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REVIEW

Molecular evolution and the latitudinal biodiversity gradient

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Species density is higher in the tropics (low latitude) than in temperate regions (high latitude) resulting in a latitudinal biodiversity gradient (LBG). The LBG must be generated by differential rates of speciation and/or extinction and/or immigration among regions, but the role of each of these processes is still unclear. Recent studies examining differences in rates of molecular evolution have inferred a direct link between rate of molecular evolution and rate of speciation, and postulated these as important drivers of the LBG. Here we review the molecular genetic evidence and examine the factors that might be responsible for differences in rates of molecular evolution. Critical to this is the directionality of the relationship between speciation rates and rates of molecular evolution.
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INTRODUCTION

> '*Animal life is, on the whole, far more abundant and more varied within the tropics than in any other part of the globe, and a great number of peculiar groups are found there which never extend into temperate regions*'
>
> —AR Wallace, 1876

One of the most striking biogeographic patterns on the planet is the uneven latitudinal distribution of biodiversity (Figure 1). A similar trend of high and low diversity is documented in all major groups of organisms, on the land and in the sea (Hillebrand, 2004).

The evolutionary and ecological factors responsible for the pattern usually referred to as the latitudinal biodiversity gradient (LBG) have been hotly debated over the past 30 years (Rohde, 1992; Gaston, 2000; Hawkins, 2004; Losos, 2008; Erwin, 2009; Gillman *et al.,* 2009). Interest in the drivers behind the LBG has expanded from biogeographers (starting with Wallace) to include ecologists, evolutionary biologists (in particular evolutionary geneticists), physiologists and palaeontologists (Rosenzweig, 1995; Allen *et al.,* 2002; Davies *et al.,* 2004; Jablonski *et al.,* 2006), however, no consensus has been reached. There is general agreement that variation in the total number of species among regions must result from variation in one or more of three basic phenomena underpinning the diversity of life on the earth: speciation rates, extinction rates and immigration rates. Regional biotas are the product of the interaction of these three processes, but understanding what factors drive these to form the LBG is problematic (Jablonski *et al.,* 2006). How far have we progressed in developing an explanation since Alfred Wallace's observation more than 130 years ago? Here we focus on recent molecular genetic studies to see whether this field, which has contributed so much to evolutionary research, has helped our understanding of the LBG.

Speciation

Differences in speciation rates have been the major focus of geneticists, ecologists and palaeontologists seeking to explain the LBG. The tropics are referred to as a cradle of diversity with high speciation rates inferred from observed high species' diversity (Stebbins, 1974; Chown and Gaston, 2000). Palaeontological data allow comparison of direct counts of species' origins (speciation) in past tropical and temperate areas and have provided the most compelling evidence for differing speciation rates (Jablonski *et al.,* 2006). Molecular data are increasingly applied to the study of speciation rates, but these analyses are not always directed specifically at the LBG (Lancaster, 2010; Lanfear *et al.,* 2010a). Although there is compelling evidence of relatively high rates of speciation in the tropics, the underlying driver(s) of this have not been identified (Martin and Mckay, 2004; Allen and Gillooly, 2006; Jablonski *et al.*, 2006; Mittelbach *et al.*, 2007; Krug *et al.*, 2009; Condamine *et al.*, 2012).

Extinction

A higher rate of extinction at higher latitudes (away from the equator) was the explanation offered by Wallace for the formation of the LBG (Wallace, 1876). Hypotheses focusing on extinction rates have often centred on the putative effects of climatic extremes in the past (for example, during the Pleistocene), which may have been felt most intensely at higher latitudes (Figure 1). In the tropics, a more uniform climate over time may have allowed the accumulation of species and these regions have thus been referred to as museums (Wallace, 1876; Wright, 1983; Guo and Ricklefs, 2000). Unfortunately, the evidence for extinction and its effect is striking by its absence in discussions on this topic, reflecting a general problem with the quantification and accommodation of extinction in biogeography (Crisp *et al.,* 2011). Only palaeontology has provided direct counts of extinction and some comparison of extinction rates, mostly in marine invertebrates. It is well recognised that climate cycling caused extinction in temperate areas during the Pleistocene, but over a longer time frame the evidence is more equivocal. Few studies find strong support for differences in extinction rates between latitudes, and the general inference is that extinction alone cannot explain the LBG

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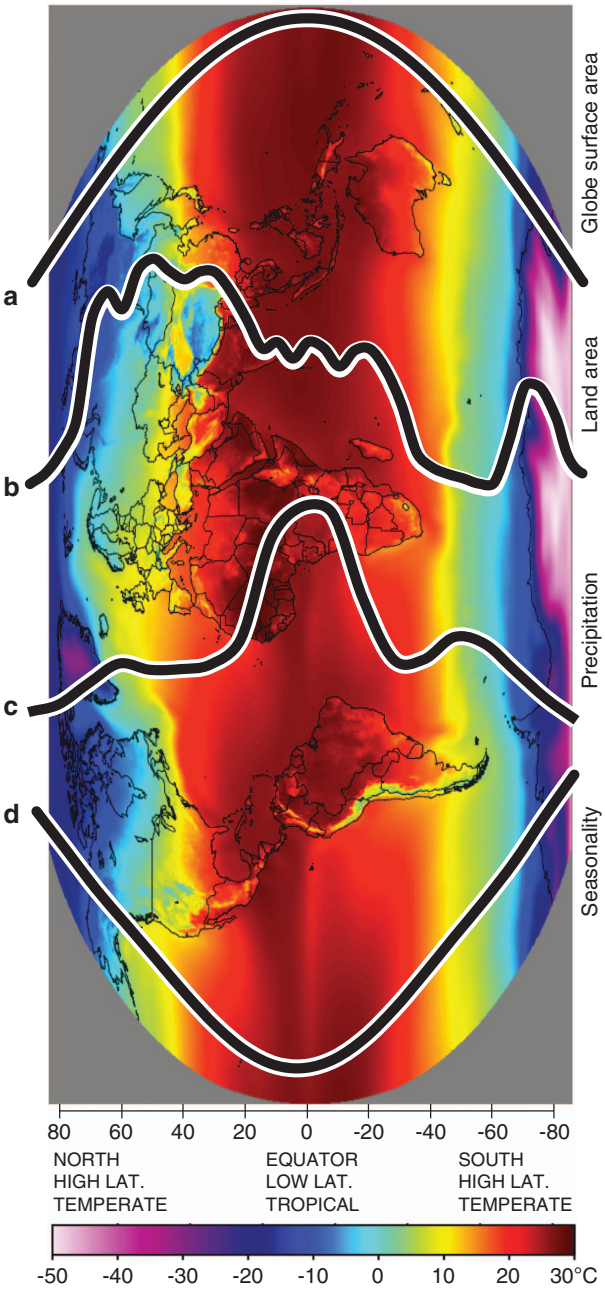

Figure 1 A latitudinal biodiversity gradient (LBG) has been identified in almost all organisms that have been investigated, on land and in the sea. The gradient involves high species' numbers near the equator (at low latitudes) and lower numbers of species at high latitudes. The question of why and how this pattern has arisen has persisted for more than 150 years. Several environmental parameters form latitudinal gradients reflecting the shape of the planet, its rotation and orientation to the sun. Although the globes' surface area (geometric calculation of a dome) is evenly graded with latitude (**a**), the extent of land area (**b**), ocean and variation in bathymetry are not graded evenly. Associated wind and ocean currents further complicate the distribution of environmental variables. Average marine and terrestrial temperatures reduce with increasing latitude (background), but precipitation (**c**) (L.D.Roper Virginia Poly. Inst. and State University) does not have a linear relationship and seasonality (for example, in terms of annual variance of insolation data from http://www.applet-magic.com/insolation.htm) increases (**d**). Correlation of these traits with the LBG does not demonstrate causation. Temperature map produced by R.A Rhode, http://en.wikipedia.org/wiki/File:Annual_Average_Temperature_Map.jpg. Details are available from the authors.

(Hawkins *et al.*, 2006; Jablonski *et al.*, 2006; Martin *et al.*, 2007; Valentine *et al.*, 2008).

Immigration

Although immigration is central to some biogeographic models (Macarthur and Wilson, 1967), it has been considered, by some, to be too weak a force to be important in as large scale a trend as the LBG (Gaston, 2000). To be relevant in this context, immigration needs to involve the permanent range change of a taxon resulting in its absence from its original range. Although evidence of large-scale species-range movement has been reported in marine bivalves, terrestrial species may be more range limited (Jablonski *et al.*, 2006; Martin *et al.*, 2007; Mittelbach *et al.*, 2007). Niche conservation has been interpreted by some as indicating a limited capacity in many plant and animal groups for substantial range (habitat) shifts (for example, Wiens and Graham 2005). Immigration has yet to be linked to molecular evolution directly, although range changes could have important implications for the assumptions within many molecular approaches. In particular, the assumption that the current range midpoint of a species represents the midpoint through most of its evolutionary history is questionable.

THE PROCESS BEHIND THE PATTERN

> '*The causes of these essentially tropical features are not to be found in the comparatively simple influences of solar light and heat, but rather in the uniformity and permanence with which these and all other terrestrial conditions have acted; neither varying prejudicially throughout the year, nor having undergone any important change for countless past ages*'.
>
> —AR Wallace, 1876

The LBG is an observable pattern that, as is typical of biogeographic patterns, does not in itself provide information about the processes involved in its formation (Figures 1 and 2). Latitude values provide a convenient scale for graphing biodiversity patterns but do not directly express the driving force(s) of the gradient (Zapata *et al.*, 2003; Currie and Kerr, 2008). In recent work, temperature has become a focal putative driver replacing an earlier emphasis on habitat area.

Palaeontological studies find evidence that environmental temperature is correlated with biodiversity through time in marine molluscs and protists (Crame, 2002; Mayhew *et al.*, 2008; but see Crampton *et al.*, 2006; where no link was found for molluscs). Most studies find the strongest correlation with temperature (or in some cases ultraviolet radiation, UV) when comparing species richness with a number of possible variables (for example, area, mid-domain effects; Clarke and Gaston, 2006). Several mechanisms have been proposed for how higher temperature might lead to greater species diversity by influencing the speed of speciation, species' physiological tolerances and extinction rates. The 'evolutionary speed' hypothesis has thus become the explanation for the biodiversity gradient preferred by many molecular biologists.

The evolutionary speed hypothesis

Rohde (1992) described the evolutionary speed hypothesis to explain the LBG in terms of a difference in speciation rates; speciation rates being influenced by a latitudinal disparity in rates of molecular evolution and selection. Any differences in rates of molecular evolution between latitudes are considered to be the products of the different environmental conditions that individuals experience at different latitudes. Differing rates of molecular evolution might influence speciation rates if the latter are determined, at least partially,

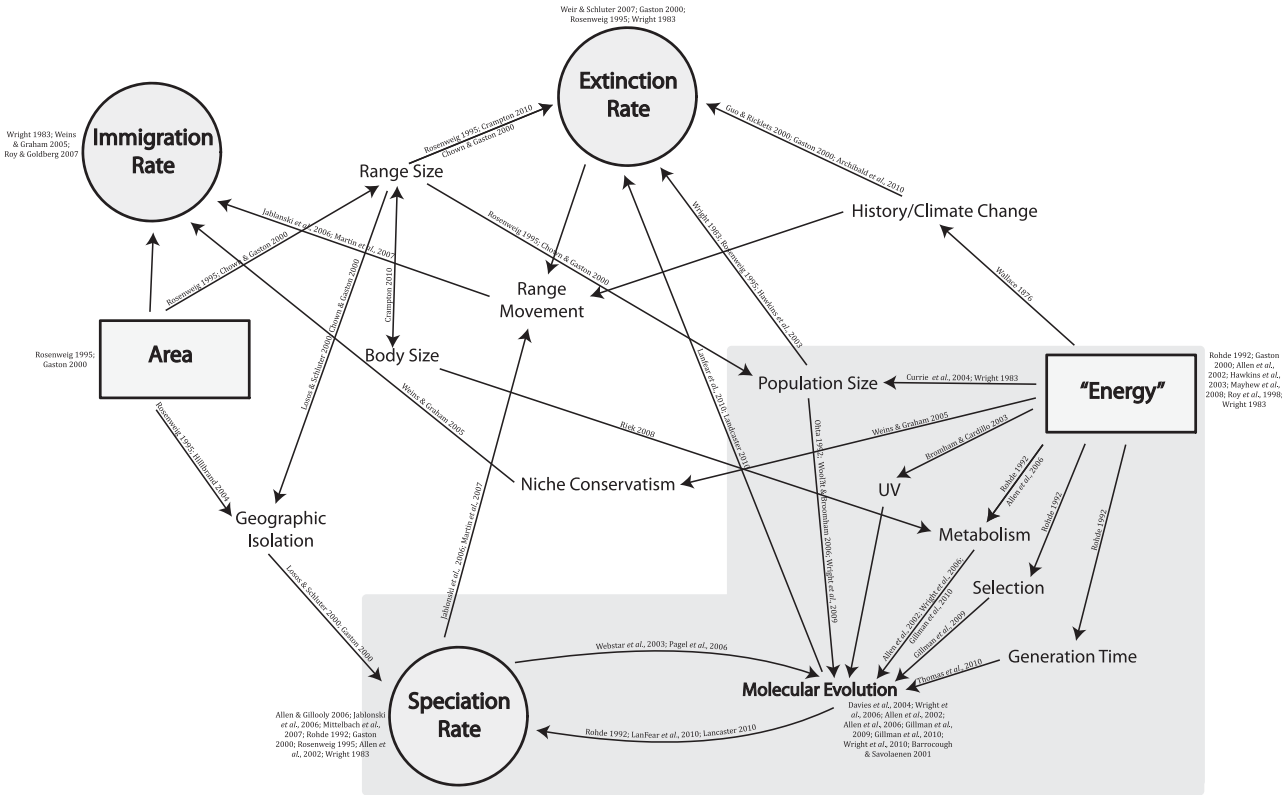

Figure 2 A multitude of hypotheses seek to explain the latitudinal biodiversity gradient (LBG). Each hypothesis aim to relate the LBG to processes that influence one or more of the three components controlling regional species diversity: immigration, extinction and speciation. The two key environmental parameters most frequently cited as underlying drivers of the LBG are area and energy (generally measured in the form of temperature). The inferred importance and directionality of effects varies among taxa, researchers and methods applied, with no clear consensus. Here we illustrate a subset of theories on the formation of the gradient that have gathered some empirical support (citations in figure) and, in particular, those that relate to molecular evolution (shaded area). A link between speciation rate and the rate of molecular evolution is widely shown, but which is the driver of the other is equivocal. A latitudinal gradient in available energy may influence many traits but these are only tentatively associated with rates of extinction and/or speciation.

by the random accumulation of genetic differences (mutation-order speciation) and natural selection (ecological speciation; Schluter, 2009; Lancaster, 2010). Allopatric speciation via genetic drift (mutation-order speciation) might result in the formation of new species just as rapidly as ecological speciation, however, the relative influence of these two processes in speciation is not known (Schluter, 2009; Nosil and Flaxman, 2011). Ecological speciation is the more readily studied of the two, and can accommodate difficulties of gene flow (Rundle *et al.*, 2000; Funk *et al.*, 2006; Langerhans *et al.*, 2007; Schluter, 2009). Mutation-order speciation might contribute to further separation of populations already partitioned by natural selection. Alternatively, it could result in faster reproductive isolation by genetic drift in geographically isolated (allopatric) populations perhaps via a Dobzhansky-Muller process (Schluter, 2009; Nosil and Flaxman, 2011).

A faster mutation rate could increase the rate of speciation (for example, see Lancaster, 2010), if mutation-order speciation contributes significantly to overall speciation rate. This presumes that all other evolutionary processes (for example, competition, character displacement, reproductive cohesion, adaptation) are limited only by mutation rate. Central to the biodiversity debate, is the conundrum: does speciation result in accelerated molecular evolution (Webster *et al.*, 2003; Pagel *et al.*, 2006) or does accelerated molecular evolution increase the chance of speciation? That even this fundamental point is subject to debate highlights just how little we understand the role of genetic variation in the LBG and speciation generally.

The evolutionary speed hypothesis predicts an elevated rate of molecular evolution towards the tropics and that this variation directly effects relative rates of speciation. A latitudinal difference in rates of molecular evolution (faster in the tropics) has indeed been found in a range of organisms using sister species comparisons of plants, fish, frogs, foraminifera and mammals (Davies *et al.*, 2004; Allen *et al.*, 2006; Wright *et al.*, 2006; Gillman *et al.*, 2009, 2010; Wright *et al.*, 2010, 2011), although the relationship for mammals may be weaker than originally reported (Weir and Schluter, 2011; Figure 3). Rohde (1992) considered three main explanations for increased rates of molecular evolution: shorter generation times; direct effects of temperature on mutation rate; and acceleration of mutation rates due to acceleration of physiological process along with an acceleration of positive selection owing to the first two. These explanations have since been elaborated and extended to include the postulated influence of: metabolic rates, generation time, UV radiation and population size (Figure 2). Here we examine these four putative drivers of differential rates of molecular evolution. To fully understand how rates of molecular evolution could be influencing the biodiversity gradient, it is important to understand how and why these drivers could affect rates of molecular evolution in the first place, providing context to studies of molecular evolution rate variation among latitudes.

Metabolic rate variation driving differential rates of molecular evolution

Work on metabolism and its influence on the LBG started as part of the 'metabolic theory of ecology' (Allen *et al.,* 2002; Gillooly and Allen, 2007). It has been suggested that the LBG arose in response to a latitudinal gradient in kinetic energy influencing rates of molecular evolution in ectotherms (Allen and Gillooly, 2006; Gillooly and Allen, 2007). Under this model, ectotherms in warmer areas have an increased metabolic rate governing consumption of oxygen and production of oxygen-free radicals that potentially increase mutation rate by damaging DNA (Allen *et al.,* 2006; Gillooly *et al.,* 2007). Comparing species with different metabolic rates is a proxy for comparison of different rates of oxygen-free radical formation (and inferred mutation), which until recently could not easily be measured directly. New sequencing technology now allows for comparison of whole genomes of parents and their offspring, which can be used to estimate *de novo* mutation rates of species (see below). In comparison to other putative divers, the concept of metabolic rates and the LBG yields clearly testable predictions, but has been heavily contested since its origin (Algar *et al.,* 2007; Hawkins *et al.,* 2007a,b; De Castro and Gaedke, 2008; Irlich *et al.,* 2009).

Unexpectedly, elevated rates of molecular evolution in the tropics compared with temperate regions have also been observed in some endotherms (for example, mammals; Gillman *et al.,* 2009), which was not predicted by the original metabolic hypothesis (Storch, 2003; Gillman *et al.,* 2009). Metabolic rates in endotherms are closely linked to body size, with larger bodies having higher absolute metabolic rates but lower rates per unit mass. As body size in endotherms tends to be greater at higher latitudes (Bergmann's rule), a counter gradient decrease in basal metabolic rate per unit mass with increased latitude is suggested (Ashton *et al.,* 2000). But Gillman *et al.* (2009) found that even when body size of mammals was accounted for, the rate of molecular evolution still increased towards the tropics. There is, however, evidence from comparative studies that basal metabolic rate is de-coupled from molecular evolution in the bird and mammal mitochondrial DNA (mtDNA) that are widely used in these studies (Lanfear *et al.,* 2007; Nabholz *et al.,* 2009; Galtier *et al.,* 2009a). Thus, the proxy for oxygen-free radicals (metabolic rate) may inform little on rates of mutation or molecular evolution, and we are no closer to understanding the driver of variation in the rate of molecular evolution and the formation of the LBG.

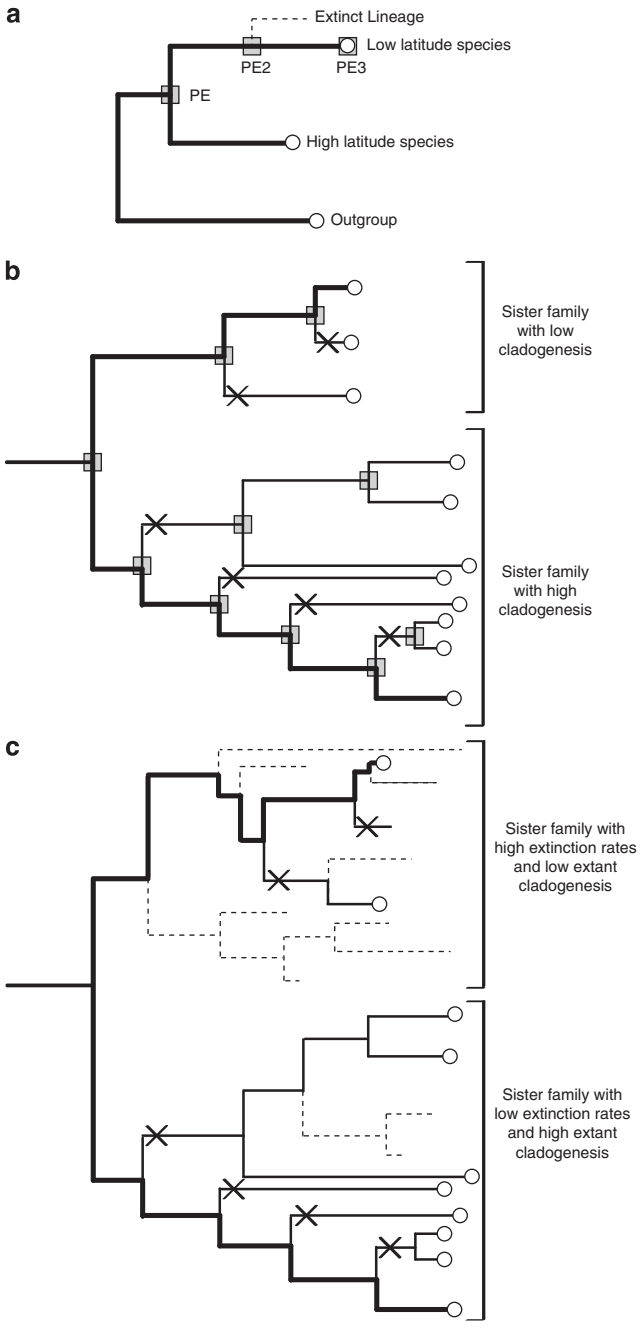

Figure 3 Evidence of variation in the rate of molecular evolution has come from two main sampling strategies that use DNA sequences from extant species (usually represented by a single individual) to infer rate differences among lineages. Possible instances of punctuational evolution at nodes (grey boxes), extinct lineages (dashed lines), pruned or un-sampled lineages (X) are shown. (**a**) The sister species comparison. Sister species are chosen to represent contrasting distributions; one at low latitude and one at high latitude (temperate versus tropical as in Gillman *et al.,* 2009). The placement of the outgroup in the phylogenetic reconstruction provides the crucial evidence for rate variation between the two ingroup lineages. By sampling only one individual per lineage, this method avoids the node-density effect that might influence estimates of molecular evolution rate (Hugall and Lee, 2007). Punctuated equilibrium (PE) predicts that acceleration in rates of molecular evolution is associated with speciation and therefore occurs at nodes. If so, observed differences in rates (different branch lengths) cannot result from PE unless speciation (PE_2) and extinction have occurred only on the branch leading to the tropical species. Hidden (or incipient) speciation (PE_3) could also explain the observed faster rate of molecular evolution if it was occurring more frequently in the tropical lineages than the temperate lineages. (**b**) Pruning large data sets. Species are selected from sister lineages with different species numbers to examine whether or not lineages with higher cladogenesis have faster rates (for example, Lanfear *et al.*, 2010a). Relatively large data sets are pruned (X) to a pair of contrasting lineages, if more sequence data are available for one family than another, to avoid the node density effect. (**c**) Pruning large data sets with extinction. Lineage extinction (dashed lines) undermines confidence in inferences about the directionality of effects. Surviving species only provide estimates of net-diversification (speciation − extinction), not rates of speciation *per se*. Insufficient knowledge of extant lineages produces the same effect as extinction suggesting that this method would only work for well-studied groups.

Generation time and longevity driving differential rates of molecular evolution

Generation times are generally assumed to be shorter in tropical organisms than their temperate counterparts. Having a short

generation time could cause lineages to accumulate more mutations in a given time period compared with lineages with long generation times (Thomas *et al.,* 2010). Although under-examined, the predicted correlation between generation time and rates of molecular evolution has been observed in some groups of invertebrates, flowering plants, host–parasite systems and mammalian nuclear sequences (Nikolaev *et al.,* 2007; Smith and Donoghue, 2008; Welch *et al.,* 2008; Thomas *et al.,* 2010), but not for other plants or mammal mtDNA (Whittle and Johnston, 2003; Nabholz *et al.,* 2008). Ohta (1992) suggested that variation in generation time and population size was the cause of the discrepancy between observed rates of protein and nucleotide evolution.

Plants (especially large, long-lived species) can be problematic subjects with regard to generation time, because mutations in reproductive tissue are also influenced by mutagenesis of somatic cells (Gaut *et al.,* 2011). Little direct testing of generation times across latitudes has been undertaken, probably because the basic biology of many tropical species is not known. Linked to this problem is the issue of longevity, which refers to the number of cell cycles an individual undergoes rather than the life expectancy of the organism. This is another life history hypothesis that has yet to be implicated in the LBG, but has a similar relationship to rates of molecular evolution as generation time. Longer-lived species (with more cell cycles per individual) are expected to be more disadvantaged by higher mutation rate, as premature aging could result from a high somatic DNA mutation rate (Nabholz *et al.,* 2008, 2009).

UV radiation driving differential rates of molecular evolution

UV is a mutagen and as a component of insolation is most constant and intense at the equator (Figure 1; Rastogi *et al.*, 2010). UV intensity has been linked to the LBG by the prediction that an increased mutation rate with higher exposure to UV (Bromham and Cardillo, 2003; Davies *et al.,* 2004; Flenley, 2011) may be correlated with speciation rate. The proposed role of UV as a producer of heritable mutations is perhaps more straightforward in animals where exposure to UV could occur during embryonic development (Epel *et al.,* 1999; Maurer *et al.*, 2011) as this could result in germline mutations. In plants, UV has been proposed as a potential mutagen of pollen grains and experimental studies have shown its effects (Ahmad *et al.,* 1991; Flenley, 2011). In mammals, a role for UV in germline mutations is less obvious but not incomprehensible (Wilson Sayres and Makova, 2011). UV levels are generally higher in the tropics than in temperate regions, however, UV radiation, unlike temperature, does not decrease with altitude and may even increase (Korner, 2007; Barry, 2008). A number of studies exploring differences in rates of molecular evolution have considered sister species pairs from different altitudes (Wright *et al.,* 2006; Gillman *et al.,* 2009, 2010; Wright *et al.,* 2010). In all cases (plants, mammals and amphibians), inferred rates of molecular evolution were higher at lower altitudes, contrary to the prediction of UV as a biodiversity driver.

Population size driving differential rates of molecular evolution

The effects of population size are complex and important as they may interact with other putative drivers of LBG (Charlesworth, 2009). There is an extensive literature relating to the influence of population size, comprising many contrasting views and interpretations. Population size is often postulated as a component of LBG hypotheses, but is variously assumed to be larger and smaller in the tropics, although evidence for either view is limited (Wright, 1983; Currie *et al.,* 2004; Gillman *et al.,* 2009). Land area of islands is commonly used as a proxy for population size in studies of rates of molecular evolution in terrestrial organisms (Woolfit and Bromham, 2005; Wright *et al.,* 2009), an assumption founded in island biogeography theory (Macarthur and Wilson, 1967).

Although there is some limited evidence for latitudinal gradients in population size on a global scale, the topic is controversial because of the contested influence of population size on rates of molecular evolution. This is frequently confounded by a lack of clarity about what estimate of molecular evolution is being used: relative branch length or population variation. Theory predicts that large populations will have more genetic diversity at any one time compared with small populations (Ellstrand and Elam, 1993). This is because smaller populations are more sensitive to genetic drift so that mutations are likely to go to fixation more quickly resulting in lower net genetic variation at the population level. Furthermore, the fewer individuals there are in absolute terms the fewer mutations there will be in the population. Thus, large populations might contain the genetic diversity required for ecological speciation. The connection between population size and relative branch length is less clear (Johnson and Seger, 2001; Woolfit and Bromham, 2005; Nikolaev *et al.,* 2007; Wright *et al.,* 2009). Branch lengths in a phylogeny, expressing local rates of molecular evolution, are influenced by mutation rate and fixation rate. Under neutral theory the ratio of non-synonymous nucleotide mutations (dN) to synonymous nucleotide mutations (dS) will be equal to their respective proportions in the genome (Kimura, 1968). Therefore, the rate of molecular evolution would not be influenced by population size under a neutral model. Synonymous nucleotide mutations do not result in amino-acid substitutions and are usually neutral with respect to natural selection, having no phenotypic effect. In contrast, non-synonymous nucleotide mutations result in amino-acid substitutions and are therefore frequently not neutral. Almost all studies investigating population size and the rate of molecular evolution violate the assumption of neutrality by studying areas of the genome that are known to be constrained by selection (for example, coding regions of mitochondrial genomes such as COI and Cyt*b*). Nearly-neutral theory was developed in response to observations of a mismatch between inferred short-term and long-term mutation rates, and predicts that slightly deleterious mutations, which would be eliminated in large populations, may tend to persist longer in smaller populations where purifying selection is more relaxed (Ohta, 1992). Thus, the nearly-neutral model predicts more non-synonymous substitutions in small populations, but synonymous substitutions should be independent of population size (Ohta, 1992). This leads to the prediction that dN/dS (ω) will differ between large and small populations, and that changes in population size (expansion and contractions) will, in theory, alter rates of molecular evolution. Some empirical evidence agrees with this model (Johnson and Seger, 2001; Woolfit and Bromham, 2005; Bakewell *et al.,* 2007; Charlesworth and Eyre-Walker, 2007; Kosiol *et al.,* 2008; Petit and Barbadilla, 2009). However, some of these studies have found the results to be weak, with one finding the reverse (dN and dS were both lower in smaller than larger populations; Wright *et al.,* 2009). Explanations proposed for these results include a role for positive selection in the larger populations, positive back-mutations during population expansion and/or linked substitutions (Charlesworth and Eyre-Walker, 2007; Wright *et al.,* 2009; Stoletzki and Eyre-Walker, 2011). Positive selection complicates matters, as the efficiency of selection in larger populations results in the retention of beneficial mutations, whereas in smaller populations beneficial mutations act more like neutral mutations (Charlesworth, 2009). In addition, these studies have tended to use a limited number of markers; often a single-mtDNA sequence, which is subject to the

Hill-Robertson effect due to selection and lack of recombination (Charlesworth, 2009). The demographic influence of population size on the gradient of molecular evolution remains unresolved, due to the continued debate and conflicting empirical evidence on the influence of population size on molecular evolution rates and uncertainty about actual population size differences between high and low latitude species.

TOWARDS A SYNTHESIS

Rates of molecular evolution and speciation

Evidence for differential rates of molecular evolution being correlated with latitude are compelling yet they lack one key feature as a model to explain the biodiversity gradient; no study has yet convincingly linked differences in rates of molecular evolution to differences in speciation, extinction or immigration rates (Wright *et al.,* 2006; Gillman *et al.,* 2009, 2010; Wright *et al.,* 2010). Although the evidence for elevated rates of molecular evolution at the tropics is substantial, it remains only a pattern. Many traits that have been linked to the biodiversity gradient, such as niche conservation, similarly describe a pattern (an outcome) rather than the process (driver) involved in generating the gradient (Wiens and Graham, 2005; Losos, 2008; Crisp and Cook, 2012). Rates of molecular evolution and numbers of species show latitudinal clines, but are they directly related to one another, or is a third independent variable implicated? For example, differences in branch length between high- and low-latitude phylogenetically independent angiosperms show higher rates of molecular evolution at low latitude, but this does not correlate with higher speciation rates. Instead, the two rates were each independently linked to an environmental variable (temperature or UV; Davies *et al.,* 2004). Therefore, an indirect link might explain the observed relationship between rates of molecular evolution and biodiversity, or one of these two traits might drive the other. Contrary to the idea that elevated rates of molecular evolution lead to increased speciation, some studies infer that elevated molecular evolution is a result of more speciation, referred to as punctuational evolution (Pagel *et al.,* 2006; Venditti and Pagel, 2010). Thus, the increased rate of molecular evolution in the tropics may be a product of speciation rather than the cause of it (Webster *et al.,* 2003; Pagel *et al.,* 2006).

Evidence of a link between rates of molecular evolution and net-diversification has been reported (Barraclough and Savolainen, 2001; Jobson and Albert, 2002; Eo and Dewoody, 2010; Lancaster, 2010; Lanfear *et al.,* 2010a but see Goldie *et al.,* 2011 where no such link was found in mammals). However, none of these studies have conclusively shown that increased rates of molecular evolution are responsible for elevated rates of speciation. Evidence that the rate of molecular evolution is driving speciation rate has been found in plants (Lancaster, 2010), but the inference relies on the approach used to model extinction (Quental and Marshall, 2010). The assumption that the plant clades studied evolved via a 'birth-death' diversification process (Lancaster, 2010) is untested, and it is possible that models of density-dependent speciation (Moran process) or pure birth (Yule process) fit the data better (Nee, 2004). Also, it is unclear whether results from a multicopy nuclear marker that evolves via concerted evolution (Internal Transcribed Spacer (ITS)) can be meaningfully extrapolated to the rest of the genome. An extensive sequence data set from extant birds (Lanfear *et al.,* 2010a) identified a link between rates of molecular evolution and net-diversification, but birds are one of the few animal groups where a latitudinal skew in molecular evolution rates has not been conclusively demonstrated (Bromham and Cardillo, 2003), even though birds do contribute to the biodiversity gradient (Hawkins *et al.,* 2003). Lanfear *et al.* (2010a) found that as dS and dN increased in bird lineages so did net-diversification, but they could not find a link between life history traits and an increased rate of molecular evolution. Within their data set there was no association between net-diversification and ω (dN/dS). This suggests that selection and population size were not influencing net-diversification rates, but that the rate of molecular evolution was the most important influence on the total number of species within a clade. Nevertheless, this result does not exclude the possibility that speciation itself accelerated the rate of molecular evolution in these birds, or even that extinction (which contributes to net-diversification) is lower where molecular rate is high (Figure 3).

Molecular evolution and extinction

As noted, analyses of molecular genetic data are limited by their inability to directly identify extinction and its effect (Weir and Schluter, 2007; Lancaster, 2010; Quental and Marshall, 2010). Molecular studies are not well suited to examining extinction rates because extinct taxa are not available for sampling. Molecular phylogenies show inferred ancestral relationships among extant (sampled) taxa and do not show how extinction (or failed sampling) influence observed branch lengths. Varying rates of speciation and extinction can leave similar molecular phylogenetic patterns (Rabosky and Lovette, 2008; Crisp and Cook, 2009), so methods such as lineage through time plots may be misleading. It is therefore difficult to disentangle the relative contribution to diversity of speciation and extinction without a good fossil record (Figure 3; Quental and Marshall, 2010).

Evidence from palaeontological studies suggests a combination of lower extinction and higher speciation rates in the tropics has driven the biodiversity gradient (Jablonski *et al.,* 2006; Martin *et al.*, 2007). If rates of molecular evolution are faster in low latitudes as many have found (Davies *et al.,* 2004; Wright *et al.,* 2006; Gillman *et al.,* 2009, 2010; Wright *et al.,* 2010), then the relationship identified in mammals and birds by Weir and Schluter (2007) is consistent, but the use of a fixed molecular clock has resulted in misleading inferences of relative speciation and extinction rates. A strict clock rate is inappropriate when it is probable that rates of molecular evolution vary among lineages. Indeed, any variation in diversification rates among lineages, which is likely to be the rule rather than the exception, will interfere with estimations of true extinction rates (Rabosky, 2010). Direct linkage of extinction rates to rates of molecular evolution has also been suggested; increased rates of molecular evolution could reduce extinction risk by increasing the number of beneficial mutations that enable lineages to persist longer (Lanfear *et al.,* 2010a). The effect of mutation rate on extinction is largely unknown, although excessive mutation rates will tend to yield a high genetic load (Butlin *et al.*, 2009; Lancaster, 2010).

Countless difficulties

> '*In the one, evolution has had a fair chance; in the other it has had countless difficulties thrown in its way*'
>
> —AR Wallace, 1876

The future role of molecular research investigating the biodiversity gradient is uncertain as studies have proven to be method-sensitive and open to variable interpretation. Considered individually, many molecular studies appear to provide compelling and relevant evidence, however, the associations they reveal rarely constitute direct evidence of a primary driver. Many studies of the LBG run parallel to one another and cannot be directly compared because they employ

very different methods. However, a consensus is developing that there is a correlation between rates of molecular evolution and species diversity; the challenge now is to identify their causal relationship.

Palaeontology has the potential to track diversity trends through time and is therefore well suited to estimate extinction, speciation and immigration rates. A recent test of the influence of seasonality on the biodiversity gradient shows the potential for palaeontology to narrow the field of possible drivers of the LBG (Archibald *et al.,* 2010). Given that sampling of DNA sequence data is limited to extant species and there are known problems with estimating extinction rates from molecular data (Rabosky, 2010), it might be argued that molecular data have a limited role in examining extinction rates, a processes central to the biodiversity gradient debate. However, analytical tools are available if appropriate questions are asked (Nee, 2004), and our ability to incorporate extinction into phylogenetic models is improving (Etienne and Apol, 2009; Morlon *et al.,* 2011; Stadler, 2011). The use of data from measurably evolving populations (for example, in rapidly evolving disease causing microorganisms; and other populations using ancient DNA approaches) will continue to enhance our ability to quantify rates of molecular change and strengthen theoretical developments (Drummond *et al.,* 2003).

Inferences about regional biodiversity and choice of entities for data sampling are both influenced by current taxonomy. In some studies, unrecognised species probably influence the conclusions drawn. It is possible that differences among the extinction and speciation rates inferred in Weir and Schluter (2007) were, in part, artefacts of more intense taxonomic splitting of high-latitude versus low-latitude biota (Tobias *et al.,* 2008). Higher within-species genetic diversity has been found in plants and vertebrates from lower latitudes, and this could be taken to mean that there are more undescribed species in the tropics, or that the tropical taxa have larger population sizes (Martin and Mckay, 2004; Eo *et al.*, 2008). Incorporation of phylogenetic information into estimates of biodiversity (phylogenetic distinctiveness metrics) is increasing (Purvis and Hector, 2000; Davies and Buckley, 2011), but phylogenetic approaches to estimating biodiversity rely in part on the same information (that is, branch length and branching pattern) being used to assess rates of molecular evolution. This circularity weakens inferences about drivers of the LBG that might be further influenced by lineage extinction and local biogeographic history.

Mitochondrial DNA has been used in several studies comparing rates of molecular evolution among lineages with speciation rates. There is some evidence that molecular evolution rates from the mitochondrial genome and the nuclear genome are subject to different pressures that might reflect a difference in proximity to cell metabolic processes or natural selection (Welch *et al.,* 2008). Mitochondrial DNA is frequently used in analyses that assume neutrality in terms of molecular evolution, but this is unlikely to be realistic (for review see Galtier *et al.,* 2009b). Given the metabolic role of mitochondria, the mitochondrial genome could be implicated in climate adaptation, and thus far from neutral in the context of the LBG. This idea has been mooted for human mitochondrial genetics (Ruiz-Pesini *et al.,* 2004; Wallace, 2005; Das, 2006; Pierron *et al.,* 2011), but remains controversial given suggestions that available methods to detect selection within populations are flawed (Kryazhimskiy and Plotkin, 2008). Nuclear 'loci' such as ITS are far from ideal given their complex and poorly understood process of sequence evolution (Elder and Turner, 1995). This could be particularly problematic in plants where hybridisation has an important role in species formation and exchange among lineages (Hegarty and Hiscock, 2005). To date, selection of DNA sequence loci has been strongly influenced by the availability of data and universal PCR primers, a problem which is being alleviated by the use of next generation sequencing (NGS) technology.

FUTURE FOCUS ON THE PROCESS

It is clear that we need approaches that avoid reliance on uncertain proxies. Tests of associations between putative drivers and speciation rates currently rely heavily on substitutes that are assumed to correlate well with the characters of interest. For example, island size is used as a proxy for population size; metabolic rate is used as a proxy for oxygen-free radicals that can influence mutations; average age of first reproduction is a proxy for average species' generation time; and current species distribution is used as a proxy for ancestral species distribution. Many of these correlations first need to be independently verified in the organisms of interest. Also, the design of many studies reflects methodological and sampling limitations, and has often dealt with comparison of just two alternative conditions (for example, big population versus small population, high latitude versus low latitude) rather than analysing gradient or transect data. Although continuous traits are often measured (for example, population size), they are then categorised into two alternatives (for example, big versus small) in order for inferences to be drawn from paired comparisons (for example, sign tests). Mutation rates vary between species, between individuals and within the genome itself, so disentangling all the drivers of mutation rates is unlikely to be easy (Hodgkinson and Eyre-Walker, 2011).

Clarity of assumptions and experimental design and application of methods at the appropriate evolutionary level are essential, and here we suggest some directions that might take us forward.

1. Many of the life-history traits that have been implicated as driving rate changes are not independent of one another and work needs to focus on taxa that allow the elimination of some of these variables. The sister species approach has some benefits over the large clade approach, but for life-history traits that cannot be adequately represented across sister species pairs, additional methods will be necessary (see Lanfear *et al.,* 2010b for an examination of different approaches to estimate rates from trees). Quantification of life history traits in specific organisms of interest is required to avoid reliance on rules of thumb and generalised concepts that are often based on limited direct evidence. An emphasis on regionally focused examples should enhance opportunities for gathering accurate life-history data, and inclusion of specific geophysical information when accommodating effects of local biogeographic history.
2. Until recently, some studies of rates of molecular evolution across the LBG have used single genes from the mtDNA genome and a single branch from each species. NGS technologies now provide multilocus data that improve confidence in estimates of relative rates of molecular evolution across the genome in sister species comparisons, even for non-model organisms. In the past, when more than one sequence was available per species the shortest branch was routinely chosen (for example, see Wright *et al.,* 2009), even though species usually contain measurable genetic diversity. NGS allows dense population sampling of sister species pairs that allows analysis of the effects of choosing the shortest branch versus other metrics, such as average branch length or random branch selection. Studies, particularly those involving high genetic divergence, may also benefit from an out-group comparison (Hugall and Lee, 2007). Population level studies have the potential to inform on speciation processes at a stage equivalent to the node in

a species tree, instead of relying on inferences about past speciation from tree tips.

3. For understanding the LBG, determining population size variation between latitudes is important. Thus, a focus on multi-locus data for taxa with sedentary lifestyles would facilitate population density comparisons. If a consistent difference in population size among latitudes exists then the interaction between population size and rates of molecular evolution becomes extremely important to the LBG debate. Multi-locus data sets exist for model organisms (for example, humans and *Drosophila*; Bakewell *et al.,* 2007; Kosiol *et al.,* 2008; Petit and Barbadilla, 2009), but it is now possible for substantial tests across the nuclear and mitochondrial genomes functional and non-functional DNA in non-model groups with NGS. However, population size may not be constant through time and methods for inferring past population size that are independent of rates of molecular evolution will be required.
4. If speciation occurs predominantly in small isolated populations (Venditti and Pagel, 2010), signal about the size of the populations during speciation may be lost through processes such as back-mutation during subsequent population expansion (Charlesworth and Eyre-Walker, 2007; Lanfear *et al.,* 2010a). Simulations using data from model organisms should make it possible to test the predicted population size effect on ω and assess the interaction of ecological speciation and population expansion. It will also be possible to estimate how long after a speciation event the population genetic signature remains detectable. Analysis of recent-past population diversity using ancient DNA methods could enhance modelling of rates of molecular evolution and population size. Similarly, studies involving ancestral gene reconstruction and resurrection will likely illuminate our understanding of the respective roles of genetic drift and selection on gene evolution (Thornton, 2004).
5. Expansion of studies involving lineages represented in the fossil record and neobiota will contribute broadly to meshing information on speciation, extinction and migration with rates of molecular evolution. In particular, such data will help explore the directionality of the relationship between rates of molecular evolution and speciation. Sequencing multiple loci from extant species that are members of fossil-rich lineages will allow extinction rates to be correlated with both speciation and molecular evolution rates. This has the potential to isolate speciation from net-diversification, an important step towards resolving the link between rates of molecular evolution and speciation. Fossil data can be used to constrain molecular clock analyses and allow rate comparisons that are not limited to sister species approaches. Measures of extinction could reveal any relationship between rates of molecular evolution and extinction.
6. Sequencing of parents and their offspring allows species-specific estimates of mutation rate, and if the necessary multiple comparisons and multiple tissues are included, the population variance in individual *de novo* mutation rate can be known (Hodgkinson and Eyre-Walker, 2011). Two experimental approaches using this method could contribute directly to the study of LBG. First, the effect of temperature and UV on mutation rate could be assessed using lab-based model organisms in controlled environments. Second, direct comparison of mutation rates of non-model organisms, where rates of molecular evolution have been shown to differ between sister species at separate latitudes, can be obtained from whole-genome parent/offspring data. This would allow discrimination between the two components of molecular evolution, variation in rates of mutation and variation in rates of fixation (Ho *et al.,* 2005). In addition, mining pedigree data sets that exist for applied research in agriculture, horticulture and conservation will allow tests for a link between longevity of individuals within species and mutation rate.

Differences in rates of molecular evolution between global regions are fascinating and warrant much more focused research. But perhaps the priority should be to find the directionality of the link, if any, between speciation and molecular evolution. Without this fundamental information the evolutionary speed hypothesis cannot provide compelling predictions to test putative drivers of the LBG.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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