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Perspective

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Revisiting the determinants of molecular evolutionary rate variation

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ABSTRACT

Rates of molecular evolution are key parameters for understanding the processes shaping biological diversity. These rates vary among lineages and loci, correlating with ecological, intrinsic biological, and genomic factors such as generation time, metabolic rate, climate, and DNA repair efficiency. Despite many correlational studies, the causal nature of these relationships remains unclear, limiting their interpretation at macroevolutionary scales. Estimating mutation rates is a difficult task that requires deep sequencing to detect germline mutations. Thus, fixed substitutions are often used as proxies for mutation rates, although they do not directly represent mutational processes due to natural selection and genetic drift. The interplay among mutation, selection, drift, and effective population size demands careful interpretation of rate variation among species. The main proposed hypotheses link life-history and environmental factors to genomic mutation, DNA repair efficiency, and fixation. However, interdependence and collinearity among these traits hinder causal inference, particularly in traditional correlational analyses. In this review, we revisit these hypotheses, highlighting their assumptions, predictions, and methodological limitations. We propose that advancing the understanding of molecular evolutionary rates requires a shift in focus: instead of seeking ultimate causes, we must identify the traits most proximately linked to the underlying mechanistic pathways, using causal models to disentangle direct and indirect effects in a standardized way across clades.

Keywords: mutation rates; neutral theory; non-synonymous substitutions; structural equation modelling; substitution rates; synonymous substitutions

INTRODUCTION: UNDERSTANDING MOLECULAR EVOLUTIONARY RATES

Rates of molecular change are central parameters in modern evolutionary theory, shaping our understanding of the patterns and processes of biological divergence and ancestry. Mutation rates, in particular, reflect both stochastic mutational events and cellular mechanisms that modulate their persistence. Nonetheless, estimating the amount of new mutations per generation is notoriously difficult due to the involved experimental requirements, especially for germline mutations that underlie heritable variation (Bergeron *et al.* 2023). To estimate this germline mutation rate, it is necessary to sequence both parents and their offspring to identify new mutations in the next generation. As a result, sequencing must be performed at extremely high coverage and precision, which increases the costs and time required to estimate the mutation rate. Despite these challenges, expanding the taxonomic breadth of *de novo* mutation rate estimates across the tree of life is essential, as these direct measurements provide the most accurate foundation for understanding the mechanistic basis of molecular evolution. Unfortunately, because this information is still not available for most taxa, researchers often opt for the substitution rate, an estimate of how frequently mutations reach fixation within lineages. Its estimation is much more straightforward as it only requires a multiple sequence alignment and, optionally, calibration times (Lanfear *et al.* 2010). However, standardizing these estimates across studies remains challenging, as different markers, genomic regions, and sampling strategies can yield different rate estimates (e.g. Santos 2012). In practice, it is important to acknowledge that substitution rates are not intrinsic traits of the studied organisms, but rather reflect data availability (i.e. the composition of the available multiple sequence alignments).

Throughout this review, we use 'rate of molecular evolution' as a general term that encompasses both mutation rates and substitution rates, indicating changes in molecular sequences over time. When discussing specific mechanisms or empirical patterns, we

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explicitly distinguish between these two processes in the text. While estimating molecular evolutionary rates through single-nucleotide substitution is the standard practice, other mutation types may also impact evolution. For instance, indels increase the probability of mutations in nearby sequences, and their fixation rate is correlated with lineage-specific evolutionary rate (Paśko *et al.* 2011). Methods for modelling and analysing indels are still uncommon, but they should not be overlooked. Other types of genomic changes may also contribute to evolutionary rate variation, including gene conversion (Daugherty and Zanders 2019), copy number variation (Lauer and Gresham 2019), structural DNA rearrangements (Näsvall *et al.* 2023), and epigenetic modifications (Habig *et al.* 2021). In this review, we focus primarily on single-nucleotide substitution rates because the hypotheses linking life-history and ecological traits to molecular evolution have been predominantly developed and tested using this metric, and comparable analytical frameworks for other mutation types in macroevolutionary contexts remain limited.

Although the mutation rate and substitution rate are positively correlated (Bergeron *et al.* 2023), they are not directly proportional. This discrepancy stems from the different types of substitutions: synonymous (dS) and non-synonymous (dN). Synonymous substitutions are determined primarily by the mutation rate, as they do not alter the amino acid sequence and are generally not subject to natural selection. Conversely, non-synonymous substitutions change amino acid sequences and are influenced by both mutation rate and selection. Still, describing the difference between dN and dS solely in terms of natural selection oversimplifies the evolutionary process leading to the observed molecular changes. According to the neutral theory, dS indeed evolves without the effects of natural selection (Kimura 1983). However, nearly neutral theory challenges this by stating that some dS have very small selection coefficients and could be influenced by natural selection (Ohta and Kimura 1971, Ohta 1973). Understanding this distinction is crucial because if both dN and dS are subject to selection, they will covary with both the effective population size (N_e) and with the traits correlated with N_e.

The Drift Barrier Hypothesis (Lynch 2007, 2010, Lynch *et al.* 2016) formalizes the relationship between dN, dS and N_e, positing that the extent to which natural selection can reduce the mutation rate is limited by the power of random genetic drift rather than by intrinsic biological constraints. According to this framework, selection can only effectively act on mutations when their fitness effects substantially exceed the power of random genetic drift ($1/2N_e$ in diploids) (Lynch 2010). Below this threshold, mutations behave as effectively neutral. This creates a drift barrier where lower mutation rates cannot evolve, and this barrier varies across species: in populations with small N_e, the barrier is high, allowing fixation of slightly deleterious mutations that natural selection is unable to avoid (Kimura *et al.* 1963). On the other hand, in populations with large N_e, even weakly beneficial mutations can be selected. The hypothesis is supported by empirical observations that mutation rates scale inversely with N_e across phylogenetic lineages (Lynch 2010), and this pattern holds across diverse organisms from bacteria to multicellular eukaryotes (Lynch *et al.* 2016). Thus, while both dS and dN may vary with N_e, dN is expected to vary more strongly due to its functional impact. Nonetheless, recognizing the complexity of these possible interactions is key to linking molecular rates to species traits and evolutionary processes.

As substitution and mutation rates are interdependent, both are influenced by species-specific factors. Importantly, the potential effect of an extrinsic or intrinsic biological variable on molecular evolution ultimately depends on its influence through three key pathways: the generation of new mutations, the repair of mutations, and their fixation within populations (Fig. 1A) (Bromham 2009). Essentially, a trait that affects the rise of new mutations and DNA repair efficiency alters the mutation rate, while influencing mutation fixation probability leads to a variation in the substitution rate. Because empirical studies typically estimate substitution rates, the underlying mechanisms are inferred by analysing correlations between traits and either dS or dN. Therefore, a trait that correlates with dS probably influences the mutation rate, while a correlation with dN could indicate a direct link to the substitution rate. Nevertheless, as stated above, evaluating dN is a more complex task because it varies with the mutation rate and the balance between drift and selection. Comparing the dN/dS ratio is helpful in these cases, as this metric is interpreted solely as the strength of selection, representing the difference between these two types of substitutions. Interpreting correlations between traits and substitution metrics can help infer which species-level (or population-level) factors influence molecular evolution and through which of the three fundamental pathways: mutation generation, DNA repair, or fixation (Fig. 1A).

Understanding the complex relationships between various levels of biological organization is crucial, particularly for determining whether and how microevolutionary processes actually influence macroevolutionary diversification. While theoretical frameworks linking molecular changes to speciation exist, empirical evidence for such direct relationships remains scarce and often contradictory (Rolland *et al.* 2023, Bromham 2024). Large-scale comparative studies have yielded inconsistent results regarding the association between molecular evolutionary rates and diversification patterns (e.g. Fontanillas *et al.* 2007, Cai *et al.* 2025), raising fundamental questions about whether macroevolutionary diversification is indeed reducible to microevolutionary processes, or if it involves emergent processes that cannot be fully predicted from molecular-level changes alone. In addition to understanding this connection, other areas of evolutionary biology can significantly benefit from a better understanding of this relationship, such as deep phylogenetic inference. For instance, there are many discrepancies between different reconstructions of the avian phylogeny, because they still do not provide a general answer as to whether the Neoaves clade arose during the Cretaceous or Cenozoic, probably due to variations in the life-history traits of birds during these periods (Field *et al.* 2019, Stiller *et al.* 2024). However, it is important to note that other factors also contribute to these phylogenetic challenges, including incomplete lineage sorting, ancient hybridization, and the rapid radiation of the Neoaves itself (Suh *et al.* 2015). Likewise, variations in molecular evolution may explain other evolutionary patterns. For example, it is still largely unknown what causes a lineage to be relict or how it has survived long periods with a low diversification rate (Caron and Pie 2022). One may argue that this is due to a slow molecular evolutionary rate, but this hypothesis has yet to be tested in the literature. In this review, we revisit key hypotheses linking species traits to molecular evolutionary rates and highlight conceptual and methodological gaps that need to be addressed to make inferences more robust and enable the field to move forward.

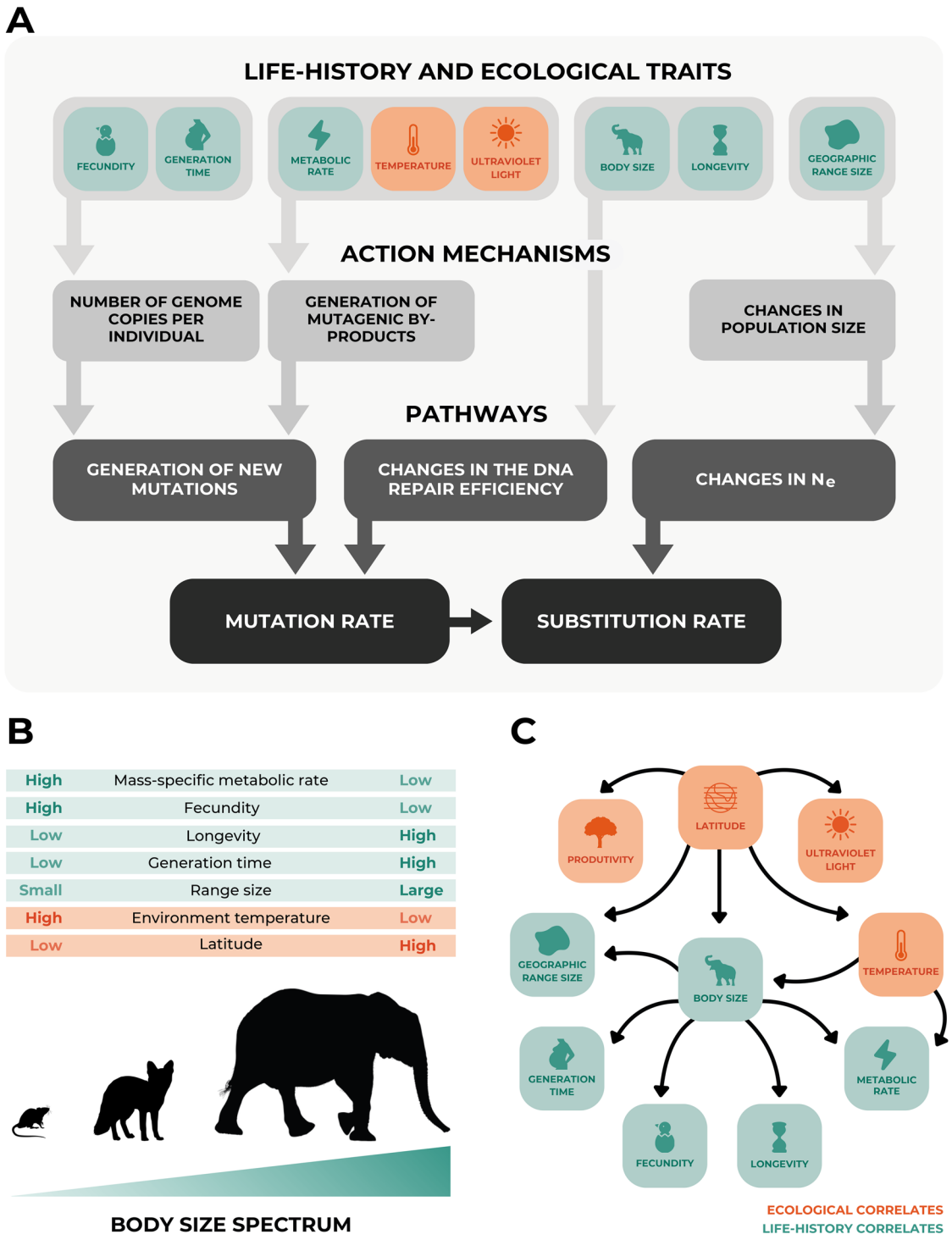

Figure 1. Correlations among life-history (green) and ecological (orange) traits and their theoretical relationships. A, mechanistic pathways and action mechanisms of life-history and ecological traits. B, life-history syndrome showing the fast–slow continuum of traits correlated with body size. C, theoretical structural equation model illustrating hypothesized direct and indirect relationships among ecological correlates and life-history traits. Arrows indicate proposed causal pathways.

WHAT WE KNOW: ECOLOGICAL AND LIFE-HISTORY CORRELATES OF RATE VARIATION

It is not surprising that a large quantity of morphological and life-history species' traits is correlated: a large species tends to have a larger home range, have fewer offspring, and live longer (Fig. 1B). Indeed, life-history traits tend to occur in syndromes and can be depicted in a fast–slow continuum of life-history characteristics (Lehtonen and Lanfear 2014; but see Del Giudice 2020 for details and potential caveats). On one side of the spectrum, fast life histories are characterized by a smaller size, shorter generation time, decreased life expectancy, higher mass-specific metabolic rate, smaller range size, and increased number of offspring (Table 1).

On the other hand, slow life histories depict the opposite pattern of trait correlation. Therefore, in this continuum, it is expected that the rate of molecular evolution will vary similarly, with higher rates on the fast side and lower rates on the slow side (Bromham 2011). Each trait can influence mutation or substitution rates through distinct pathways. However, as previously mentioned, they often converge on being correlated with three main pathways (Fig. 1A): (i) the generation of new mutations (e.g. through the number of genome copies per individual or the generation of mutagenic by-products); (ii) changes in the DNA repair efficiency; or (iii) changes in N_e (see following sections).

Because life-history traits are central in molecular evolution, other traits that correlate with them may also predict molecular evolutionary rates. In fact, even ecological correlates, which are known to influence molecular evolution, are often hypothesized to do so because life-history traits themselves vary with the environment (but see the following section, 'Environmental predictors'). Essentially, any variable that serves as a good proxy for life-history traits has the potential to predict the rate of molecular evolution. For instance, molecular evolution was found to be associated with habitat type in deep-sea invertebrates (Weiss and Berv 2025, Caron *et al.* 2026) and with distance of migration in birds (Pegan *et al.* 2024). That said, one should be cautious when using these secondary factors to predict molecular evolution directly. These variables are proxies for life-history traits, which in turn are proxies for the processes of mutation generation and fixation. Following this chain of inference may, in some instances, lead to the reliance on variables with additional confounding factors that can obscure the 'true' relationship between species' traits and molecular evolution.

Life-history traits

When discussing the fast–slow continuum, it is essential to emphasize that these hypotheses refer to the average life-history traits of species, not individuals. At the individual level, the continuum often does not hold, as many idiosyncrasies come into play, and factors such as density dependence and environmental variability strongly shape life-history variation (Del Giudice 2020, Van de Walle *et al.* 2023). Nevertheless, emergent properties at the species level allow for the development of hypotheses linking life-history traits to molecular evolution. Importantly, these traits rarely act in isolation (Fig. 1C). Instead, they may exert synergistic effects on the tempo of molecular evolution. For instance, one explanation proposed for why species on the slower side of the spectrum tend to evolve more slowly is the correlation between parental care and other life-history traits (Bromham 2011). Parental care refers to the energy invested by parents in raising their offspring after birth. Species that produce fewer offspring generally devote more time and energy to ensure offspring survival, thereby increasing the likelihood that each descendant reaches maturity. By investing more resources into the survival of fewer offspring, these species may also experience slower molecular evolution. This illustrates how combinations of life-history traits, rather than the isolated effect of each one, shape the tempo of evolution.

Looking more closely at each correlate, some life-history traits are thought to influence the pathway of new mutation generation (Fig. 1A). Two action mechanisms can be identified within this category: changes in the number of genome copies per individual and the production of mutagenic by-products (Fig. 1A). First, two life-history correlates are hypothesized to influence the number of genome copies per individual: fecundity and generation time (Table 1). High fecundity increases the chance of producing new mutations by generating more gametes, and thus more genome copies, potentially elevating dS. Indeed, correlations between fecundity and molecular evolution have been reported (e.g. Bromham 2002, Welch *et al.* 2008, Santos 2012, Hua *et al.* 2015, Bergeron *et al.* 2023, Cai *et al.* 2025), and this association depends on the gamete output of a species or lineage, because of the required increase in cell divisions. For example, compared to their non-social relatives, social hymenopterans exhibit a higher rate of molecular evolution, in which continuous oogenesis sustains a high rate of gamete production (Bromham and Leys 2005). The variation in the number of genome copies probably explains why males and females may differ in their rates of molecular evolution (Bergeron *et al.* 2023). As male gametes are produced much more frequently than female gametes, the male germline is more prone to accumulating mutations. Nonetheless, this interpretation has been challenged by the 'faulty male hypothesis' (developed primarily for mammals; Hahn *et al.* 2023), which attributes the male bias to intrinsically less accurate DNA replication or repair mechanisms in male germlines, independent of gamete production rates.

Generation time has been hypothesized to play a role similar to fecundity: shorter generations result in more frequent genome replications and, therefore, greater opportunities for mutations (therefore affecting dS). Three main explanations have been proposed for this association (Lewin and Eyre-Walker 2025): (i) mutations may occur more frequently in early stages of gametogenesis, which have a stronger impact in short-lived organisms; (ii) shorter generations entail faster cell divisions, increasing replication errors; and (iii) organisms with shorter generation times may be exposed to greater nonreplicative damage, either via higher lesion rates or less accurate repair. However, evidence is mixed for each mechanism and even for the overall link between generation time and molecular rates (see, e.g., Thomas *et al.* 2010, Bergeron *et al.* 2023, Lewin and Eyre-Walker 2025 for supporting evidence; Bromham 2002, Welch *et al.* 2008, Lanfear *et al.* 2010, Cai *et al.* 2025 for contrary evidence). Notably, these associations are expected to be weaker for mitochondrial genes, as mitochondrial replication depends not only on cell division but also on cellular activity (Bromham 2002).

The second action mechanism through which a trait can affect the generation of new mutations and influence molecular evolution is the production of mutagenic by-products (Fig. 1A), represented by a single life-history trait: metabolic rate (Table 1). Smaller organisms exhibit higher mass-specific metabolic rates, which can increase the production of DNA-damaging metabolites, increasing the mutation rate (and also dS). This mechanism may help explain why ectotherms generally have lower molecular rates than endotherms and why the mitochondrial genome usually evolves faster than the nuclear genome (Bromham 2002). However, evidence supporting this hypothesis remains scarce (Lanfear *et al.* 2007; but see Gillooly *et al.* 2005, Santos 2012). Several factors have been suggested to account for this lack of a clear association: (i) differences in ATP generation efficiency between species, which lead to variable production of free radicals

Table 1. Main hypotheses on the determinants of molecular evolutionary rate variation.

Category	Hypothesis	Rationale	Assumptions	Predictions	Targeted rate	Selected references
Life-history correlates	Fecundity	Higher fecundity increases the number of times the genome is copied per generation	Increased production of gametes has to be accomplished through more cell divisions, and genome replication has to be associated with the mutational load	As fecundity increases, the rate of molecular evolution increases	Mutation rate	(Bromham 2002, Bromham and Leys 2005, Welch *et al.* 2008, Santos 2012, Hua *et al.* 2015, Bergeron *et al.* 2023, Cai *et al.* 2025)
	Generation time	Species with faster generation times copy their genomes at a higher frequency, accumulating more DNA replication errors per unit of time	Generation time has to be associated with the general rate of genome replication, and genome replication has to be associated with the mutational load	As generation time decreases, the rate of molecular evolution increases	Mutation rate	(Bromham 2002, Welch *et al.* 2008, Lanfear *et al.* 2010, Thomas *et al.* 2010, Bergeron *et al.* 2023, Cai *et al.* 2025, Lewin and Eyre-Walker 2025)
	Metabolic rate	Higher mass-specific metabolic rates produce a higher concentration of damaging metabolic by-products, such as free radicals, which can accumulate more DNA damage per unit of time	Rate of ATP generation has to be similar in species with similar metabolic rates, and oxidative stress has to be directly associated with germline mutation rates	As mass-specific metabolic rate increases, the rate of molecular evolution increases	Mutation rate	(Gillooly *et al.* 2005, Lanfear *et al.* 2007, Santos 2012, Qiu *et al.* 2014, Jing *et al.* 2025)
	Longevity	Long-lived species have more investment in DNA repair mechanisms	Ageing may not be associated with more DNA damage (without repair)	As longevity decreases, the rate of molecular evolution increases	Mutation rate	(Nabholz *et al.* 2008, Welch *et al.* 2008, Lourenço *et al.* 2013, Hua *et al.* 2015, Bergeron *et al.* 2023, Cai *et al.* 2025, Jing *et al.* 2025)
	Geographical range size	Species with larger range sizes have bigger and more balanced populations, reflecting larger effective population size and genetic variation	Demographic processes have to covary with the species' geographical range size	As geographical range size increases, the rate of molecular evolution increases	Substitution rate	(Ivan *et al.* 2022, Amador *et al.* 2025)
	Body size	Larger size is related to increased cell divisions for growth, maintenance, and reproduction, and larger species have more investment in DNA repair. It can also be related indirectly through metabolic rate, fecundity, longevity, generation time, or range size	If an indirect relationship is expected, body size has to be directly related to the species' life-history traits	As body size increases, the rate of molecular evolution decreases	Mutation and/or substitution rate	(Bromham 2002, Thomas *et al.* 2006, Fontanillas *et al.* 2007, Welch *et al.* 2008, Lanfear *et al.* 2010, 2013, Gillman *et al.* 2012, Santos 2012, Qiu *et al.* 2014, Bromham *et al.* 2015, Hua *et al.* 2015, Nabholz *et al.* 2016, Barrera-Redondo *et al.* 2018, Ivan *et al.* 2022, Bergeron *et al.* 2023, Cai *et al.* 2025, Jing *et al.* 2025, Weiss and Berv 2025)
Ecological correlates	Ultraviolet light	UV light causes specific classes of mutations, such as general DNA damage	Germline cells must be directly or indirectly exposed to UV, and unrepaired lesions must be transmitted as heritable mutations	As the incidence of UV light increases, the rate of molecular evolution increases	Mutation rate	(Davies *et al.* 2004, Lanfear *et al.* 2013, Bromham *et al.* 2015)
	Temperature	Higher temperatures induce faster biochemical reactions, generating more free radicals that cause DNA damage	Germline cells have to be consistently impacted by the production of free radicals	As temperature increases, the rate of molecular evolution increases	Mutation rate	(Wright *et al.* 2003, Davies *et al.* 2004, Rolland *et al.* 2016, Barrera-Redondo *et al.* 2018, Ivan *et al.* 2022, Zhao *et al.* 2024, Cai *et al.* 2025)
	Productivity	Productivity may produce faster metabolic rates, increasing the production of mutations (see below)	Productivity has to be related to the species' metabolic rate	As productivity increases, the rate of molecular evolution increases	Mutation rate	(Barrera-Redondo *et al.* 2018)
	Latitude	Low latitudes have higher temperatures and productivity, which promotes faster molecular evolution. The same applies to the latitudinal variation of body size and range size	A species' latitudinal distribution has to covary with temperature, productivity, body size, and/or range size	As latitude decreases, the rate of molecular evolution increases	Mutation and/or substitution rates	(Wright *et al.* 2006, Gillman *et al.* 2009, 2012, Lourenço *et al.* 2013, Bromham *et al.* 2015, Rolland *et al.* 2016, Ivan *et al.* 2022, Zhao *et al.* 2024, Cai *et al.* 2025)

even at similar metabolic rates (Lanfear *et al.* 2007); (ii) limited diffusion of free radicals, reducing their impact on nuclear DNA (Lanfear *et al.* 2007); (iii) oxidative stress appears not to increase germline mutation rates (Lanfear *et al.* 2013); and (iv) insufficient metabolic rate data, the over-reliance on proxies such as body size, or covariation with other life-history traits (Qiu *et al.* 2014, Jing *et al.* 2025).

The second pathway through which life-history traits may influence molecular evolution is via enhanced DNA repair efficiency, particularly in relation to longevity and body size (Fig. 1A; Table 1). For longevity, it has been proposed that long-lived species undergo more rounds of replication and therefore should accumulate more mutations (increasing dS) (Medawar 1952). Yet evidence for this is weak, and often the opposite trend is found, leading to the reformulated view that long-lived species invest more in repair mechanisms, as the risk of harmful mutations rises with age (the mutation accumulation theory; Charlesworth 2000). Support for this last correlation has been reported across diverse taxa (e.g. Nabholz *et al.* 2008, Welch *et al.* 2008, Lourenço *et al.* 2013, Hua *et al.* 2015, Jing *et al.* 2025; but see Bergeron *et al.* 2023, Cai *et al.* 2025). Body size is often placed under the same mechanism: larger species undergo more cell divisions for growth, maintenance, and reproduction, and therefore are expected to evolve more efficient repair systems (decreasing mutation rate and dS). Consistent evidence supports this prediction (e.g. Bromham 2002, Fontanillas *et al.* 2007, Welch *et al.* 2008, Santos 2012, Lanfear *et al.* 2013, Bromham *et al.* 2015, Hua *et al.* 2015, Barrera-Redondo *et al.* 2018, Ivan *et al.* 2022, Cai *et al.* 2025, Jing *et al.* 2025; but see Thomas *et al.* 2006, Lanfear *et al.* 2010, Gillman *et al.* 2012, Bergeron *et al.* 2023, Caron *et al.* 2026, Weiss and Berv 2025), with some studies suggesting the effect is stronger in the mitochondrial genome, where links between mutation and carcinogenesis have been reported (Welch *et al.* 2008). While this hypothesis is compelling, it is important to note that body size covaries strongly with other life-history traits, which may obscure the causal chain of events underlying its relationship with molecular evolutionary rates (see section 'Why do we still not know what drives molecular rates?'). Importantly, repair efficiency has become a recurrent explanation in studies of molecular evolution, not limited to longevity or body size. For longevity, the mechanism is plausible and supported by evidence that enhanced repair serves as a longevity assurance system (MacRae *et al.* 2015, Popov *et al.* 2024). However, in other cases, it is often invoked *post hoc*, especially when predicted correlations are absent or reversed, as commonly observed in studies of body size (Welch *et al.* 2008). Moreover, if enhanced repair efficiency were truly a general mechanism, one should expect that it could also have evolved in traits associated with high mutation input, such as high fecundity or short generation time. Yet, these lineages typically show elevated rather than reduced molecular rates. This highlights the need for more rigorous testing of the DNA repair hypothesis, rather than relying on it as a universal explanation.

The third and last pathway through which traits can affect molecular evolution is by influencing N_e (Fig. 1A). This mechanism does not act on the mutation rate, but directly on the substitution rate. Range size is often considered a primary correlate in this category (Table 1), under the rationale that species occupying larger areas tend to maintain larger, more stable populations, leading to higher nucleotide diversity (Amador *et al.* 2025). However, the theoretical implications for overall rates of molecular evolution are complex. While larger N_e generally allows for more efficient selection, potentially leading to faster adaptive evolution, the nearly neutral theory posits that if most mutations are weakly deleterious, species with smaller N_e might exhibit higher overall substitution rates due to increased genetic drift (Lanfear *et al.* 2010, Ivan *et al.* 2022). Moreover, traits promoting wide distribution, such as high fecundity and niche generalism, do not necessarily translate into higher local abundance, which is more constrained by ecological and energetic limitations (Novosolov *et al.* 2017). In fact, range size and local population density can even be negatively correlated (Novosolov *et al.* 2017). These complexities suggest that the link between range size, N_e, and substitution rates may be more complex than previously thought. Yet, studies explicitly addressing this relationship remain limited.

Environmental predictors

Climatic variables are thought to influence the pathway of generation of new mutations through the production of reactive metabolites (Fig. 1A). Among these variables, environmental temperature stands out (Table 1), as higher temperatures can accelerate biochemical reactions, increasing the formation of free radicals that damage DNA (Allen *et al.* 2006). In theory, this effect should occur across all organisms. However, it should be more pronounced in ectotherms than in endotherms, whose body temperature fluctuates with the environment, possibly making them more susceptible to such damage. While this reasoning is plausible, empirical evidence shows that temperature-related effects on molecular evolution are observed in ectotherms (e.g. Ivan *et al.* 2022, Cai *et al.* 2025; but see Rolland *et al.* 2016), in endotherms (e.g. Zhao *et al.* 2024, Cai *et al.* 2025), and also in plants (e.g. Wright *et al.* 2003, Davies *et al.* 2004, Barrera-Redondo *et al.* 2018). A straightforward explanation is that the influence of temperature may not depend on an organism's ability to regulate its body temperature. Alternatively, the effect in endotherms has been linked to the Red Queen hypothesis; that is, if ectotherms evolve faster in warmer environments, endotherms may also be selected for elevated rates of molecular evolution (Gillman *et al.* 2009). Nevertheless, it is difficult to disentangle the direct effect of temperature from a Red Queen scenario.

Similarly, UV light has also been proposed to influence the generation of free radicals and increase molecular evolutionary rates (Table 1). UV exposure is known to cause specific types of DNA damage, as well as more general genomic lesions (Bromham 2024). Nonetheless, this effect has been tested in detail in plants (Davies *et al.* 2004, Lanfear *et al.* 2013, Bromham *et al.* 2015), with findings suggesting that, in some cases, high UV incidence has been associated with lower rates of molecular evolution (Hua and Bromham 2017). Proposed explanations for this pattern fall within the argument that it may result from the evolution of more efficient DNA repair systems (see section 'Life-history traits'). Notably, the influence of these factors (i.e. UV light and temperature) underlies broader theories, such as the explanation for higher species richness in the tropics (Gillman and Wright 2014). In this context, greater UV exposure and elevated temperatures in tropical regions could lead to higher mutation rates. Consequently, the production of new mutations (i.e. the molecular evolutionary rate)

could be directly linked to the generation of new species. However, while some evidence supports this idea (e.g. Lanfear *et al.* 2010, Iglesias-Carrasco *et al.* 2019), the relationship between molecular evolution and diversification remains highly debated.

Finally, two additional predictors are thought to indirectly correlate with molecular evolutionary rates: productivity and latitude (Table 1). Environmental productivity reflects the amount of available energy in a system, commonly measured as net primary productivity (NPP). Its potential influence would be mediated through metabolic rate, which could increase as more energy becomes available (Barrera-Redondo *et al.* 2018). However, evidence for this effect remains very limited. Latitude, in turn, is among the most frequently tested predictors of molecular evolution (Wright *et al.* 2006, Gillman *et al.* 2009, 2012, Lourenço *et al.* 2013, Bromham *et al.* 2015, Rolland *et al.* 2016, Ivan *et al.* 2022, Zhao *et al.* 2024, Cai *et al.* 2025). Yet, by itself, a correlation with latitude provides little insight into the underlying evolutionary process. Instead, it may indicate the role of variables that show a strong latitudinal gradient, which could drive heterogeneity in molecular rates (see section 'Why do we still not know what drives molecular rates?').

WHY DO WE STILL NOT KNOW WHAT DRIVES MOLECULAR RATES?

Nearly every macroevolutionary study that has tested the relationships between life-history/ecological traits and molecular evolutionary rates used multiple linear regression models, whether accounting for phylogenetic history or not. As mentioned earlier, the main obstacle with this approach is that life-history traits do not vary in isolation in nature but occur in integrated syndromes (Fig. 1B, C). Consequently, it is fundamentally difficult to isolate any single variable as the primary cause of variation in molecular evolution. Even when multiple predictors are included in a statistical model, it remains challenging, for example, to determine whether body size emerges as the best predictor due to its own direct mechanistic action or simply because it covaries with all other traits in the syndrome. Hence, this analytical approach leads to a cycle of redundant or, sometimes, spurious explanations. While a life-history trait often emerges as the best predictor, cautionary calls are frequent because it may indeed covary with other variables and thus its association with the molecular evolutionary rate may be indirect. Therefore, the critical question that needs to be reframed is not which trait is the ultimate cause of variation in molecular evolution, but rather which trait is most proximately related to the underlying mechanistic pathway in a given evolutionary context.

In essence, the concern goes beyond the raw correlation between the predicting traits, and involves the association of these variables conditional on other variables in the model. The consequence of performing a multiple linear regression analysis is that the correlation structure is not taken into account (Fig. 1C), making the model's outcome unreliable (McElreath 2020). For this reason, testing for statistical multicollinearity (and possibly excluding variables from the model) is insufficient, as the biological interdependence among variables intrinsically exists. For instance, if a regression model identifies a significant association between molecular evolution and body size, replacing body size with a correlated trait like fecundity will often yield a similar significant result. This analytical interchangeability raises a critical question: what does such a pattern truly reveal about the drivers of molecular rate variation? Is it caused directly by body size, by fecundity, or is it the effect of their shared covariation with a third variable (e.g. longevity)? Standard regression frameworks cannot distinguish between these competing hypotheses, as they are designed to identify predictors, not to depict the underlying causal structure of the data.

Given this context, it should not be surprising when a specific life-history/ecological trait fails to correlate with molecular evolution. On the contrary, even within rigorous theoretical frameworks that predict the correlation, its absence should indeed be expected. For instance, within a specific clade, fecundity may be much more variable and informative than body size, and thus it may correlate more strongly with the rate of molecular evolution. That is not to say that body size does not affect molecular evolution in this clade, but that some other factor is governing the variation of the rate of molecular evolution. In other words, the trait with the most explanatory variation will best capture the association with molecular evolution, given the evolutionary history and phenotypic variability of the lineage. More importantly, all these life-history traits serve as proxies for the underlying pathway of action (Fig. 1A). Therefore, it is likely that in some clades certain traits may serve as better proxies than others for the same underlying mechanism. That is why considering the causal structure of these life-history syndromes cannot be understated: if this structure is ignored, any causal inference drawn from correlational patterns remains speculative at best, as the identified 'best predictor' may merely be the variable with the most favourable statistical properties rather than the one with the greatest biological relevance.

MODELLING RATE VARIATION WITH DIRECTION AND STRUCTURE

To account for directionality and indirect relationships when testing predictors of molecular evolution, it is necessary to use methods that not only address multicollinearity but also incorporate structural assumptions during hypothesis testing. Structural equation modelling (SEM) offers one such solution (Fan *et al.* 2016). SEM differs from multivariate linear regression in its ability to test hypotheses involving both direct and indirect relationships among variables. Although some authors describe SEM as modelling causality, this characterization is misleading as no statistical method can definitively distinguish between association and causation (Pearl 2012). Establishing causality among predictors requires controlled laboratory experiments. However, such experimental approaches are only feasible for a limited number of clades, making it essential to employ analytical frameworks that at least account for the underlying data structure. Therefore, 'true' causal links will emerge primarily in laboratory studies rather than, it appears, in macroevolutionary research.

Although SEM has its limitations, its application in biological sciences is definitely highly valuable. Its power stems from integrating two key functionalities within a single framework. First, and perhaps most importantly in this context, SEM enables testing complete hypotheses about causal relationships (e.g. Fig. 1C). In

doing so, it simultaneously estimates direct, indirect, and total effects of multiple variables, accounting for their interdependencies (Fan *et al.* 2016). Second, SEM permits the use of latent variables, which are unmeasured variables that may influence the model but for which insufficient data exist (e.g. metabolic rate). This modelling approach allows inclusion of these variables by estimating their values based on how other variables in the model affect them. Due to these features, SEM is more suitable for testing hypotheses when there are multiple complex relations predicted within a system.

Although SEM is the primary method discussed here for addressing the challenges in molecular evolutionary predictor hypotheses, alternative approaches deserve consideration. Commonality analysis (CA), for instance, offers a complementary framework for decomposing regression effects in the presence of multicollinearity, allowing researchers to partition variance into unique and shared components among predictors (Ray-Mukherjee *et al.* 2014). This method provides valuable insights into how different evolutionary factors contribute individually and jointly to molecular evolutionary patterns. However, unlike SEM, CA focuses primarily on variance decomposition rather than testing comprehensive hypotheses about direct and indirect causal pathways. Other statistical methods could be applied to these problems, but the essential consideration remains recognizing these methodological challenges and selecting approaches that account for the complex relationships among evolutionary predictors.

CONCLUSIONS AND HOW TO MOVE FORWARD

Studies of molecular evolution have advanced considerably over the past decades, revealing intricate relationships between life-history traits, environmental factors, and the tempo of molecular change. Yet, significant challenges remain in establishing clear causal links between species characteristics and molecular evolutionary rates. The pervasive covariation among life-history traits creates several interdependencies that multiple linear regression models are unable to disentangle. While we have identified plausible mechanistic pathways, the relative importance of these processes varies across lineages and evolutionary contexts. Moving forward, it will be fruitful to embrace analytical frameworks that explicitly model these complex relationships rather than relying on multiple linear regression approaches that treat predictors as independent entities.

Another important issue that should not be overlooked is the lack of standardization when comparing predictors of molecular evolution across lineages. Frequently, studies use various markers (e.g. mitochondrial, nuclear, chloroplastidial) along with different sets of predictor variables. Different markers are thought to be influenced in distinct ways by life-history traits, depending on the mechanistic pathways through which the trait acts. For instance, mitochondrial genes may be more sensitive to metabolic rate due to their proximity to oxidative stress sites within the cell (Fontanillas *et al.* 2007, Lanfear *et al.* 2007). Similarly, the number of analysed loci, the targeted genomic regions (coding vs. non-coding), and sampling strategies (number of individuals, populations, or species) vary considerably across studies, potentially affecting rate estimates and their correlations with life-history traits. Therefore, researchers must carefully consider these consequences when testing evolutionary hypotheses. An additional issue is that the same life-history trait is often quantified using different proxies across studies, largely because no single metric is universally applicable or available for all clades. For instance, generation time can be estimated as the age of first reproduction, the age at last reproduction, or adult survivorship. This means that apparent differences between clades might reflect measurement inconsistencies rather than genuine biological variation. Systematically addressing these methodological challenges will certainly help clarify the drivers of molecular evolutionary rate across the tree of life.

A crucial development that significantly advanced this endeavour was the shift from single-gene to genome-scale data in the last few decades (Lynch 2010). In particular, recent genomic technologies allow for accurate estimates of the germline mutation rate (Bergeron *et al.* 2023). Moreover, the increasing availability of whole-genome datasets now offers unprecedented power and resolution to address many analytical challenges. More specifically, this transition allows greater power to detect correlates of molecular rate variation, disentangle correlated biological and environmental predictors, and compare patterns across loci and genomic compartments (e.g. mitochondrial, nuclear, plastid). With many loci, it becomes possible to test whether trait effects are consistent genome-wide or concentrated in specific genome compartments or functional classes. However, the adoption of genome-scale data also introduces new challenges, including substantial computational demands for analysis.

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AUTHOR CONTRIBUTIONS

Fernanda S. Caron (Conceptualization [Equal], Investigation [Lead], Project administration [Equal], Visualization [Lead], Writing—original draft [Lead], Writing—review & editing [Equal]) and Fabricius M.C.B. Domingos (Conceptualization [Equal], Project administration [Equal], Supervision [Lead], Writing—review & editing [Equal]).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

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