Review

The genome as a life-history character: why rate of molecular evolution varies between mammal species

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DNA sequences evolve at different rates in different species. This rate variation has been most closely examined in mammals, revealing a large number of characteristics that can shape the rate of molecular evolution. Many of these traits are part of the mammalian life-history continuum: species with small body size, rapid generation turnover, high fecundity and short lifespans tend to have faster rates of molecular evolution. In addition, rate of molecular evolution in mammals might be influenced by behaviour (such as mating system), ecological factors (such as range restriction) and evolutionary history (such as diversification rate). I discuss the evidence for these patterns of rate variation, and the possible explanations of these correlations. I also consider the impact of these systematic patterns of rate variation on the reliability of the molecular date estimates that have been used to suggest a Cretaceous radiation of modern mammals, before the final extinction of the dinosaurs.

Keywords: mutation; substitution; generation time; longevity; metabolic rate; molecular clock

1. MOLECULAR EVOLUTION IS A LIFE-HISTORY TRAIT IN MAMMALS

If you give me a mammal in a box, but you do not tell me what the species is, I can make a fair guess at many of its characteristics based on the size of the box. A mammal in a small box is probably going to have a high metabolic rate, a short generation time, relatively high reproductive output and probably lives in reasonably large populations in the wild. Not only that, I can also take a guess at the relative rate of molecular evolution: a gene in a mammal in a small box is likely to evolve faster than the same gene in a larger mammal.

This predictable scaling of species characteristics with body size has been termed the 'fast–slow continuum' of mammalian life history [1]. At one end of the continuum is the strategy of many small mammals to live fast, die young and have lots of babies (e.g. mice), at the other end is the strategy of many large mammals to live long, mature late and have few babies (e.g. elephants). Body size is not a perfect predictor of life history [2,3], and the fast–slow continuum is an oversimplified categorization of life-history variation [4]. But, on the whole, many characteristics in mammals have a tendency to vary with body size.

Intriguingly, one of the factors that scales with body size in mammals is the rate of molecular evolution (the number of changes in the nucleotide sequence of the genome per unit time). Why would rate of molecular evolution scale with size? To answer this question, we need to consider many aspects of mammalian biology that influence molecular evolution, affecting either the mutation rate or the rate at which these mutations go to fixation in the population.

Mutation is sometimes treated by biologists as if it were an abiotic process, contributing random variation at a uniform rate across the genome, among individuals, between lineages or over time. But the per-base mutation rate of DNA is modulated by biological features of the organism. Point mutations arise when a change to a nucleotide sequence, caused by damage or replication error, is imperfectly repaired such that the base sequence is permanently and heritably changed. So the rate of mutation depends on the accuracy of DNA replication and the efficiency of DNA repair. Both replication accuracy and efficiency of repair are controlled by cellular mechanisms, which can vary in efficiency over time or between species. For example, mutation rate can vary during an individual's lifetime, increasing with age [5], or with poor condition [6]. Mutation rate can also vary heritably between individuals or lineages, and therefore may evolve [7–9]. Variation in mutation rate between mammal species can be studied directly, for example, by direct sequencing of genomes within known pedigrees [10] or by monitoring the by-products of DNA repair [11]. But most estimates of mammalian mutation rates are made from comparisons of homologous DNA sequences from different species.

Comparative analysis of DNA sequences has a number of advantages, not least of which is the practicality of being able to compare rates of molecular

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evolution in the wide range of species for which sequence data are available [12]. Comparative analysis also allows, to some extent, the different forces acting on the rate of molecular evolution to be dissected, because the mutation rate and the substitution rate can be estimated separately by comparing different classes of substitutions that are expected to differ in their exposure to selection.

A mutation that alters the DNA sequence in an individual genome becomes a substitution when it increases in frequency in the population until it replaces all other alternative alleles. Neutral mutations that have no effect on fitness can be fixed by drift. The rate at which neutral mutations become fixed in the population is dependent only on the mutation rate [13]. In mammals, synonymous nucleotide changes in protein-coding sequences—those that do not alter the amino acid sequence of the protein—are generally assumed to be neutral, so the mutation rate is commonly gauged by estimating the synonymous substitution rate. Recent analyses have suggested that selection may operate on some synonymous sites in mammals [14,15], calling this assumption of neutrality into question. However, if selective co-efficients of synonymous sites are generally near zero, and if most mammalian species have relatively small effective population sizes, then it seems reasonable to suppose that most synonymous mutations will behave as if neutral, so that differences in the synonymous mutation rate between species will predominantly reflect differences in mutation rate. Further investigation of the rates and patterns of substitution at sites commonly considered to be neutral would be valuable, particularly if compared across species.

While the rate of neutral substitutions is dependent on the mutation rate, the rate of non-neutral substitutions is determined by the interaction between mutation rate, selection and drift. Strongly deleterious mutations will not become substitutions, because, by definition, they tend not to be passed on to successful offspring, so they will not generally be observed in comparative analyses. But the rate at which slightly deleterious mutations are fixed is determined by both their impact on fitness and the effective population size. Mammal species have comparatively small effective population sizes, so random sampling effects (genetic drift) can overwhelm selection on non-synonymous mutations that are only mildly deleterious or slightly advantageous [16]. Since strongly deleterious mutations are removed by selection, and fixation of advantageous alleles is considered to be relatively rare across the genome, most non-synonymous substitutions will fall into this 'nearly neutral' category. So the non-synonymous substitution rate is expected to increase, relative to the mutation rate, in smaller populations [17,18]. Therefore, comparison of synonymous and non-synonymous substitution rates can tell us something about the causes of species-specific differences in rate of molecular evolution, by allowing us to distinguish changes in mutation rate from changes in population size or selection.

Many aspects of a species' biology can influence its rate of molecular evolution by affecting the mutation rate or the substitution rate. One of the most notable trends in rate of molecular evolution is the body-size effect in vertebrate molecular evolution: small-bodied species generally have faster rates of molecular evolution than their large-bodied relatives [19]. This pattern has been detected in total genetic distance and synonymous substitution rates in both mitochondrial and nuclear sequences [20,21] and in non-synonymous substitutions in nuclear sequences [22]. But the cause of this body-size pattern in mammalian molecular evolution continues to be debated, because there are many possible mechanisms. In mammals, many life-history traits tend to scale with body size, so the various hypotheses are difficult to tease apart, and may operate in concert to produce characteristic rates of molecular evolution [23].

(a) *Generation time and the copy error effect*

One of the most widely accepted hypotheses is that the body-size pattern in rate of molecular evolution in mammals is driven by differences in generation time. Evidence for a correlation between generation time and rates of molecular evolution has been provided by many different studies for a large number of mammal species across a wide range of orders [20,21,24–26]. This pattern is evident when highly divergent mammal lineages are compared [27], but it is also observed in comparisons between more closely related species [20,28].

The observed correlation between molecular rates and generation time in mammals is commonly assumed to be due to species with shorter generation times copying their genomes more often per unit time, thereby accumulating more DNA replication errors. Consistent with this hypothesis, the generation time effect has been noted for synonymous substitution rates, which should reflect the mutation rate, in both mitochondrial and nuclear sequences in mammals [20,22,29]. The generation time effect has also been detected for non-synonymous changes for nuclear sequences [22], which may be a reflection of the influence of mutation rates on the rate of nearly neutral substitutions in small populations.

But although there is a clear correlation between generation time and rate of molecular evolution, the explanation of this pattern in terms of number of genome replications is somewhat problematic. Rates clearly do not scale linearly with differences in generation time. For example, mice can go through 50 generations for every one human generation, yet their rates of molecular evolution are only several times faster [27,30]. At the other end of the size spectrum, baleen whales have slower rates of molecular evolution than hominids despite roughly similar generation times [31]. If differences in the mutation rate were solely owing to differences in the number of generations per unit time, and if all species had the same number of genome copies per generation and the same error rate per replication, then we would expect a log linear relationship between generation time and mutation rate, with a slope of approximately one (J. J. Welch 2010, personal communication) . The negative relationship between log rates and log generation time is approximately linear, but the estimated slope is much less than one, having been estimated at approximately −0.15 [22], or between −0.34 and −0.44 [29].

There are many possible reasons why the observed slope is less than one.

Measurement error could play a role in clouding the generation time effect. For example, it may be that estimates of mutation rate, typically based on estimating the synonymous substitution rate, may systematically underestimate the true mutation rate, or that measurements of generation time, typically based on age at first breeding rather than average age at reproduction, are significantly biased. In addition, molecular rates are effectively estimated over the whole lineage, but generation time values are taken at the tips of the phylogeny from the extant species [12,29]. If generation time varies between extant species, then it must have changed along at least some lineages, so the value at the tips does not represent the generation time at all points on the lineage [32]. However, it seems more probable that these measurement biases would add imprecision to the detection of a relationship between generation time and rates rather than having a systematic dampening effect.

An alternative explanation is that the absence of a simple scaling between rate of molecular evolution and generation length may be due to a number of complicating factors that weaken the connection between species' generation length and the number of DNA copies per unit time. One complicating factor is that the number of germline cell divisions per generation varies between mammal species. For example, it has been estimated that there are on average 31 cell generations in the human female germline, compared with only 25 in mice [33], so there should be more opportunities for copy errors to occur per generation in humans than in mice. The differences for the male germline are more striking: 401 in humans to 62 in mice. This acts to dampen the difference between these species in the expected number of germline DNA copies per year: if mice and humans had the same rate of copy error per replication then we would expect around seven times as many copy error mutations per year in mice than in humans in the male germline (the difference is much larger for the female germline, but the majority of the germline mutations in mammals are expected to occur in males [34]). But this is still larger than typical estimates of the difference in synonymous substitution rate between rodents and primates, so it does not explain why the generation time effect is of a much lower magnitude than expected on the basis of a copy error effect alone.

Although it does not provide a full explanation of the generation time effect in mammals, the copy error effect does influence mammalian molecular evolution in other ways, most notably the impact of 'male-driven evolution' [34–36]. The difference in germline copy number between males and females arises from the way that gametes are produced: in mammals, ova are produced early in development by symmetrical divisions (each germline cell divides to produce two more germline cells) whereas sperm are produced throughout adult life by asymmetrical divisions (each germline cell gives rise to one gamete-producing cell and one germline cell which will then divide again: see [37]). Male germline cells divide again and again to keep producing large numbers of sperm cells, so the average number of cell divisions to produce gametes is much higher than in females, and increases with male age. Because of this, the potential for mutation owing to copy errors is much higher in the male germline than in females. Comparative studies have shown that DNA sequences that spend more time in males (such as those on the Y-chromosome) have a higher mutation rate than those that spend more time in females (such as X-chromosomes) [34].

In addition to the sex-biased copy effects in the nuclear genome, the number of copies per generation differs between mitochondrial and nuclear sequences. Nuclear sequences are expected to be copied once per cell generation, but mitochondrial sequences are likely to be copied more than once per cell generation, with the number of replications depending to some degree on the activity of the cell. Consistent with a copy error effect, mitochondrial genomes in mammals have higher mutation rates than the nuclear genome. This may be because mitochondria, copied multiple times per cell division (and at lower replication fidelity), accumulate more copy errors per generation than the nuclear genome. However, the copy error effect may not influence mitochondrial molecular evolution to the same extent as nuclear if additional mechanisms operate, particularly at cell division, to purge mitochondria with a high mutational burden [38], reducing the overall germline mutation rate per generation. Mitochondrial sequences are also unlikely to be subject to male-driven evolution in mammals, since the mitochondria from sperm are typically discarded from the fertilized zygote [39]. There are other possible explanations of high mutation rate in mitochondria, such as increased damage from metabolic by-products or lower efficiency of DNA repair.

So while generation time correlates with substitution rates in mammals, it does not provide a simple scaling factor for rates of molecular evolution. It may be that additional factors moderate the difference in number of DNA copy errors per unit time: for example, if the number of germline cell divisions per unit time is itself influenced by generation length, or if copy error rates are shaped by life history (see below). Or it may be that the DNA copy effect is not the sole or primary cause of the generation time effect, which may instead be a reflection of some other causal factor that scales with generation time. For example, a generation time effect has been reported for mitochondrial sequences in mammals [20], but subsequent analyses suggest that this association is due to covariation between generation time and longevity [22,29].

(b) *DNA damage arising from metabolism or environmental energy*

An alternative explanation of the body-size effect in rates of molecular evolution is that smaller mammals have higher mass-specific metabolic rates. This means that each cell in a small-bodied mammal produces more by-products of metabolism, which could generate greater rates of mutation through DNA damage.

Support for this hypothesis has been drawn from a number of observations. Firstly, endothermic 'warm-blooded' animals that use metabolism to maintain a constant body temperature (birds and mammals) have higher absolute rates of molecular evolution than poikliothermic vertebrates, such as reptiles and fish, with environmentally determined body temperatures [19,40,41]. Secondly, comparisons between some mammal species have indicated that those with higher mass-specific metabolic rates have higher rates of DNA repair, which is interpreted as a sign of greater rates of oxidative damage to their DNA [11,42,43]. Thirdly, the much higher rate of mutation in mitochondria compared with the nuclear genome has been attributed, at least in part, to the mitochondrial genome being at the site of oxidative phosphorylation, as well as being relatively unprotected, compared with the nuclear genome [41]. Fourthly, the decline of mitochondrial function with age has been considered to be a consequence of cumulative metabolic damage [44].

Some comparative studies have used a correlation between body size and rate of molecular evolution as evidence in favour of the metabolic rate hypothesis [45]. But given that many life-history traits scale with body size in mammals, the correlation between size and substitution rates cannot, by itself, be considered evidence for a causal role for by-products of metabolism in rate of molecular evolution in mammals. Indeed, studies that have specifically compared the explanatory power of metabolic rate with body size or other life-history traits in mammals have not found any significant role for metabolic rate as a driver of variation in rate of molecular evolution, beyond its covariation with other life-history traits [20,28,46]. This does not negate the mitochondrial ageing hypothesis in general: the decay in mitochondrial macromolecules with accumulated activity (and hence age) has been well documented. But there is currently a lack of compelling evidence that mass-specific metabolic rate is a key driver of lineage-specific variation in rate of molecular evolution in mammals.

An idea related to the metabolic rate hypothesis is that environmental energy influences mutation rate [47]. This hypothesis has been promoted by Rohde [48,49], who suggested that higher temperatures and increased levels of solar radiation could have a direct mutagenic effect on DNA, and that this would speed the rate of accumulation of genetic differences between populations, accelerating speciation. Rohde also suggested an indirect link between climate and rate of molecular evolution: higher temperatures could lead to shorter generations, which could increase the rate of substitution through selection (shorter generations would also be expected to lead directly to increased mutation rate [50]). The direct action of climate on mutation rates, or the indirect effect through elevated growth rates, is most plausible for primary producers [51], but has been hypothesized to have 'knock-on' effects on consumers including mammals [49,52]. A large study of pairs of mammal species that differ in their geographical ranges found higher substitution rates in the cytochrome b gene of species in lower latitudes or altitudes [52]. The cause of the relationship is not clear: this study did not estimate

synonymous rate, which would allow effects of mutation rate to be detected, and there was no significant difference in the ratio of non-synonymous to synonymous changes (dN/dS) across the dataset, which would be expected if the pattern was due to selection, or reduction in population size. To clarify both the generality and cause of this pattern, the effect of climate or distribution on rates of molecular evolution in mammals should be investigated further.

In addition to the possibility of a direct impact of climate on mutation rate, there could be indirect causes of the relationship between latitude or altitude and rates of molecular evolution. For example, body size in mammals varies with latitude [53,54], so, given that rates of molecular evolution scale with body size in mammals, this could cause an indirect pattern in molecular rates with latitude (though Gillman *et al.* [52] found that body size did not explain the pattern in their dataset). Similarly, if diversification rate is linked to rate of molecular evolution (see below), then rates of molecular evolution could show a latitudinal gradient, since diversification rates have been shown to increase with decreasing latitude in some taxa [55]. This area deserves further investigation, preferably with a large dataset that allows accurate estimation of both synonymous and non-synonymous substitution rates.

(c) Body size, longevity and the role of selection in shaping rates of molecular evolution

The correlation between body size and rate of molecular evolution in mammals has typically been interpreted as an indirect effect of the covariation of size with other life-history traits that are thought to influence the rate of molecular evolution, such as generation time or metabolic rate. However, it is possible that body size itself influences mutation rates, by placing a premium on the reduction of mutation in both somatic and germline cells. To examine this possibility, we need to consider the possible role of selection in shaping differences in mutation rate between species.

In asexual microbes, mutation rates can clearly be shaped by selection. In a population under strong selective pressure, a mutation that raises the mutation rate may generate novel traits that are selected for, indirectly promoting the linked 'mutator' allele [56]. But the fitness benefits of the occasional advantageous mutation seems unlikely to play a significant role in the evolution of mutation rates in mammals, because their small population sizes make advantageous mutations rare and any mutator alleles will become unlinked from the beneficial mutations they generate through sexual reproduction [7]. The evolution of mutation rates in mammals seems more likely to be driven primarily by the competing costs of mutation and repair. Mutation imposes a significant cost on mammals in terms of reduced fitness, either by causing somatic damage to the individual or by the production of offspring with harmful mutations [57]. DNA repair, either of incidental damage or copy errors, must impose a cost in terms of resources used that could otherwise have been directed to growth or other functions. So selection may act on the balance between the cost of repair and the cost of mutation, and the

optimum balance may differ between species depending on the relative costs.

The relative costs of mutation and repair may vary with life history in mammals. A large-bodied mammal must make more cells in its lifetime than a smaller mammal, which means it must make more genome copies per generation. So for the same level of DNA replication fidelity, a large-bodied animal would be expected to accumulate more DNA copy errors, in both germline and somatic cells. In addition to the increased opportunity for replication errors, a large mammal has more genome copies in its body that must be maintained against DNA damage. A mutation in a somatic cell, acquired either through damage or replication error, could lead to cell failure or, much worse, formation of a tumour cell that replicates at the expense of the organism [58]. In short, large size provides more opportunities for things to go wrong: more cell generations that create opportunities for DNA replication errors, and more cells that are targets for incidental mutation owing to damage. So to achieve the same lifetime chance of harmful mutation as a small-bodied organism, large-bodied organisms must have a lower per genome mutation rate, paid for by a higher investment in DNA repair [59].

Consistent with this hypothesis, long-lived, large-bodied mammals do appear to have adaptations to maintain more genome copies over a longer lifespan. There is some evidence that large-bodied mammals can have more effective mechanisms to suppress tumour formation, such as a reduction in telomerase activity. Telomerase activity repairs telomeres eroded by cell divisions: without it, cells suffer replicative senescence so that they cannot divide indefinitely. Telomerase activity has been shown to decrease with increasing body size in rodents, consistent with the prediction that larger animals should invest more in the prevention of cancers [60]. Longer lived species may also reduce the damage due to metabolism by producing fewer reactive oxygen species (ROS) than shorter lived species [61]. For example, bats have unusually long lifespans for their body size, and at least some species seem to suffer less damage from ROS [62]. Furthermore, measurements of the by-products of DNA excision repair have been found to be lower in some long-lived, large-bodied mammals than in smaller mammals [11,42]. But while there is evidence that larger, longer lived mammals suffer relatively less DNA damage, it is less clear the role that variable rates of DNA repair play in this pattern. Rates of DNA repair do vary between mammal species, at least at the broad scale: for example, rats and mice lack some of the excision repair mechanisms found in primates. But the extent to which repair rates are fine-tuned in different species in response to different costs of mutation and repair is not currently known.

While DNA repair rates are difficult to directly compare between species, comparative analyses have revealed species-specific patterns of mitochondrial mutation rates that provide indirect evidence of the influence of selection on mutation rates. There is a growing body of evidence that mitochondrial mutation rates are correlated with longevity [22,29]. The mitochondrial genome is more vulnerable to metabolism-related DNA damage than the nuclear genome, because it is located at the site of oxidative phosphorylation, and lacks the protective structures of the nuclear genome [41]. Rates of synonymous substitution in mitochondrial sequences are significantly correlated with longevity in mammals, and longevity provides much greater explanatory power for variation in mitochondrial synonymous rates than body size or generation time [22,29]. While longevity scales with rate of molecular evolution in the nuclear genome, this relationship is not significant when other life-history factors are taken into account, particularly generation time and fecundity [22]. This pattern is consistent with the evolution of mechanisms that reduce the mutation rate in mitochondria in order to maintain function over a longer lifespan. However, the mechanism for this hypothesized reduction is not known.

(d) *Fecundity and the rate of molecular evolution in mammals: a pattern in search of an explanation*

The observed association between fecundity (average number of offspring per year) and rates of molecular evolution in mammalian nuclear sequences remains unexplained. Welch *et al.* [22] found that fecundity was strongly positively correlated with both synonymous and non-synonymous rates for six nuclear genes, above and beyond its correlation with body size, generation time and maximum lifespan. One possible explanation for this pattern is that the number of genome copies per generation scales with fecundity, thereby generating more opportunities for copy error mutations in species with higher fecundity.

Britten [63] suggested that the number of gametes produced per generation could influence the rate of molecular evolution. This prediction has not been widely tested though it has been shown that social hymenoptera have a higher rate of molecular evolution than their non-social relatives, possibly owing to massively increased gamete production in social queens [64]. In mammals, female gamete production seems unlikely to have a large impact on species-specific rates of molecular evolution, since female mammals produce an excess of ovules by symmetric division before the onset of reproduction. But if fecundity scales with the average number of cell generations in sperm production, then it is possible that the fecundity effect may be an indirect measure of the number of male gametes produced per year in species with polyandry, or where older fathers contribute disproportionately to the pool of offspring. For example, it has recently been demonstrated experimentally that polyandry leads to the evolution of greater sperm production in mice, and to elevated fertilization success in multiple matings [65]. However, it is not clear whether there would be a more general relationship between fecundity and higher average number of cell generations in sperm in mammals, when compared across many species.

Alternatively, the association between fecundity and mutation rate may arise as a consequence of variation in parental investment. 'Slow' life histories in mammals are typically characterized by having fewer offspring per lifetime, but investing more resources

per offspring in order to increase the average chance of survival of each offspring, thus trading quantity of off-spring against quality [66,67]. Parental investment typically includes protection and provisioning, but could investment in offspring also extend to DNA repair? Reduced mutation rate through increased investment in DNA replication fidelity and repair would reduce the chance of producing low fitness off-spring owing to de novo deleterious mutations. The relative benefit of mutation rate reduction may be higher for a low fecundity mammal.

The correlation between fecundity and rate of molecular evolution in mammals might be the indirect result of correlation with other life-history traits that scale with substitution rate. For example, larger litter size in mammals has been associated with higher diversification rates [68,69]. It has been suggested that rates of molecular evolution in mammals are linked to diversification [52], although a recent study failed to find evidence for this link for either mitochondrial or nuclear sequences [70]. At present, there is a lack of information to distinguish the possible causes of the link between fecundity and rates of molecular evolution, and there is a clear need for studies that contrast selective explanations (parental investment) against mechanistic causes (copy error effect), taking into account possible indirect causes (such as diversification rate).

2. THE INFLUENCE OF POPULATION SIZE AND DIVERSIFICATION RATE ON THE RATE OF MOLECULAR EVOLUTION

One of the most pervasive effects on the rate and pattern of substitutions is the effective population size, which reflects the effect of random sampling on gene frequencies in populations [71]. As effective population size decreases, the relative influence of random sampling on allele frequencies increases, so that more slightly deleterious mutations will be fixed by drift rather than eliminated by selection. The practical upshot of this is that the rate of nearly neutral substitutions—such as non-synonymous substitutions of small selective effect—will increase as effective population size decreases. Effective population size influences the substitution rate, not the mutation rate, so small populations should have relatively higher rates of non-synonymous substitutions compared with synonymous substitutions (dN/dS or ω).

Mammals generally have much smaller effective population sizes than invertebrates or unicellular organisms, and this is reflected in a lower efficiency of selection operating on mammalian genomes [16]. There have been surprisingly few empirical tests of the influence of effective population size on rates of molecular evolution among mammal species, but they have generally confirmed the pattern predicted by theory. Effective population size is linked to life history in mammals [72]: larger mammal species are likely to have smaller effective population sizes, and therefore have an increased dN/dS ratio [25,73]. But many other factors could also influence the effective population size in mammals, such as sex ratio, variation in number of offspring between individuals, sub-population structure and the demographic history of the population [72,74].

The population size effect has been proposed as one potential driver of a fascinating pattern in rates reported in a number of comparative studies: lineages with a larger number of extant species have faster rates of molecular evolution. This pattern has been observed by comparing branch lengths in sister clades that differ in extant species diversity in plants [75,76], invertebrates [77] and birds [78]. The link between diversification rate and rate of molecular evolution was also supported by studies that compared root-to-tip path lengths through molecular phylogenies with the number of nodes traversed [79].

Three reasons have been put forward for greater rates of molecular evolution in rapidly diversifying clades [75]. Firstly, the process of speciation may cause an increased rate of molecular evolution. For example, population subdivision accompanying speciation may result in reductions in long-term average effective population size, increasing the rate of substitution of nearly neutral mutations [80]. However, this seems unlikely to provide a full explanation of the pattern, which has been detected for synonymous substitutions in birds, which should reflect the mutation rate and be relatively unaffected by population size [78]. Secondly, there could be a direct causal link between molecular change and diversification. Higher rates of molecular evolution could drive more rapid speciation by accelerating the accumulation of genetic differences between separated populations. A raised mutation rate could provide both the fuel for selection for differential adaptation or reproductive isolating mechanisms, by increasing the amount of standing variation [81], or could contribute to a drift-mediated accumulation of incompatible genetic changes [82]. Thirdly, there may be an indirect link between diversification rate and rate of molecular evolution, if factors that impact on molecular rates also influence diversification rates. For example, generation time in plants has been linked to both rates of molecular evolution [83] and diversification rates [84], so could potentially provide a bridge between the two. There is some evidence that mammalian lineages with 'fast life history' (large litters, rapid gestation and short inter-birth intervals) have higher diversification rates [68], so life history could potentially provide a link between diversification rate and rate of molecular evolution in mammals. An indirect link might also be found through the possibility of environmental energy effects [52]: if tropical clades that have higher diversification rates also have increased mutation rates then molecular evolution rates will be correlated with net diversification even if there is no direct causal link between the two.

However, a recent study of rates of molecular evolution across both mitochondrial and nuclear genomes of mammals failed to find any evidence of a significant association between mutation rates or substitution rates and net diversification rate [70]. Why do mammals show no evidence of a pattern of rate variation that has been detected in other taxa? Clearly, mammal lineages do vary significantly in diversification rate [85], so the failure to detect any relationship with a large dataset suggests that any link between diversification rate and rate of molecular evolution is either absent or very

weak in mammals. This may be because the mode of speciation is different in mammals, for example, if reproductive isolation in mammals is generally driven by few changes to key genes rather than the gradual accumulation of hybrid incompatibilities across the genome. Or it may be that a link in the causal chain between diversification and molecular evolution is absent in mammals. For example, small body size and short generation time scale with both molecular rates and diversification rate in birds potentially creating an indirect link between the two, but although small size and short generations are predictors of fast molecular rates in mammals they do not seem to be associated with greater species richness [85].

3. EVOLVING MOLECULAR CLOCKS AND THE MAMMALIAN RADIATION

We have seen that a number of life-history characteristics are associated with variation in rates of molecular evolution in mammals, including body size, generation time, fecundity, longevity and population size. Owing to the covariation of mammalian life-history traits, these characteristics tend to scale together: small mammal species tend to have shorter generations, more offspring, briefer lives, larger populations and faster molecular rates than their larger relatives. Indeed, the covariation of rate with life history suggests that rate of molecular evolution itself should be viewed as a life-history characteristic [23]. Smaller animals that copy their DNA more often will collect more replication errors per unit time, but their shorter lives and higher fecundity may allow them to bear the cost of this higher mutation rate. Larger animals may have fewer germline replications per unit time, but they must copy their genome more times per generation in order to make body cells or gametes, and they must maintain a larger number of genome copies against harmful mutation over a longer reproductive lifespan. In addition, their reproductive output relies on the success of a smaller number of offspring. So for a given rate of mutation, a large, long-lived animal has a greater chance of deleterious mutation reducing its fitness. It is possible, then, that the balance of life-history traits in the overall reproductive strategy of mammalian species includes the rate of molecular evolution, which might be modulated by selection acting on the relative investment in DNA protection and repair. On a practical level, these patterns of rate variation may impinge on the use of DNA sequence analysis to study mammalian evolution. In particular, the correlation of rate of molecular evolution with life history in mammals is potentially of great importance to attempts to date key events in mammalian evolutionary history using molecular data.

(a) The changing picture of the mammalian radiation

The picture of early mammalian evolution has changed dramatically over the last two decades. The 'traditional' model of mammal evolution assumes that, although mammalian lineages were present throughout the Cretaceous, they were restricted to the role of small-bodied generalists until the extinction of the dinosaurs. However, recent fossil finds show that early mammals occupied a wide range of niches including semi-aquatic mammals, gliders, ant-eaters and carnivores [86–90]. But since there is insufficient fossil evidence to unambiguously place the modern crown groups of Monotremata, Marsupialia and Placentalia before the Cretaceous–Tertiary (KT) boundary [91], this ecological diversity in itself does not challenge the notion that the adaptive radiation of modern mammals was limited until the final extinction of the dinosaurs at the end of the Cretaceous.

A more radical rewrite of mammalian evolution has been suggested on the basis of molecular analyses. Molecular phylogenies have had two important impacts on the interpretation of the ordinal-level radiation of modern mammals. Firstly, molecular phylogenies have overturned established phylogenetic relationships and shaken the mammalian tree [92]. Some of these hypotheses have become widely accepted, such as the close relationship between hippos and whales [93], and the basal position of the 'Afrotheria', a clade including elephants, golden moles, tenrecs and aardvarks [94]. Other groupings suggested on the basis of molecular phylogenies are more controversial, such as the 'Pegasoferae', a clade containing bats, horses, carnivores and pangolins [95].

Secondly, estimates of divergence dates from molecular data have placed the origin of mammalian orders deep in the Cretaceous, long before the earliest fossil evidence for these lineages [96–99]. Molecular dating studies infer time since divergence from differences between homologous DNA or protein sequences from different species. Published date estimates vary substantially with method and data used, but most have placed the divergence between ordinal lineages, such as the split between rodents and primates, long before the final extinction of the non-avian dinosaurs at the KT boundary, 65 million years ago [100]. For example, a recent study that used a multiple-gene alignment to estimate the divergence dates on a large phylogeny of mammals concluded that the base of the radiation of modern mammals was in the Cretaceous, with the major ordinal lineages arising $100-85$ million years ago [99]. Furthermore, by plotting the origin of extant lineages over time, they found that lineage origination rates did not significantly increase directly after the KT boundary, despite this period being traditionally considered as the trigger for the explosive radiation of mammalian diversity.

So the picture that has been emerging from several decades of molecular dating studies is that of a Cretaceous diversification of the major mammalian lineages, followed by a radiation of sub-ordinal lineages in the Eocene [99]. The Cretaceous ordinal divergence dates were initially treated with some scepticism, but as more studies have emerged that reach similar conclusions using a range of molecular data and increasingly sophisticated methods, there has been a growing acceptance of the molecular estimates for the Cretaceous origin of many modern mammalian lineages. However, as there is still no unambiguous fossil evidence to support this new view of the mammalian ordinal radiation, it is pertinent to ask what confidence we should place in the molecular date

estimates. A consideration of the biological influences on rate of molecular evolution in mammals is clearly relevant to assessing the reliability of molecular dating.

(b) *The effect of life-history variation on molecular dates*

Early attempts to use molecular data to estimate dates of divergence in mammals assumed a uniform molecular clock. When it became clear that the assumption of uniform rates in all lineages was not met by the data, and that this could lead to misleading date estimates, rate variable dating methods were developed. These used various methods to allow multiple rates along a phylogeny, through the *a priori* definition of rate categories [97], estimation of local clocks [99] or using a statistical model of rate change to assign rates to branches [96].

Many people feel that a 'relaxed clock' method that allows each branch to take a different rate is a more realistic approach to the data. But these methods make complex statistical assumptions about the data; these assumptions are not always transparent to the end user, and may not provide an adequate description of the data [101]. In particular, it is currently not known how well these relaxed clock methods deal with systematic biases in rates over a phylogeny [102]. We know that rates of molecular evolution vary between mammalian lineages, and that a significant part of this rate variation is connected to species' life history. This means that rates of molecular evolution may change over the mammal tree as life-history traits, such as body size and generation time, evolve along lineages [32]. Importantly, this could create directional trends in rates over time, not simply the random variation that is described by many of the stochastic models employed in molecular analyses. Furthermore, species-specific rates could potentially generate bias in all date estimates, no matter which data or methods are used, making concordance between different studies less convincing as an argument for veracity. If all studies are prone to shared biases, then consistency across studies is not, by itself, sufficient cause for confidence.

Systematic biases in rates could arise from the link between molecular evolution and life history. Many eutherian mammal orders show an increase in average body size from their first appearance in the fossil record to the present day. This suggests that there could also have been a shift in life-history traits such as generation time and longevity from the base of the mammalian radiation to the present [32]. Since body size and its life-history correlates are linked to rate of molecular evolution in mammals, it is possible that many mammalian lineages have experienced a slow-down in molecular rates compared with the earliest periods of mammalian radiation. The extent of this effect, and its impact on date estimates, is unknown. Fast rates early in the mammalian radiation could make molecular date estimates systematically too old [32].

There is a clear need for analyses that test the robustness of mammalian molecular date estimates to life-history rate variation. Springer *et al.* [96] conducted their dating analysis on a general dataset, and

then on a subset of small-bodied lineages that they considered would not show a marked increase in body size over the mammalian radiation. The small-bodied dataset produced younger date estimates for divergence times between placental mammal orders (though still pre-KT), perhaps illustrating the potential for life-history rate variation to impact on molecular dates. It would be interesting to use information on change in body size over time obtained from the palaeontological record to model and test the impact of life-history rates on molecular date estimates [32]. Ideally, information on life history could be directly incorporated into date estimation as a prior distribution on rate variation. In any case, the more we know about the way rates of molecular evolution vary between mammal species, the wiser we will be when we try to read their evolutionary history from the traces left in mammalian genomes.

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