has occurred over the ~220 million year history of this group (Meredith *et al.* 2011). The rate of diversification varies greatly across different branches as can be seen in the family-level mammalian phylogeny (figure 1). Among the orders with the slowest sequence evolution rate are the Perissodactyls (odd-toed ungulates), today represented only by three families: Equidae (horses, zebras and asses), Tapiridae (tapirs) and Rhinocerotidae (rhinos). This order originated 56.8 million years ago (Mya), yet underwent extensive phenotypic divergence in conjunction with their explosive taxonomic radiation during the Eocene. Indeed, there are many more extinct than extant perissodactyls, particularly among the equids and rhinos: 14 perissodactyl families are extinct compared to only 3 that are extant (McKenna and Bell 1997).

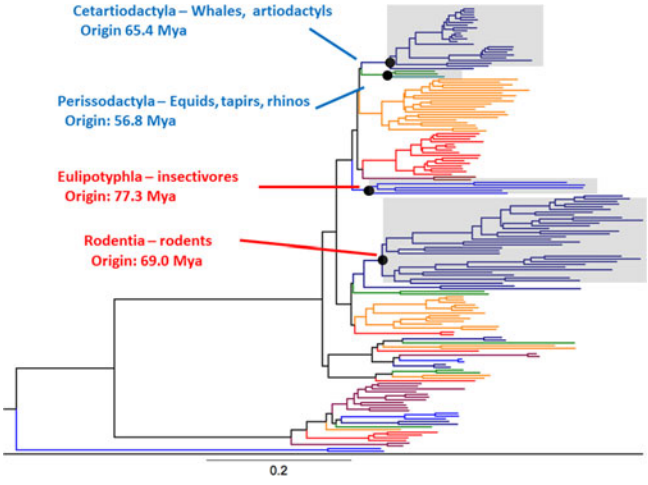

Figure 1. Maximum likelihood family-level phylogeny of Mammalia (reduced data set from Meredith *et al.* 2011). Each order is illustrated with a different color. Four groups that show great disparity between molecular and phenotypic divergence are highlighted.

Another laurasiatherian group with intermediate sequence diversification rates is Cetartiodactyla, which originated around 65.4 Mya (figure 1). Yet within this group there were also extensive adaptive radiations that resulted in incredible morphological, physiological and behavioural modifications. One lineage, the cetaceans (origin 33.9 Mya), has undergone among the most drastic phenotypic divergence of any mammals, yet also happens to have the potentially slowest nucleotide substitution rate (Meredith *et al.* 2011). Even after the shift to an obligate aquatic lifestyle, there have been additional adaptive radiations within this group (Deméré *et al.* 2005).

The phenotypic divergence described above stands in stark contrast to the relative phenotypic conservatism within certain mammalian clades having much faster nucleotide substitution rates. Among these are some lineages within the Eulipotyphla (insectivores) and Rodentia (rodents) orders. Both of these groups are older (77.3 Mya and 69.0 Mya, respectively) and are characterized by very rapid sequence evolution rates compared to both Perissodactyls and Cetartiodactyls (Meredith *et al.* 2011). Despite their age, faster rates and, in the case of rodents, much greater species diversity, many of their lineages have remained relatively conserved in life history, ecology, and behaviour. There are a few rodents and insectivores that do have very divergent phenotypes; for example, some of the fossorial species including the naked mole rat (Rodentia) and the moles (Eulipotyphlans), however, these have among the slowest nucleotide substitution rates among their orders.

6. Mechanisms behind phenotypic evolution

Molecular and morphological evolutionary rates are either not correlated or negatively associated (Bromham *et al.* 2002; Davies and Savolainen 2006; Goldie *et al.* 2011). As we describe above, in mammals some of the greatest phenotypic divergence occurs among groups that have lower rates of nucleotide substitution. Since mutations are ultimately the source of heritable variation, this suggests three things. The first is that we are yet to characterize genomic regions that lead to the remarkable phenotypic divergence of lineages such as Perissodactyls and Cetartiodactyls. Second, most of the mutations examined to date are neutral or nearly neutral. Third, the processes that lead to nucleotide versus phenotypic divergence likely differ.

One potentially important factor may be effective population size (N_e). As described above, there appears to be a disassociation between phenotypic evolution and sequence evolution; in many cases the 'slow' lineages are the ones that have the most drastic phenotypic change. Often these are larger-bodied animals with smaller N_e, which means that

more mutations will become fixed by drift and with a smaller influence from selection. In species with small N_e, the fixation of slightly deleterious mutation is more likely (Woolfit 2009). Since deleterious mutations are by definition the ones that negatively affect the function of proteins, it follows that more mutations that influence the phenotype would become fixed. This could theoretically result in greater phenotypic variation in small populations that may become advantageous after a change in selection regimes.

If balancing selection is important in maintaining species shape and morphology, then small population size would enable more stochastic variation in mutations affecting form and function (Bromham 2009). In a variable environment, the new phenotypes that are either neutral, or slightly deleterious, may suddenly give a selective advantage and become exaptations (Gould and Vrba 1982). Is it possible that functional diversifications may have been initiated after fixation of mutations that were initially deleterious? Potentially. Once a novel phenotype arises, it may enable the species to fill unoccupied niches or escape competition, and subsequently become the basis for additional adaptations. Because of the effect population size has on drift, one can predict there will be more phenotypic divergence in species with smaller N_e, even though the neutral substitution rate would be slower. Consistent with the prediction, species with larger body size (a proxy for N_e) have been observed to undergo more rapid phenotypic evolution, although this needs to be rigorously tested (Meredith *et al.* 2009). Furthermore, large species also have higher nonsynonymous (dN)/synonymous (dS) ratios than smaller taxa (Popadin *et al.* 2007; Goldie *et al.* 2011; Lartillot and Poujol 2011).

Although there must be a molecular basis to phenotypic evolution, not all molecular changes affect form or function. Across large parts of the genome there appear to be relatively few areas that have major effects on phenotypes, and specifically morphology. Therefore, only small subsets of mutations alter phenotypic traits. It then follows that on any given phylogenetic branch most mutations are irrelevant in terms of functional evolution. In a way, this is a reaffirmation of the neutral theory stating that a vast majority of genetic changes are neutral, or nearly neutral, and therefore most mutations have no selective advantage or disadvantage (Kimura 1983).

If most mutations examined thus far are neutral, and there is a decoupling of molecular and morphological evolutionary rates, how can we identify genetic alterations that lead to major adaptive radiations, i.e. dynamic evolution? The leading hypothesis postulates that mutations in regulatory elements play the key role (Carroll 2008). One of the most important observations from which this arose described cooption of conserved proteins for different and independent functions across disparate tissues and organisms (McMahon *et al.* 2003). This strongly suggests that changes in regulation of proteins can produce unique structures with different functions. Such regulatory changes can also alter fixed morphological traits. Fondon and Garner (2004) found indels of trinucleotide repeats in the coding regions of regulatory genes that influence skull morphology and polydactyly in the domestic dog. There was strong evidence that these mutations arose after the formation of the breeds, over the course of only 50–100 years. This is possible because of the much higher mutation rates of microsatellite indels compared to substitutions. Insertions and deletions that affect phenotypes are not restricted to short tandem repeats. For example, the three independent mutations that cause melanism in felids are deletions within exons of genes (Eizirik *et al.* 2003). Additional indels and substitutions can occur in 5′ and 3′ untranslated regions and *cis*- and *trans*-regulatory elements, and noncoding mRNAs that would also affect regulation of genes (Carroll 2008).

The patterns in phenotypic divergence and the genetic variants affecting form have three very important implications for evolutionary biology. First, nucleotide substitutions, which represent the variation predominantly used to reconstruct phylogenies and estimate evolutionary rates, may not be the class of mutations that produces the vast majority of functional changes we are interested in understanding. Second, there are classes of mutations much more common than SNPs and these may be a more important source of adaptive variation. Third, the types of mutations that may most affect phenotypes are often not present in sequence datasets because the sites where they occur are usually discarded from phylogenetic and timetree analyses.

7. Conclusions and future prospects

The patterns observed in previous studies and a family-level phylogeny of mammals suggest that the predominant processes affecting phenotypic changes are not tied directly to nucleotide substitutions rates. The most likely mechanisms are indels in coding and noncoding regions of genes, duplications and deletions, gene losses, gene gains, and substitutions that affect regulatory elements, and changes in expression patterns (Carroll 2008).

One of the first steps that would increase our understanding of phenotypic evolution would be more exhaustive analyses of the associations between nucleotide substitution rates and phenotypic divergence. Although there is strong evidence that these are not directly linked, the relationship between them could shed light on what natural history traits and environmental factors influence them. Ideally the analyses would incorporate fossil records to avoid biases introduced from ancestral state reconstruction. Stem fossils for the respective lineages could be used to more accurately infer the amount of phenotypic divergence in each branch, so that it

could be compared to sequence evolutionary rate. We now have unprecedented sampling of taxa for both molecular and morphological data, which allows us to select groups that would provide the most power for comparative analyses.

The second important step in understanding phenotypic evolution would be to construct genomic datasets specifically for the purpose of capturing variation that likely explains phenotypic divergence. Next-generation sequencing technologies could be leveraged to produce the large amounts of data that this would require. Initially, RNA-Seq could be used to sequence cDNA libraries from different embryonic and adult tissues in several representatives of two groups. The first would have great functional divergence but slow rate of nucleotide substitutions and the second group would have little functional divergence but very fast substitution rate. The differences in gene expression, sequence variation and splice variants could be used to identify candidate gene networks that may drive phenotypic divergence. Next, capture arrays could be designed to re-sequence these regions in additional individuals and species from both groups, to execute a comprehensive exploration of genomic factors driving functional diversification.

There are many remarkable organisms that thrive across our planet, inhabiting nearly every corner, from the mildest environments to the most extreme. The *Origin of Species* described how life evolves and developed a framework for understanding adaptive radiations. With the recent developments in genomics, we can now decipher the mechanisms behind these adaptive radiations. There have been many population-level studies that identified causal genetic variants that influence phenotypes. In parallel, phylogenetic research has resolved many relationships among species and the timing and pattern of radiations. Now is the time to merge these approaches into a comprehensive study that will elucidate evolutionary mechanisms responsible for the remarkable phenotypic diversity we see today.

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References

Adachi J, Cao Y and Hasegawa M 1993 Tempo and mode of mitochondrial-DNA evolution in vertebrates at the amino-acid-sequence level - rapid evolution in warm-blooded vertebrates. *J. Mol. Evol.* **36** 270–281

Adams DC, Berns CM, Kozak KH and Wiens JJ 2009 Are rates of species diversification correlated with rates of morphological evolution? *Proc. R Soc. B Biol. Sci.* **276** 2729–2738

Beall CM, Almasy LA, Blangero J, Williams-Blangero S, Brittenham GM, Strohl KP, Decker MJ, Vargas E, Villena M, Soria R, Alarcon AM and Gonzales C 1999 Percent of oxygen saturation of arterial hemoglobin among Bolivian Aymara at 3,900–4,000 m. *Am. J Phys. Anthropol.* **108** 41–51

Bleiweiss R 1998 Relative-rate tests aid biological causes of molecular evolution in hummingbirds. *Mol. Biol. Evol.* **15** 481–491

Britten RJ 1986 Rates of DNA-sequence evolution differ between taxonomic groups. *Science* **231** 1393–1398

Britten RJ and Davidson EH 1971 Repetitive and non-repetitive DNA sequences and a speculation on origins of evolutionary novelty. *Quart. Rev. Biol.* **46** 111–138

Bromham L 2009 Why do species vary in their rate of molecular evolution? *Biol. Lett.* **5** 401–404

Bromham L 2011 The genome as a life-history character: why rate of molecular evolution varies between mammal species. *Philos. Trans. R Soc. B Biol. Sci.* **366** 2503–2513

Bromham L, Rambaut A and Harvey PH 1996 Determinants of rate variation in mammalian DNA sequence evolution. *J Mol. Evol*. **43** 610–621

Bromham L, Woolfit M, Lee MSY and Rambaut A 2002 Testing the relationship between morphological and molecular rates of change along phylogenies. *Evolution* **56** 1921–1930

Brown WM 1983 Evolution of animal mitochondrial DNA; in *Evolution of genes and proteins* (ed) RK Koehn (Sunderland, MA: Sinauer) pp 62–88

Carroll SB 2008 Evo-devo and an expanding evolutionary synthesis: A genetic theory of morphological evolution. *Cell* **134** 25–36

Collar DC, Schulte JA and Losos JB 2011 Evolution of extreme body size disparity in monitor lizards (Varanus). *Evolution* **65** 2664–2680

Cooper N and Purvis A 2009 What factors shape rates of phenotypic evolution? A comparative study of cranial morphology of four mammalian clades. *J Evol. Biol.* **22** 1024–1035

Cooper N and Purvis A 2010 Body Size Evolution in mammals: Complexity in tempo and mode. *Am. Nat.* **175** 727–738

Davies TJ and Savolainen V 2006 Neutral theory, phylogenies, and the relationship between phenotypic change and evolutionary rates. *Evolution* **60** 476–483

Deméré TA, Berta A and McGowen MR 2005 The taxonomic and evolutionary history of fossil and modern balaenopteroid mysticetes. *J Mamm. Evol.* **12** 99–143

Douzery E, Lebreton JD and Catzeflis FM 1995 Testing the generation time hypothesis using DNA/DNA hybridization between artiodactyls. *J Evol. Biol.* **8** 511–529

Echols H and MF Goodman 1991 Fidelity mechanisms in DNA-replication. *Annu. Rev. Biochem.* **60** 477–511

Eizirik E, Yuhki N, Johnson WE, Menotti-Raymond M, Hannah SS and O'Brien SJ 2003 Molecular genetics and evolution of melanism in the cat family. *Curr. Biol.* **13** 448–453

Elmer KR, Fan S, Gunter HM, Jones JC, Boekhoff S, Kuraku S and Meyer A 2010 Rapid evolution and selection inferred from the transcriptomes of sympatric crater lake cichlid fishes. *Mol. Ecol.* **19** 197–211

Fondon JW and Garner HR 2004 Molecular origins of rapid and continuous morphological evolution. *Proc. Natl. Acad. Sci. USA* **101** 18058–18063

Galtier N, Blier PU and Nabholz B 2009 Inverse relationship between longevity and evolutionary rate of mitochondrial proteins in mammals and birds. *Mitochondrion* **9** 51–57

Gillooly JF, Allen AP, West GB and Brown JH 2005 The rate of DNA evolution: Effects of body size and temperature on the molecular clock. *Proc. Natl. Acad. Sci. USA* **102** 140–145

Gillooly JF, Brown JH, West GB, Savage VM and Charnov EL 2001 Effects of size and temperature on metabolic rate. *Science* **293** 2248–2251

Gillooly JF, Charnov EL, West GB, Savage VM and Brown JH 2002 Effects of size and temperature on developmental time. *Nature* **417** 70–73

Goldie X, Lanfear R and Bromham L 2011 Diversification and the rate of molecular evolution: no evidence of a link in mammals. *BMC Evol. Biol*. **11** 286

Gould SJ and Vrba ES 1982 Exaptation - A missing term in the science of form. *Paleobiology* **8** 4–15

Hart RW and Setlow RB 1974 Correlation between deoxyribonucleic-acid excision-repair and life-span in a number of mammalian-species. *Proc. Natl. Acad. Sci. USA* **71** 2169–2173

Janecka JE, Helgen KM, Lim NT, Baba M, Izawa M, Boeadi and Murphy WJ 2008 Evidence for multiple species of Sunda colugo. *Curr. Biol.* **18** R1001–R1002

Janecka JE, Miller W, Pringle TH, Wiens F, Zitzmann A, Helgen KM, Springer MS and Murphy WJ 2007 Molecular and genomic data identify the closest living relative of primates. *Science* **318** 792–794

Jeschke JM and Kokko H 2009 The roles of body size and phylogeny in fast and slow life histories. *Evol. Ecol.* **23** 867–878

Kimura M 1983 *The neutral theory of molecular evolution* (Cambridge: Cambridge University Press)

King MC and Wilson AC 1975 Evolution at two levels in humans and chimpanzees. *Science* **188** 107–116

Kornberg A 1980 *DNA Replication* (San Francisco, CA: WH Freeman)

Kunkel TA 1992 DNA-replication fidelity. *J Biol. Chem*. **267** 18251–18254

Laird CD, McConaug BL and McCarthy BJ 1969 Rate of fixation of nucleotide substitutions in evolution. *Nature* **224** 149–154

Lanfear R, Ho SYW, Love D and Bromham L 2010 Mutation rate is linked to diversification in birds. *Proc. Natl. Acad. Sci. USA* **107** 20423–20428

Lanfear R, Thomas JA, Welch JJ, Brey T and Bromham L 2007 Metabolic rate does not calibrate the molecular clock. *Proc. Natl. Acad. Sci. USA* **104** 15388–15393

Lartillot N and Poujol R 2011 A phylogenetic model for investigating correlated evolution of substitution rates and continuous phenotypic characters. *Mol. Biol. Evol.* **28** 729–744

Li WH, Ellsworth DL, Krushkal J, Chang BHJ and Hewett Emmett D 1996 Rates of nucleotide substitution in primates and rodents and the generation time effect hypothesis. *Mol. Phylogenet. Evol*. **5** 182–187

Li WH, Tanimura M and Sharp PM 1987 An evaluation of the molecular clock hypothesis using mammalian DNA-sequences. *J. Mol. Evol.* **25** 330–342

Mahler DL, Revell LJ, Glor RE and Losos JB 2010 Ecological opportunity and the rate of morphological evolution in the diversification of greater Antillean anoles. *Evolution* **64** 2731–2745

Martin AP and Palumbi SR 1993 Body size, metabolic-rate, generation time, and the molecular clock. *Proc. Natl. Acad. Sci. USA* **90** 4087–4091

McKenna MC and Bell SK 1997 *Classification of Mammals above the species level* (New York: Columbia University Press)

McMahon AP, Ingham PW and Tabin CJ 2003 Developmental roles and clinical significance of hedgehog signaling. *Curr. Topics Dev. Biol.* **53** 1–114

Meredith RW, Gatesy J, Murphy WJ, Ryder OA and Springer MS 2009 Molecular Decay of the Tooth Gene Enamelin (ENAM) mirrors the loss of enamel in the fossil record of placental mammals. *PLoS Genet.* **5** e1000634

Meredith RW, Janecka JE, Gatesy J, Ryder OA, Fisher CA, Teeling EC, Goodbla A, Eizirik E, *et al.* 2011 Impacts of the cretaceous terrestrial revolution and kpg extinction on mammal diversification. *Science* **334** 521–524

Mooers A and Harvey PH 1994 Metabolic rate, generation time, and the rate of molecular evolution in birds. *Mol. Phylogenet. Evol*. **3** 344–350

Murphy WJ, Pevzner PA and O'Brien SJ 2004 Mammalian phylogenomics comes of age. *Trend. Genet.* **20** 631–639

Murphy WJ, Pringle TH, Crider TA, Springer MS and Miller W 2007 Using genomic data to unravel the root of the placental mammal phylogeny. *Genome Res*. **17** 413–421

Nabholz B, Glemin S and Galtier N 2008 Strong variations of mitochondrial mutation rate across mammals - the longevity hypothesis. *Mol. Biol. Evol*. **25** 120–130

Nabholz B, Glemin S and Galtier N 2009 The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evol. Biol.* **9** 54

Ohta T 1993 An examination of the generation-time effect on molecular evolution. *Proc. Natl. Acad. Sci. USA* **90** 10676–10680

Oli MK 2004 The fast-slow continuum and mammalian life-history patterns: An empirical evaluation. *Basic Appl. Ecol.* **5** 449–463

Omland KE 1997 Correlated rates of molecular and morphological evolution. *Evolution* **51** 1381–1393

Orr HA and Turelli M 2001 The evolution of postzygotic isolation: Accumulating Dobzhansky-Muller incompatibilities. *Evolution* **55** 1085–1094

Pagel M, Venditti C and Meade A 2006 Large punctuational contribution of speciation to evolutionary divergence at the molecular level. *Science* **314** 119–121

Poisot T, Verneau O and Desdevises Y 2011 Morphological and molecular evolution are not linked in Lamellodiscus (Plathyhelminthes, Monogenea). *PLoS One* **6** e26252

Popadin K, Polishchuk LV, Mamirova L, Knorre D and Gunbin K 2007 Accumulation of slightly deleterious mutations in mitochondrial protein-coding genes of large versus small mammals. *Proc. Natl. Acad. Sci. USA* **104** 13390–13395

Purvis A, Fritz SA, Rodriguez J, Harvey PH and Grenyer R 2011 The shape of mammalian phylogeny: patterns, processes and scales. *Philos.Transac. R. Soc. B Biol. Sci*. **366** 2462–2477

Read AF and Harvey PH 1989 Life-history differences among the eutherian radiations. *J. Zool.* **219** 329–353

Rowe DL and Honeycutt RL 2002 Phylogenetic relationships, ecological correlates, and molecular evolution within the Cavioidea (Mammalia, Rodentia). *Mol. Biol. Evol.* **19** 263–277

Safi K, Cianciaruso MV, Loyola RD, Brito D, Armour-Marshall K and Diniz JAF 2011 Understanding global patterns of mammalian functional and phylogenetic diversity. *Philos.Transac. R. Soc. B Biol. Sci*.**366** 2536–2544

Schluter D and Conte GL 2009 Genetics and ecological speciation. *Proc. Natl. Acad. Sci. USA* **106** 9955–9962

Seligmann H 2010 Positive correlations between molecular and morphological rates of evolution. *J. Theor. Biol.* **264** 799–807

Simpson GG 1953 *The major features of evolution* (New York: Columbia University Press)

Soltis PS, Soltis DE, Savolainen V, Crane PR and Barraclough TG 2002 Rate heterogeneity among lineages of tracheophytes: Integration of molecular and fossil data and evidence for molecular living fossils. *Proc. Natl. Acad. Sci. USA* **99** 4430–4435

Stanley SM 1973 Effects of competition on rates of evolution, with special reference to bivalve mollusks and mammals. *System. Zool.* **22** 486–506

Steiper ME and Seiffert ER 2012 Evidence for a convergent slowdown in primate molecular rates and its implications for the timing of early primate evolution. *Proc. Natl. Acad. Sci. USA* **109** 6006–6011

Teeling EC, Springer MS, Madsen O, Bates P, O'Brien SJ and Murphy WJ 2005 A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* **307** 580–584

Tsantes C and Steiper ME 2009 Age at first reproduction explains rate variation in the strepsirrhine molecular clock. *Proc. Natl. Acad. Sci. USA* **106** 18165–18170

Venditti C and Pagel M 2010 Speciation as an active force in promoting genetic evolution. *Trend. Ecol. Evol*. **25** 14–20

Welch JJ, Bininda-Emonds ORP and Bromham L 2008 Correlates of substitution rate variation in mammalian protein-coding sequences. *BMC Evol. Biol*. **8** 53

Welch JJ and Bromham L 2005 Molecular dating when rates vary. *Trend. Ecol. Evol.* **20** 320–327

Woolfit M 2009 Effective population size and the rate and pattern of nucleotide substitutions. *Biol. Lett*. **5** 417–420

Zuckerkandl E and Pauling L 1965 Molecules as documents of evolutionary history. *J. Theor. Biol.* **8** 357–366