The role of ecology and molecular evolution in shaping global terrestrial diversity

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Abstract

The density of species varies widely across the earth. Most broad taxonomic groups have similar spatial diversity patterns, with greatest densities of species in wet, tropical environments. Although evidently correlated with climate, determining the causes of such diversity differences is complicated by myriad factors: many possible mechanisms exist to link climate and diversity, these mechanisms are not mutually exclusive, and they may overlap in the patterns they generate. Further, the importance of different mechanisms may vary between spatial scales. Generating uneven spatial diversity patterns in regions that are below equilibrium species richness requires either geometric or historical area effects, or regional differences in net diversification. Here, I investigate the global climate correlates of diversity in plants and vertebrates, and hypotheses that could link these correlates to net diversification processes, in particular through climate-linked patterns of molecular evolution. I first show strong climate–diversity relationships only emerge at large scales, and that the specific correlates of diversity differ between plants and animals. For plants, the strongest large-scale predictor of species richness is net primary productivity, which reflects the water–energy balance at large scales. For animals, temperature seasonality is the strongest large-scale predictor of diversity. Then, using two clades of New World passerine birds that together comprise 20% of global avian diversity, I investigate whether rates and patterns of molecular evolution can be linked to diversification processes that could cause spatial diversity patterns in birds. I find that most substitution rate variation between phylogenetically independent comparisons of avian sister species appears to result from mutation rate variation that is uncorrelated with climate. I provide evidence of nearly neutral effects in mitochondrial coding sequences, finding a significant, negative correlation between non-synonymous substitution rates and population size. Using phylogenetically independent comparisons, I also find that birds in low temperature seasonality, and isothermal environments, and birds with small elevational ranges have increased non-synonymous substitution rates, indicative of relaxed purifying selection. Other climate variables have no direct effect on molecular evolution. Molecular evolutionary patterns are dominated by mutation rate variation. Recovered patterns were stronger when mutation rate variation was controlled, indicating

that such variation is a source of noise in analyses, and may be generally problematic across short genetic distances for analyses using mitochondrial genes. I bring these findings together with emerging literature to outline a framework for understanding net diversification patterns. Maintaining adaptations to climate, and the limits of those adaptations have population-genetic consequences that can affect lineage persistence and the processes of speciation and extinction in a fashion that is consistent with observations at multiple levels of diversity.

Table of contents

Abstract ..... **ii**
List of figures ..... **vii**
List of tables ..... **xii**
Attestation of authorship ..... **xiv**
Co-authored works ..... **xv**
Acknowledgements ..... **xvi**

1 Introduction **1**
1.1 Background 1
1.2 Study purpose and rationale 6
1.2.1 Terrestrial ecoregion diversity *7*
1.2.2 Ecological correlates of molecular evolution *8*
1.3 Thesis layout and overview 12

2 Literature review **18**
2.1 Overview 18
2.2 Development of molecular evolutionary theory 20
2.2.1 The origins of the neutral theory of molecular evolution *20*
2.2.2 Development of evolutionary theory in the molecular era *21*
2.2.3 Patterns predicted for genetic diversity *28*
2.2.4 Subsequent criticisms of neutral theory *31*
2.2.5 Moving beyond a dichotomy *37*
2.3 Species diversity patterns and their potential causes 40
2.3.1 Species diversity gradients *40*
2.3.2 Diversity across time and space *42*
2.3.3 Diversity across spatial scales *43*
2.3.4 Causes of species richness variation *46*
2.3.5 Prominent hypotheses for the latitudinal diversity gradient *59*
2.3.6 Net diversification mechanisms under population-genetic principles *72*
2.4 Molecular evolutionary rate variation 77
2.4.1 Phylogenetic estimates of molecular evolutionary rates *77*
2.4.2 Factors that affect the substitution rate *83*
2.4.3 Empirical tests of the N_e—molecular-rates relationship *88*
2.4.4 Range size as a surrogate measure of population size *95*
2.4.5 Factors affecting species' range sizes *98*
2.4.6 Comparative methods in phylogenetics—sister species contrasts *100*
2.4.7 Mitochondrial DNA as a molecular evolutionary marker *101*
2.5 Birds as an evolutionary model system 104
2.5.1 New World oscines and suboscines as a study system *105*

3 Methods **109**
3.1 Plant terrestrial ecoregion diversity patterns 109

3.1.1 Analysis *112*
3.2 Animal population and diversity patterns 114
3.2.1 Animal terrestrial ecoregion diversity *114*
3.2.2 Correlates of vertebrate diversity *114*
3.2.3 Range sizes and latitudinal ranges in animals *115*
3.2.4 Population size–range size covariation *118*
3.2.5 Variation in population density with latitude and elevation *120*
3.3 Effect of range size on molecular evolutionary rates and patterns in New World birds 122
3.3.1 Sequence dataset assembly *122*
3.3.2 Phylogenetic and ecological data *124*
3.3.3 Dataset quality controls *129*
3.3.4 Analysis *131*
3.3.5 Potentially confounding factors *132*
3.4 Effect of climate and elevation on New World bird evolution 135
3.4.1 Climate *135*
3.4.2 Elevation *135*
3.4.3 Apparent mutation rate variation *136*

4 Results 137
4.1 Plant terrestrial ecoregion diversity patterns 137
4.1.1 Plant diversity patterns *137*
4.2 Animal population and diversity patterns 145
4.2.1 Animal terrestrial ecoregion diversity *145*
4.2.2 Correlates of vertebrate diversity *148*
4.2.3 Range sizes and latitudinal ranges in animals *154*
4.2.4 Population–range size correlations *158*
4.2.5 Variation in population density with latitude and elevation *163*
4.3 Effect of range size on molecular evolutionary rates and patterns in New World birds 169
4.3.1 Sequence dataset assembly *169*
4.3.2 Phylogenetic and ecological data *170*
4.3.3 Dataset quality controls *171*
4.3.4 Analysis *172*
4.3.5 Potential confounding factors *179*
4.4 Effects of climate, energy, and elevation on New World bird evolution 184
4.4.1 Climate *184*
4.4.2 Elevation *187*
4.4.3 Apparent mutation rate variation *190*

5 Discussion 196
5.1 Plant terrestrial ecoregion diversity patterns 196
5.1.1 Plant diversity patterns *196*
5.2 Animal population and diversity patterns 202
5.2.1 Animal diversity patterns *202*

5.2.2 Correlates of vertebrate diversity in large ecoregions *203*
5.2.3 Range sizes and latitudinal ranges in animals *205*
5.2.4 Population–range size correlations *207*
5.2.5 Variation in population density with latitude and elevation *208*
5.3 Effect of range size on molecular evolutionary rates and patterns in New World birds 212
5.3.1 Sequence dataset assembly *212*
5.3.2 Analysis *212*
5.3.3 Potentially confounding factors *216*
5.3.4 Comparisons with prior studies *217*
5.3.5 Use of range size as an N_e estimator *221*
5.4 Effect of latitude, elevation, climate and migratory status on New World bird evolution 223
5.4.1 Latitude *223*
5.4.2 Climate *224*
5.4.3 Elevation *228*
5.4.4 Apparent mutation rate variation *230*
5.5 Implications for understanding diversity, diversification, and evolution 233
5.5.1 Using mtDNA as a molecular marker *233*
5.5.2 The evolutionary speed hypothesis *237*
5.5.3 Towards a framework for understanding spatial diversity patterns *239*
5.6 Limitations of study 253
5.6.1 Effective population size approximation *253*
5.6.2 Restriction to mitochondrial housekeeping genes *254*
5.6.3 Taxonomic breadth *255*
5.6.4 Sister species comparisons *256*
5.7 Directions for future study 258
5.7.1 Understanding mt mutation rate variation *258*
5.7.2 Better approximations of N_e *258*
5.7.3 Investigate more explicit links between multiple diversity levels *259*
5.7.4 Latitudinal variation in minimum viable populations *259*
5.7.5 Latitudinal variation in population structure and dynamics *259*

6 Conclusion 261

References 263

Appendices 298
Appendix 1: Effect of data quality on plant PSRs in terrestrial ecoregions 298
Appendix 2: Data quality and outlier analyses for range size–population size study ..301

List of figures

Figure 1. Model of the processes that theoretically combine to generate patterns of species richness. Red arrows represent hypothesised positive relationships between variables, while blue arrow represent hypothesised negative relationships.....16
Figure 2. Disparities in the latitudinal gradients of (a) hottest and (b) coldest months of the year in the New World. Points represent average values within 10,000km^2 equal-area hexagonal cells across continental North and South America up to 60°N and below 500 metres above sea level.....50
Figure 3. A conceptual problem with identifying causal patterns in net diversification.....73
Figure 4. Relationship between the study clades used in molecular analyses.....106
Figure 5. Relationships between families in the New World suboscines.....107
Figure 6. Relationships between families in the New World suboscines.....108
Figure 8. Map of WWF terrestrial ecoregions.....110
Figure 9. Map of the 8 WWF biogeographic realms.....110
Figure 10. Map of the 14 WWF biogeographic biomes.....111
Figure 7. Example of a pair of sister species included in analyses.....128
Figure 11. Relationship between modelled net primary productivity and plant species richness of global terrestrial ecoregions.....137
Figure 12. Relationship between modelled net primary productivity and plant species richness across sizes classes of global terrestrial ecoregions.....140
Figure 13. Strength of relationship (linear regression adjusted R^2) between species richness and: (a) modelled mean annual net primary productivity; and (b) ecoregion latitudinal midpoints.....142
Figure 14. Area distribution of ecoregions across quartiles of net primary productivity...143
Figure 15. Relationship between plant species richness and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2.....144
Figure 16. Relationship between total vertebrate phylogenetic diversity and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2.....150

Figure 17. Relationship between bird species richness and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2.....151
Figure 18. Relationship between mammal species richness and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2.....152
Figure 19. Relationship between (a) plant species richness and Faith's vertebrate phylogenetic diversity; and (b) the relationship between the residuals of the relationship in (a) and net primary productivity. Data points represent individual terrestrial ecoregions larger than $10^{5.5}$ km^2.....153
Figure 20. Scaling of latitudinal range with range size for mammal species in three latitudinal bands in the northern hemisphere. Species were assigned to latitudinal bands by: (a) the latitudinal midpoint of their range, or (b) their maximum latitude.....156
Figure 21. Scaling of latitudinal range with range size for non-migratory passerine bird species in three latitudinal bands in the northern hemisphere. Species were assigned to latitudinal bands by: (a) the latitudinal midpoint of their range, or (b) their maximum latitude.....157
Figure 22. Matrix of regression variable relationships in predicting population size from range size (extent of occurrence) and body mass.....161
Figure 24. Relationship between range size (extent of occurrence) and population size across the IUCN Redlist categories for oscine and suboscine passerine birds of the New World, where: (a) all species are included; and (b) endangered/critically endangered species are excluded.....163
Figure 25. Latitudinal variation in (a) nucleotide diversity and (b) the ratio of nucleotide diversity to nucleotide divergence in a global dataset of passerine birds for all species with 10 or more ND2 sequences available.....167
Figure 26. Relationship between the residuals of the population–range size relationship and (a) species' absolute latitudinal centroid, and (b) species' latitudinal centroid for passerine birds.....168

Figure 27. Relationship between the residuals of the population–range size relationship and average elevation, for montane passerine bird species (a) within 20° of the equator and (b) outside of 20° from the equator. 168
Figure 28. Relationship between transformed contrasts of range size and ω for a) contrasts that were excluded from the final dataset as low-power, and b) contrasts included in the final dataset. 172
Figure 29. Relationships between relative substitution rates and relative range size for all sister species pairs of New World passerine birds in the raw dataset. Relative rates of (a) non-synonymous substitutions, (b) synonymous substitutions, (c) ω, and (d) the substitution rate are show 174
Figure 30. Relationships between relative substitution rates and relative range size for all sister species pairs of New World passerine birds in the final dataset.. Relative rates of (a) non-synonymous substitutions, (b) synonymous substitutions, (c) ω, and (d) the substitution rate are shown 176
Figure 31. Relationships between ω and range size contrasts for sister species pairs from each of nine families of New World passerine birds in the final dataset 177
Figure 32. Accumulation of (a) non-synonymous and (b) synonymous mutations in sister species, contrasted between the smaller-ranged and larger-ranged species in each pair ... 178
Figure 33. Effects of migration on the ω–range size relationship, shown on sister species pairs of New World passerine birds where (a) the larger-ranged species is migratory and the smaller-ranged species is not and (b) the smaller-ranged species is migratory and the larger-ranged species is not 181
Figure 34. Variation in range sizes across latitude for 414 species of New World passerine bird in the final dataset, presented using (a) untransformed range sizes, and (b) log-transformed range sizes. 182
Figure 35. The ω–range size relationship for sister species contrasts of New World passerine birds (a) inside 20° of the equator (tropical) and (b) outside of 20° of the equator (extratropical) 183
Figure 36. Relationships between log-transformed ω contrasts and four significant climate predictors for sister species of New World passerine birds: (a) latitude, measured as the difference in absolute latitudes of resident/breeding range centroids, (b) log-transformed

isothermality contrasts, (c) log-transformed temperature seasonality contrasts, and (d) log-transformed contrasts of net primary productivity 187
Figure 37. Relationship between ω and two measures of elevation for sister species of New World passerine birds. For (a) and (c) differences in minimum elevation are used, and (b) and (d) differences in elevational range are used 189
Figure 38. The relationship between d_S variance and time and genetic distance between sister species pairs of New World passerine birds 192
Figure 39. Diagnostics of d_S variation. (a) Histogram of the distribution of $|d_S$ contrasts$|$, showing a biomodal distribution and a break at a value of 0.1 (b) Rank-order $|d_S$ contrasts. 192
Figure 40. A comparison of ω–range size relationships in two subsets of the final dataset for sister species pairs of New World passerine birds. (a) Data exclude comparisons with large |ds contrast| values, and (b) data exclude comparisons where both species are within 20° of the equator 193
Figure 41. A comparison of ω–temperature seasonality relationships in two subsets of the final dataset for sister species pairs of New World passerine birds. (a) Data exclude comparisons with large |ds contrast| values, and (b) data exclude comparisons where both species are within 20° of the equator 195
Figure 42. Maximum value for temperature seasonality in sister species contrasts of New World passerine birds in (a) both species in pairs within 20° of the equator,, and (b) with one or both species within pairs outside of 20° of the equator 195
Figure 43. Relationship between square-root-transformed modelled net primary productivity and log-transformed plant species richness across sizes classes of global terrestrial ecoregions 197
Figure 44. Tropical and temperate species differences in (a) the range size–population size relationship of passerine birds, and (b) the distribution of range sizes for almost all recognised bird species. 227
Figure 45. Relationship between the number of substitutions per site and absolute latitude for all species in the final dataset 257
Figure 46. Relationship between modelled net primary productivity and plant species richness across sizes classes of global terrestrial ecoregions for ecoregions with upper

quality estimates (quality categories 1 and 2 in Kier *et al*. 2005): (a) small ecoregions between 10^3 and 10^4 km^2; (b) medium ecoregions between 10^4 and 10^5 km^2; (c) large ecoregions between 10^5 and 10^6 km^2; and (d) the largest ecoregion subset.....299
Figure 47. Relationship between modelled net primary productivity and plant species richness across sizes classes of global terrestrial ecoregions for ecoregions with lower quality estimates (quality categories 3 and 4 in Kier *et al*. 2005): (a) small ecoregions between 10^3 and 10^4 km^2; (b) medium ecoregions between 10^4 and 10^5 km^2; (c) large ecoregions between 10^5 and 10^6 km^2; and (d) the largest ecoregion subset.....300
Figure 48. Two analyses for outliers. Cook's distance detects leverage of data points, while studentised residuals show the standardised magnitude of deviation from predicted values. 301
Figure 49. Influence of number of bases on variance in the substitution rate in (a) the raw dataset and (b) the final dataset.....302

List of tables

Table 1. List of the 14 WWF biogeographic biomes (WWF, 2012). 112
Table 2. AIC-based model selection amongst linear regression models of the productivity-plant species richness relationships within terrestrial ecoregion size classes, controlling for $\log_{10}$area, realm and biome type.....143
Table 3. Comparison of linear regression models of plant species richness and NPP for all terrestrial ecoregions, and the subset of tropical and subtropical moist broadleaf forest ecoregions across four ecoregion size classes.....144
Table 4. AIC-based model selection amongst linear regression models of the productivity-species richness relationships for birds within terrestrial ecoregion size classes, controlling for area, realm and biome type. Evidence ratios are provided for the ratio of Akaike weights between the first and second models. 150
Table 5. AIC-based model selection amongst linear regression models of the productivity-species richness relationships for mammals within terrestrial ecoregion size classes, controlling for area, realm and biome type.. 151
Table 6. OLS multiple regression model explanatory power for NPP and area in predicting species richness in moist broadleaf forest ecoregions only.....152
Table 7. Median range sizes of non-migratory passerine birds and mammals distributed across three latitudinal bands in the northern hemisphere. 159
Table 8. Distribution of bird range sizes and IUCN conservation categories for the dataset of species for which population size and body mass were available, and for all birds.....165
Table 9. Within-family tests of the population size–range size relationship for all families with at least 10 genera 166
Table 10. Sequence availability for mitochondrially encoded protein-coding genes across all bird species 175
Table 11. Distribution of species ranges in the raw dataset for sister species comparisons across nine families of passerine birds.....177
Table 12. Ordinary least squares results for the regression of range size contrasts against the contrasts of four evolutionary metrics for sister species of New World passerine birds 184

Table 13. Regression slopes of the ω –range size relationship for nine families of passerine birds. 186
Table 14. Correlation matrix of evolutionary metrics and range size, showing Pearson's r for the final dataset. 189
Table 15. Test for confounding effects from factors that could influence rates of molecular evolution. Bivariate Pearson correlation coefficients are shows for two evolutionary metrics, and range size 190
Table 16. Bivariate correlations between six climate variables (plus latitude), and three evolutionary metrics across sister species contrasts of New World passerines. For each combination, the climate variable is estimated first for pairs of birds that both have resident ranges 195
Table 17. Effects on absolute latitude as a predictor of ω and substitution rate from the inclusion of isothermality, temperature seasonality, and NPP in OLS regression models. 195
Table 18. Results of regression models predicting three evolutionary metrics (ω, d_S, substitution rate) from elevational contrasts of sister species of New World passerine birds 200
Table 19. Results of regression models predicting three evolutionary metrics (ω, d_S, substitution rate) from elevational contrasts of sister species of tropical New World passerine birds that occur within 20° of the equator. 201

Attestation of authorship

I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made.

Signed: 

Co-authored works

The sections relating to the plant terrestrial ecoregion diversity study (2.3.3 Diversity across spatial scales; 3.1 Plant terrestrial ecoregion diversity patterns; 4.1 Plant terrestrial ecoregion diversity patterns; 5.1 Plant terrestrial ecoregion diversity patterns) are derived from the following publication:

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P.M. & J.C. conceived the idea. P.M. conceived the analysis, and collated and analysed the data. All authors contributed to the writing of the manuscript.

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1 Introduction

1.1 Background

Ecology and evolution interact to create uneven distributions of species densities at both small and large spatial scales. The principal example is the near-universal gradient of increasing species richness from the poles to the equator—the latitudinal diversity gradient (LDG) (Hillebrand, 2004). Species richness peaks in the warm, wet tropics, gradually declining through temperate, and more seasonal environments to the species-poor poles. The generality of the LDG is such that it occurs in plants and animals, in endotherms and ectotherms, and in terrestrial and marine environments. The well-recognised species-level phenomenon is paralleled not only by similar patterns at higher taxonomic ranks, but also by patterns within species of increasing genetic diversity (Adams & Hadly, 2013), genetic divergence (Martin & McKay, 2004), and subspecies richness (Martin & Tewksbury, 2008). If these various phenomena are causally linked, then studies of molecular evolution may reveal what bridges the gap between them.

Understanding the causes of the LDG has been a sustained goal of ecology for 200 years (Hawkins, 2001), over which time much progress has been made (e.g., Dowle, Morgan-Richards, & Trewick, 2013; Mittelbach et al., 2007; Rohde, 1992). There is compelling evidence that diversity patterns correlate with environmental energy (Allen, Brown, & Gillooly, 2002), the water-energy balance (Hawkins et al., 2003), and time-integrated area (Fine, Ree, & Burnham, 2008). However, the causes of the LDG remain incompletely known. In addition to different responses between climate and diversity between taxonomic groups (e.g., Currie, 1991), it is as yet unestablished whether genetic diversity, subspecies richness, and species richness all increase towards the tropics because they are directly causally linked to each, because they are indirectly linked through sharing a common cause, or if these patterns are simply coincidental. At least three major issues have contributed to our lack of understanding. Firstly, there has been a lack of data to explore possible pattern explanations with sufficient breadth and depth to be both

generalisable and able to account for the complex ecological patterns of diversity that comprise the LDG. Secondly, there has been a focus on finding an ultimate explanation for the LDG (e.g., Rohde, 1992) without establishing that one necessarily exists, contributing to an imbalance between hypothesis generation and testing (Palmer, 1994). Thirdly, there has been a lack of integration between possible causes, which have been compartmentalised in their treatment. While the first issue is increasingly being resolved, the latter two have been the subject of less direct consideration.

Ecology and evolution are disciplines that are often thought of as exploring phenomena on different timescales, but that nevertheless interact. At the population level, ecology considers phenomena such as demographic fluctuations in response to biotic and abiotic factors, including climate variation and predator/prey interactions. Short-term fluctuations might occur within a single generation or over a small number of generations, while longer-term, climate-linked fluctuations might cover periods of thousands or tens of thousands of years. Evolution is linked to ecology through population genetic processes, such as allele frequencies that fluctuate in response to these ecological variations. However, many critical evolutionary processes occur on much longer timescales: for example, speciation occurs over much greater periods of time, given that typical species lifespans in vertebrates are 1 or 2 million years (Webb, 2013). More fundamentally, in the absence of positive selection, genetic drift takes tens of thousands of generations to fix a *de novo* nuclear mutation within a vertebrate population (Kimura & Ohta, 1969). Therefore, the timescales for fixation of neutral mutations can be orders of magnitude longer than ecological phenomena (Halligan, Oliver, Eyre-Walker, Harr, & Keightley, 2010), with the exceptions of the longest term climate cycles, such as Milankovitch and glacial cycles (Doughty et al., in press). As such, many ecological changes are likely to have occurred in a lineage during a putatively neutral mutation's passage to fixation. The differences in relevant timescales between ecology and evolution have meant that the disciplines have often been considered in isolation. For example, ecological responses to environmental change (e.g., behavioural changes, or range shifts), and more recently epigenetic modifications (e.g., gene expression regulation via DNA methylation), might be expected to precede evolutionary ones in species with long generation times and small population

sizes, and might therefore guide directions taken by researchers. However, there is also emerging evidence that speciation can occur on short timescales on the order of tens or hundreds of generations when driven by selection (Hendry, Nosil, & Rieseberg, 2007; Nosil, 2012)

The principal example of the deep linking and dynamic feedback between ecological and evolutionary phenomena is the degree to which organisms are adapted to their environments. Such adaptation is classically understood in terms of natural selection acting on genetic variation within populations in response to ecological feedback, resulting in changing allele frequencies (Fisher, 1930; Fisher & Ford, 1947), although many factors beyond genetics are also critical in shaping phenotypes (Goodwin & Trainor, 1985; Griffiths & Gray, 1994), and both organismal and genetic complexity can be constructed in the absence of selection (Lynch, 2007a; Lynch, 2007b; Stoltzfus, 1999, 2012). There are other key interactions between ecological and evolutionary phenomena. For example, fluctuations in population size may affect levels of standing genetic diversity (Bonnell & Selander, 1974; O'Brien, 1994), habitat density and connectivity may affect both gene flow (Williams, Brawn, & Paige, 2003) and effective population size (Keller & Largiadèr, 2003). Effective population size (N_e) is a key evolutionary parameter that estimates the population-genetic behaviour of a population as its attributes deviate from an ideal Wright-Fisher population (Charlesworth, 2009; Wright, 1931). N_e is of fundamental importance as it affects the intensity of natural selection (Nabholz, Uwimana, & Lartillot, 2013; Weber, 1990; Weber & Diggins, 1990). Some of these effects have been predicted since the early population genetics literature (Wright, 1931, 1943), even if they have not always featured prominently (Gould, 1981).

There are major implications that come from separately considering ecological and evolutionary explanations for understanding species diversity and diversification, highlighted in the context of present work by the tendency to contrast different classes of explanations for global diversity patterns (Currie et al., 2004; Rohde, 1992). Even when the distinction is apparently clear—for example, when contrasting speciation and dispersal as explanations for the diversity of a post-glaciation biota—the confluence of ecological and

evolutionary factors weaken such broad distinctions. Not only does speciation occur in an ecological context, the capacity for dispersal is evolved. Further, potential explanations for macro-scale diversity patterns are rarely mutually exclusive, and so multiple conceptually indistinct explanations may causally overlap and be consistent with the observed patterns concurrently. Moving forward requires investigating and contrasting mechanistic explanations for diversity patterns with increasing sophistication by exploiting the rapidly expanding data availability. This should involve making distinctions on the basis of temporal and spatial scales rather than attempting to categorise overlapping processes as being of an ecological or an evolutionary nature.

As with the contrast of ecology and evolution, observed evolutionary patterns are typically complex and do not neatly fit the classical selection-based models of evolution under the modern synthesis, nor the neutral models that were developed subsequently. Early studies of heterozygosity in a wide range of organisms suggested that values were at once too high to be readily interpreted under selection theory (Lewontin, Ginzburg, & Tuljapurkar, 1978) and too invariable across a wide range of distantly related groups to be readily interpreted under neutral theory (Lewontin, 1974). More recently, patterns of putatively neutral and non-neutral substitutions and polymorphisms have been investigated in a range of species using the McDonald-Kreitman test, which is used to test for positive selection. In some of these tests, a greater proportion of non-neutral substitutions than polymorphisms are observed (e.g., Fay, Wyckoff, & Wu, 2002; McDonald & Kreitman, 1991), which is often interpreted as evidence of positive selection in a selectionist paradigm. However, this pattern can also be interpreted as consistent with nearly neutral evolution in a neutralist paradigm (Ohta, 1993), with differences predicated on past population size, which is often poorly understood. This, and other facets of the selectionist–neutralist debate are discussed in more detail in the literature review (2.2 *Development of molecular evolutionary theory*). This debate affects the interpretation of the molecular evolutionary results in the present work. Further, at least in part, the debate also forms the foundation of the conflict between many potential explanations of broad-scale diversity patterns.

Not only do conflicting paradigms present challenges for interpreting evolutionary patterns, detecting the patterns themselves can be difficult due to the complexity of temporally heterogeneous ecological effects. Detectable signals of contemporary ecological phenomena on patterns of DNA sequence evolution can be obscured by conflicting past fluctuations in ecological phenomena that cause different evolutionary patterns, for example by varying the strength of purifying selection. Unfortunately, only certain past fluctuations are independently known—for example, patterns of climate variation—while many others must be inferred using sequence data that is subject to paradigm-specific interpretation. For a small number of species, fossil data may be available to recreate variation in historical ranges; however, for most, this can only be inferred from habitat changes, or from population-genetic inferences based on SNPs or allele frequencies.

Low statistical power, small effect sizes and limited model explanatory power are all common in ecology and limit the scope to test theory under classical statistical frameworks (Møller & Jennions, 2002). Indeed, ecological and evolutionary studies typically explain less than 5% of data variation (Møller & Jennions, 2002), and therefore detected patterns are prone to over-interpretation. However, there are several avenues to address the problem of low statistical power, through increasing sample size, and making more accurate measurements of factors that contribute noise. In the case of examining the effect of population size and climate on the rates and patterns of molecular evolution, this leads to several courses of action that may help to better examine the relationship.

The work presented here attempts to make steps towards resolving several of the above issues. Methods are developed to improve statistical power. I attempt to integrate the findings of past macroecological research and my own findings in a population-genetic context to reduce the set of possible explanations. Finally, I propose a framework within which the LDG can be understood that is consistent with current knowledge.

1.2 Study purpose and rationale

This project aims to establish which climate-related factors can influence rates of molecular evolution, and determine the extent to which these factors play a role in net diversification differences across biodiversity gradients such as the latitudinal diversity gradient (LDG). Establishing such effects on the processes of molecular evolution is fundamental when determining the foundations of causal, mechanistic links and feedbacks that affect population genetics, and diversity patterns. Such foundations allow more rigorous exploration of evolutionary scenarios that will allow us to look into the past to establish the causes of diversity patterns, and perhaps to look into the future as we assess our effects on species.

A further aim is to develop novel methods for the management and exploration of larger data sets than have been common in the past. Much past exploration of these and related evolutionary questions has involved small amounts of data, with moderate or low taxon sampling. The creation of the main DNA sequence database presented in this thesis involves the collation of almost 100,000 mitochondrial DNA sequences across nearly 7,500 species. Appropriate methods are therefore needed to streamline selection of representative sequences for subsequent phylogenetic analysis. A pipeline approach to sequence selection, alignment, and model-testing was therefore developed. My intention is to develop more robust methods for taxon selection, ensuring phylogenetic methods meet data quality standards to improve parameter estimation and, in turn, produce better estimates of topology and branch lengths.

The thesis is divided into two major components. Approximately half of the work examines macroecological patterns of diversity and species' ranges and population sizes are explored in a number of ways. The purpose of these analyses is to establish a basis to interpret the other half of thesis, in which patterns of molecular evolution are sought over macroecological scales. These two components are described below, with hypotheses for the work outlined for each.

1.2.1 Terrestrial ecoregion diversity

Hypotheses:

- **The major determinants of plant and animal species richness differ across spatial scales, and these effects can be observed by using ecoregions as natural sampling units.**
- **Plant and animal species richness patterns correlate strongest with measures of biologically available energy at large spatial scales where long-term, net diversification processes are causal.**
- **There is a causal link between biologically available energy and net diversification rates.**

Initial macroecological analyses of diversity patterns was performed using terrestrial ecoregions as sampling units. As an approach to macroecological studies of diversity, this approach provides a perspective that is different from grid cells. A typical macroecological analysis would divide continents or the global landmass into 1 degree cells, or an equal area equivalent, and studies often include analyses at more than one grain size to understand how relationships vary with scale. However, two issues arise—when grain size is sufficiently small, many neighbouring cells lack spatial independence. Conversely, when grain size is large, many cells might cross the boundaries of habitat types and therefore contain an undesirably heterogeneous combination of environments. By using ecoregions as sampling units, boundaries have been carefully considered on the basis of plant and animal species composition, reducing undesirable heterogeneity, and spatial dependence. Further, the more than 800 terrestrial ecoregions span five orders of magnitude variation in area, allowing unique approaches to the effect of scale to be investigated. Thus, a terrestrial ecoregion approach to diversity patterns can complement traditional macroecological analyses.

It is worth noting that the boundaries between ecoregions are frequently indistinct and represent compromises between the distributions of different sets of species. While there should be greater independence in species composition between ecoregions than between randomly placed, neighbouring grid cells, there is certainly overlap—especially with large-

ranged generalist species. Also, the diversity of small-island and montane ecoregions that contain endemics are undoubtedly influenced by the source ecoregions from which ancestral species derive. Nonetheless, such an approach may be able to offer insights about diversity patterns not readily derived from traditional approaches.

1.2.2 Ecological correlates of molecular evolution

Hypotheses:

- **Rates of molecular evolution at loci evolving under purifying selection are faster in small populations in accordance with nearly neutral theory.**
- **Rates of molecular evolution at loci evolving under purifying selection follow a latitudinal gradient that mirrors the latitudinal diversity gradient.**
- **Purifying selection is relaxed on average in the tropics, providing a mechanistic link between molecular evolution and large scale diversity patterns through varying strengths of climate-linked selective constraint.**

The idea that molecular evolutionary rates and patterns might underpin broad diversity gradients was firstly clearly expressed by Rohde (1992), but has at least a partial history dating to the 1950s. Both Dobzhansky (1950) and Fischer (1960) argued that biotic interactions were more important inside than outside of the tropics, leading to a latitudinal gradient of biotically driven natural selection. Rensch (1959) linked climate to the strength of natural selection, which he saw as a major avenue to the creation of mosaics of geographic races. Rensch also described the occurrence of shorter generation times in tropical species, a mechanism that if generally true could lead to greater effective evolutionary time in the tropics (see Rohde, 1992). Also, Federov (1966) argued that genetic drift could play a larger role in tropical species, due to smaller population sizes, that could in turn explain the morphological variation seen in tropical species. Each of these theories implies a key role for population-genetic processes in causing the LDG. Rohde (1992) made the link explicit, by arguing that greater effective evolutionary time (evolutionary speed) could stand as a unifying explanation for the LDG, by causing differences in the natural selection regime, and the mutation rate. Rohde's predictions are discussed in detail in the literature review, as are the developments of this theory in contemporary frameworks (e.g.,

Gillman & Wright, 2014), that integrate water, energy and area into a predictive framework for diversity patterns.

However, to test the role of evolutionary speed in generating the LDG, we must establish the strength of any links between abiotic factors, molecular evolution, and diversity patterns, as well as exploring a range of possible confounding factors. To achieve this, I first investigate the links between diversity and both net primary productivity and area by looking at relationship between species richness and productivity across a global dataset of terrestrial ecoregions that vary in size by more than three orders of magnitude. I initially establish the patterns for plant species and then show that related patterns exist in both birds and mammals. However, patterns are more complicated for animals than for plants, suggesting a simple relationship with productive energy alone may not causally underpin the LDG.

Range sizes of species may covary with latitude: in particular a greater latitudinal extent is predicted to occur at high northern latitudes, a pattern called Rapoport's rule (Stevens, 1989). This could happen because high-latitude organisms tolerate a wider range of climate variation, by virtue of living in highly seasonal environments, or because they could be better dispersers in response to abiotic selection pressures. It has been argued that such a pattern could cause the LDG because smaller tropical ranges have higher ratios of perimeter to area, and therefore could have more incidentals occurring outside of their normal range (Stevens, 1989). In addition to potentially driving or contributing to the LDG, such a pattern could also have implications for molecular evolutionary patterns, because it implies average population size covaries with latitude. While there is some evidence for larger ranges in some higher-latitude species, it is not clear if this is caused by Rapoport's rule or by other phenomena (Rohde, 1999). An alternative explanation is that greater longitudinal land extents at high northern latitudes could cause larger ranges independent of latitudinal span. I investigate these two alternatives by investigating the relationship between latitudinal span and range size in three latitudinal bands for birds and mammals. I show that regardless of whether latitudinal bands are defined by latitudinal mid-point or maximum latitude, in both taxa the shallowest relationship occurs in high latitudes,

indicating that for equivalent range sizes, high latitude species have small latitudinal ranges.

Even if population range sizes do not increase towards the poles because of greater latitudinal spans, population densities are likely to be lower in the tropics because the total density of individuals in most broad taxonomic groups increases towards the equator at a lower rate than the density of species within those groups (Storch, 2003). While Federov's (1966) proposal that genetic drift due to lower population densities in the tropics is circular as a cause of the LDG (Schemske, 2002), the increased role for genetic drift in small populations might still confound the search for other relationships between ecological factors and rates of molecular evolution, because smaller populations might experience accelerated molecular evolution (Ohta, 1972b). Therefore, I investigate the influence of population size on molecular evolution. To do so, I first establish that commonly available estimates of range size provide a useful first-order approximation of population size, so that this relationship can be investigated over a wide range of species.

I explore the relationship between a number of climate variables that have been variously proposed to correlate with patterns of net diversification. I find that there is a relationship with climate but that is not mediated by mean annual temperature nor net primary productivity as previously proposed. Instead, the strongest relationship is with isothermality, a measure of the evenness of temperature that correlates with seasonality. The relationship is largely orthogonal to that of population size.

Finally, I synthesise these findings in an outline for a framework that may contribute to our understanding of the LDG, through a reconsideration of net diversification. A number of studies have examined net diversification rates by decoupling speciation and extinction rates. This decoupling provides a flexible approach to net diversification, as it allows multiple possible causes for diversity differences, and does not force a dichotomy between the 'cradle' and 'museum' hypotheses of tropical diversity. Nonetheless, it may be impossible to decouple extinction from speciation. Several studies have found that speciation and extinction rates are higher in the tropics, but that the gap between

speciation and extinction rates (i.e., net diversification) is greater in the tropics (and one study found the reverse in recently diverged taxa).

1.3 Thesis layout and overview

This thesis follows a traditional layout, being arranged into Introduction, Literature Review, Methods, Results, and Discussion chapters at the top level, with the studies that comprise the thesis as sections within these chapters. These sections are described below.

1. Plant terrestrial ecoregion diversity patterns

In this section I demonstrate the critical role of scale dependence in the relationship between net primary productivity and plant species richness in terrestrial ecoregions. Across all terrestrial ecoregions there is only a weak upper limit on diversity set by productivity. However, this is because the strength of the relationship between species richness and productivity scales with ecoregion size. In the largest terrestrial ecoregions, there is a strong, monotonic, positive relationship. I argue that this pattern reflects the role of net diversification in causing large-scale diversity patterns. Therefore, there is strong evidence that the ecological correlates of net primary productivity relate to diversification in plants. However, in small ecoregions, while diversity is limited by net primary productivity, actual diversity levels are set by a confluence of other factors.

2. Animal diversity, range, and population size patterns

In this section, the analyses conducted with plant diversity are initially repeated with vertebrate diversity. I demonstrate that in terrestrial ecoregions similar patterns are evident for bird and mammal diversity, but the relationship with net primary productivity is substantially weaker, and indirect. Instead, biome and realm are more strongly related to animal diversity. Given these results, I undertake some exploratory analyses and show that diversity responses are fundamentally different between ecoregions that typically experience frosts and those that do not. Temperature seasonality emerged as a strong predictor of vertebrate diversity, and gradient of the relationship was steeper in ecoregions that do not experience frosts.

Temperature seasonality has been linked to diversity gradients through animal latitudinal ranges being limited by differences in climate tolerances, a phenomenon referred to as Rapoport's rule. I investigate the potential for reduced tropical latitudinal ranges to have

been caused by climate tolerances. I show that it is instead more likely that latitudinal variation in species' range sizes relates to land area than to physiological tolerances of climate. This is consistent with several other studies that have challenged the generality of Rapoport's rule and its usefulness in explaining diversity gradients.

While range sizes vary greatly across latitudes, median range sizes in animals are smaller in the tropics than in temperate regions. Given that range size and population size covary, this factor along with typically lower population densities in the tropics suggest smaller tropical population sizes on average. As a major focus of the thesis is to account for covariates of molecular evolutionary rates that might cause diversity differences, population size must also be considered. Previously, formal explorations of range size–population size variation have modelled the relationship on the area of occupancy of relatively intensively studied species. Here, I show that the widely available range size measure—extent of occurrence—also forms a log-linear relationship with population size within certain bounds. I further show that in addition to this relationship, densities of bird populations decrease on average towards the equator and at higher elevation. Nevertheless, an additional exploratory analysis shows that there is a latitudinal gradient in nucleotide diversity within species, with diversity peaking at the equator. This indicates that factors beyond population size influence nucleotide diversity.

3. Effect of range size on rates of molecular evolution

In this section, I show for the first time in a study of sister species contrasts that there is a negative correlation between population size and the rate of molecular evolution. This relationship holds for mitochondrial protein-coding genes in two large clades of New World passerine birds—the nine-primaried oscines and the suboscines. The pattern is present in the total dataset, and is stronger in a dataset that uses a priori filters to remove low-power comparisons. Within this refined dataset, the negative relationship is present independently in both subclades and eight of the nine included bird families, with the only exception being a family with only four comparisons. I find no evidence of a mutation-rate effect on these molecular evolutionary patterns. In smaller populations the rate of non-synonymous substitution is accelerated, which as a whole-gene effect on housekeeping

mitochondrial genes is most likely to reflect relaxed purifying selection, as predicted under nearly neutral theory, the expansion of the neutral theory proposed by Tomoko Ohta, which focuses on the role of slightly deleterious mutations in evolution. The methods developed for this study improve on those in previous studies, by being more systematic; all available sister species produced on whole family phylogenies are included, rather than building a dataset by individually selecting sister species pairs.

4. Effect of climate and elevation on rates of molecular evolution

In this section, climate variables are investigated for correlations with the rate of molecular evolution. I demonstrate that the strongest molecular evolution covariate is isothermality, a measure of temperature evenness that is itself a close correlate of temperature seasonality. Isothermality data are derived from the BIOCLIM dataset (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005), where it is defined as the ratio of mean diurnal temperature range to the annual temperature range. The effect, as with range size, is on accelerating non-synonymous substitutions, an effect that appears to reflect relaxed purifying selection. It is difficult to conceptually separate two possible causes for this effect. On one hand, purifying selection could be weaker because of a population size effect, as tropical population densities are lower; on the other, climate-linked background selection could be reduced in the tropics. Although it has been proposed previously, there is no direct evidence for the latter possibility at the population genetic level, although it is consistent with the otherwise anomalous result that nucleotide diversity is greater in tropical species.

Mean annual temperature has a separate effect from other climate variables, as a positive relationship was detected with the synonymous mutation rate. This pattern is linked to an acceleration of the mutation rate. A latitudinal mutation rate effect on endotherms has been detected before, although this is the first study to detect this in birds. However, the pattern is weak, and would be difficult to link causally to diversity patterns.

I bring these results together to reflect on hypotheses for the latitudinal diversity gradient. I outline a framework that is consistent with these results that provides a simple, climate-based explanation for processes that might have generated the latitudinal diversity

gradient through effects on net diversification (Figure 1). This model consists of a number of environmental inputs and factors that have positive and negative effects on the genetic and ecological traits of populations. These effects combine to affect the formation and persistence of subpopulations, and in turn alter the propensity for speciation and extinction. I argue through the course of the thesis that the conditions in the tropics, where abiotic selection is weaker on average than in the extratropics, are biased towards increasing net diversification through positive effects on speciation, and negative effects on extinction. In the final section, I develop this model by arguing that the processes of speciation and extinction are conceptually linked, such that they should not be viewed wholly distinct processes.

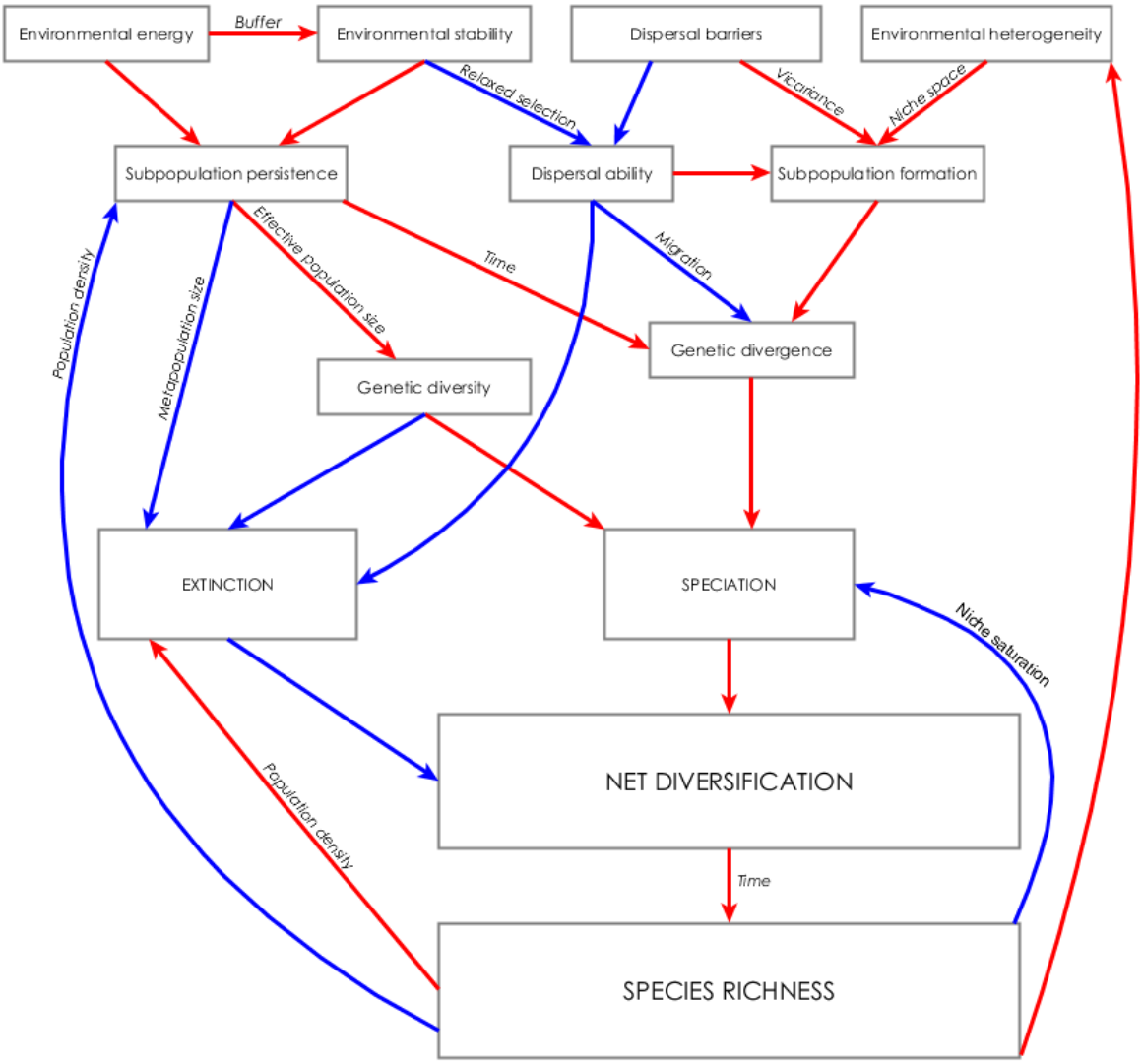

Figure 1. Model of the processes that theoretically combine to generate patterns of species richness. Red arrows represent hypothesised positive relationships between variables, while blue arrow represent hypothesised negative relationships. Explanations are provided in text.

Electronic data is available for some components of this thesis. The database of avian mitochondrial sequences generated in this work is available here:

- http://dx.doi.org/10.6084/m9.figshare.1236602

Phylogenetic input and output files and sister species data are available here:

- http://figshare.com/s/9ef2e8dc785811e5994206ec4b8d1f61

Note, it is not possible to list the authors of all of the sequences collated in the avian mitochondrial sequence database given it contains only slightly fewer than 100,000 gene sequences. Also note that the database in its raw form is compiled without additional verification that the species identified is correct. Methods are presented below to minimise the risk of using misidentified, low-quality, or otherwise unrepresentative sequences when multiple sequences are available for a species (by identified the sequence of median length to congeners). It is recommended that such methods are applied whenever the database is used.

2 Literature review

2.1 Overview

This literature review covers a range of topics across macroecology, population genetics, and phylogenetics. I begin with a history of the development and contemporary interpretation of evolutionary theory with particular reference to the latter half of the twentieth century, during which the neutral theory of molecular evolution emerged. The neutral theory has a central place in the development of contemporary evolutionary theory and in explaining molecular evolutionary patterns. As described in the literature review, despite its explanatory power, the neutral theory has been inconsistently integrated into widely supported evolutionary paradigms by some workers. One cause appears to be continued misunderstandings of the neutral theory and its relationship to natural selection (Hughes, 2007), a problem arising when the roles of drift and selection are not evaluated with reference to population genetics. Also, neutral and selection theories have been established as opposing paradigms, whereas it may be more accurate to view evolution as a plurality of adaptive and non-adaptive forces operating concurrently (Lynch, 2007b). The persistence of these issues highlights a need for better understanding of the historical and contemporary perspectives of molecular evolution and population genetics to be integrated into broader evolutionary thinking. I review and discuss the original observations that formed the basis for accepting the neutral theory, as these observations remain relevant as evidence for the operation of non-adaptive evolutionary forces. The developments of modern selection theory also offer important new insight into molecular evolutionary processes, and additional ways of interpreting the rapidly expanding data that are available. Although a narrative review of the development of molecular evolution crosses into the history and philosophy of science, the inclusion of this review serves a function by discussing the development of the two major opposing paradigms in molecular evolution that have acted as lenses on decades of results in the field. Both

paradigms have guided theory towards somewhat disparate approaches to understanding spatial diversity patterns.

Following the review of molecular evolutionary theory, I discuss large-scale spatial diversity patterns, with particular reference to the latitudinal diversity gradient. Ecological, evolutionary, and historical explanations for diversity gradients are discussed with particular reference to the covariation between species richness and latitude. The importance of evolutionary mechanisms that operate to produce diversity gradients via differences in net diversification will be emphasised because of the evidence that such mechanisms continue to operate (e.g., Martin & Tewksbury, 2008; Rolland, Condamine, Jiguet, & Morlon, 2014; Svenning, Borchsenius, Bjorholm, & Balslev, 2008). The theorised connections between molecular evolution and species, sub-species, and genetic diversity will then be explored. Mechanisms that could affect the rate of molecular evolution to cause these patterns are discussed, as are confounding factors that could produce similar patterns without being causally connected to net diversification. Particular emphasis is given to the role of population size in causing molecular evolutionary rate differences. This emphasis is important because population size has been theorised to be linked to molecular evolutionary rate differences (Ohta, 1972b, 1992), and average population sizes are likely to vary with latitude because species densities increase more than individual densities towards the tropics (Storch, 2003), indicating the potential for a spurious link. Mechanisms for net diversification are then evaluated in light of population genetics.

Finally, the methods and metrics used to investigate molecular evolutionary rate differences are discussed. Limitations in previous methods will be outlined, as well as the difficulties that are inherent in interpreting various evolutionary metrics. Approaches that could address those limitations are explained, which form the basis of methods used in the molecular evolutionary component of this thesis. Factors that can affect the rate of molecular evolution are given consideration, and results of previous investigations of those effects are summarised. Decisions made in the selection of study taxa, and other choices in data selection are discussed and justified.

2.2 Development of molecular evolutionary theory

2.2.1 The origins of the neutral theory of molecular evolution

In stark contrast to the selection-driven evolutionary models that preceded it, the neutral theory of molecular evolution states that polymorphisms and substitutions at the DNA level primarily result from mutation and genetic drift (Kimura, 1983). Under this theory, the bulk of evolutionary change is comprised of effectively neutral mutations that 'escape' natural selection because of their negligible effect on organismal fitness, and therefore are only subject to chance extinction or fixation. While the subject of decades-long controversy (e.g. Hahn, 2008; Kreitman, 1996; Lewontin, 1974; Milkman, 1976; O'Brien, 1994), the neutral theory has been a broadly successful predictive paradigm for understanding molecular evolutionary change (Lynch, 2007b; Lynch, Bobay, Catania, Gout, & Rho, 2011; Nei, Suzuki, & Nozawa, 2010), and key tenets of the neutral theory have been subsumed by selectionist models of evolution, even if they are not always recognised as such (Hughes, 2007). For example, it is widely accepted that the sequences of functionally important gene domains remain conserved over evolutionary time. Yet, the idea that the main mode of natural selection is negative rather than positive is opposite to selectionist predictions from the pre-molecular era (Hughes, 2007). Under the selectionism of the pre-molecular era (e.g., Fisher, 1930), loci that do not contribute to fitness were expected to change slowly, whereas it is now firmly established that such loci evolve rapidly when compared to functionally important genomic regions, and such selective constraint is now a hallmark of important biological function (e.g., Rands, Meader, Ponting, & Lunter, 2014). Hence, tenets of neutral theory have contributed importantly to our understanding evolution, regardless of paradigm.

The development of the neutral theory relates in large part to our changing understanding of natural selection and genetic drift. In the pre-molecular era of the modern evolutionary synthesis, while purifying selection was tacitly accepted as a filter for maintaining function—the "rejection of injurious variation" (Darwin, 1859, p. 81)—the main focus regarding natural selection was on positive selection, often referred to as Darwinian selection. Positive selection was widely viewed as the cause of both evolutionary change, and much of the variation observed within populations. Fisher's (1930) geometric model,

for example, views evolution as a progression of slightly advantageous mutations gradually accumulating via natural selection acting as a filter on variation with infinitesimal fitness effects. The accumulation of infinitesimal differences is precisely what Darwin had postulated, updated by Fisher to be consistent with Mendelian particulate inheritance. For panselectionists such as Fisher, neutral variants were expected to arise, but to be of little consequence to evolution (e.g., Fisher, 1929; Fisher & Ford, 1947), and mutations were necessary for evolution but did not influence its direction (Fisher, 1934). During the same period Wright (1931, 1943, 1948) developed a theoretical argument for the importance of genetic drift in small populations, influencing allele frequencies by sampling effects independent of natural selection. Fisher (1922) had also considered the effects of genetic drift in small populations, but he did not accept that species effective population sizes were anything less than their global population sizes (Skipper, 2008). Indeed, Fisher considered natural populations sufficiently large to model them with mathematically convenient infinite population sizes, in which random genetic drift does not affect allele frequencies. This view was at least partly justified by empirical observations of fluctuating allele frequencies in a population of moths that were not readily explained by genetic drift (Fisher & Ford, 1947). Throughout the pre-molecular era, Fisher's evolutionary views were substantially more influential than Wright's, and the role of genetic drift was largely ignored in the progression of the modern evolutionary synthesis, despite the pluralism of the modern synthesis when it emerged in the 1940s (Dietrich, 1994; Gould, 1981).

2.2.2 Development of evolutionary theory in the molecular era

Because several decades of theoretical population genetics pre-date the molecular evolutionary era of the 1960s, there was a broad foundation from which to interpret new types of evolutionary data as they began to emerge. As Lewontin (1974, p. 189) notes, prior to the molecular era, population genetics was "like a complex and exquisite machine, designed to process a raw material that no one had succeeded in mining". In fact, the period lacked even for a concrete concept of the gene. Morgan (1933, p. 315) encapsulated the theoretical nature of population genetics in the first half of the twentieth century when he stated that there was "no consensus of opinion amongst geneticists as to what the genes are—whether they are real or purely fictitious".

The molecular evolutionary era that begun in the second half of the twentieth century was characterised by rapid advances in both understanding and technology. The determination of DNA structure (Franklin & Gosling, 1953a, 1953b; Watson & Crick, 1953) was followed by a number of key technological advances that finally allowed access to the evolutionary raw material. By the early 1960s, development of high-resolution gel electrophoresis (e.g., Hubby, 1963) enabled allelic differences in proteins to be rapidly detected, and intensive methods developed earlier to sequence amino acid chains were being applied to haemoglobins across multiple species (reviewed in Schroeder, 1963). Although initially collected by molecular biologists, the newly available empirical data soon began to be imported into population genetics to test evolutionary predictions.

Although the theoretical basis for the development of neutral theory arose from models of genetic drift in finite and subdivided populations that were developed during the pre-molecular era (e.g. Wright, 1931, 1943), as noted above Fisher's influence had meant that drift was largely ignored leading into the molecular era (Gould, 1980, 1981). Instead, the population-genetic theory at the beginning of the molecular era was dominated by panselectionism (Dietrich, 1994), under which allele fixations and polymorphisms were expected to be predominantly caused and maintained by natural selection. Initial studies of protein variation indicated many loci were highly and unexpectedly polymorphic (Harris, 1966; Lewontin & Hubby, 1966), the implications of which are discussed in more detail below (2.2.3 Patterns predicted for genetic diversity). Theory at this time also predicted that the substitutional load (the 'cost' of selection) set an upper limit on the rate of fixation under natural selection (Haldane, 1957). When it became possible to compare interspecific protein sequences, rates of genetic change could be estimated by comparing the number of amino acid differences between protein orthologues for species pairs whose divergence times had been established in the fossil record (Margoliash, 1963; Zuckerkandl & Pauling, 1965). Kimura (1968a) calculated that these rates corresponded to approximately one nucleotide substitution every two years for a typical mammalian genome—substantially higher than Haldane's substitution limit of one allele per 300 generations. Kimura concluded that such rates excluded Darwinian selection as a cause of

most amino acid substitutions. The bulk of substitutions instead appeared to be selectively neutral. However, workers immediately raised objections to Kimura's conclusion of predominant neutrality because the limits derived from Haldane's cost of selection assumed selection necessarily reflected differential death rates for genotypes, and acted on single loci even if multi-locus characters were under selection (e.g., Maynard Smith, 1968; Moran, 1970; Sved, 1968). In fact, several such critiques of Haldane's cost of selection had already been made prior to the advent of the neutral theory (Dodson, 1962; Van Valen, 1963), but were not addressed by Kimura (1968a). As Kimura and Maruyama (1969) showed, within finite populations any upper limit on selection-driven substitutions would have to reflect the strength of selection on mutants: as selection coefficients approach effective neutrality, the upper limit on substitution rates increasingly reflect the mutation rate rather than the substitutional load. While this approach reflects a balance between selection and drift in determining the fate of many mutations, it further blurs any distinction between substitutions that could be attributed to either positive selection or genetic drift. Such observations can be interpreted in favour of either selection or neutrality, depending on emphasis. Thus, while amino acid substitution rates appeared higher than had been predicted by early neo-Darwinian population genetics, and neutral evolutionary dynamics provided a legitimate explanation (O'Donald, 1969), additional observations were required to popularly establish the neutral theory.

Independent of population-genetic arguments, the biochemical evidence indicated that much evolutionary change was undirected by natural selection (Kimura, 1968b; King & Jukes, 1969). In addition to Kimura's (1968a) work on amino acid divergence, Walker (1968) found large divergences in DNA between seemingly closely related species that exceeded Haldane's limit. Walker reported a 13% divergence between rat and mouse DNA, leading him to conclude that "the genomes of related organisms are unexpectedly different." (p. 231). Walker also noted that Kimura's calculations for rates of amino acid sequence divergence were substantially lower than the rates of DNA divergence he estimated using DNA–DNA homology. If both results were correct, it appeared that many of the changes in DNA sequences did not alter the amino acid sequence. While Walker dismissed Kimura's conclusions about neutral evolution because of the critiques levelled at

Kimura's reliance on the cost of selection, Walker proposed that this discrepancy would be resolved if many of the DNA differences occurred at largely synonymous third codon positions. However, substitution rate differences between codon positions can only be readily explained by either mutation rate differences, or differences in the patterns of natural selection. As King and Jukes (1969) note, there is no reason to expect a mutation rate difference between codon positions. Also, synonymous mutations are more likely to be neutral than non-synonymous changes a priori, because they do not alter the amino acid sequence (although selection may occur for other reasons; see Chamary & Hurst, 2005). Therefore, the elevated rate of synonymous substitutions is indicative of neutral evolution.

The biochemical argument for predominantly neutral evolution is further strengthened by studies of mutation and substitution in *Escherichia coli* (King & Jukes, 1969). Cox and Yanofsky (1967) showed that in an *E. coli* mutator strain with a GC mutation bias, GC content increased proportionally to their expectations under the bias in the mutation rate, and that occurred without any obvious fitness effects, indicating that much of the change was selectively neutral. While Cox and Yanofsky suggest at least some of these mutations might have fixed under positive selection, the broad shift in genome composition reflects the mutational spectrum. Similarly, using Dayhoff and Eck's (as cited in King & Jukes, 1969) amino acid frequency data, King and Jukes showed that, barring the single outlier of arginine, the expected frequencies of amino acids calculated from random permutations were strongly correlated with observed frequencies (R^2 = 79%). These lines of evidence all suggest that evolution mirrors the random process of mutation, suggestive of the prevalence of "non-Darwinian evolution" (King & Jukes, 1969, p. 788).

A related observation indicating widespread neutral evolution was the substantial numbers of substitutions between homologous housekeeping genes despite a highly conserved function. For example, despite hundreds of millions of years of evolutionary divergence, sequences for the cytochrome *c* gene from birds, fish and mammals can be expressed by yeast, functioning with reduced expression levels but biological activity up to 66% of that of the yeast sequence (Hickey et al., 1991). This level of function occurs despite

a 40% difference in amino acid residues between vertebrate and yeast cytochromes *c* (Clements, O'Connell, Tsunasawa, & Sherman, 1989). In a further study of yeast cytochrome *c* variation, Hampsey, Das, and Sherman (1988) found that of the amino acid sequence's 108 residues, only 21 were evolutionarily invariable, and another 12 were evolutionarily conserved, leaving nearly 70% of residues with greatly reduced specificity. Such studies indicate that the scope for largely neutral evolution is substantial even within protein-coding loci, and that the bulk of evolutionary change in housekeeping genes can occur without functional differentiation. Nevertheless, positive selection can play a role in mtDNA evolution without causing major functional divergence, potentially contributing compensatory mutations in expanding populations (Charlesworth & Eyre-Walker, 2007), and at times for adaptation to climate (Balloux, Lawson Handley, Jombart, Liu, & Manica, 2009; Foote et al., 2011; Meiklejohn, Montooth, & Rand, 2007).

A further major line of evidence that supported the development of the neutral theory concerns the constancy of the substitution rate over evolutionary time. Margoliash (1963) showed that the number of amino acid substitutions in cytochrome *c* that had occurred between pairs of eukaryotes (including mammals, birds, fish and fungi) was approximately proportional to the time since the pair's evolutionary divergence as estimated in the fossil record. Zuckerkandl and Pauling (1965) found similar patterns in haemoglobin proteins, concluding that there may be "a molecular evolutionary clock" (p. 148). Classically viewed, positive selection could only produce such a clock-like pattern if all lineages fixed approximately the same number of beneficial mutations at approximately evenly spaced periods of time. Yet, such constancy across the eukaryotic tree from humans to yeast seems implausible, even if linked to changes in species' environments, as different species inhabit environments with vastly different stability. More importantly, as noted above, the function of cytochrome *c* shows no evidence of change across eukaryotes and the majority of its residues are unspecified, suggesting little opportunity for positive selection. In contrast, this clock-like pattern is consistent with substitution mirroring the discrete, stochastic process of mutation—the hallmark of neutral evolution (Kimura, 1983). While the mutation rate and strength of natural selection varies between species, across genomes, within genes, and within codons (e.g. Bromham & Penny, 2003) the molecular

clock still performs well when this variation is averaged out by sampling across long periods of time and large sections of genomes (Nei et al., 2010). However, we do observe variation in molecular rates that depart from the molecular clock on temporal or taxonomic scales where important variation such as life history traits are unequal on average between compared species or groups (Bromham, 2011). While some molecular rate variation in such comparisons is noise caused by the stochastic nature of substitution (Weir & Schluter, 2008), other causes include: factors involving natural selection affecting the fixation rate either directly (Charlesworth & Eyre-Walker, 2007; Gillman, Keeling, Ross, & Wright, 2009; Ohta, 1972b) or indirectly (Gillespie, 2000); variation in the mutation rate (Lanfear, Ho, Love, & Bromham, 2010); and the relationship between the proportion of polymorphisms in pairwise sequence distances and time (Ho, Phillips, Cooper, & Drummond, 2005; Peterson & Masel, 2009). Despite the emphasis given in the literature, particularly by Kimura, the neutral theory does not directly predict a universal molecular clock, and would not be falsified by its absence (Nei et al., 2010). Variation in the mutation rate, or the strength of purifying selection, can cause deviations from a clock-like substitution rate, but are consistent with neutral theory *sensu lato*, although not as formulated by Kimura (Nei, 2013). However, the neutral theory can explain the existence of a molecular clock across a wide range of organisms if effectively neutral mutation rates are similar in calendar time (Nei, 1975).

Finally, King and Jukes (1969) note the great variation in genome content across vertebrates, coupled with low gene density. It was already known by the late 1960s that a typical mammalian genome such as the human genome would contain little more than about 1% protein coding DNA (Muller, 1967, as cited in Ohno, 1972), and it was also known that lungfish had a genome 17 times larger than humans. Coupled together, King and Jukes found it implausible that function could explain the vast and variable amounts of non-coding DNA. Consistent with the inference that a large amount of vertebrate genomes is unlikely to be functionally important, Ohno (1972) argued that regardless of genome size a typical mammal genome could not contain more than approximately 30,000 functional loci of 1000bp, because the known mammalian mutation rate would cause an intolerable number of deleterious mutations. On the basis of genetic load, Ohno argued

that approximately 90% of the human genome must be 'junk' DNA that was not subject to purifying selection. This population-genetic argument has not been falsified, although the upper limit on the deleterious mutation rate is made indistinct by other evolutionary phenomena such as soft selection, epistasis, which may decrease population-wide effects on fitness from deleterious mutations (Agrawal & Whitlock, 2012). As such, the effect of a mutation cannot be estimated outside of its genomic or ecological contexts. Also, as Agrawal and Whitlock point out, empirically estimating the mutational load is fraught with challenges, as it requires a reference genome without mutation. Recent gene counts for the human genome have settled on figures fewer than 20,000 coding loci (Ezkurdia et al., 2014) with a recent estimate that 8.2% is constrained by purifying selection (Rands et al., 2014), which is consistent with Ohno's prediction. Ohno's prediction relies entirely on a population-genetic argument from genetic load, rather than knowledge of mammalian genomic content (Ohno incorrectly guessed most 'junk' would be pseudogenes). However, attributing this consistency of this prediction to genetic load requires addressing the theoretical and empirical challenges above.

The initial authors of the neutral theory gathered a wealth of evidence. In summary, lines of evidence supporting the neutral theory included that: the scope for neutral evolution of proteins was considerable; putatively neutral sites such as the third codon position experienced a substantially higher substitution rate than missense substitutions; evolution occurred faster than predicted by classical panselectionism; clock-like substitutions appeared to reflect mutation-driven, rather than selection-driven processes; and, genomes had both low gene density and high haploid size variation without varying greatly in obvious function or complexity.

Although there were immediately strong lines of support for neutral theory, it was not widely embraced by evolutionary biologists at the time of its proposal, as many advocated panselectionism at all levels of biological organisation. The reverse was not necessarily true for neutralists: Kimura (1983), for example, believed that there was a disconnection between organismal and molecular evolution, arguing that at the phenotypic level, selection rather than neutrality predominated, allowing for adaptation in a Darwinian

fashion. Instead, Kimura opposed what he termed "naive panselectionism and its intrusion into the realm of molecular evolutionary studies" (Kimura, 1976, p. 152). Continuing Fisher's tradition of translating Darwinian thought in the terms of a new era, other evolutionary biologists argued that selection prevails as the major determinant of evolutionary patterns at all levels, including the molecular level (Milkman, 1976; Simpson, 1964). Milkman's views of evolution almost precisely mirror Darwin's Malthusian views, extended to the molecular level in a population-genetic framework:

> "individuals are ranked competitively as to overall phenotype, and those favored by genes, environment and luck gain the available places, just as in animal or plant breeding, though not quite so rigidly…It takes very little selection to obliterate random genetic drift, and so the advantageous choice will be made almost every time." (Milkman, 1976, p. 153).

Hence, while Milkman also argued that selection operates at the phenotypic level, he did not allow for the same disconnection between genotypic and phenotypic evolution that Kimura had. Instead, for Milkman, the reach of selection is so great that it easily overwhelms drift, an idea that was commonly held by panselectionists in this era (Gould & Lewontin, 1979). However, this assertion lacks an equal weight of evidence in its support.

2.2.3 Patterns predicted for genetic diversity

Beyond what Kimura considered naïve panselectionism, there have been a range of other criticisms of the neutral theory. Most of the evidence that strongly supported neutral theory considered differences that occur over long periods of time, such as amino acid composition of polypeptides, and amino acid and DNA divergence between species. One of the major lines of evidence that challenged the neutral theory regarded much shorter time periods: genetic variation within populations.

A tenet of population-genetic theory is that for a neutral locus at mutation-drift equilibrium, there is a relationship between heterozygosity and effective population size (N_e). N_e estimates the equivalent effective breeding size of a population if it were an ideal Wright-Fisher population (Charlesworth, 2009; Wright, 1931). N_e differs from the entire adult breeding population size because multiple factors reduce panmixia, cause

fluctuations in size through though, and selection and linkage cause deviations from mutation-drift equilibrium. At mutation-drift equlilibrium, $H_e = 4N_e\mu/(4N_e\mu + 1)$, where H_e is expected heterozygosity and μ is the per-generation mutation rate (Kimura, 1968b). From this equation, provided there is not a strong, negative relationship between N_e and μ, we can expect heterozygosity to scale positively and non-linearly with population size for such loci. However, early investigations found a range of heterozygosities that corresponded to only a fourfold variation in N_e in species ranging from wild grasses and fruit flies to humans (Lewontin, 1974). To explain such results under neutral theory at mutation-drift equilibrium, either mutation rates negatively covary with N_e, or N_e genuinely only varies fourfold across these organisms. While Lewontin (1974, p. 210) referred to the latter proposition as a "patent absurdity", there is now some evidence for the former (Lynch, 2010). More recent data show that across a much broader range of species, from bacteria to vertebrates, only two orders of magnitude variation in diversity exist, despite presumably much greater variation in N_e (Lynch, 2006). However, if many loci are not at mutation-drift equilibrium, then observed heterozygosity will be lower than otherwise predicted. Low levels of recombination, rather than non-neutral dynamics, were identified as a cause of less-than-expected heterozygosity in *Escherichia coli*, because reduced recombination affects the ability of metapopulations to reach such an equilibrium (Selander & Levin, 1980). In other situations where restricted gene flow occurs between subpopulations, species-wide levels of heterozygosity will also be lower than that expected for a panmictic population of the same size (Maruyama & Kimura, 1980). Similarly, heterozygosity might be reduced through periodic population bottlenecks, which can have lasting effects particularly for populations with low intrinsic growth rates (Nei, Maruyama, & Chakraborty, 1975), resulting in a reduction of long-term N_e, and insufficient time to reach mutation-drift equilibrium. Several of these factors have been given insufficient consideration in recent criticisms of neutral theory, where it has been stated that neutral theory predicts an unqualified correlation between N_e (or N) and genetic diversity (Bazin, Glémin, & Galtier, 2006; Hahn, 2008; Stoeckle & Thaler, 2014). This is exactly equivalent to stating that neutral theory predicts all loci are at mutation-drift equilibrium. The importance and extent of non-equilibrium dynamics may have been poorly understood at the time of the development of neutral theory, and given little or no emphasis at that time.

However, given that deviations can be caused by many phenomena that are not inconsistent with neutral theory, including background negative selection (Charlesworth, Morgan, & Charlesworth, 1993), occasional positive selection (Ohta & Kimura, 1971), population substructure (Maruyama & Kimura, 1980), or population size dynamics (Nei et al., 1975), such deviations cannot justify the outright rejection of neutral theory.

Classical selection theory also provides two contrasting expectations for genetic variation within populations, developed as theoretical explanations for heterosis (i.e., hybrid vigour, Shull, 1914) in the pre-molecular era (see Crow, 1948). While the limited range of observed heterozygosity was considered problematic for neutral theory, heterozygosity patterns also challenged the expectations held under selection theory for either the classical or balance models. The classical model predicts that genetic diversity should generally be low in populations because heterosis observed for the offspring of two inbred lines is predicted to be caused by the hybrid being heterozygous at loci where either of the parental lines were homozygous for recessive deleterious alleles. Fitness is therefore rescued by dominant alleles. The result is low heterozygosity, because the one advantageous allele should quickly fix in the population. Under the balance model, heterosis instead occurs because certain heterozygous combinations of alleles have an advantage over the fittest homozygote (overdominance, versus dominance in classical theory). Therefore, at loci where overdominance occurs, balancing selection maintains multiple alleles in a population at once, increasing genetic diversity. Therefore, of the two selectionist views, the balance model is capable of producing more genetically diverse populations. However, Lewontin et al. (1978) showed that even under the balance model, heterosis could not maintain as many segregating alleles as had been observed in natural populations. Additional restrictions on heterozygote fitness could inflate the number of segregating alleles under heterosis, although such restrictions were improbable to be generally true (Lewontin et al., 1978). The only proposed selectionist mechanism that could potentially maintain greater genetic diversity was local adaptation to different niches that might exist across a population's range (Gillespie, 1977; Lewontin et al., 1978). Consistent with this possibility, a number of mechanisms can maintain population differentiation in the face of gene flow (e.g., Morgans, Cooke, & Ord, 2014; Saint-Laurent, Legault, & Bernatchez, 2003).

However, under selectionist theory, this explanation would also require that the polymorphic loci under investigation were either themselves direct targets of selection, or in close linkage with a direct target of selection. The first of these possibilities is unlikely for the alleles studied in the 1970s since most were ubiquitous proteins and were not chosen for study as predicted targets of ecological selection. The second possibility could only account for genetic diversity patterns in the absence or near-absence of recombination. Also, recent evidence suggests ecological differentiation can occur with minimal genetic structure across populations (Deane, McCoy, Robertson, Birt, & Friesen, 2013; Morgans et al., 2014), indicating local adaptation is further unlikely as a general explanation for genetic diversity patterns. While neither the originally formulated selectionist nor neutralist theories predicted observed heterozygosity patterns, either can produce results consistent with those patterns either in part or under particular circumstances. Given that the degree of observed genetic diversity commonly falls in between predictions for neutral loci at equilibrium and loci under balancing or classical selection, genetic diversity patterns are apparently consistent with an evolutionary model in which both selection and neutral dynamics can play important roles at the molecular level.

2.2.4 Subsequent criticisms of neutral theory

By the 1990s, two decades of modelling evolutionary processes (e.g., Gillespie, 1994; Kingman, 1978; Ohta, 1992; Ohta & Gillespie, 1996 and references therein) had produced several lines of evidence that led to prominent calls for the abandonment of strictly neutral models of evolution (Kreitman, 1996). One issue noted by Kreitman was the neutral theory—specifically, what constituted a neutral mutation—was imprecisely defined. Kimura (1983) defined neutral mutations as those for which $s \ll 1/2N_e$, where s is the selection coefficient. As Kreitman (1996) notes, this definition differs from the one Kimura gives later, where $s < 1/2N_e$ (Kimura, 1986). However, while Kreitman is critical of this inconsistency, any mutation with a non-zero selection coefficient is technically non-neutral, and Kimura needed to define an arbitrary limit on what should be reasonably considered neutral along a gradient of increasing selection effect; both cases refer to mutations for which any direct effect of selection is small. Neutrality is more liberally defined by Li (1978) as $s < 1/N_e$. While a non-arbitrary cut-off for neutrality may not be possible, the

situation is less clear still for near-neutrality, as it requires two arbitrary bounds. The uncertainty in this has led to wholly qualitative definitions of near neutrality (Ohta, 1992), and qualitative definitions of neutrality that subsume near-neutrality (Nei, 2005).

Despite the neutral theory having been declared "dead" almost two decades ago (Kreitman, 1996), attempts have been made to periodically re-slay it since. Recently, researchers involved in the DNA barcoding of fish suggested the wholesale rejection of neutral theory on the basis of low genetic diversity in the 648 base-pair barcode region (Stoeckle & Thaler, 2014). However, the barcode region is a section of the COI gene located within the mitochondrial genome—an essentially non-recombinant locus of 37 genes fundamental to energy metabolism, and subject to strong purifying selection (Edgar et al., 2009; Stewart et al., 2008). These factors cause strong background selection on mtDNA, reducing N_e because virtually all lineages carrying deleterious mutations will ultimately be purged from the population in the absence of fitness-restoring recombination (Charlesworth et al., 1993). Also, being uniparentally transmitted in most eukaryotes, we expect mtDNA to have smaller N_e than autosomal nuclear loci. Low N_e should result in low genetic diversity in well-mixed populations, although we might also expect rapid divergence between populations in the absence of interbreeding (e.g., Naka, 2010). However, Stoeckle and Thaler reject the entire notion of N_e affecting genetic diversity, claiming ad-hoc adjustments have been made to neutral theory when predictions have failed. They argue that the census population size (N_c) is the relevant population size metric with which to understand genetic diversity, and propose a radically different explanation for low genetic diversity at COI: strong purifying and adaptive selection govern both diversity and substitution across the mitochondrial genome, including at four-fold synonymous sites. Stoeckle and Thaler suggest that the approximate mitochondrial molecular clock across animal species results from strong co-evolution, although it is unclear why recurrent, synchronised positive selection on housekeeping genes would occur. Maintaining function that is intermittently eroded through slightly deleterious substitutions is plausible although such a process, even if it were clock-like, is at odds with Stoeckle and Thaler's position because it relies on a combination of adaptive and non-adaptive evolution. Continuous adaptive change in response to environmental

fluctuations could also be plausible provided there was evidence that environmental shifts regularly alter preferred mtDNA genotypes that could be corrected by positive selection. However, even if there was a basis to accept this, the majority of substitutions would still have to hitchhike to fixation because of population genetic theory governing DNA in linkage (Birky & Walsh, 1988), and the substitutional load based on the known mtDNA substitution rate (Haldane, 1957; Kimura, 1968a). Further, the prevalence of third-codon-position substitutions is difficult to explain without invoking neutrality. As such, Stoeckle and Thaler's hypothesised explanation for a deterministic molecular clock fails on multiple levels.

A wide range of additional criticisms have been made of the neutral theory as a result of increasingly available genomics data. Prominently, Hahn (2008) argues that two major tenets of the strictly neutral theory are systematically violated. Hahn states the first neutral theory tenet is the claim that the majority of polymorphisms and substitutions are not under direct selection, and cites a number of McDonald-Kreitman (MK) tests as evidence of its violation. The MK test (McDonald & Kreitman, 1991) examines the ratios of synonymous to non-synonymous differences for substitutions and polymorphisms (d_N/d_S and P_N/P_S respectively). In the test's original form, if d_N/d_S exceeds P_N/P_S, it is taken as evidence of positive selection driving evolution, the converse is taken as evidence of purifying selection acting on segregating variation, and no difference would be expected in the instance of strict neutrality. Positive results for modified versions of the MK test have been obtained when excluding polymorphisms at frequencies below an arbitrary cut-off that has ranged from 12.5% to 33% (Charlesworth & Eyre-Walker, 2006; Fay et al., 2002; Zhang & Li, 2005). Hahn reports that species with large N_e (e.g., *Drosophila*) have been found to have evidence of positive selection when low-frequency polymorphisms are excluded, while species with small N_e (e.g., humans) lack evidence of positive selection. Unfortunately, there is no way from the results of the MK test to distinguish between apparent positive selection and relaxed purifying selection, as has previously been shown in Hawaiian *Drosophila* that have experienced population bottlenecks (Ohta, 1993). Also, fluctuating population sizes can also cause elevated non-synonymous substitution through consecutive phases of slightly deleterious fixations under relaxed selection and

compensatory positive selection (Charlesworth & Eyre-Walker, 2007; also see Goldstein, 2013). If species histories are well documented, it may be possible to avoid such error (Fay et al., 2002), although this is rarely the case. At the same time, MK tests—even when modified to remove lower-frequency polymorphisms—may lack power to detect relatively rare positive selection, such as would be expected for species with lower N_e (Charlesworth & Eyre-Walker, 2008; Gossmann, Keightley, & Eyre-Walker, 2012). A further consideration is that the MK test may not be valid on theoretical grounds; d_N/d_S was developed for species-level comparisons and behaves differently to equivalent selection pressures from its within-species analogue P_N/P_S (Kryazhimskiy & Plotkin, 2008), and stochastic fluctuations under neutrality can substantially bias estimates on short timescales (Mugal, Wolf, & Kaj, 2014).

The interpretation of MK test results as evidence for fixation predominantly by positive selection contrasts with other approaches to d_N/d_S that suggest a prevalence of nearly neutral evolution. Populations with presumed smaller N_e, for example, larger-bodied versus smaller-bodied mammals, endosymbiotic versus free-living organisms, and, in some cases island versus mainland species (see 2.4.3 Empirical tests of the *Ne*—molecular-rates relationship), have been found to have elevated d_N/d_S ratios relative to their counterparts (Eyre-Walker, Keightley, Smith, & Gaffney, 2002; Johnson & Seger, 2001; Woolfit & Bromham, 2003, 2005) more frequently than the reverse (Wright, Gillman, Ross, & Keeling, 2009). Ohta (1972a) initially noted that the ratio of cistron divergence to total DNA divergence correlates negatively with generation time, indicating a larger proportion of nearly neutral amino acid substitutions occur in organisms with long generation times, which typically have smaller N_e. Indeed, relative to small mammals, large mammals also experience elevated d_N/d_S, and more radical amino acid substitutions (Popadin, Polishchuk, Mamirova, Knorre, & Gunbin, 2007), for which the physicochemical properties of the original and substituted amino acids are different. Given that the rate of adaptive evolution is likely to be either uncorrelated with population size (Adams et al., 1991; Galtier, 2016), or positively correlated if adaptive evolution is mutation limited, these various results indicate that elevated d_N/d_S is primarily the result of the proportion of slightly deleterious mutations that fix in a population. It is difficult to reconcile these

results with those of Sawyer, Parsch, Zhang, and Hartl (2007), who estimated that almost 95% of amino acid replacements that had fixed in *Drosophila* had positive selection coefficients, based on a model derived in part from the MK test. Sawyer et al. (2007) acknowledge that the mean selection coefficient for these amino acid substitutions is small ($s = 2.5 \times 10^{-6}$, scaled selection coefficient $N_e s = 2.5$), and therefore a substantial proportion of replacements are nearly neutral, although not slightly deleterious. Because it is unlikely that the estimates of N_e are categorically wrong, and also unlikely that small populations evolve faster than large populations due to positive selection, other MK model assumptions are likely to be the cause of the contradictions. Although rarely discussed in the literature, two of the major assumptions of the MK test are potentially responsible: that sites evolve independently, and that selection pressures are invariant (Fay, 2011). These assumptions require that N_e does not vary over time (but see Li & Durbin, 2011), hitchhiking does not occur (but see Chun & Fay, 2011), and genomes are at mutation-drift equilibrium (but see Nei et al., 1975). While positive selection would explain elevated d_N/d_S relative to P_N/P_S if these assumptions were met, if systematically violated, a wide range of interpretations of the same patterns is possible, including being the result of relaxed purifying selection. Also, non-equilibrium P_N/P_S values can result from hitchhiking. These difficulties limit the value in the MK test, and given the availability of other tests for positive selection (Fay, 2011), it is almost certainly better to use alternative methods.

Hahn's second tenet of the neutral theory is that linked selection affects few loci in the genome. Linked selection would not occur if there was never any positive or negative selection in the genome. Indeed, Kimura's work demonstrates that he expected genetic diversity to scale with population size without linked selection effects, as it would at mutation–drift equilibrium. However, this was because it represented the simplest case and there was a lack of evidence to the contrary during the bulk of his career. As such, the absence of linked selection is a simplifying assumption of some neutral models, but not a tenet of the neutral theory. Given that Ohta and Kimura (1971) had calculated that approximately 10% of amino acid substitutions could result from positive selection while still being consistent with neutral theory, and that purifying selection is a key facet of the neutral theory, both hitchhiking (Maynard Smith & Haigh, 1974) and background selection

(Charlesworth et al., 1993) have clear scope for reducing diversity within a neutral theory framework. Kimura (1983) did not find hitchhiking to be a plausible explanation for substitution patterns because the synonymous substitution rate did not appear to vary with the non-synonymous rate. Birky and Walsh (1988) later gave a mathematical explanation for why this would happen: theoretically, hitchhiking accelerates fixation of neutral mutations, but the probability of a neutral allele being fixed is the probability that it co-occurs with the positively selected mutation, and hence is exactly equal to its frequency in the population. Since the probability of fixation of a neutral allele is its frequency (Kimura, 1983), a result that is empirically robust despite the imperfect molecular clock (Bromham & Penny, 2003; Nei et al., 2010), linkage should not affect the long-term neutral substitution rate. Kimura instead argued that many large populations would not be at mutation-drift equilibrium, which would have the effect of reducing N_e. For example, a *Drosophila* species with a very large natural population (10^9) could be expected to have a substantially smaller effective size because population fluctuations reduce N_e, and, because the time taken to reach mutation-drift equilibrium is proportional to population size, equilibrium may never be met because it requires longer periods of population stability than could normally be expected (Ohta & Kimura, 1973). Indeed, even Drosophila populations with populations believed to be as large as 10^{14} have effective sizes on the order of 10^6 (Kaplan, Hudson, & Langley, 1989), which is only an order of magnitude higher than large mammal populations (Hawks, Hunley, Lee, & Wolpoff, 2000). Ecological traits may also impact N_e: Turner, Wares, and Gold (2002) found that effective and census sizes diverged by three orders of magnitude in a common marine fish that relies on estuaries for reproduction, while empirical studies of other species often find a single order of magnitude difference. Collectively, these observations suggest that predictions about diversity under neutral theory must be made with caution. While many basic neutral models describe phenomena at mutation-drift equilibrium, neutral theory itself does not predict that such an equilibrium should generally exist—nor what the ratio of N_e:N should be. The use of such models should be limited to cases in which equilibrium conditions can be demonstrated.

An additional observation that challenges the validity of the neutral theory is the over-dispersed mitochondrial molecular clock (Kimura, 1983; Langley & Fitch, 1974; Ohta & Kimura, 1971). Substitution events in coding sequences occur with greater variance in timing than expected under a random Poisson process, as would be expected in the simple case of a locus that is unaffected by positive selection (Fitch, 1971). Bursts of positive selection causing episodic fixation could account for the over-dispersion pattern (Gillespie, 1984a, 1984b), although other explanations are also possible. For example, Cutler (2000) showed that a deleterious mutation model could cause patterns consistent with patterns of molecular clock over-dispersion. Cutler's modelling also showed that the patterns produced by selective sweeps were inconsistent with an over-dispersed clock unless additional forces cause those sweeps to be irregular. Further work has shown that when protein-folding thermodynamics are taken into consideration, the number of neutral neighbouring sequences varies substantially along neutral evolutionary trajectories (Bastolla, Porto, Roman, & Vendruscolo, 2002). As changes in the number of available neutral sites means shifts in the neutral mutation rate, deviations can be expected through time from Poisson process expectations based on the average neutral mutation rate. Also, shifts in the strength of selection due to ecological, environmental, or demographic changes in a population can affect the regularity of fixation events without strong, positive selection (Bromham & Penny, 2003). Therefore, the over-dispersion of the molecular clock challenges the application of simple, strictly neutral models in which selection is absent, but does not give strong evidence in favour of a positive selection model over a nearly neutral model.

2.2.5 Moving beyond a dichotomy

The position that both adaptive and non-adaptive processes are essential to evolution has become widely accepted amongst the practitioners of many disciplines within evolutionary biology. This shift is illustrated by Hahn (2008) who, while proposing a selection-centred interpretation of molecular evolution, emphasises that "whatever the proximate causes of deviations from neutrality, the ultimate results are likely to be the retardation of adaptation and the fixation of mildly deleterious mutations" (p. 262). Regardless, some workers have continued to maintain a dichotomy between selection and

neutral theories, albeit through approaches that focus on redefinition rather than mechanism. For example, Ohta (1992, 2002) defines nearly neutral mutations as all those on which both selection and drift act, rather than mutations that fall into a bounded range of $N_e s$ products where population size influences the effectiveness of natural selection above a threshold value. Because genetic drift acts on all mutations in finite populations, Ohta thereby defines molecular evolution on mutations with non-zero selection coefficients as being governed by nearly neutral theory, resulting in a nearly neutral theory that is indistinct from wider evolutionary theory. Conversely, other workers interpret genomic patterns that stray from strict neutrality as evidence for the importance of selection at the genomic level: Hurst (2009), for example, describes the 'reach' of selection as the areas in a genome with patterns that have been affected by selection processes. This approach places an unjustifiable primacy on selection as both stochastic and deterministic forces act in tandem, yet stochastic forces are only considered important in the absence of selection. Also, this approach does not distinguish between positive and negative selection, yet negative selection is an integral part of neutral theory.

No mutation can be precisely neutral ($s = 0$), nor is any mutation unaffected by stochastic factors ($N_e \to \infty$). On these grounds, it has been argued philosophically that attributing fixation to selection or drift is incoherent for the fate of individual mutations (Clatterbuck, Sober, & Lewontin, 2013). Nevertheless, models can make population-level predictions about evolution's probabilistic processes at which level causality can be disentangled. Lynch, for example, makes distinctions between adaptive and non-adaptive processes (rather than selection and drift), and by formulating models for the interaction of mutation, selection and N_e has made valuable insights about the structure of genomes and genetic networks, and genetic and biochemical complexity (Lynch, 2006, 2007a; Lynch, 2007b; Lynch et al., 2011; Lynch & Conery, 2003; Lynch, Koskella, & Schaack, 2006). By considering adaptive and non-adaptive processes within a pluralistic evolutionary framework, rather than contrasting selectionist and neutralist theories, progress towards a fuller understanding of evolution's critical processes can be made.

The balance between stochastic and deterministic factors varies not only between loci, but at a single locus with time, ecological circumstance, or genetic background. Considering these well-recognised principles, there is little value in treating neutral or selection theories in isolation as complete models of genomic evolution. While, at their extremes, perhaps it remains fair to say that the "two schools of thought … could not be more antithetical" (Hurst, 2009, p. 2), at their extremes, both are mathematically convenient theories that represent idealised fragments of a biological reality. As such, they can be better viewed as complementary ideals rather than antithetical paradigms. Neutralism and selectionism should not be lenses through which to filter biology, nor hammers with which to pound ill-fitting results into predetermined shapes.

Our focus should turn to better understanding the loci and circumstances under which neutral or non-neutral dynamics are more likely to be prominent, and how the genomic forces shaping divergence and diversity patterns interact. While the majority of mutations that fix in a population may be effectively neutral, the stochastic mechanism that has fixed these mutations may vary across the genome and with N_e. We also need to better understand the genome-wide influence of selection, how populations respond to the dynamics of fluctuating sizes, and the proportion of mutations that have selection coefficients such that they are nearly neutral (i.e., those for which fixation probability is sensitive to population size change). There are also formidable and exciting challenges to link these molecular processes to other biological phenomena of interest such as speciation, extinction, and wider biological diversity patterns.

2.3 Species diversity patterns and their potential causes

2.3.1 Species diversity gradients

The study of global biotic diversity is a richly historied area of eco-evolutionary research. The longest-recognised pattern in macroecology is the latitudinal diversity gradient (LDG): the pattern of declining biodiversity from the tropics to the poles across a wide range of biomes, taxa, taxonomic ranks, and spatial scales (Hillebrand, 2004; Willig, Kaufman, & Stevens, 2003). The LDG fascinated the naturalists who founded modern evolutionary theory (Darwin, 1859; Wallace, 1878), yet determining its causes remains one of science's most prominent u nresolved challenges (Pennisi, 2005). As an almost-universal pattern (Hillebrand, 2004), and the subject of focussed, long-term scientific scrutiny (Palmer, 1994; Rohde, 1992), much is known of the LDG's many potential causes, allowing for increasingly specific hypothesis testing by contemporary researchers. In addition to the well-known latitudinal gradient in species richness, patterns are also evident within species (intraspecific genetic diversity, and numbers of subspecies), and taxonomic ranks above species (genera and families).

The LDG appears to be a long-term feature of Earth's biogeography. While the strength of the LDG has fluctuated through time and space, a discernible LDG has been detected in the fossil record since at least the Cambrian period (Powell, 2009). However, some evidence suggests it may have been recurrent rather than consistently present throughout the Phanerozoic (Mannion, Upchurch, Benson, & Goswami, 2014). Changes in the strength of the LDG through time may relate to changes in available biome area, or climate (Archibald, Bossert, Greenwood, & Farrell, 2010; Mannion et al., 2012; Powell, 2009). Indeed, the challenge for potential explanations for the LDG is to provide a framework consistent with the wide range of observations and scales over which observations have been made.

While Johann Forster (1778) wrote the earliest-known account of the LDG, in which he related his observations to climate, the first detailed description of latitudinal vegetation differences appears in Alexander von Humboldt's 1808 volume, *Views of Nature*. Von Humboldt's (1808/1850) observations deserve recognition for their depth. He noted that

changes in vegetation occurred across a gradient related to climate, stating that "organic development and abundance of vitality gradually increase from the poles toward the equator, in proportion to the increase of animating heat" (p 217). Although the notion of an "abundance of vitality" is vague, in other passages von Humboldt specified more precisely what he thought to be the causes of a relationship between climate and species richness. For example, he was convinced that the physiological consequences of seasonality, and below-freezing minimum annual temperatures, limited the taxonomic range and the forms that survived in temperate regions:

> "Nature undergoes a periodic stagnation in the frigid zones; for fluidity is essential to life. Animals and plants, excepting indeed mosses and other Cryptogamia, here [in the temperate north] remain many months buried in a winter sleep. Over a great portion of the earth, therefore, only those organic forms are capable of full development, which have the property of resisting any considerable abstraction of heat, or those which, destitute of leaf-organs, can sustain a protracted interruption of their vital functions. Thus, the nearer we approach the tropics, the greater the increase in variety of structure, grace of form, and mixture of colours, as also in perpetual youth and vigour or organic life." (p 215).

Interestingly, von Humboldt also noted that the high-elevation zones of the Neotropics contained many of the groups found in northern temperate regions, including close allies of European species. From this, he concluded that temperate biota were a subset of tropical biota, lacking what he considered to be the tropics' most remarkable and beautiful forms, such as palms, tree-ferns, and bananas. Indeed, he remarked that while "nature has permitted the native of the torrid zone to behold all the vegetable forms of the earth without quitting his own clime" (p 231), temperate inhabitants require "the glowing fancy of the poet, and the imitative art of the painter" to "enable the imagination to depict in vivid colours the images of an exotic Nature" (p 231). Thus, although a pre-evolutionary explanation, von Humboldt's observations of the LDG were set in a framework that incorporates many of the fundamental ideas that persist in our current understanding of the pattern, such as the influences of average temperature, temperature extremes, and seasonality. Further, by defining temperate biota as a subset of tropical biota that possess

specific 'properties' or adaptations to seasonal climates, he also identified a role for what is now referred to as niche conservatism in the LDG.

The generality of the LDG in time and space is uncommon for patterns within ecology. In a meta-analysis of approximately 600 latitudinal gradients, Hillebrand (2004) found the LDG persists across a wide range of eukaryotic taxa, biomes, regions, spatial extents, and ecological characteristics. LDGs were broadly similar regardless of hemisphere, dispersal mode, thermoregulation, and trophic level. Hillebrand also found that non-significant studies still had negative correlations between latitude and diversity, indicating a possible lack of power rather than fundamentally different processes. However, Hillebrand's meta-analysis also identified some variation: regional LDGs had larger effect sizes than local LDGs; terrestrial and marine LDGs had larger effect sizes than freshwater LDGs; and LDGs for large-bodied organisms had larger effect sizes than those for small-bodied organisms.

2.3.2 Diversity across time and space

Although latitude *per se* cannot cause the LDG, it is a convenient proxy for the many potentially causal environmental factors that change from the tropics to the poles. The environmental gradients that are hypothesised to play more direct roles in causing differential diversity, including: mean annual temperature, biologically available energy, primary production, seasonality, climatic harshness, and area (Currie et al., 2004; Mittelbach et al., 2007). Because globally these factors vary not only in space but in time, long-term, paleontological studies can offer particular insight into the constancy of the relationship between these factors and the diversity of various biotic groups. These deep-time approaches can help to decouple effects of factors that currently covary.

Studies of the LDG within the paleontological record have been divided on whether the LDG has been consistently present or has recurred multiple times through geological time during colder or more seasonal global conditions (Mannion et al., 2014). Mannion et al. point to several methodological issues in studies that have found a persistent LDG, and point out that the studies that have addressed those issues have instead reported periods

either without a strong LDG or with a temperate diversity peak. These variations have indicated the potential importance of area and temperature seasonality as drivers of the LDG over the long term. Further, Archibald et al. (2010) find that New World insect and dicot diversity during the Eocene followed a temperature seasonality gradient, rather than a mean annual temperature gradient.

Paleontological studies such as those reviewed by Mannion et al. (2014) raise a key consideration for those who wish to draw general conclusions about the LDG from contemporary climate and diversity data. While there is currently a strong relationship between mean annual temperature and diversity for many taxa, mean annual temperature and temperature seasonality are also currently strongly correlated. Given that during periods of globally reduced seasonality the LDG has been less distinct, and there may have been global peaks in diversity in temperature regions for some taxa (Mannion et al., 2012), the importance of the link between mean temperature and diversity in generating the LDG could potentially have been overstated in previous studies. This consideration is important because temperature seasonality and mean annual temperature are mechanistically distinct as causes of diversity patterns.

2.3.3 Diversity across spatial scales

The effect of scale on ecological patterns has become a central issue over the last several decades (Levin, 1992). Even if the effect of scale is not under direct investigation, studies of phenomena that can be observed at different scales should give explicit consideration of the focal scale to ensure that appropriate explanatory mechanisms are considered, and that results are not inappropriately extrapolated beyond the scale at which observations were made. The focal spatial scale of a study determines a range of factors that may not be immediately obvious, including the temporal scale on which observed patterns have been formed (Whittaker, Willis, & Field, 2001). Patterns found at local-to-landscape spatial scales typically correspond to short temporal scales, and tend to be formed by biotic interactions such as competition, locally relevant environmental gradients such as topography, and contingencies such as disturbance. At larger regional to continental scales, climatic factors and broad habitat availability may shape species occurrences, and

evolutionary mechanisms may affect the number and type of interacting species. Changes in such factors occur via mechanisms that operate over substantially longer timescales (Whittaker et al., 2001).

Accordingly, spatial scale is a primary consideration in studies of biological diversity. The most widely investigated diversity relationships is the (net primary) productivity–species-richness relationship (PSR). It is worth noting that net primary productivity (NPP) is a complex variable, which is influenced strongly by climate at large spatial scales (e.g., Nemani et al., 2003), where there is considerable variation in water and energy availability, and accordingly NPP correlates with the water-energy balance (Lieth, 1976). At small scales, productivity reflects a range of biotic and abiotic factors, many of which are unrelated to climate (Grace, Adler, Harpole, Borer, & Seabloom, 2014). At regional and continental extents, at coarse grain sizes, and amongst both plants and animals, positive PSRs are common (Cusens, Wright, McBride, & Gillman, 2012; Gillman & Wright, 2006). The methods and, consequently, the results of synthetic reviews and meta-analyses of PSRs have been diverse and have resulted in divergent opinion regarding the form and generality of PSRs (see Gillman & Wright, 2010; Gurevitch & Mengersen, 2010; Lajeunesse, 2010; Whittaker, 2010 and related papers). In particular, while at large-scales terrestrial PSRs are typically positive, it is unclear to what extent small-scale patterns are congruent (Adler et al., 2011). Given the above, even when large-scale and small-scale studies recover similar forms of the PSR, they are unlikely to have the same causes. Specifically, as grain size increases, the measure of diversity itself changes from alpha, or point, diversity to gamma diversity, and the ecological interactions that shape local diversity patterns appear to be contributors rather than ultimate explanations of global diversity patterns (Currie et al., 2004; Rohde, 1992). Instead, complex, emergent processes involving net diversification and historical contingency are likely to be increasingly important at larger scales, reflecting the influence of longer time scales on patterns at larger spatial scales (Whittaker et al., 2001). Despite this, small-scale ecological mechanisms have been invoked previously to explain global diversity patterns at the broadest scales. For example, Huston (1999 and references therein) argues that global plant diversity gradients can be explained by the local-scale mechanisms of competition and competitive exclusion.

The central importance of scale to diversity patterns can be illustrated by considering the contrasting effects of dispersal on species richness at different scales. At small spatial and temporal scales, dispersal can have a positive effect on diversity, through increased immigration rates. Even at regional scales, dispersal can boost the diversity of an initially depauperate biota, which can also have long-term effects on subsequent diversity (Buerki et al., 2012). On these scales, the local species pool is increased, and therefore dispersal has a positive diversity effect through an ecological mechanism. However, on evolutionary timescales, coarse-scale diversity can instead be elevated in a biota comprised of poor dispersers: low migration between subpopulations reduces gene flow, and increases the rate of speciation. Provided incipient and new species do not quickly go extinct, this pattern increases net diversification and elevates species richness (Jocque, Field, Brendonck, & De Meester, 2010; Salisbury, Seddon, Cooney, & Tobias, 2012). Indeed, diversification in the Amazon basin is linked to the formation of the Amazonian river system, which reduced dispersal between previously connected populations across the basin (see 2.3.4 Causes of species richness variation: Population structure).

At large scales, productivity has been invoked to explain global gradients in species richness, and is amongst a suite of metrics related to water, energy and climate that are strongly correlated to macro-scale diversity. There are several alternate theories that invoke environmental energy or biologically available energy at large scales as a causal factor in shaping species richness patterns such as the LDG. However, many of those theories are either unsupported by the available evidence or have yet to be clearly and mechanistically tested (Currie et al., 2004; Evans, Warren, & Gaston, 2005). As with smaller scales, it is unclear what the link is between species richness and key climatic factors such as temperature, rainfall, evapotranspiration, and productivity. Because these variables correlate with each other, untangling cause from spurious correlation is challenging. Further, how these patterns change with scale remains incompletely explored. The role of energy is explored in more detail below.

2.3.4 Causes of species richness variation

Macro-scale species richness variation is ultimately linked to abiotic factors such as climate and area that cause differences in either ecological limits to species richness, time for diversification, or the rate of net diversification—the difference between speciation and extinction rates (Mittelbach et al., 2007). The difficulties in separating these causes lies in several key factors. An overarching consideration is of the abiotic factors themselves. A widely recognised abiotic covariate of species richness is environmental energy, which is frequently causally linked to the LDG through various potential mechanisms (Evans, Warren, et al., 2005). The contemporary species richness correlation with climate is substantially stronger than the correlation with area, but it is not clear if contemporary or historical area should be more important to species richness patterns (Fine & Ree, 2006). Similarly, historical climate could be more important than contemporary climate (Wiens & Donoghue, 2004). And, while energy is strongly correlated with species richness, the potential effects of solar energy input fall into at least three distinct categories: solar energy controls the total resource availability to communities that derive their energy from photosynthesis; solar energy is a primary influence on ambient temperature, which in turn controls a wide range of biologically relevant chemical reaction rates; and, the variation in energy flux, considered through measures such as temperature seasonality and isothermality, controls temperature stability and the portion of the year in which temperatures are above freezing, preventing tissue damage and enabling biochemical processes to occur. Each of the effects of energy could affect species richness through different mechanisms, including controlling the number of individuals in communities, setting the 'pace of life', or shaping adaptive evolution. Not only can abiotic factors affect more than one of the causes of species richness variation, these causes are not mutually exclusive. Finally, the causes, too, may overlap mechanistically even though they are often treated as being separate (e.g., Evans, Warren, et al., 2005). For example, ecological limits to species richness affect net diversification rates if species richness approaches those limits, creating a dynamic equilibrium (Rabosky & Hurlbert, 2015). A mechanistic distinction can only be consistently maintained if we limit the consideration of net diversification rate as a cause of species richness patterns under non-equilibrium conditions, for example when

energy directly causes a net diversification difference by affecting mutation rates, and in turn speciation rates.

While a range of ecological factors can affect species richness, those that do not affect net diversification rates (e.g., coexistence, or immigration rates) fail to predict the degree of disparity in species richness between tropical and temperate biotas (Currie et al., 2004). For example, while tropical communities may harbour more individuals than temperate communities, the increase in species richness towards the equator is steeper than the increase in individuals, indicating the total density of individuals alone is not causal (Storch, 2003). However, ecological limits to diversity can affect net diversification rates through diversity-dependent feedbacks to speciation or extinction rates (Rabosky, 2009; Rabosky & Glor, 2010; Rabosky & Hurlbert, 2015), provided there is sufficient species saturation for these effects to occur. Currently, there is not strong or consistent evidence that continental species richness is sufficiently close to a limit for density-dependent effects to play an important role in net diversification (Harmon & Harrison, 2015). Also, the importance of biotic interactions in the tropics can lead to a competition–dispersal trade-off, where net diversification is increased in the tropics due to lower migration rates and stronger subpopulation structure (Jocque et al., 2010; Salisbury et al., 2012). Hence, ecological factors can play a role in generating species richness variation, but do so by mediating the effects of climate differences on net diversification rates.

The mid-domain effect

Given a bounded geographic area, a gradient in species richness towards its centre mass may arise through the interaction of the geometric constraints of the landmass with species ranges. Current landmass distributions might therefore cause a latitudinal gradient in species richness without an effect of climate (Colwell & Lees, 2000). The mid-domain effect is a null model for species richness because it excludes climate factors as determinants of species richness patterns to establish if patterns are explainable in their absence. Although there has been a growing body of evidence that mid-domain effects cannot explain the LDG (see Hawkins, Diniz-Filho, & Weis, 2005 and references therein), as Colwell, Rahbek, and Gotelli (2004) point out, the purpose of a null model is not to explain all or none of a

phenomenon, but to establish the contribution of different effects. Therefore, geometric constraints may play a role in shaping the LDG in addition to the effects of climate and other factors (but see Hawkins, Diniz-Filho, & Soeller, 2005; Zapata, Gaston, & Chown, 2005).

Time and area

The tropics have covered a larger extent than the temperate regions for long periods of evolutionary history, notably through the Eocene. This gives rise to the time and area hypothesis, in which there has been greater evolutionary opportunity for diversification in the tropics (Mittelbach et al., 2007), reinforced by evolutionary lags caused by tropical niche conservatism (Wiens & Donoghue, 2004). This may cause differences in species richness because, with all else being equal, larger areas can harbour more species than smaller areas (Coleman, Mares, Willig, & Hsieh, 1982; MacArthur & Wilson, 1967). As such, this is a particular case of ecological limits to diversity, where limits are directly set by area rather than being set directly by climatic factors relating to available energy, as might otherwise be posed for the LDG (see Wright, 1983). Further, with all else being equal, older areas can harbour more species than younger areas (Borges & Brown, 1999). Variation in relative tropical and temperate area (and the intensity of temperature seasonality) over geological time may cause periods of elevated extinction as local climates change, suitable habitat shifts latitudinally, and biome areas expand and contract. For example, during the warmer periods leading into the Eocene, temperate diversity was restricted due to the contraction of temperate extent, and during the transition to a cooler climate during Eocene seasonality was lower than present seasonality, affecting the strength of the LDG and the groups of organisms present in temperate latitudes (Archibald et al., 2010). As such, the current extent and climate conditions of Earth's temperate conditions are relatively recent, and must be populated with those clades that inhabited the reduced temperate extents previously, or those that escape their tropical climate niches.

If the time and area effect is a primary cause of the LDG rather than being incidental or having a secondary, reinforcing importance, it would occur with no intrinsic difference in the capacity of equal-area tropical and temperate regions to support diversity. Therefore,

we might expect to see reversals of the LDG in the deep past after long cold periods in which the temperate extent has been greater than the tropical extent. Clades arising in temperate periods should have mid-latitudinal diversity peaks. Also, given current land area distribution there should be at least equal per-lineage net diversification in temperate and tropical regions. On the first point, there is limited support (Mannion et al., 2014), although the strength of the LDG has certainly fluctuated over geological time. On the second point, clades arising during colder periods have shallower LDGs than clades arising during warmer periods (Romdal, Araújo, & Rahbek, 2013), although there are a paucity of clades that do not exhibit at least a weak LDG (Hillebrand, 2004), and when such cases have been studied, there is evidence of reduced tropical diversification rather than increased temperate diversification (Krug, Jablonski, & Valentine, 2007). The third point is contradicted by current evidence of greater tropical origination of recent clades (Jablonski, Roy, & Valentine, 2006) and ongoing higher temperate extinction rates (Weir & Schluter, 2007). In fact, despite the likely importance of niche conservatism, most clades arise in the tropics, with some subsequently expanding their ranges into temperate zones, while clades of temperate origin rarely invade the tropics (Jablonski et al., 2013; Jablonski et al., 2006). Given that summer temperatures are relatively invariable up to 40 degrees from the equator (Figure 2a), while winter temperatures follow a strong latitudinal gradient (Figure 2b), climate niche conservatism should play a lesser role in temperate-to-tropical expansions than the reverse. Hence, other factors—most likely biotic—must play a more substantial role in restricting such expansions than niche conservatism. Nevertheless, because the earth has been predominantly tropical over the history of multicellular life, most clades for which we have LDG data arose during tropical periods, and there has been greater opportunity for diversification in tropical environments (Romdal et al., 2013). As such, time and area, reinforced by niche conservatism, almost certainly play an important role in shaping diversity patterns, but do not stand as a complete explanation.

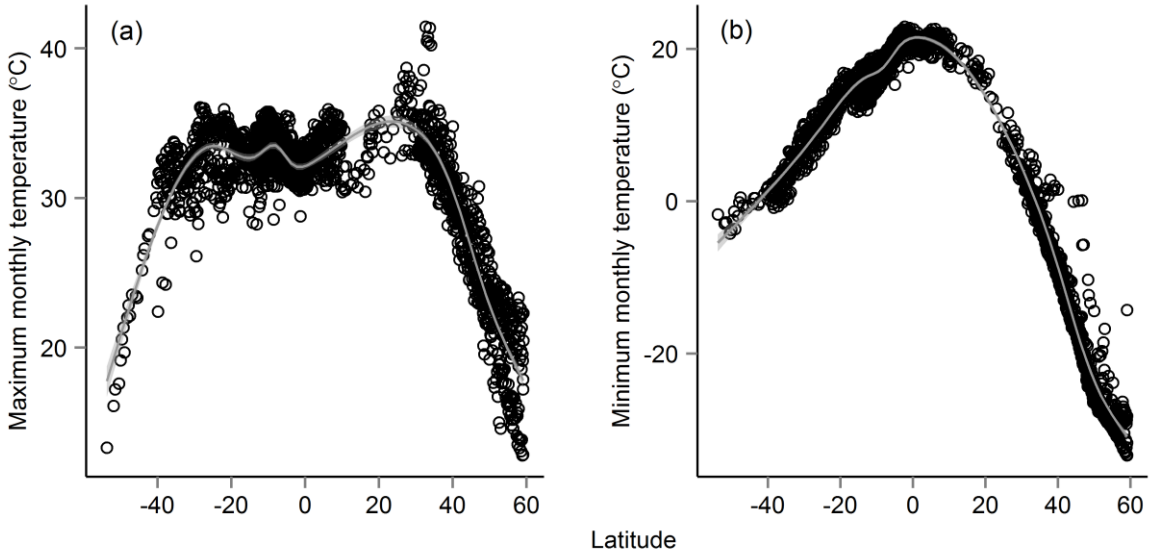

Figure 2. Disparities in the latitudinal gradients of (a) hottest and (b) coldest months of the year in the New World. Points represent average values within 10,000km^2 equal-area hexagonal cells across continental North and South America up to 60°N and below 500 metres above sea level. Estimates were derived in ArcGIS 10.1 (ESRI, 2011). Temperature data derived from BIOCLIM dataset (Hijmans et al., 2005) as raster data in 2.5 arc-minute resolution.

Net diversification rates

A latitudinal gradient in rates of net diversification has been demonstrated using diverse phylogenetic methods in a wide range of taxa, such as palms (Svenning et al., 2008), flowering plants (Jansson & Davies, 2008), bivalves (Jablonski et al., 2006), butterflies (Condamine, Sperling, Wahlberg, Rasplus, & Kergoat, 2012), birds (Cardillo, Orme, & Owens, 2005), amphibians (Wiens, 2007), and squamates (Pyron, 2014). Nonetheless, not all studies have consistently found elevated tropical net diversification rates. For example, a study of 111 phylogenies across mammals, birds, insects, and angiosperms found that transitions between tropical and temperate environments typically led to decreased diversification regardless of direction, rather than increasing after temperate-to-tropical transitions (Jansson, Rodríguez-Castañeda, & Harding, 2013). A further study detected no latitudinal difference in net diversification for 232 mammalian genera (Soria-Carrasco & Castresana, 2012), and suggested either small, undetected differences existed or other factors such as dispersal dynamics may be important in generating diversity patterns (also see Roy & Goldberg, 2007). Such patterns could be particular to mammals as they show only a weak LDG for orders other than Chiroptera (Buckley et al., 2010). However,

subsequent research has detected higher tropical net diversification in mammals at taxonomic ranks higher than genus (Rolland et al., 2014). While methods differences may underpin these divergent results, net diversification rates may also vary between taxonomic levels, and may not be consistent between all clades.

While patterns of net diversification rate variation have been detected in many taxa, the underlying patterns of speciation and extinction are not necessarily consistent. Indeed, the net diversification patterns that have been detected in mammals are both variable and method-dependent. Of three recent studies that employed phylogenetic methods to estimate speciation and extinction in mammals, two studies have found elevated net diversification in tropical taxa (Rolland et al., 2014; Weir & Schluter, 2007), and one found no difference (Soria-Carrasco & Castresana, 2012). However, different patterns of speciation and extinction rates underlie the former two studies. Rolland et al. (2014) found elevated net diversification in families and orders of mammals was driven by both elevated speciation and reduced extinction in the tropics, while Weir and Schluter (2007) found both speciation and extinction rates to be lower in recent tropical taxa, with a larger gap between the rates in the tropics resulting in higher net diversification. Weir and Schluter also found tropical taxa to be older on average than temperate species, and thus it is unclear to what extent their result was driven by the systematic undersplitting of tropical species (Tobias, Bates, Hackett, & Seddon, 2008). Weir and Schluter examined New World mammals and birds, for which there is evidence of substantial taxonomic undersplitting in the tropics. Mitochondrial divergence is greater than 10% within numerous Amazonian bird populations currently recognised as single species (Naka, Bechtoldt, Henriques, & Brumfield, 2012). Although Weir and Schluter found elevated temperate speciation rates, they were examining recent rates from sister species, which could be elevated in temperate zones following Pleistocene glacial extinctions. Also, their model assumes constant speciation and extinction rates, and when this assumption is relaxed, no speciation rate difference is detected (Rabosky, Title, & Huang, 2015). Consistent with this, Pyron (2014) also found higher extinction rates in temperate squamates were the cause of the LDG, rather than elevated tropical speciation rates, while Pyron and Wiens (2013) found that both higher speciation and lower extinction rates in the

tropics were causal in the LDG of amphibians. High diversity in the Californian flora has also been linked to low extinction rates (Lancaster & Kay, 2013). While some phylogenetic methods that estimate extinction rates from extant species produce unreliable results if diversification rates vary across the phylogeny (Rabosky, 2010), or through time via ecological limits (Rabosky, 2009), many recent net diversification studies have used more flexible methods to address this issue by allowing rate variations across trees, and allow negative diversification rates (Lancaster & Kay, 2013; Pyron, 2014; Pyron & Burbrink, 2013; Pyron & Wiens, 2013). Nevertheless, extinction rate estimates have wider confidence intervals than speciation rate estimates, even when all model assumptions are met (Rabosky, 2014).

Adding to uncertainty over the accuracy of phylogenetic estimates of speciation and extinction rates, phylogenetic rate estimates can differ substantially from those inferred from the fossil record (Quental & Marshall, 2010). Typically, the fossil record shows higher rates of extinction than do phylogenetic methods (Morlon, Parsons, & Plotkin, 2011). However, as the divergent results for speciation and extinction rates across phylogenetic mammal studies show, patterns of net diversification can vary by taxonomic level. Morlon et al. (2011) find that cetacean diversification is similar between fossil and phylogenetic estimates provided phylogenetic patterns are investigated at the family level, and net diversification is allowed to vary across the phylogeny. However, when studied as a whole, cetacean phylogenetic estimates of net diversification suggest low extinction rates because the signal of extinction is lost under rapid radiations of certain clades. Hence, appropriately selected taxonomic levels and flexible analytic methods allow phylogenetic estimates of diversification that are consistent with the independent patterns in the fossil record.

Similar to phylogenetic studies, studies of net diversification in the fossil record show variable patterns of extinction and speciation contributing to overall net diversification differences. A study of the Neogene fossil record in Old World mammals indicated greater net diversification in small versus large mammals was linked to lowered extinction rates in small mammals, despite higher origination rates in larger mammals (Liow et al., 2008).

Liow et al. suggest that high turnover rates in large mammals lead to a truncated distribution of ages, and hence lower overall diversity. Also, using the bivalve fossil record, Jablonski et al. (2006) show that the bivalve LDG is a result of net diversification processes where both speciation rates are higher in the tropics, and extinction rates lower. Jablonski et al. find that bivalve genera and subgenera originate more often in the tropics, and subsequently disperse into temperate zones, while retaining their tropical range. This model has also received phylogenetic support from a study finding that latitudinal expansions into different climates happen frequently in New World birds (Jansson et al., 2013).

While net diversification rates studies are largely consistent in finding elevated topical net diversification rates, consistency of the speciation and extinction rates that comprise net diversification are somewhat less clear. Determining these rates requires substantially more assumptions, which may explain the variation across studies. Nevertheless, the evidence converges on elevated tropical net diversification resulting from lowered tropical extinction rates, with more equivocal evidence for elevated tropical speciation rates.

Geography and population structure

As a component of speciation and extinction dynamics, population substructure is a further component of net diversification needing consideration. It has long been appreciated that geographic barriers play important roles in ecology and evolution by influencing population structure, and that this could be important for understanding tropical diversity (Wallace, 1878). Geographic barriers may restrict ranges and the formation of barriers may subdivide populations, reducing gene flow and increasing genetic divergence between demes. The reduced N_e caused by population subdivision and the resultant increased inbreeding may further promote rapid change and karyotypic variation (Bush, Case, Wilson, & Patton, 1977). However, the evolutionary dynamics of these subdivided populations remains poorly understood, and the roles played by natural selection and genetic drift in their divergence have yet to be well established (Coyne & Orr, 2004; Gavrilets, 2003; Mayr, 1963), and may vary with circumstance. Regardless of the precise mechanism, it is generally accepted that given sufficient time and sufficiently

reduced migration, geographical barriers to gene flow are reinforced by more permanent biological barriers, resulting in bifurcation or multifurcation of species and therefore contributing to patterns of species richness. Therefore, if there is a greater propensity for population subdivision in the tropics, this could be a contributing factor to the elevated species richness found there (Barraclough & Savolainen, 2001; Davies, Savolainen, Chase, Moat, & Barraclough, 2004).

The influence of substructure on natural selection in a metapopulation depends on migration rates between demes, and the size and number of demes. Glémin, Ronfort, and Bataillon (2003) developed a metric for the effective population size of selection, N_{e} for the effect of deleterious mutations. In the case of the infinite island model, where a metapopulation comprises an infinite number of demes, $N_{e} = N(1+m/hs)$, where N is deme size, m is migration rate, and h and s are the dominance and selection coefficients of arising deleterious mutations respectively. Following this definition, $N_{e} > N$ for non-neutral mutations given some degree of migration ($m > 0$). Therefore demes experience greater selection efficiency than would independently evolving populations of the same size (i.e., the case when $m = 0$, $N_{e} = N$). In the case of finite demes, Glémin et al. also show the difference between demic N_{e} and N is minor when there are few demes, and N_{e} is always lower in the metapopulation than it would be in a panmictic population. Hence, as the greater the isolation of demes, the more they evolve like separate, small populations.

Because demes are subsets of a metapopulation, they are expected to have elevated extinction risk relative to a single, unstructured population. The occurrence of such extinctions also reduces metapopulation N_{e} (Nei, 1976). Cherry (2004) shows that as extinction and recolonisation events increase between demes, the effectiveness of selection is reduced in a similar fashion to small, unstructured populations. While on one hand genetic diversity can be expected to increase in a metapopulation as gene flow decreases (increasing at least one measure of N_{e}), on the other demic extinction also reduces genetic diversity (Maruyama & Kimura, 1980). As stochastic events, demic extinctions further reduce the effectiveness of selection (Cherry, 2004).

In addition to demic extinction, metapopulations as a whole may also stand a greater risk of extinction than equivalently sized, panmictic populations. Attempting to realistically simulate metapopulation extinction risk, Higgins and Lynch (2001) modelled population structure, demographic and environmental stochasticity, and genetic variation to determine their joint effects on effective population size and selection efficiency. Their model suggests that highly structured metapopulations with small dispersal ranges are at additional risk of extinction compared to panmictic populations of equivalent sizes. Their simulations indicate that demographic fluctuations are important causes of extinction in small metapopulations, while the accumulation of mildly deleterious alleles in larger metapopulations poses a risk because of reduced selection efficiency. Even large metapopulations have relatively small N_e when they are highly structured, and therefore are predicted to experience relative high extinction risk compared to panmictic populations.

Dispersal ability affects the frequency of migrants between subpopulations, and therefore the effectiveness of selection. Following Glémin et al. (2003), a linear relationship between $N♀$ and migration rate is expected when other variables are static, seen by expanding the expression for $N♀$ given above into a linear form, $N♀ = mN/hs+N$. However, while migration reflects dispersal ability, dispersal ability itself probably has a complex relationship with the environment, such as non-linear relationship with distance between demes. Moore, Robinson, Lovette, & Robinson (2008) experimentally investigated dispersal abilities of ten tropical bird species across distances of 100m, 200m and 300m. Their results show that dispersal success for species with low or intermediate dispersal ability decayed rapidly and non-linearly as dispersal distance increased (their Figure 1). This non-linear pattern and its effect on N_e is exemplified in the simulations of Higgins and Lynch (2001), who find that threshold levels exist in dispersal distances that substantially affect metapopulation N_e. When demes are separated by distances that exceed certain critical, threshold distances determined by dispersal ability, migration levels reduce substantially and demic populations began to accumulate slightly deleterious mutations more rapidly.

The flora and fauna of northern Amazonia divide into well-recognised zones of endemism (Cracraft, 1985). Zones of endemism are defined as areas where multiple species share distinct range boundaries, often marked by defined geographic features, such that there is usually a substantial turnover in species found in different zones. While Cracraft (1985) formalised the Amazonian areas of endemism, the relationship between species' distributions and geographic features in the region have been recognised for much longer. In his early biogeographical work, Wallace (1852) noted the extent to which large Amazonian rivers impose range limits on various groups, describing in particular the divide in primate species on either sides of Rio Negro. Wallace further stated that local Amazonian peoples were aware of the sharp species boundaries imposed by rivers in the region.

Various hypotheses have been proposed for the formation and maintenance of species distributions in these zones, and these hypotheses have often been causally linked to the overall regional diversity. Haffer (1969) identified Pleistocene climate change as a driver of Amazonian bird richness. Haffer proposed that Pleistocene dry periods reduced Amazonian forest to isolated patches that acted as refugia for forest-dependent species. While these refugia were proposed to be periodically reconnected during the Pleistocene's more humid periods, Haffer argued that their isolation was sufficient to promote allopatric speciation and therefore act as centres of speciation. Haffer's explanation contrasted with previous explanations for Amazonian plant diversity that had assumed widespread, long-term forest stability (e.g. Fedorov, 1966) and low extinction rates (Ashton, 1969) were causal mechanisms. Although Haffer initially proposed the refuge hypothesis as an explanation for animal distributions, similar distributions patterns were also identified in several plant families (Prance, 1973, 1982). However, plant sampling efforts have been unevenly distributed across the region, and as Nelson, Ferreira, da Silva, & Kawasaki (1990) point out, the rarity of many Amazonian species is likely to give highly studied regions an appearance of significant floristic diversity that may be no more than a sampling artefact. More directly problematic, the refugia hypothesis predicts the Pleistocene to be a period of elevated origination amongst species yet in a review of the origination of 1400 Neotropical species and reciprocally monophyletic, genetically distinct

subspecies, no such pattern is evident (Rull, 2008), and molecular dating indicates the splitting of sister species of the western Amazonian lowlands frequently pre-date the Pleistocene (Moritz, Patton, Schneider, & Smith, 2000). Lowland diversification rates may, in fact, have slowed in Neotropical birds during the Quaternary (Weir, 2006). Further, species have individualistic responses to climate change, rather than ecosystem or community responses, and there is a lack of palynological evidence for widespread loss of forest cover in response to reduced rainfall during the Pleistocene (Bush, 1994; Colinvaux, De Oliveira, & Bush, 2000; Colinvaux, de Oliveira, Moreno, Miller, & Bush, 1996).

The major period of uplift in the northern Andes is fundamental to the broad picture of Amazonian species richness. The lower western slopes of the Andes contain the zones of highest avian diversity in the Neotropics (Hoorn et al., 2010). The lowland zones of endemism across the Amazon basin appear to be strongly influenced by the riverine barriers of the wide, lower reaches of the major rivers, or by marine incursion (Ayres & Clutton-Brock, 1992; Capparella, 1991). Ribas, Aleixo, Nogueira, Miyaki, and Cracraft (2012) found that the phylogeographic history of the trumpeters (Aves: *Psophia*) is consistent with the proposed development of the Amazonian river basin during the Pliocene and Pleistocene (e.g. Latrubesse et al., 2010) rather than an earlier commencement during the late Miocene (e.g. Figueiredo, Hoorn, van der Ven, & Soares, 2009). The trumpeters comprise eight species that diverged in vicariant events relating to the development of the Amazonian river system. The progressive, western-to-eastern development of Amazonian tributaries south of Rio Amazonas formed the dispersal barriers that divide the zones of endemism identified by Cracraft (1985), in which the trumpeter species are found. Further subdivision is evident for at least one species within the zone of endemism it inhabits (i.e., *P. napensis* within the Napo region), although no additional population substructure was found within the Guianan species (*P. crepitans*) despite its range spanning the Rio Branco, which acts as an important barrier for a number of bird species inhabiting the Guiana Shield (Naka, 2011).

Amazonian birds are typically more sedentary than temperate birds (Mayr, 1969), and migratory behaviour is less prevalent in species with tropical breeding ranges, although

migration within the tropics does occur and has received less attention than Nearctic–Neotropical migration (Rappole, 1995). In the wood-warbler family (Parulidae), breeding latitude is strongly associated with migratory behaviour (Winger, Lovette, & Winkler, 2012), which is an evolutionarily labile behaviour, and not strongly phylogenetically constrained within the wood warblers. Basal and peak metabolic rates are lower in tropical than temperate birds (Wiersma, Chappell, & Williams, 2007; Wiersma, Muñoz-Garcia, Walker, & Williams, 2007), and tropical species have smaller flight muscles, hearts and lungs (Wiersma, Nowak, & Williams, 2012). In a study of dispersal ability, a number of tropical forest bird species were unable to consistently traverse a 100m water barrier, and half of tested species failed to disperse across 300m in every trial (Moore et al., 2008). Develey and Stouffer (2001) note that rarely used roads that have canopy breaks of 10–30m form dispersal barriers to understory birds, indicating an important behavioural component in addition to the physiological component for reduced tropical species vagility, a point also noted in island birds (Diamond, 1981). Furthermore, high tropical diversity correlates with the prevalence of niches in the tropics that are associated with limited dispersal (Salisbury et al., 2012). These factors provide several lines of evidence that dispersal barriers are indeed greater in the tropics, and at least partly for intrinsic ecological reasons (Janzen, 1967).

While oceans, the lower reaches of major rivers, and mountain ranges have long been recognised as important dispersal barriers, it has been recently demonstrated that relatively small geographic barriers may also substantially reduce migration and affect population structure in tropical birds (Naka, 2011). Such barriers include breaks in continuous forest cover, medium rivers, and individual mountains. For 14 bird species with ranges divided by the Rio Negro, genetic divergence between divided populations was similar at its widest reaches and its headwaters, despite the comparative ease with which the latter could presumably be crossed (Naka, 2010). This suggests that relatively fragmented tropical landscapes may have important effects on the population structure of their inhabiting species and their evolution.

Naka, Bechtoldt, Henriques, and Brumfield (2012) identified distinct eastern and western avian faunas within the Guiana Shield, northern Amazonia that are divided either by Rio Branco or Rio Negro, or that replace one another in Branco–Negro interfluvium. Although not classically considered a major river barrier to the extent of the better-known rivers in the region, Naka's (2011) analysis of barriers to avian dispersal in the Guiana Shield indicates Rio Branco is an important dispersal barrier. The western region of the Guiana Shield is marked by numerous dispersal barriers within zones of endemism, while the eastern region is relatively contiguous and has probably been historically more stable (Fouquet et al., 2012; Naka, 2011). In addition to river and other landscape dispersal barriers, Fouquet et al. (2012) note the importance of quaternary forest refugia to explain the phylogeographic patterns within eastern Guiana Shield frogs.

Hence, certainly for the Amazonian biota, there is evidence that the high levels of species richness are at least partly influenced by the barriers to dispersal, and the propensity for limited dispersal in many Amazonian organisms. Given that the N_e of selection should decrease under such circumstances—thereby increasing extinction risk—further consideration should be given to understanding how the persistence of such Amazonian species occurs such that species richness is substantially elevated.

2.3.5 Prominent hypotheses for the latitudinal diversity gradient

The LDG has generated an abundance of explanations that employ the above and additional causal mechanisms. Palmer (1994) identified 120 hypotheses in the literature that he considered plausible. Despite 200 years of inquiry, almost three-quarters of these were proposed within the decade before Palmer's publication. Although they have not been enumerated in the same way since, many additional hypotheses have been added in the two decades after Palmer's paper, resulting in a literature that has been weighted towards continuous speculation rather than systematic testing. However, as the premises of these many hypotheses are related, they may be grouped in classes that allow for more systematic analyses (Rohde, 1992). Because of the large numbers of hypotheses, and because many of these are considered circular, inconsistent with data, or limited in their scope, I will restrict the discussion to a smaller number of prominent hypotheses. It is not

within the scope of this review to re-consider hypotheses that already either have been falsified or shown to insufficiently generalise, and indeed detailed reviews of many other hypotheses are available in the literature (Currie et al., 2004; Dowle et al., 2013; Evans, Warren, et al., 2005; Palmer, 1994; Pianka, 1966; Rohde, 1992; Schemske, 2002; Willig et al., 2003). Because the time/area hypothesis has already been discussed above and deemed unlikely as the principal cause of the LDG, it will not be explored separately here, although additional discussion is provided in the environmental harshness section below.

Species–energy theory

One of the most intuitive concepts regarding diversity gradients is that we would expect to find more species in areas where there is more biologically available energy, because a greater energy supply can support more individual organisms. Given more individuals, more minimum viable populations can be supported, allowing the stable co-existence of a greater density of species. This mechanism under species–energy theory is referred to as the more-individuals hypothesis (Hurlbert & Jetz, 2010; Wright, 1983). Wright's (1983) initial formulation of species–energy theory is a modification of the equilibrium model of island biogeography (MacArthur & Wilson, 1967), which models species richness as a balance between immigration and extinction rates controlled by island isolation and area respectively. Because area can be considered a surrogate for resource and habitat availability, Wright modelled species richness as proportional to per-unit-area energy production × area, such that species richness is predicted by available energy, which he defined as the rate of resource production for a target taxon. In this sense, Wright's model recalls the original ideas of Forster (1778), who linked diversity to both climate and area. Hurlbert and Jetz (2010) noted that while Wright's model recognises that energy is an important correlate of species richness, it assumes equivalence between energy and area. However, species–energy curves are typically steeper than species–area curves (Hurlbert & Jetz, 2010; Storch, Evans, & Gaston, 2005; Wylie & Currie, 1993), indicating unequal effects of energy and area on species richness. Equal effects would be expected if the effect on species richness was mediated by an increase in the number of individuals (hence, Wright's formulation is known as the more-individuals hypothesis). However, Hurlbert and Jetz (2010) note than area and energy might affect species richness differently even if

those effects are mediated through increased population sizes: energy affects population size via population density, whereas no density effects occur with area. Schuler, Chase, and Knight (2015) show that in experimental zooplankton communities, energy affects both species–area relationships (SARs) and densities of individuals, and that an important interaction between energy and area occurs. In high energy environments, they found the density of individuals and number species both increased with area, while in low-energy environments, neither of these factors were affected by area.

Within broad clades, there must be upper limits on density above which energy cannot continue to have an effect. Indeed, while increased resource availability appears to increase abundances at local scales where energy is limiting (notably in ants, Kaspari, O'Donnell, & Kercher, 2000), there are also cases in which there is no definitive association between macro-scale energy availability and population densities. For example, tree species richness—but not stand densities—are substantially higher in the tropics than temperate zones (Currie et al., 2004). More generally, Currie et al. show that there is a stronger correlation between available energy and species richness than between either available energy and individuals, or individuals and species, indicating that there is not a prominent causal pathway from energy to individuals to species richness as predicted by the more-individuals hypothesis. Effects of climate on species richness are not consistently mediated through increased population densities, and—at least in some clades—generate higher species richness in energetic areas despite lower population densities (Storch, 2003).

Terborgh et al. (1990) found similar overall breeding bird densities in Peruvian tropical forest and North American temperate forest. At the tropical site alpha diversity peaked at five times the level of temperate uniform forest diversity, average body mass was higher, and entire feeding guilds were present that were absent in the temperate forest. Many of the tropical species were rare, with a third having densities lower than 1 breeding pair per square kilometre. Similar individual densities and proportions of rare species have been found in other Amazonian forests (Thiollay, 1994, 1999). While breeding season bird densities may be relatively invariant, it is also worth noting that results are season dependent: there is a positive productivity–density relationship in the non-breeding, or

wintering, ranges of North American birds (Meehan, Jetz, & Brown, 2004). Nonetheless, changes in bird species densities are substantially greater than individual densities between low and high energy environments. Tropical species have smaller populations on average than temperate species, in contradiction to the more-individuals hypothesis, under which tropical and temperate population sizes should be similar. Thus, increases in species richness are not mediated by numbers of individuals, but by other mechanisms.

If densities in broad taxonomic groups such as trees and birds are relatively invariant with environmental energy, the fate of additional energy becomes of interest: why does more energy fail to translate into more individuals in the tropics? First, for trees, additional tropical energy largely manifests as lower intra-annual energy variation rather greater peak energy flux, and therefore causes a longer growing season. Thus, rather than accommodating more individuals, this energy is used year-round by existing individuals. Boreal and temperate trees may be of similar size to tropical trees, but their annual production is substantially lower (Luyssaert et al., 2007). The growing season follows a latitudinal gradient, and because canopy trees all live on timescales much greater than these annual cycles, lower intra-annual variation can only result in longer metabolically active periods for existing individuals. Second, for both trees and birds, there are resource limits to densities in addition to energy requirements: trees are limited by the physical packing of their crowns in competition for light, although tropical forests may have more vertical structure (Terborgh, 1985). Two factors could influence tropical bird densities. First, territoriality in birds also place upper limits on their population densities (Brown, 1969), such that area rather than energy limits populations in some high energy regions, although territory size does also vary with resource concentration (Craig & Douglas, 1986; Gass, Angehr, & Centa, 1976). Interspecific competition means that such territoriality affects the populations of multiple species, although the extent that this affects total community density is unclear. Second, resident tropical bird densities are likely to also be limited by the total density of birds when migrants are included. These migrations are common to all of the major tropical realms (Karr, 1976). However, the extent of any such effect is unclear because migrants do not simply enter the habitats of resident species and

compete for the same resources, and many exploit seasonal resources that may not sustain year-round populations (Rappole, 1995).

An interesting consideration is that in situations where energy does not limit density, excess biologically available energy could act as a buffer against extinction. Minor reductions in production would not have the same immediate impact of density that they would in energy-limited environments. Given that small populations face greatest extinction risks, a buffering effect of this nature would be consistent with the preponderance of rare species in Amazonian forest. More detailed analyses of energy budgets would be needed to determine if such an effect occurs to an important degree. However, if so it would have a long-term effect on species richness that is caused by energy supply but not mediated by a total number of individuals.

Climate tolerance

Early naturalists such as Alexander von Humboldt associated tropical species richness with a perceived benevolence of the climate in the first descriptions of the LDG. The concept of a harsh climate, for von Humboldt (1850), related to the availability of liquid-phase water. He contrasted a warm, wet tropical climate lacking hard physiologically derived limitations with a seasonally cold, north-temperate climate in which water is frozen for part of the year. Thus, the first concept of the LDG related diversity to the biological availability of water and energy, and the physiological limits of life. However, this concept of harshness is both incomplete and arbitrary, and a range of other definitions are possible.

Adequately, and non-circularly, defining environmental harshness is challenging. Informally, harsh environments are associated with low species richness, and might include, for example, those with high concentrations of heavy metals, pH extremes, temperature extremes, or extremes of nutrient concentration. However, because these are relatively rare environments, low species richness does not necessarily reflect reduced net diversification resulting from conditions that approach immutable physiological limits for organisms. Instead, low species richness could reflect the limited area of such

environments, and the dispersal difficulties imposed by their isolation from similar areas (Colinvaux, 1993). Were this the case, environmental harshness would be indistinguishable from the time/area hypothesis (see 2.3.4 Causes of species richness variation). Also, to avoid circularity, environmental harshness cannot be defined by species richness if it is to be used in a causal explanation of diversity patterns. The circularity in such definitions of environmental harshness has been noted before (e.g., Rohde, 1992, and references therein).

Because species can adapt to the putatively harsh aspects of environments, some workers have argued that definitions of harshness fail to generalise across species. For example, polar bears, Antarctic penguins, desert plants, and extremophile microbes survive in environments that would be lethal to other organisms, while themselves experiencing strain from commoner environments (Thiery, 1982). Considered by itself, this could lead to the conclusion that no environment could be formally considered harsher than another. However, in the case of temperature, if the only barrier to colonising cold temperate regions was adaptation to abiotic conditions, then the climate harshness hypothesis would again be a particular case of niche conservatism. Once formerly tropical species adapt to the cold temperate environment, there should be no latitudinal variation in net diversification rates between them and species that have retained their ancestral tropical niche (Wiens & Donoghue, 2004). However, as already described, there is substantial variation in net diversification rates across latitudes (see 2.3.4 Causes of species richness variation: Net diversification rates). Indeed, as described by Thiery (1982, p. 699), we must shift our focus from "the trite observation that penguins and toucans find each other's natural habitat harsh but find their own habitats benign" to explaining "why toucans and penguins are not capable of tolerating each other's habitat". Given the latitudinal asymmetry in net diversification rates, there are reasons to investigate possible climate-related differences in selective pressures that could be related to net diversification. If such climate effects can be shown to be universal, they could form an adequate definition of climate harshness.

Given life's universal need for energy and water, cold or seasonally cold climates can be considered harsh in two respects: resource limitations and the physiological effects of low

temperature. In the classic, three-way adaptive strategy model for plants developed by Grime (1977), adaptations to resource-linked stressors are not simply traits that can be gained without cost, but are trade-offs that reduce competitive ability. Within Grime's framework, these trade-offs allow a range of species to co-exist across a landscape where stress and disturbance vary in space and time. However, in regions that have widespread resource limitations, all species must share these trade-offs, potentially reducing the range of life strategies that can succeed over evolutionary time in that region. Such adaptive strategies include inducing states of reduced metabolism, such as hibernation, or deciduousness. In addition to resource limitation, low temperatures also induce physiological challenges for living cells. During below-freezing conditions organisms must avoid death and tissue damage through, for example, the expression of anti-freeze proteins (Davies & Hew, 1990; Griffith & Yaish, 2004), other means of water supercooling (García Bañuelos, Vázquez Moreno, Winzerling, Orozco, & Gardea, 2008), or cellular dehydration (Pearce, 2001). Collectively these two features of cold environments mean that physiological adaptations must be maintained by purifying selection for survival. It has long been thought that natural selection is more strongly abiotically driven in temperate than tropical zones (Dobzhansky, 1950), giving rise to a latitudinal gradient in the importance of biotic interactions. Dobzhansky argued that the increased importance of biotic interactions in the tropics could result in narrower tropical niches. Schemske (2002, 2009) extended the idea of a gradient in the importance of factors causing selection to suggest that in temperate regions climate imposes 'fixed' targets for natural selection, while in tropical regions, biotic interactions cause 'shifting' targets for natural selection, as species co-evolve. Strong purifying selection in temperate regions could plausibly reduce long-term N_e, and reduce genetic diversity through background selection (Charlesworth et al., 1993; Hudson & Kaplan, 1995). Indeed, a reduction of hard abiotic selection can be inferred in tropical birds through their reduced peak metabolic rates, smaller organs, lower flight capacity, reduced sustained metabolic output, and reduced feather mass (Wiersma, Chappell, et al., 2007; Wiersma, Muñoz-Garcia, et al., 2007; Wiersma et al., 2012). Because reduced selection is inferred as the cause of these patterns, tropical species should have elevated d_N/d_S ratios in housekeeping metabolic genes, such as mitochondrial housekeeping genes relative to temperate species of equivalent population sizes.

In addition to relaxed selection, two climate-related factors could affect panmixia and deme formation, with implications for the speciation rate. Because species experience a wider temperature range in seasonal, temperate regions there could be increased selection for dispersal ability (Dynesius & Jansson, 2000; Janzen, 1967). Further, increased biotic selection in the tropics could result in a trade-off in organismal traits between competition and dispersal (Jocque et al., 2010; Pellissier, 2015). The strong subdivisions in species across the suture zones in Amazonia, even in volant taxa (Naka et al., 2012), supports the inference that dispersal differs between tropical and extratropical organisms (e.g., Salisbury et al., 2012).

It has previously been shown that low seasonal variation in temperate zones correlates with elevated temperate species richness in the fossil record (Archibald et al., 2010), indicating a correlation between seasonality and net diversification. In addition to potentially reducing speciation by increasing selection for dispersal, strong temperature seasonality could also increase extinction rates by several mechanisms. Firstly, in seasonal environments there is an increased reliance on production during short growing seasons. Sufficiently marginal growing season productivity appears to set absolute limits on some groups of organisms. Alpine treelines, for example, are affected not only by minimum annual temperatures but also summer temperatures, the length of the growing season, and productivity (Körner, 2012). In areas with short growing seasons, there is greater reliance on productivity during that season to produce energy for maintenance and growth. Several consecutive poor years can cause widespread mortality, although species with high seasonal mortality may also be relatively well-adapted to fasting (Lindstedt & Boyce, 1985). While mortality can be offset by a range of adaptations, although any such adaptations must also be maintained by purifying selection. Secondly, species must adapt to a wider range of temperatures in temperate regions. In vertebrates, for both ectothermic (Sperry, Blouin-Demers, Carfagno, & Weatherhead, 2010) and endothermic (Lindstedt & Boyce, 1985) groups, mortality peaks in winter, indicating the limits of climate tolerances. The effects of severe winter storms on mortality rates have been documented in the literature for a long time (e.g., Bumpus, 1899). As such, temperate populations may be

subject to more 'Court Jester' and less 'Red Queen' evolution (Barnosky, 2001) than tropical populations—that is, temperate evolution is shaped to a greater extent by stochastic events such as rapid climate fluctuations, inducing mortality with less species specificity.

Climate stability hypotheses have traditionally been considered ecological hypotheses, because stable climates could plausibly cause narrower niches in species (Klopfer & MacArthur, 1960), or enable species to tolerate greater overlap in niches (Klopfer & MacArthur, 1961). A greater number of species could co-exist under these scenarios in a stable environment, provided such a climate permitted differences in realised niches. However, the Court Jester hypothesis sets climate stability in a context where it plausibly causes a latitudinal gradient in net diversification. Considering the additional effects of climate harshness described above, climate tolerances are linked to broad-scale species richness through several potentially concurrent effects on net diversification rates. Further, climate stability creates a constancy of resource supply over evolutionary timescales—separate from instantaneous rates of energy supply, or short-term averages—which enable the evolution of complex trophic webs, more specialisation, and the stability of higher trophic levels amongst consumers (Brown, 1981; Hutchinson, 1959).

Many of the potential effects of climate tolerances on net diversification require exploration. To date, these effects have been given little consideration in the literature. For example, in a wide-ranging review of climate hypotheses for diversity patterns, Currie et al. (2004) only consider climate tolerances in the ecological terms of their influence of species distributions, rather than on net diversification. One specific prediction can be made here: given both the Court Jester hypothesis and the prediction of stronger temperate purifying selection, we should expect to see lower N_e in temperate than tropical populations for a given N_c. This could manifest as greater nucleotide diversity in tropical populations.

Evolutionary speed

The effective evolutionary time hypothesis, also referred to as the evolutionary speed hypothesis (ESH, but see Rohde, 2013) proposes that increases in ambient temperature

(Rohde, 1992) or biologically available energy (Gillman & Wright, 2014) cause elevated rates of molecular evolution, thereby increasing net diversification through higher origination rates. Rohde (1992) proposed that evolutionary speed was accelerated in the tropics by shorter generation times, higher mutation rates, and faster positive natural selection. The relative contributions from each of these factors has not been established, nor has their generality. Establishing the importance and generality of these factors is challenging as there could be variation between species or clades, and there are multiple possible causes for mutation rate differences and variation in the strength or effectiveness of natural selection. However, at the broad level, the resulting framework predicts a three-step pathway from climate to rates of molecular evolution, to the speciation rate, to species richness (Gillman & Wright, 2014). ESH is a hypothesis of species richness that is controlled by climate primarily through accelerated speciation. Like the species-energy hypothesis its mechanism derives from energy, either through the kinetics of elevated ambient temperatures in the tropics or resource supply from elevated tropical production.

An appealing feature of ESH is that, regardless of the underlying mechanisms, the pathway by which it is proposed to operate is readily testable. First, there should be a correlation between either temperature or biologically available energy and rates of molecular evolution. Second, therefore should be a correlation between the rate of molecular evolution and species richness (or more precisely to net diversification). Finally, because the pathway from energy to species richness is mediated by molecular evolutionary rates, a correlation between energy and species richness that omits the intermediate step should be weaker than either of the two directly predicted correlations, provided energy does not have other effects on species richness that are not mediated by rates of molecular evolution. To date, these predictions have received minimal testing. Davies, Savolainen, Chase, Moat, and Barraclough (2004) investigated the relative strengths of the two directly predicted relationships against the relationship between climate and species richness in flowering plants. While they found some evidence of the first step—a correlation between energy and molecular evolutionary rates—they found no clear and strong evidence of the second step, between the rate of molecular evolution and species richness. Also, the direct link Davies et al. found between energy and species

richness was substantially stronger than the link between energy and molecular evolutionary rates. These results suggest that energy's effect on species richness is not mediated by the rate of molecular evolution. However, the molecular evolutionary rates used by Davies et al. were estimated from the substitution rate of single species from sister families. Such low-density taxon sampling can produce unreliable phylogenetic estimates (Heath, Hedtke, & Hillis, 2008). Even an accurate rate estimate for a single species using such a method cannot be considered representative of the family as there can be substantial rate variation within plant families (Doebley, Durbin, Golenberg, Clegg, & Ma, 1990). As such, family molecular evolutionary rates were likely to have been estimated with more error than energy in the study. Given unequal error, the finding that molecular rates were a poor or non-significant predictor of species richness in multiple regression models containing energy terms cannot be used to definitively conclude that species richness is mediated by energy independent of molecular evolution. However, Davies et al. also found that latitude significantly predicted the rate of molecular evolution in sister families, and this relationship was stronger than that between the rate of molecular evolution and species richness. Such a correlation indicates that error in the evolutionary rates estimated by Davies et al. may not be the primary reason for the lack of the predicted correlation with species richness. Latitudinal variation in evolutionary rates is a prediction of ESH because environmental energy declines with increasing latitude. A stronger correlation with latitude than energy suggests energy is not the main latitudinal correlate that affects rates of molecular evolution. There is a distinct need for more testing of these pathways to investigate the viability of ESH as an explanation of global diversity patterns, as well as other potential molecular evolutionary mechanisms and patterns that might relate to global diversity patterns.

While Davies et al. (2004) found evidence of the first step of ESH—accelerated molecular evolutionary rates in high energy environments—not all studies have successfully recovered this pattern. Amongst plants, latitudinal differences in molecular evolutionary rates has been consistently recovered (Davies et al., 2004; Gillman, Keeling, Gardner, & Wright, 2010; Wright, Keeling, & Gillman, 2006). However, among vertebrates, the pattern has been considerably more varied. In one study of birds a latitudinal difference was

found (Gillman, McCowan, & Wright, 2012), while in another no pattern was recovered (Bromham & Cardillo, 2003). Studies of fish (Wright, Ross, Keeling, McBride, & Gillman, 2011), mammals (Gillman et al., 2009), and amphibians (Wright, Gillman, Ross, & Keeling, 2010) have all recovered significant patterns with latitude. However, in the case of mammals, while the original analysis showed that in sister species pairs of mammals, a greater proportion of the lower latitude species in the pairs had elevated substitution rates (Gillman et al., 2009), an additional analysis of the same data by Weir and Schluter (2011) found that no correlation existed between the magnitude of rate differences and the size of the latitudinal gap between sister species. Finally, a study of turtles identified a significant correlation between latitude and substitution rates for mtDNA, but not nuclear DNA when independent contrasts were used (Lourenço, Glémin, Chiari, & Galtier, 2013). Many of these studies have used small numbers of comparisons and often single genes, indicating a need for more comprehensive studies. Further, patterns have been sought with latitude, across which multiple environmental and historical factors change. Relationships with direct measures of proposed causal factors, such as temperature, seasonality, or productivity would advance a mechanistic understanding of these relationships.

ESH has recently been proposed to exist in a wider framework referred to as the integrated evolutionary speed hypothesis (IESH), in which productivity and area have dual causal effects on rates of molecular evolution. These effects are proposed to cause higher origination rates in large, productive areas, leading to a more rapid accumulation of species over time (Gillman & Wright, 2014). Within IESH secondary effects on evolutionary rates are caused by biotic feedbacks, environmental heterogeneity and rates of environmental change. Because IESH predicts more factors than energy to cause increased origination rates, and the only mechanism proposed within the framework to drive net diversification differences is the rate of molecular evolution (see Figure 2, Gillman & Wright, 2014), the direct link between climate/energy and speciation should be weaker than the link between rates of molecular evolution and speciation, provided IESH accounts for most of the relationship between energy and species richness. However, there are two caveats: firstly, while molecular rates–speciation relationship should be strong

under IESH, accurately estimating the rate of speciation is not straightforward (Pyron & Burbrink, 2013); and secondly, as IESH could operate in tandem with other net diversification mechanisms, the molecular rates–speciation relationship may be obscured by these other mechanisms. On the second point, careful testing may be able to separate the effects of different causal mechanisms.

A principal concern for IESH is that although there is a clear empirical link between productivity and species richness, there is a limited mechanistic scope for a causal relationship that is mediated by rates of molecular evolution. Hurlbert and Stegen (2014) outline the circumstances under which productive energy could limit species richness, and therefore cause net diversification differences. Under their model, productivity primarily acts on species richness levels by increasing population sizes and decreasing extinction. However, it is proposed that IESH operates primarily by accelerated speciation caused by greater tropical mutation rates or shorter tropical generation times, without these mechanisms being clearly linked to productivity. Hurlbert and Stegen further argue that the necessary and sufficient conditions include having reached equilibrium species richness so that population sizes are meaningfully controlled by productivity. However, IESH is proposed to operate at a disequilibrium, and although it does not exclude the possibility of equilibrium species richness (Gillman & Wright, 2014), such an equilibrium is at odds with the theory as originally formulated (Rohde, 1992).

Other workers have emphasised the potential role for the metabolic theory of ecology to explain the LDG within a framework consistent with ESH. Under such a model, energy operates as a catalyst for biochemical processes, rather than a resource supply. Brown (2014) demonstrates that there is a close connection between environmental energy and species richness when energy is temperature expressed as an Arrhenius equation, such that temperature is explicitly related to reaction rates. The slope of this relationship is close to that predicted by the activation energy of biologically important processes, which may be meaningful in the context of the strong link between temperature and species richness (Brown, 2014; Storch, 2012). However, as Brown (2014) notes, there is as yet no mechanistic link between the temperature dependency of metabolic processes and alpha or beta species

richness on ecological or evolutionary levels, placing it in a similar position as productivity-based explanations of species richness. Brown and others (e.g., Gillman & Wright, 2013) suggest that the Red Queen hypothesis (Van Valen, 1973, 1977) could transfer a temperature-dependent relationship with species richness to endothermic organisms that do not themselves experience strong temperature dependence for metabolic kinetics. While these ideas are plausible and the correlation itself compelling, the lack of a currently known mechanistic link to diversity undermines the metabolic theory's importance as an explanation rather than a correlate of species richness patterns alongside other energy-based explanations.

2.3.6 Net diversification mechanisms under population-genetic principles

To a certain extent, net diversification theories of global species richness patterns recapitulate the broader evolutionary debate between selectionism and neutralism. Does the greater diversity—and perhaps specialisation—in the tropics demand a view of finer tropical adaptation, or can non-adaptive processes account for this diversity? As an example, a selectionist viewpoint of tropical diversity might encompass several ideas focusing on positive selection driving diversification by elevating speciation rates. Operationally, tropical speciation could be elevated as a result of greater tropical specialisation, and 'isolation by adaptation' effects (Nosil, Egan, & Funk, 2008), potentially resulting from more raw material for positive selection to act upon being available in the tropics if tropical mutation rates are higher (e.g., Rohde, 1992). An opposing neutralist viewpoint could encompass ideas that differ by focusing on relaxed purifying selection. For example, tropical extinction rates can be lowered by reduced abiotic selection, and tropical speciation rates can be elevated by lower tropical dispersal. Where it occurs, specialisation could also result through relaxed selection, as plausible models exist for the evolution of complexity in eukaryotes via constructive neutral evolution (Gray, Lukeš, Archibald, Keeling, & Doolittle, 2010; Stoltzfus, 1999).

In some instances, both selectionist and neutralist interpretations can be made for a single process that is capable of generating net diversification differences. Therefore, some consideration has to be given to meaningfully separating more specific predictions of these

frameworks, or reinterpreting the broad patterns under a singular, pluralistic framework. Moreover, because generalised effects like the mutation rate and N_e affect both speciation and extinction (Figure 3) additional consideration must be given to how their overlapping effects resolve at different strengths in different circumstances (i.e., at different latitudes) to generate net diversification differences.

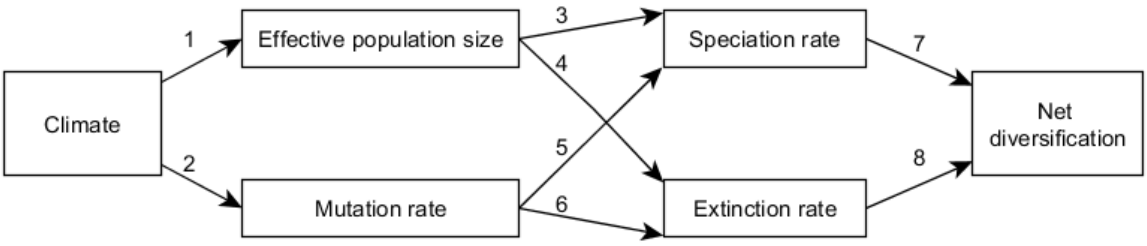

Figure 3. A conceptual problem with identifying causal patterns in net diversification. Climate has a range of putative effects that can be summarised as affecting N_e or the mutation rate (1, 2). Effective population size affects both the speciation and extinction rates (3, 4), as does the mutation rate (5, 6). These in turn dually affect net diversification (7, 8). Justification for the arrows are provided in text.

Mutation rate

The difference in neutralist and selectionist positions can be further illustrated through the effect of mutation rate on speciation. Firstly, as argued within ESH, higher mutation rates can lead to accelerated speciation through increased positive selection (Gillman & Wright, 2014; Rohde, 1992). For example, ecological speciation could be accelerated if divergent selection is mutation-limited. Secondly, higher mutation rates, as predicted in tropical ectotherms under a metabolic theory framework, could be driven neutrally (Brown, 2014), as reproductive isolation will evolve more rapidly in absence of direct selection. However, in either case, an elevated mutation rate is also expected to increase genetic load, and therefore extinction risk (Lancaster, 2010). The risk of extinction through mutational meltdown is probably restricted to particularly small populations ($N_e \approx 100$, $N_c \approx 1000$; Lynch, Conery, & Burger, 1995), although increased mutation must increase the long-term N_e threshold required to maintain a viable population through population size fluctuations. As the mutation rate is evolvable, there is therefore selective pressure that must theoretically limit mutation rate increases, at least within the limits that selection can

act (Lynch, 2010). Therefore, the influence of mutation cannot be understood through its influence on speciation alone.

Given the opposing pressures placed by the mutation rate on net diversification, we can now consider how mutation rate variation could contribute to spatial variation in diversity. Because the mutation rate is evolvable, the mutation rate can be elevated by three mechanisms: 1) a hotter ambient climate induces either more DNA damage or a faster rate of cell division, in either case leading to a higher mutation rate because of the trade-off with metabolic costs of DNA repair; 2) selective pressure for more rapid adaptation favours increased mutation rates, despite the increased genetic load; or 3) mutation rates are higher in areas where extinction risk from genetic load is reduced, reaching a different equilibrium through selection–drift balance. Under the first mechanism, the mutation rate for ectotherms would be increased (Allen et al., 2002; Timofèëff-Ressovsky, 1934), while endotherms would be unaffected. Under the second mechanism, the effect would also be extended to endotherms because the mutation rate is increased by intrinsic factors. This is the hypothesised Red Queen effect implicated in faster mammalian substitution rates at lower latitudes (Gillman et al., 2009). Both of the first two mechanisms involve fitness costs caused by increased genetic load, and therefore reduce genetic diversity, unless generation times are reduced such that per-generation mutation rates do not increase. However, it is possible that these mechanisms could increase species richness if the extinction rate does not increase equally, or if there is a sufficient lag time between speciation and extinction that species richness is inflated by ephemeral species. If caused by a smaller increase in the extinction rate than the speciation rate, then genetic load does not limit species richness, and it is unclear how the mutation–cost-of-repair trade-off is reached. If caused by a lag time effect, this would result in an excess of young species. Under the third mechanism, all species would experience species-specific extrinsic or intrinsic increases in their mutation rate, tied to their extinction risk from genetic load. This mechanism does not lead to higher overall selection pressures where mutation rate is elevated, and therefore should not affect genetic diversity.

Effective population size

As with the mutation rate, variation in N_e can be examined in a population genetic framework relevant to spatial diversity patterns. Given reduced negative selection from climate in the tropics, it has long been proposed that biotic selection pressures are stronger in the tropics (Dobzhansky, 1950)—that is, intra- and inter-specific competition partially or wholly replace the reduction in abiotic selection. Indeed, a wide-ranging review found substantial evidence for a greater importance of biotic interactions in the tropics (Schemske, Mittelbach, Cornell, Sobel, & Roy, 2009). However, in the case of plants, a meta-analysis showed little overall evidence that herbivory is elevated in the tropics, and instead found more chemical defences in high latitude than low latitude plants (Moles, Bonser, Poore, Wallis, & Foley, 2011). If there are indeed gradients in biotic and abiotic selection pressures are separate, there is no reason for average selection on species to be equal, or even necessarily similar, across a range of latitudes. Although there is no definitive work addressing the question of the total strength of selection, within vertebrate species, lower latitude population have greater genetic diversity than higher latitude populations, consistent with weaker total selection (Adams & Hadly, 2013).

Selection decreases N_e, which could be understood as decreasing the ratio N_e:N_c. Given reduced hard selection in the tropics, and greater N_e:N_c, a tropical population with a similar census size as a temperate population could be expected to have a larger N_e, which would give a population-genetic basis for understanding the prolonged survival of tropical species in smaller populations. Further, Federov (1966) postulated that greater genetic drift could drive greater tropical diversification, although he only considered this via small tropical populations. However, given that there are different balances of biotic and abiotic selective pressures inside and outside of the tropics (Dobzhansky, 1950; Schemske, 2002, 2009), it is also plausible that there is reduced total selection in the tropics. Were this the case, genetic drift could play the role that Federov suggested, but without the circularity of requiring initially small population sizes. Other factors might also affect N_e:N_c; for example the lower average dispersal capacity of tropical species may decrease the ratio.

Linking speciation and extinction rates

The factors that control the speciation rate have been recently formalised in an explicit framework (Dynesius & Jansson, 2014). In their framework, Dynesius and Jansson identify three elements that contribute to the speciation rate: the rate at which lineages split, the time for speciation to occur in those lineages, and the persistence of the lineages. Of these, Dynesius and Jansson note that the persistence of lineages has been underappreciated in the study of speciation. For example, clades could have both copious lineage splitting and minimal speciation if the newly arising lineages have persistence times substantially shorter than the time to speciation.

Complementary to this framework, an additional consideration makes it explicitly relevant to net diversification rates. In principle, there is little to distinguish a diverging lineage from a small population of a reproductively isolated species. Three fates are available to the subspecific lineage: merging with its parent lineage, extinction, and speciation. Of these, the first two have the same effect on net diversification, and so do not need to be considered separately. Therefore, the two distinct fates for the lineage—extinction and persistence to speciation—are identical to the distinct fates of a small, post-speciation population. Given the similarity, there is a conceptual linkage between both speciation and extinction. This may contribute to explanations for the frequent covariation in speciation and extinction rates (see 2.3.4 Causes of species richness variation). It would also explain why speciation rates in the tropics appear to continue to be higher than in temperate regions, while already supporting a distribution of species that has a long tail of small population sizes.

2.4 Molecular evolutionary rate variation

2.4.1 Phylogenetic estimates of molecular evolutionary rates

The substitution rate

In molecular phylogenetics, the substitution rate measures the rate at which sequence differences accumulate along branches of a phylogenetic tree. The rate may be described in absolute terms (e.g., substitutions site^{-1} MY^{-1}) in a time-calibrated phylogeny, or in relative terms (substitutions site^{-1}) from an internal node to respective tips along their untransformed branches. Substitutions include fixations, polymorphisms, and mutations, the proportions of which depend on the genetic distances between compared entities (i.e., the time available for fixations to have accumulated), as well as factors affecting genetic diversity such as direct or linked natural selection, and demographic factors such as population bottlenecks.

In a species-level gene tree, neighbouring tips coalesce to a node that approximates the ancestral allele. This node might also approximate the timing of speciation in some cases, although species and genes have distinct histories (Ho et al., 2011; Rogers & Gibbs, 2014). Furthermore, except in rare cases such as polyploidy, speciation is not a point event but rather occurs over a protracted time period (Rabosky & Matute, 2013). The discrepancy between the timing of speciation and gene tree coalescence is important when geological or biogeographic dates are used to calibrate substitution rates, although multiple methods are available to incorporate uncertainty in calibration points (Forest, 2009; Ho & Phillips, 2009).

Although time calibrations do not feature in the present work, it remains of substantial interest that absolute substitution rates show time dependence (Ho et al., 2005). Substitution rates are accelerated when measured over short time periods, a pattern which might reflect the effect of deleterious mutations yet to be purged from populations (Ho et al., 2011; Ho et al., 2005), standing genetic variation present before speciation (Peterson & Masel, 2009), or one of several other causes, including sequencing errors (Ho et al., 2011). Given that fundamental evolutionary metrics such as d_N/d_S were developed for the population genetics of fixed substitutions, and behave fundamentally differently when

applied to polymorphisms (Kryazhimskiy & Plotkin, 2008), understanding the contributions of fixations and standing variation to substitution rates is important.

In addition to time dependence, substitution rates are influenced by an array of neutral and non-neutral selection factors, aspects of ecology and population, and molecular biology. Some principal influences include N_c, mutation rates, and life history traits. Each of these will be discussed below. Some effects can be universal across genome and circumstance, while others can be variable, and context specific. Because these effects may resolve differently on ecological and evolutionary timescales, patterns observed may depend on the taxonomic level of comparisons.

Estimating evolutionary branch lengths using phylogenetics

In general terms, contemporary molecular phylogenetic estimates are commonly made using distance (e.g. neighbour joining), maximum likelihood, or Bayesian methods. These methods simultaneously estimate a range of model parameters including tree topology and branch lengths, assuming a supplied model of nucleotide substitution. Of the common types of tree-building methods, both maximum likelihood and Bayesian approaches to phylogenetics are capable of employing complex models believed to produce the most accurate phylogenetic reconstructions, while distance methods have the benefit of being substantially faster. Other approaches are also used (e.g., maximum parsimony), but will not be discussed here. A number of reviews on the differences between the numerous approaches and their statistical/philosophical bases are available (Barker, 2015; Felsenstein, 1988; Holder & Lewis, 2003; Whelan, Liò, & Goldman, 2001; Yang & Rannala, 2012).

Bayesian phylogenetic inference, which uses Markov Chain Monte Carlo algorithms to estimate the joint posterior probabilities of model parameters, is a relatively recently developed method (Rannala & Yang, 1996; Yang & Rannala, 1997). The Bayesian approach allows for sophisticated analyses that can incorporate prior knowledge. Because Bayesian mathematical philosophy includes the principle of incorporating prior knowledge, Bayesian phylogenetic methods can include fossil-based calibrations of node ages, morphological trait data, and many variations on the molecular clock. However, for

simpler tasks that involve branch length estimation and topology reconstruction maximum likelihood inferences are at least as suitable as Bayesian inferences. Bayesian inferences require priors on many parameters, not all of which are explicitly defined. The implications of these priors are not necessarily obvious, and they may affect inferences whether informative or uninformative priors are given (Barker, 2015). A commonly given example of this phenomenon is that an uninformative prior on topology (all topologies are equally likely) favours large and small clades, and disfavours clades of intermediate size (Randle & Pickett, 2010). While it is difficult to generalise about the precise effects of these priors on topology and branch length estimation, branch lengths for complex simulated data are more accurately estimated under maximum likelihood than Bayesian inference (Schwartz & Mueller, 2010), although they perform similarly when Dirichlet priors are used instead of exponential priors (Zhang, Rannala, & Yang, 2012). This, alongside the practical and philosophical implications of priors may give impetus to use a maximum likelihood inference in the cases where it is sufficient (Barker, 2015).

In the present study, branch lengths and topology are of principal interest, and none of the additional model complexities available to Bayesian inference are required. Therefore maximum likelihood was chosen for the phylogenetic inferences presented. Note, that the phylogenetics pipeline developed in the present work can produce phylogenies using both MrBayes (Bayesian inference; Ronquist & Huelsenbeck, 2003), and GARLI (maximum likelihood inference; Zwickl, 2006).

Data sufficiency in estimating molecular rates

Because phylogenetic models must simultaneously estimate an often large number of parameters, sufficient data must be supplied for robust estimates. In the case of a single-gene, species-level phylogeny, the amount of data is controlled by the number of species included. Additional data can also be provided through concatenating multiple genes (Rokas & Carroll, 2005; Rokas, Williams, King, & Carroll, 2003). The amount of data needed will vary with model complexity, as simpler models have fewer parameters and need less data to adequately parameterise. When data are not homogenous, more complex models can be used that can allow for rate variation between sites and between particular

nucleotide substitutions. Additional models may also be applied to partitions of data that are expected to evolve differently, such as by codon position in coding sequences. Selecting between models and partitioning schemes can be achieved using various model selection tools that penalise model complexity, such as the Akaike Information Criterion (Akaike, 1974).

The number of species required to ultimately derive adequate estimates of branch lengths remains incompletely understood. Sullivan, Swofford, and Naylor (1999) found that with an alignment of 1,307 bp of contiguous mtDNA comprising coding and tRNA genes, sequences for at least 20 species (with one sequence per species) were needed to stabilise their estimates of the gamma shape parameter and proportion of invariable sites in an unpartitioned phylogenetic model. The maximum number of species they included in their estimates was 40, and although above 20 species parameter estimates fell within the 95% confidence interval limits of their estimated values, parameter estimates appeared to further stabilise beyond 20 species. If the estimates derived from 40 species represented true values for the model parameters, then it would appear that approximately 40 species might be needed for good estimates. However, within a model where sites are permitted to vary in their evolutionary rates following a discrete approximation of a gamma distribution and a proportion of the total sites are determined to be invariable, the gamma shape parameter and proportion of invariable sites are non-independent parameters. Sullivan et al. (1999) found the parameters strongly and positively covaried once at least 10 species were included, indicating models based on similar alignments may have more accuracy than implied by parameter stability alone. While 20 species are sufficient for parameter estimation on an unpartitioned GTR+I+Γ model for 1307 bp of shark mtDNA, requirements may increase for any factor that reduces the number of characters that inform each parameter estimate: data partitioning, shorter alignments, the use of DNA with a lower mutation rate, or shorter genetic distances between comparisons. Even within mtDNA, different genes may have different capacities to resolve phylogenies (Omland, Lanyon, & Fritz, 1999).

Although Sullivan et al. (1999) are able to provide a guide to the number of species required for constructing a parameter-stable, maximum likelihood phylogenetic tree, the number of species required will also vary with phylogenetic proximity. By definition, closely related species will provide fewer unique, variant sites that inform the model. Nevertheless, dense taxon sampling is important for accurate phylogenetic reconstruction (Agnarsson & May-Collado, 2008; Heath et al., 2008; Poe & Swofford, 1999), indicating that higher quality analyses are possible for more species-rich clades, even if more species are required for parameter estimates. Although the marginal benefits of including increasingly more taxa diminish to zero after parameters are stable, there are no negative effects from doing so, apart from exponentially increasing processor time. The only exception would be including distantly related species with long branches, which may be worse than including fewer species for certain methods of phylogenetic inference (Kim, 1996). Thus, including as many closely related species as possible—approaching complete taxon sampling where possible—should result in robust phylogenies.

In addition to long-branch attraction, several potential problems arise from including deep divergences in phylogenetic trees. Perhaps the best understood of these additional problems is site saturation: over deep time, multiple substitutions may have happened at sites, making accurate reconstructions difficult even when statistical corrections can be made (Philippe et al., 2011). Current phylogenetic models can account for model and rate variation between sites, and for clock variation across trees. However, given sufficiently deep comparisons, other parameters that are treated as homogeneous over the tree—such as substitution rate matrices, gamma rates parameters, and proportions of invariable sites—may also vary (Gillman, McBride, Keeling, Ross, & Wright, 2011). For example, a mitochondrial gene tree across mammals and birds (320 million year divergence; Meredith et al., 2011; Shedlock & Edwards, 2009) may suffer from the substantial difference in selective constraint on mitochondrial genes between the two taxa, even without the problem of saturation (see 2.4.7 Mitochondrial DNA as a molecular evolutionary marker). The extent of these problems increases towards particularly deep comparisons (Philippe et al., 2011), and it remains unclear to what extent these problems exist for more proximate comparisons.

Given the above considerations, priority for clade selection in the present study was given to families of birds that approach complete taxon sampling. Complete or near-complete taxon sampling is possible for mitochondrial genes for many passerine families. Although the boundary of family is ultimately arbitrary, it is also conservative as it is unlikely that the problems arising from distant comparisons should be experienced within any family-level comparison on birds. Families are also approximately the highest taxonomic rank where complete taxon sampling can be expected. Further details on taxon selection can be found below (2.5.1 New World oscines and suboscines as a study system).

Use of the ω ratio to investigate natural selection

The ratio of non-synonymous substitutions per non-synonymous site (d_N) to synonymous substitutions per synonymous site (d_S) ($d_N/d_S = \omega$) is a commonly used metric to understand the effects of natural selection on coding sequence evolution. The principle is that because non-synonymous mutations alter the amino acid structure of their resulting proteins, they should be subject to more purifying and positive selection than synonymous mutations, which do not alter a protein's amino acid sequence (King & Jukes, 1969). This greater degree of selection could manifest as a slower rate of substitution at variable non-synonymous than synonymous sites and a larger proportion of non-synonymous than synonymous sites. This should be true for most genes that evolve under purifying selection, even if they experience bursts of positive selection. Indeed, across hundreds of orthologues compared between s at a range of evolutionary distances, almost all genes have ω values substantially below 1 (Nei et al., 2010; Sibley & Monroe, 1990).

Synonymous mutations are often referred to as 'silent', even though it is an oversimplification to treat non-synonymous mutations as non-neutral and synonymous mutations as neutral. Not only are many non-synonymous mutations effectively neutral because they do not affect any important features of their gene products (see 2.2.2 Development of evolutionary theory in the molecular era), but also some synonymous changes can have non-neutral effects of comparable magnitudes (Fay et al., 2002), and many more of smaller magnitudes still sufficient to be subject to selection (Chamary &

Hurst, 2005; Parmley, Chamary, & Hurst, 2006; Shields, Sharp, Higgins, & Wright, 1988). The distribution of fitness effects for synonymous and non-synonymous mutations in two non-essential proteins were found to be similar in a study of *Salmonella* (Cox & Yanofsky, 1967). In at least rare instances, synonymous substitutions can affect protein function: Kimchi-Sarfaty et al. (2007) identified a synonymous mutation affecting substrate specificity in a mammalian membrane transport protein. They attribute this effect to altered timing of protein folding caused by codon usage. In general, codon usage is expected to affect translational efficiency as transfer RNAs can differ substantially in their availability (Bromham & Penny, 2003). Therefore, selection can act to bias codon usage in highly expressed genes, although the strength of such selection is typically considered to be weak. In addition to codon usage, synonymous variants can also affect mRNA stability (Duan et al., 2003), and in some cases, the accuracy of splicing (Chamary, Parmley, & Hurst, 2006). Collectively, these observations give cause to doubt the wholesale neutrality of synonymous mutations.

Although there is not a neutral/non-neutral dichotomy between synonymous and non-synonymous substitutions, such a dichotomy is not required to validate the use of ω branch analysis to detect purifying selection. Provided proportionately many more synonymous substitutions are effectively neutral than non-synonymous substitutions, then purifying selection can be detected with error provided sufficient codons are analysed. As already noted, virtually all genes have $\omega < 1$, with an average of 0.21 across 15,350 human–mouse orthologues (Nei et al., 2010), indicating that purifying selection acts more consistently on non-synonymous mutations. Further, time-dependent substitution rates appear to affect non-synonymous substitutions in primate mt genomes, but not synonymous substitutions (Subramanian & Lambert, 2011).

2.4.2 Factors that affect the substitution rate

Population size

Census population size (N_c), the total number of individuals in a population, controls the total mutational input entering populations. As such, larger populations have greater pools of variation for adaptation, and more positively selected mutations arise within such

populations. However, if larger populations are spread over more heterogeneous environments (i.e., they are larger through range expansions rather than higher densities) there may be less opportunity for universally advantageous mutations (Ohta, 1972b). Nevertheless, we could expect larger populations to have elevated rates of adaptive substitutions if adaptation is mutation-limited (Gossmann et al., 2012). This expectation has been borne out in *Drosophila* (Weber & Diggins, 1990), in plants (Siol, Wright, & Barrett, 2010), and in humans in the population expansion that followed the late Pleistocene bottleneck (Hawks et al., 2000; Hawks, Wang, Cochran, Harpending, & Moyzis, 2007).

In addition to increasing the capacity for adaptation, larger populations experience selection pressures differently than smaller populations. As natural selection is stronger in larger populations (Fisher, 1930), a greater proportion of slightly deleterious mutations are purged from such populations. However, the extent to which this affects the substitution rate depends on the distribution of fitness effects for new mutations (Woolfit, 2009), as the rate shift depends on the proportion of mutations that fall into deleterious, nearly neutral and effectively neutral categories (Lanfear, Kokko, & Eyre-Walker, 2014). Because fewer mutations are effectively neutral in large populations, background selection (Charlesworth et al., 1993) should increase with population size. Background selection reduces N_e at linked loci, providing an additional reason why N_e and N_c scale asymptotically rather than linearly (also see 2.2.4 Subsequent criticisms of neutral theory).

In the strictly neutral case, the time to fixation for individual mutations is slower in larger populations, and the chance of fixation for individual mutations is reduced. The total substitution rate is unaffected by these differences, in the latter case because the change in the chance of individual mutations being fixed is exactly countered by the greater number of mutations entering the population (Kimura, 1983). Further, the strictly neutral substitution rate is theoretically unaffected by positive or negative selection in the long run (Birky & Walsh, 1988).

This section has only given brief consideration to N_c. More can be said regarding population size, although it is N_e rather than N_c that is the principally relevant

evolutionary measure of population size. The role of N_e specifically is discussed below (2.4.3 Empirical tests of the N_e—molecular-rates relationship).

Mutation rate

The mutation rate is the per-base rate at which uncorrected alterations to DNA sequences accumulate within genomes. In metazoans, with separate somatic and germ cell lines, the germline mutation rate is the rate at which such errors are transmitted between generations, and therefore is of direct relevance to substitution rates. However, the somatic mutation rate can also be relevant, as it may be under selection (Galtier, Jobson, Nabholz, Glémin, & Blier, 2009). Although the mutation rate varies across the genome for a number of reasons, it is common to refer to a single mutation rate, representing an average rate of error accumulation for a locus or genome, often only taking point mutations into consideration. As comparisons of substitution rates concern point mutations at one or more loci, this subset of the mutational spectrum is sufficient for current consideration. What is of principal importance for the present work is that the mutation rate is an evolvable species trait (Bromham, 2011; Thomas & Hahn, 2014), and that is subject to both selection and drift (Lynch, 2010).

Because most point mutations occur as copy errors during replication, the mutation rate can change by differences in the rate of germline cell division, or in the rate of replication error repair. The former could change as the result of selection corresponding to life-history traits, or on a per-generation mutation rate basis, while the latter may reflect a balance between the cost of error repair and the cost of mutation (Bromham, 2011; Lynch, 2010). If metabolic activity increases the rate of DNA damage, or there is a correlation between environmental energy and DNA damage (Allen et al., 2002; Gillooly, Allen, West, & Brown, 2005), then factors such as climate could also affect mutation rates. However, because all of these factors—selection, drift, and endogenous and exogenous mutagens—can act simultaneously and can interact, it is far from trivial to separate causality.

In one respect, increasing the mutation rate is comparable to increasing population size, as it affects the total mutational input into populations. Therefore, if adaptation is mutation-

limited, an elevated mutation rate can accelerate adaptive evolution. However, under an increased mutation rate, the total mutation density is changed: individuals carry more mutations on average, with a concomitant increased genetic load (Drake, Charlesworth, Charlesworth, & Crow, 1998). Because maintaining fitness under genetic load requires negative selection, increased mutation rates at loci evolving under purifying selection decreases N_e.

Evolution under high mutation rates can be studied in animals using the mt genome. Because the mutation rate in animal mt genomes is approximately tenfold higher than the nuclear rate (Lynch et al., 2006), strong selection is needed to maintain fitness under a relatively high mutational load. However, as a non-recombinant, uniparentally inherited molecule, mtDNA has low N_e, making purifying selection less effective than in the nuclear genome. In mammals, at least, there is evidence that transmission is complex, with a level of intraorganismal selection against amino acid substitutions occurring during transmission, and severe bottlenecking in copy number (Stewart et al., 2008; Stewart & Larsson, 2014; Wai, Teoli, & Shoubridge, 2008). Therefore, only part of the genetic load consequences of the mt mutation rate is mitigated through hard selection.

The result of elevated mutation rate—as demonstrated by comparisons between substitution rates in animal nuclear and mt genomes—is an increase in the effectively neutral substitution rate. Because selection at coding loci disproportionately affects non-synonymous substitutions, an increased mutation rate may have a modest effect on the amino acid substitution rate (but see Nei et al., 2010), and the effect will be locus-specific. As a larger proportion of synonymous substitutions are minimally affected by selection, the effect of the mutation rate is evidenced in an elevated synonymous substitution rate.

Life history

A range of species' life history traits correlate with substitution rates. However, many of these life history traits themselves covary, making causal distinctions difficult to draw. As Bromham (2011) discusses, the well-known mammalian body size correlation with the

substitution rate (e.g., Martin & Palumbi, 1993) is difficult to interpret because body size also correlates with traits such as metabolic rate, generation time, fecundity, and longevity.

Both body size and longevity are both potentially linked to the germline mutation rate evolution through the trade-off between the costs of somatic mutations and DNA repair (Nabholz, Glémin, & Galtier, 2008; Promislow, 1994). Body size selection can occur in response to climate, as well as competition. Given such selection, lower per-replication mutation rates are needed to maintain large-bodied organisms. Large-bodied mammals have adaptations consistent with reducing costs associated with high rates of cell replication, including reduced telomerase activity, which prevents cell line immortality, and therefore cancer risk (Shay, Zou, Hiyama, & Wright, 2001). However, although substitution rates per unit time are lower in large-bodied species, it is unclear the extent to which DNA repair versus other factors such as mutagen production causes this difference (Bromham, 2011). Large-bodied organisms also tend to be long-lived. Longevity could also be either a target of selection or the result of selection on the mutation rate. For example, bats and volant birds are both long-lived compared to similarly sized non-volant mammals and birds (Healy et al., 2014). Healy et al. principally relate their finding to predator avoidance, with selection to maintain fitness as a response to increased longevity in volant species. Some secondary selection for increased longevity may result in this way. However, given that strong selective pressure is needed to maintain flight-capable mitochondria (Shen, Shi, Sun, & Zhang, 2009), and mutation accumulation is linked to ageing, selection for flight capability (e.g., for migration and resource access) also directly increases longevity, regardless of predation.

Generation time and fecundity are additional factors that correlate with molecular evolutionary rates. However, unlike body size and longevity—where higher DNA replications per unit time result in selection lowering the mutation rate—short generation times and high fecundity result in higher per-unit-time mutation rates and substitution rates (Thomas, Welch, Lanfear, & Bromham, 2010). The effects of generation time and fecundity also differ conceptually from those of body size and longevity, as any direct selection on the mutation rate is transgenerational. Generation time and fecundity are

themselves linked through the r-K continuum, as species with short generations also typically have high fecundity. Thomas et al. (2010) found evidence that non-synonymous substitution rates were elevated in invertebrates with shorter generations, indicating weaker selection. This would be consistent with high fecundity resulting in reduced parental care, and indeed Bromham (2011) proposed that this reduced parental care could potentially be extended to DNA repair.

A final life history trait that correlates with rates of molecular evolution is metabolic rate. The mass-specific metabolic rate generally correlates positively with the mutation rate (Martin & Palumbi, 1993), and has been associated with DNA damage caused by metabolic by-products, such as reactive oxygen species (ROS). However, across a wide range of taxa, and both nuclear and mt loci, there is no evidence of a direct relationship between mass-specific metabolic rate and the rate of molecular evolution (Lanfear, Thomas, Welch, Brey, & Bromham, 2007). Lanfear et al. point out that metabolic rate is decoupled from ROS production in several ways. Because DNA damage invoked by ROS is repairable, and other mechanisms can prevent damage, it is unlikely that evolutionary rates passively reflect metabolic rate. A study in poison dart frogs found that active, rather than resting, metabolic rate correlated with substitution rates in both mt and nuclear genes (Santos, 2012). It was not clear if this is an effect of metabolism directly (i.e., ROS damage), or of a correlated, but unmeasured, life history trait (e.g., longevity was not measured). The effect extended to nuclear loci, which is unexpected for ROS damage, and in mammals, at least, there is little evidence that ROS damage is the cause of mtDNA mutations (Kauppila & Stewart, 2015).

2.4.3 Empirical tests of the N_e—molecular-rates relationship

In developing the nearly neutral theory, Ohta (1972b) first proposed that smaller populations might evolve faster because of reduced natural selection. In the simplest case, functional loci under purifying selection should experience a negative Population-size–Evolutionary-rates Relationship (PER), because on average a non-neutral change to a functional locus will worsen, not improve its function. However, there are many complicating factors in this relationship, leading to a range of additional predictions. More

precisely, a negative PER can be predicted when the expected number of slightly deleterious substitutions scales more steeply with N_e than the expected number of slightly advantageous substitutions. Therefore, when this is true will depend on the shape of the distribution of fitness effects (DFE), background selection and linkage effects, and the population sizes under consideration.

Accordingly, there are circumstances under which a positive PER is predicted. For example, Charlesworth and Eyre-Walker (2007) show species recolonising mainlands from islands experience short-term increases in their evolutionary rates, which they attribute to fitness-compensating back-substitutions in expanding mainland populations. Loci that do not predominantly evolve under purifying selection may also show positive PERs.

In addition to negative and positive PERs, there are also some predictions that PERs are either relatively flat, or only elevated following population size fluctuations. Flat (i.e., unresponsive to population size variation) PERs could be expected when only a small proportion of mutations fall into the nearly neutral category. Indeed, the views of Ohta and Kimura—representing the nearly neutral, and neutral theories respectively—diverged on the shape of DFEs for the mutations entering populations (Woolfit, 2009). Ohta (1977) favoured an exponential distribution that produces a greater proportion of slightly deleterious mutations than the gamma distribution of Kimura (1979). Estimates of DFEs across a range of species have produced variable results (Eyre-Walker & Keightley, 2007), and do not clearly support either an overwhelming prevalence or paucity of nearly neutral mutations, leaving the mutational spectrum an indefinite avenue to address the generality of negative PERs. Further, using a computational model of thermodynamic protein stability beginning with randomly generated coding sequences, Goldstein (2013) found evidence for differences in DFEs between small and large populations because the better-adapted proteins of large populations were more robust to mutation. Mutational robustness lessens the effects of mutation, such that mutational fitness effects were of similar magnitudes across population size classes. Goldstein further found that population size changes only caused temporary substitution rate shifts, indicating oscillating

population sizes caused by periodic bottlenecks and recoveries would be required to cause a prolonged negative PER.

To determine the factors that underpin molecular evolution, and to ultimately test the nearly neutral theory, Kreitman (1996, p. 683) argued that we need "better molecular data from closely related species, which should allow more informed interpretations of evolutionary rate differences". However, despite such clearly stated needs and the conflicting outcomes of theoretical studies, there have been few large-scale attempts to empirically analyse how population size affects DNA substitution rates. Studies to date have either compared small numbers of species for which power to detect differences might be limited, distantly related species for which consistent population size differences may be challenging to infer, or indirect proxies for population size for which a number of other biologically and evolutionarily important factors might also change.

Ohta (1972a) undertook an early test of the prediction that smaller populations experience a relaxed purifying selection regime. She created ratios of DNA substitution rates (as inferred through DNA renaturation experiments, e.g. Laird, McConaughy, & McCarthy, 1969) to enzyme substitution rates between pairs of species, finding that against the background DNA substitution rate, smaller populations appear to accept a greater proportion of protein-coding changes. However, the evolutionary distances between the comparisons were large and generation times—the surrogate used for population size—were not evenly distributed across comparisons, making causal inferences difficult to establish. While the comparative methods were innovative within the limitations of the time they were undertaken, they require verification using more direct tests, and more closely related species.

De Salle and Templeton (1988) investigated evolutionary rate variation in mt restriction sites between Hawaiian species of *Drosophila* that are known to have had historically different population sizes. They found restriction sites evolved faster in species that had undergone repeated population size bottlenecks. However, although seven species of *Drosophila* were included, all of the large-population species form a monophyletic clade.

Hence, the study includes only a single phylogenetically independent contrast, limiting its generality.

Advances in sequencing technology, phylogenetic techniques and computing power have allowed for larger and more focused comparisons. For decades, ω has been a widely used metric for evaluating selective constraint and positive selection in protein-coding DNA sequences (reviewed in Yang & Bielawski, 2000). As many synonymous mutations are believed to be effectively neutral in most (although some experience purifying selection, see Chamary et al., 2006) and many non-synonymous mutations are subject to selection (Tamuri, dos Reis, & Goldstein, 2012), ω provides a gauge of selective constraint on genes, following the same rationale that Ohta (1972a) used. Because relaxed selective constraint is the expected signature of accelerated molecular evolution in small populations, the use of ω allows for direct tests of the consequences of population size variation. Specifically, small populations should experience an overall acceleration of nucleotide substitutions in non-neutral genomic loci, such as protein-coding sequences, through an increased non-synonymous substitution rate, elevating ω in those sequences.

Although there are limited species-level data on directly measured census or effective population sizes, there are several ways of making N_e estimates with varying degrees of directness. Firstly, certain ecological traits can be used to delineate N_e categories (e.g., free-living versus endosymbiotic species, large-bodied versus small-bodied species, or eusocial versus non-social species). These categories provide restricted means for quantifying N_e differences, with the exception of body size, for which average differences between contrasted species can be quantified. However, a N_e–body size relationship cannot be assumed with certainty to occur on modest scales, because although population densities typically decrease as body size increases (Damuth, 1981), this may be partly or fully compensated by larger ranges. Several studies have created paired contrasts based on the above characteristics, and provide some evidence for a negative correlation between N_e and evolutionary rates and patterns. For example, highly social Hymenoptera and social parasites have faster nucleotide substitution rates than non-social species (Bromham & Leys, 2005), endosymbiotic bacteria have a higher rate of radical amino acid substitution

than related gamma-Proteobacteria (Wernegreen, 2011). Endosymbiotic bacteria and fungi also experience elevated substitution rates than their free-living relatives (Woolfit & Bromham, 2003), and species with larger body masses experience an elevated rate of radical amino acid substitutions compared to smaller-bodied species for birds (Weber, Nabholz, Romiguier, & Ellegren, 2014), and mammals (Popadin et al., 2007). Finally, there is also evidence that ω is higher in larger mammals (Lartillot & Poujol, 2011).

A second category of study for the effect of N_e difference uses comparisons between island and mainland species under the rationale that the physical limits of island size should limit island population size. This appears to be a sound assumption, as microsatellite sequence diversity in endemic island bird species is lower than mainland species, suggesting that insular lineages do experience long-term population size reductions (Hughes, 2010). However, islands and mainlands also differ in a number of other ways (e.g., climate, predator abundance, population density, salt exposure, and resources), and it is likely that population size is not the only factor to systematically change in such comparisons. The consequences of these correlated changes are unknown, but there is the potential for substantial effects. In particular, limited competition, diversity, and predation elevate the population densities of many island species above that of comparable mainland species. Across 334 species, Buckley and Jetz (2007) found an order of magnitude difference in lizard densities between mainland populations (average density, 128 individuals ha^{-1}) and neighbouring island populations (average density, 1920 individuals ha^{-1}). The phenomenon of island density compensation has also been observed in birds, although differences are below an order of magnitude and frequently within twofold (e.g., Blondel, Chessel, & Frochot, 1988; George, 1987; MacArthur, Diamond, & Karr, 1972). Studies that have contrasted molecular evolutionary rates using the island–mainland criterion are described below.

Johnson and Seger (2001) compared nine mainland–island pairs of bird taxa (species or subspecies), of which five comparisons were phylogenetically independent. They found that ω was elevated in island species, although their methods have been criticised for lacking formal criteria to establish population size differences between island and

mainland lineages (Wright et al., 2009). For example, five of the nine island species were distributed across either large island archipelagos or Madagascar, which covers an area (587,000 km^2) larger than the extents of many mainland bird species. Also, while elevated ω is indicative of relaxed purifying selection, mainland species had a greater proportion of unpreferred codons in five of the nine comparisons, despite their presumably larger populations. While this result is expected if synonymous substitutions are effectively neutral, four of the five island species with lower proportions of unpreferred codons also had elevated synonymous substitution rates relative to their mainland counterparts. This result is at least theoretically consistent with compensatory mutations, which would be expected in larger populations following an expansion (Charlesworth & Eyre-Walker, 2007). However, the causes of these patterns are intractable without larger samples sizes, study designs that specifically address these possibilities, and stricter control on population size inclusion criteria.

More recently, Woolfit and Bromham (2005) independently contrasted coding sequences for 44 island–mainland pairs of vertebrate and invertebrate taxa. They found that 61% of island taxa had elevated ω, (although the result was only significant using a one-tailed test), but experienced no overall increase in substitution rate. Presumably, non-synonymous substitutions were a minor component of the total molecular rate, and this difference was subsumed within the greater observed synonymous rate variation. Further, Woolfit and Bromham did not find any relationship between island size and ω. If island size approximated population size, then this lack of pattern would challenge the inference that relaxed purifying selection is causal in the variation in evolutionary patterns between island and mainland species. However, across a range of vertebrates and invertebrates, population densities would need to be considered in addition to island size before generalisations about population size could be made.

Critical of previous studies, Wright et al. (2009) contrasted 48 island–mainland (and in some cases, small-island–large-island) bird pairs, using stricter selection criteria than had previously been applied. Unlike previous studies, they found a slower tempo of molecular evolution (measured as relative substitution rates between neighbouring species) in

smaller island populations than in mainland species, and also found comparable ω in island and mainland lineages. If their island species indeed have smaller N_e than their mainland species, these findings are inconsistent with theoretical predictions under nearly neutral theory, and are instead consistent with higher mainland mutation rates. These results are given a more detailed examination in the context of the present work's results (see 5.3.4 Comparisons with prior studies).

Reconciling the varying results of these studies is challenging. In addition to method differences, the lack of strength and consistency in recovered patterns could have been caused by incorrect phylogenetic inferences, as comparisons between species with either large genetic distances or comparisons with inappropriate outgrouping can affect the accuracy of molecular branch lengths and other evolutionary metrics (Heath et al., 2008). Dense taxon sampling improves tree topography when limited DNA sequence data is available (Agnarsson & May-Collado, 2008). Conversely, trees produced with sparsely sampled taxa may have inaccurate branch lengths caused by substitution saturation (Hugall & Lee, 2007), or incorrect node placement (Heath et al., 2008). Further, a lack of sufficient genetic sequence data per species may weaken the observed relationships and if the signal-to-noise ratio is low this could result in type II errors. Improvements across these areas may provide more consistent results.

An additional method for comparing molecular evolutionary rates and patterns resulting from N_e differences contrasts sister flighted and flightless sister lineages. Loss of flight should result in reduced N_e by reducing dispersal capacity, and therefore population mixing, and also because flightless bird lineages tend to be found on islands (Shen et al., 2009). Indeed, there is evidence of accelerated rates of molecular evolution and elevated ω in nuclear genes of flightless insect lineages (Mitterboeck & Adamowicz, 2013) and mitochondrial genes of flightless and weakly flying birds (Shen et al., 2009). However, there are two weaknesses to this approach. Firstly, relaxed selection resulting from reduced energy requirements stands as an additional explanation for elevated ω in flightless lineages. This is particularly a problem for interpreting Shen et al. (2009), as they investigate housekeeping genes that are integral in energy production from the

mitochondrion. Secondly, smaller flightless populations are assumed based on insularity, but are not formally tested in these studies. The issues and potentially confounding factors associated with island-mainland comparisons have already been addressed.

To my knowledge, only a single sister taxon contrast studies of the population size–evolutionary rates relationship to date has attempted to quantify population size differences between contrasts beyond landmass size. In a study investigating the effect of body size on rates of molecular evolution in birds, Gillman et al. (2012) tested a subset of 41 species pairs for which population or range size data were available. They found no significant difference occurred, although data on the magnitude of the contrasts makes it unclear what power the study had to detect an effect. This leaves a distinct gap in the literature that requires addressing. Because birds have been the long-term focus of much attention in ecology and evolution, they make an excellent taxonomic group to investigate this question in new ways. Population size estimates are available for some species, and range size estimates (as extent of occurrence) are available for all species. If range size can be used as a surrogate measure for population size similar to island/mainland size, then the reliance on island lineages for small populations can be eliminated. The potential for range size to be used as to estimate population size is discussed below.

2.4.4 Range size as a surrogate measure of population size

The range of a species is amongst its most fundamental characteristics. A species with a larger range could be expected to have a larger population than a smaller-ranged species if their densities and occupancies are the same across their respective ranges. However, it is understood that local abundances vary between species due to many facets of ecology. In addition to direct intraspecific competition and predation, population densities are affected by factors such as: body size (Damuth, 1981), territoriality (Grant & Kramer, 1990), apparent competition (Holt, 1977), resource density (Enoksson & Nilsson, 1983), migratory behaviour (Gaston & Blackburn, 1996a), and habitat niche breadth (Brown, 1984). Further, some of these factors, such as niche breadth and body size are determinants of range size, creating potential confounds in the use of range size as a population size surrogate. At regional scales, determinants of coarse-grain population densities override those at local

scales, with broad habitat availability, and climate becoming principal factors (e.g., Sherry & Holmes, 1988).

The range size of a species can be defined in multiple ways. Where range size refers to area of occupancy (AOO; commonly, an estimate by presence or absence in cells across a spatial grid), it is commonly used as a proxy for population density and population size at local scales (Hanski, 1982), although substantial variation exists in these relationships (Holt, Lawton, Gaston, & Blackburn, 1997). However, due to the intense sampling efforts required to estimate it, AOO has usually only been derived at local scales for partial extents, and for smaller-ranged species to assess their conservation status. Instead, the extent of occurrence (EOO commonly, a polygon covering the geographic extent, including AOO) is much more widely estimated. While the occupancy-abundance relationship has been widely studied on local scales (Holt, Gaston, & He, 2002), the relationship between AOOs and population sizes at larger scales, across heterogeneous landscapes has been given less attention, although direct scaling appears to be inappropriate (Hui et al., 2009). Moreover, the relationship between population size and EOO, is poorly understood, although the positive relationship between local densities and range size do not appear to be upheld as they are when occupancies are used (Harcourt, Coppeto, & Parks, 2005), and EOO is believed to correlate weakly with population size compared to AOO (Gaston & Fuller, 2009).

It has previously been demonstrated that there is a strong log-log correlation between site occupancy and population size in the Anseriformes (Gaston & Blackburn, 1996a), British breeding birds (Blackburn, Gaston, Quinn, Arnold, & Gregory, 1997), and a wide range of non-avian organisms (see Holt et al., 2002 and references therein). The relationship is considered sufficiently strong that occupancy is the basis for determining population-based conservation statuses in the IUCN Red List (Baillie, Hilton-Taylor, & Stuart, 2004). Gaston and Blackburn (1996a) also show that at the ordinal level, population size also correlates with body mass and latitudinal midpoint of species range. As commonly recovered in occupancy-abundance relationships, population size increased faster than area of occupancy, indicating that larger-ranged species also had higher population

densities. This may be caused by the array of species traits factors that influence geographic ranges, including fecundity, dispersal ability, and habitat niche breadth (Laube et al., 2013).

The relationship between AOO and EOO as two distinct measures of range size has not been given sustained consideration in the literature. Where the relationship has been investigated (e.g., Gaston & Fuller, 2009), the ratio of occupancy to extent appears to decline with increasing extent—i.e., smaller proportions of large extents are occupied than of small extents. However, when excluding colonial seabirds, many of which have small breeding grounds relative to their extents, there is a strong log-log relationship between AOOs and EOOs (Gaston & Fuller, 2009). If this relationship remains strong for species with large extents, for which AOOs are typically unestablished, then EOOs may act as useful range size measures for first-order population size estimates. Because sister species are more likely than more distantly related species to share similar habitat and climate niches, relative contrasts of their range sizes might also be more likely to reflect population size than absolute values across a wide range of species.

Therefore, the use of range size may provide a substantially better approximation of population size than island size and mainland size. Although AOO would provide a stronger correlate of population size, EOO may also be valuable when some controls are applied in its use. The use of range size eliminates the need of introducing the potential bias of having large and small populations in necessarily different environments. In addition to island density compensation, island colonisation from mainland populations might produce population bottlenecks, as well as introducing the immigrants to a marine-influenced environment that may have differences in predators, habitat, climate, and food resources. Mainland colonisation from island populations can result in population expansions, with short-term increases in substitution rates (Charlesworth & Eyre-Walker, 2007).

2.4.5 *Factors affecting species' range sizes*

Given that population size can affect the rates and patterns of molecular evolution, even if those patterns are not consistent, some consideration must be given to factors that could bias population size across latitudes. With respect to causes of the LDG, some factors are of no interest. For example, Federov (1966) argued for an increased importance of genetic drift in the tropics because greater species density reduces average population density. While the persistence of those smaller populations is of interest, their existence is an effect rather than a cause of the LDG. Range size is an additional strong correlate of population size. Unlike population density, range size could plausibly vary with latitude without a pre-existing LDG.

Mechanistically, climate tolerances have been linked to range size evolution. Stevens (1989) proposed that species co-existence in the tropics could be explained by patterns of geographic range size, naming this explanation Rapoport's rule for the ecologist who first provided evidence of the pattern. Specifically, Stevens proposed that extended latitudinal ranges can arise in temperate species, because they are capable of tolerating broader climatic conditions than are tropical species. This leads to the prediction that temperate species have larger latitudinal ranges than tropical species, which could cause a latitudinal gradient in species richness through increased beta diversity in saturated communities, or as Stevens proposes, could lead to increased tropical alpha diversity because of a greater proportion of incidentals occurring outside of their range caused by an increased edge-to-range ratio. Rapoport's rule has been variously criticised for a lack of generality on empirical (Rohde, 1999) and theoretical (Šizling, Storch, & Keil, 2009) grounds. Nonetheless, the rule has received sustained interest in the literature, and the pattern emerges under restricted circumstances (Veter et al., 2013). Further, whether Rapoport's rule stands as an explanation for the LDG or not, the effect of climate tolerances on range size (e.g., Pither, 2003) could still cause a latitudinal gradient in population size.

In addition to climate affecting the latitudinal ranges of species, land area places longitudinal geometric constraints on possible range sizes. Therefore, in regions where there is limited longitudinal extent of continental land masses, or where large proportions

of species are insular, median range sizes are smaller (Orme et al., 2006). There are latitudinal patterns in land extent, but not a simple, symmetrical latitudinal gradient: land extent is greater in the northern than the southern hemisphere, peaking at mid to high latitude. Thus, the effect of geometric constraints on range size can be separated from the effects of Rapoport's rule.

Species attributes and tolerances must also shape their range sizes. Generalist species, on average occupy larger areas than specialists, not only for climate (as noted above), but for ecological aspects such as habitat and dietary preferences (Pyron, 1999; Ruokolainen & Vormisto, 2000). Given that it has been predicted that biotic interactions are greater in the tropics, gradients in proportions of specialists might not passively reflect greater tropical trophic stability, but might also feed back into evolutionary processes through secondary range size effects.

Avian range sizes are not static over species' evolutionary histories. Further, unlike other species traits, such as body size, ranges do not evolve under Brownian motion (Diniz-Filho & Tôrres, 2002). Webb and Gaston (2000) present evidence for a general pattern within birds whereby range sizes expand after speciation, and subsequently decline over the "lifespan" of the species. Some clades in their analysis only showed evidence of a decline. This gives rise to the possibility that range size could be latitudinally biased by differences in species age distributions. Indeed, there could be a latitudinal bias in species age as a result of the effect of climate during the Pleistocene on speciation (Avise & Walker, 1998; Weir & Schluter, 2007).

There are therefore several reasons to expect some variation in species range sizes across latitude that might interact with climate. At least some of these reasons could be important for understanding causes of the LDG. Consideration of range size must be made for analyses that seek climate-related patterns, where those patterns could also relate to range or population size.

2.4.6 *Comparative methods in phylogenetics—sister species contrasts*

Any study that investigates relationships between species traits and rates of molecular evolution must adequately account for the lack of phylogenetic independence across a phylogenetic tree (Harvey & Pagel, 1991). Failing to account for non-independence causes the same kinds of statistical problems as other forms of pseudoreplication, including the problems caused by spatial non-independence in macroecological analyses. As such, phylogenetic non-independence can cause spurious relationships to emerge, or obscure true relationships (e.g., Cardillo, 2002).

Felsenstein (1985) proposed a method for creating phylogenetically independent contrasts (PICs) that removes phylogenetic signal, resulting in a contrasts free of autocorrelation due to proximity. In doing so, PICs relate the expected variances in traits to those in branch lengths. The validity of this approach is based on characters evolving under Brownian motion (Felsenstein, 1985; Harvey & Rambaut, 2000). Any violation of this assumption requires additional consideration of the appropriate statistical approach for analysis.

While the assumption of Brownian motion is appropriate for many species traits, geographic range size does not demonstrate the phylogenetic autocorrelation expected under Brownian motion evolution (Diniz-Filho & Tôrres, 2002). This is unsurprising, given that range size evolves across the lifespans of species in similar ways, rather than diverging between sister species towards distinct values (Webb & Gaston, 2000). The effect of incorrectly using whole-tree PICs on a dataset exploring range size would therefore be to spuriously assume correlations in the deeper nodes where none is likely to exist. This problem would exist for any other method that assumed ancestral range size patterns could be modelled from current range size data, such as, phylogenetic least squares regression (Grafen, 1989).

In the same paper that he proposed the PIC method, Felsenstein (1985) also described an alternative method that uses a reduced dataset comprised only of closely related pairs of species. By removing additional comparisons, uncertainty can be addressed in the phylogeny. This method would also be suitable for contrasting traits such as range size,

which have current values of interest that relate to other important biological traits such as population size, but that do not evolve under Brownian motion. The present work relies on this method.

By contrasting only tips connected by single nodes on a phylogeny with complete or near-complete species-level taxon sampling, sister species contrasts are generated. Although the phylogenetic dataset is approximately halved from whole-phylogeny PICs, there is no data loss for traits that cannot be reliably reconstructed for deeper nodes. Also, contrasts have the desirable properties of having phylogenetic independence, and making few other assumptions. For example, no assumptions are needed about species' traits prior to speciation. In the case of range size, the only assumption is that the differences between sister species' current ranges approximately reflect average differences since speciation.

2.4.7 Mitochondrial DNA as a molecular evolutionary marker

For decades, the mitochondrial (mt) genome has been the commonest locus for phylogeographic and phylogenetic analyses of animals (Ballard & Whitlock, 2004). Avise et al. (1987) argued that animal mtDNA is an ideal population-genetic marker for animal phylogeography because mt genes are ubiquitous across metazoans, structurally simple, easily isolated and amplified, transmitted simply and without recombination, and quickly evolving under a high mutation rate. Such characteristics are also desirable for phylogenetics, as some of the evolutionary complexities of the nuclear genome are avoided, and many parsimony-informative characters are available between closely related species. Nonetheless, many of the attributes described by Avise et al. are not absolute: recombination rates are low but probably non-zero (Awadalla, Eyre-Walker, & Smith, 1999; Kraytsberg et al., 2004), transmission is complex and can be heteroplasmic (Hill, Chen, & Xu, 2014; Stewart & Larsson, 2014), and is not strictly maternal nor uniparental in all organisms (Wolff, Nafisinia, Sutovsky, & Ballard, 2013; Zouros, 2013).

One of the purported benefits of using the mt genome for animal phylogenetic analyses is that its evolutionary pressures are thought to be well understood. Although often assumed to evolve neutrally in population genetic studies (Ballard & Kreitman, 1995), it is now

widely accepted that the mt genes evolve under strong purifying selection (Popadin, Nikolaev, Junier, Baranova, & Antonarakis, 2013), with evidence of positive selection (Meiklejohn et al., 2007). This mode of evolution makes mtDNA inappropriate for many population-genetic applications, such as using mt nucleotide diversity to estimate N_e (Galtier, Nabholz, Glémin, & Hurst, 2009). Galtier et al. (2009, p. 4546) describe mtDNA as "the worst population genetic and phylogenetic molecular marker we can think of", a claim that is remarkably distant from the earlier assessment of mtDNA as a marker that fulfils the "wish list" of "an ideal molecular system for phylogenetic analysis" (Avise et al., 1987, p. 492). As a phylogenetic marker, mtDNA is prone to some degree of topological error because the effect of hybridisation at an effectively non-recombinant locus is the complete erasure of evolutionary history. Nonetheless, the negativity of Galtier et al.'s assessment of mtDNA as a phylogenetic marker is hyperbolic: the low N_e of mtDNA relative to nuclear DNA reduces coalescence time, and therefore the chance of incomplete lineage sorting; and, the high mutation rate of mtDNA relative to nuclear DNA improves phylogenetic resolution over short evolutionary distances (Moore, 1995). Most apparent paraphyly in bird mtDNA systematics is caused by taxonomic errors that have been exposed by the analysis, rather than reflecting the insufficiency of mtDNA as a marker (McKay & Zink, 2010). Further, across broad groups of mammals single mt gene trees produce reliable species phylogenies (Agnarsson & May-Collado, 2008). As such, mtDNA has some distinct advantages over nuclear DNA for certain applications.

Although the limitations of mtDNA have been discussed prominently for many years (Ballard & Whitlock, 2004; Galtier, Nabholz, et al., 2009), studies that require a high degree of taxon sampling of molecular markers frequently rely solely on mtDNA. There are practical reasons for this: for birds, almost 100,000 mtDNA coding gene accessions are available on GenBank (see 3.3.1 Sequence database assembly). While there is a current project to sequence the nuclear genomes of the approximately 10,000 described bird species (Bird 10K Project; http://b10k.genomics.cn/), at present only mt genes are widely sequenced across the avian tree of life. In February 2014, there were three mt genes for which GenBank held between 20,000 and 30,000 avian accessions—an average of 2 to 3 accessions per species. While not all species are sequenced, approximately 75% of

recognised bird species have at least one mt accession in GenBank. More than 300 species have 20 or more accessions of a single gene, allowing for a range of population-genetic phenomena to be investigated. Even commonly sequenced nuclear loci (e.g., RAG1) lack coverage that approaches this breadth and depth (11% species have GenBank accessions for RAG1).

The strength of purifying selection in the mt genome may relate to taxon-specific attributes. For example, birds and mammals appear to have different degrees of selective constraint on the evolution of mt genes (Stanley & Harrison, 1999), which presumably relate to metabolic differences. Further, the greater metabolic demands of flighted versus flightless birds (Shen et al., 2009), flighted versus flightless insects (Mitterboeck & Adamowicz, 2013), and migratory versus non-migratory fishes (Sun, Shen, Irwin, & Zhang, 2011) have been invoked as causes of reduced ω in the mt exome of those groups. Given that tropical birds appear to have reduced selection for metabolism relative to temperate birds (Wiersma, Chappell, et al., 2007), there may be ω differences that can be detected across a latitudinal gradient in birds.

2.5 Birds as an evolutionary model system

Scientists and enthusiasts have shared an unparalleled, long-term fascination with avifauna. As a result, birds are an exceptionally well-studied class of organisms. In June 2014 there were more than 240 million occurrence records across the 14 classes of chordate animals in the Global Biodiversity Information Facility (GBIF) database (http://www.gbif.org). More than 210 million (87%) of these were for birds alone. Mammals, which one might naively assume would be the best-studied class for our own place within it, had fewer than 10 million occurrence records—less than 5% of the avian record, despite having 55% of the number of species. The subsequent addition of eBird (http://ebird.org/) data to GBIF in the latter part of 2014 has further increased the disparity between birds and other organisms.

Although birds offer many advantages as a study taxon, the large proportion of migratory species (19% of bird species, Kirby et al., 2008) also presents an analytical challenge. Migratory birds may comprise a relatively small component of tropical rainforest species richness, as tropical resident species substantially outnumber migrant species, but temperate and boreal species richness can vary greatly between breeding and non-breeding seasons. Bird abundances vary seasonally across all regions with large-scale migration involving billions of individuals (Rappole, 1995). Hence, the instantaneous climate and range size of a migrant species is seasonally dependent, as is the strength of the avian LDG.

The convention in avian macroecology is to analyse breeding bird ranges as the range that represents species' ecologies, including when range dynamics or species richness is being investigated. Not only do few studies provide any justification for their use of breeding ranges, few provide any evidence of having treated it as a decision where the validity of using breeding or non-breeding ranges was given consideration. Unless migratory species are excluded, this lack of consideration may be problematic for inferences relating species richness to environmental variables that have seasonal components, such as temperature or primary production. For migratory birds such as Nearctic–Neotropical migrants that do not experience temperate winter, the mean annual temperature, winter minimum, and

temperature range of their breeding range is of little direct biological significance. However, the importance of these factors may still bear on migrants by shaping the non-migratory biota across the breeding range. Further, ecological traits can be affected by both breeding and non-breeding season climate conditions (Rushing, Dudash, Studds, & Marra, 2015). Establishing the effects and appropriate use of breeding and non-breeding ranges may have lacked emphasis in the past for reasons of historical bias. Northern hemisphere naturalists describe the ranges of migratory birds as 'wintering' and 'breeding' ranges, implying a priority to the breeding range, to which the non-breeding range acts as a temporary interruption. Yet, migratory bird species typically spend more than half—and some up to three-quarters—of the year in their non-breeding range (Rappole, 1995).

2.5.1 New World oscines and suboscines as a study system

The principal advantage of working with birds for macroecology and evolution is the abundance of ecological and molecular data available. However, these data are not distributed evenly between clades. Because sister species comparisons are used as the basis for molecular evolutionary rate analyses, dense taxon sampling was prioritised for clade selection. Due to the previously extensive focus on New World birds, coupled with the extraordinary species richness of New World passerines, the major clades selected for study were: the New World nine-primaried oscines (Emberizoidea, excluding the Old World family Emberizidae; Barker, Burns, Klicka, Lanyon, & Lovette, 2013) and the New World suboscines (Tyrannides; Ohlson, Irestedt, Ericson, & Fjeldså, 2013)—that together comprise about 20% of global bird species. The relationship between the two clades is shown below (Figure 4). The relationships between the families within each are also shown, including between study taxa used in this thesis (Figures 5 & 6).

Several factors underlie the decision to use these two broad New World clades. First, as both are clades of passerine birds, many major aspects of life history should be broadly similar, and variation in body size and habitat are more restricted than across the broader avian tree. Second, the distribution of land in the New World allows for interesting tests of biogeographic hypotheses of diversification, as the two landmasses of the New World are large, topographically varied and have experienced long periods of separation. Third, the

two clades themselves have different histories in the New World. The nine-primaried oscines entered the Nearctic from boreal Europe and only recently diversified in the Neotropics, while the suboscines have a long tropical history and have only spread marginally into the Nearctic. Hence, use of these clades controls for factors that might affect the relationship between an approximation of population size (range size) and rates of molecular evolution, such as broadly different patterns of natural selection resulting from stark environmental differences experienced by shorebirds or seabirds compared to forest species, or from differences in mating systems, or various other factors. However, use of these clades also ensures that effects are not simply reflective of a single radiation that may reflect an historical biogeographic effect rather than a more general process.

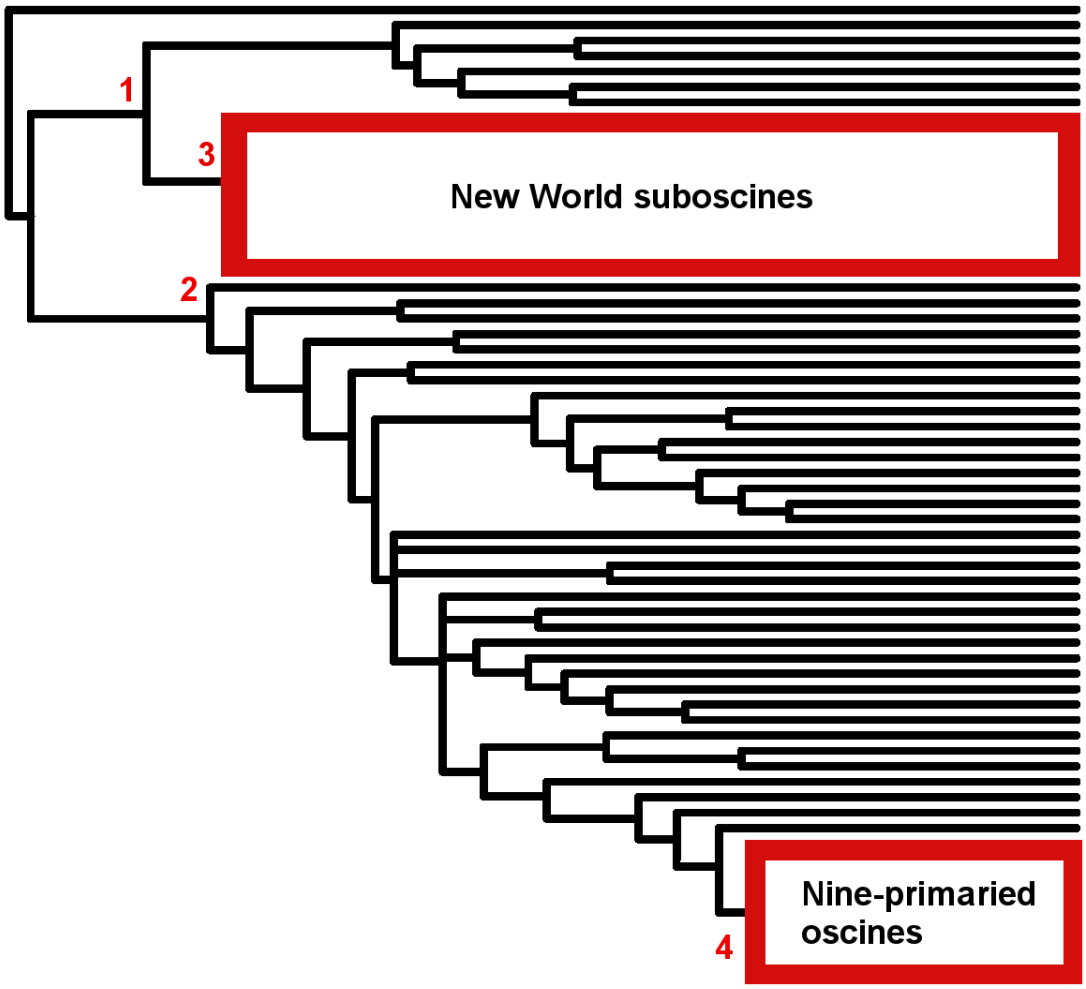

Figure 4. Relationship between the study clades used in molecular analyses. Red numbers represent 1) the origin of the suboscines, 2) the origin of the oscines, 3) the origin of the New World suboscines and, 4) the origin of the nine-primaried oscines. Adapted from (Ericson, Klopfstein, Irestedt, Nguyen, & Nylander, 2014).

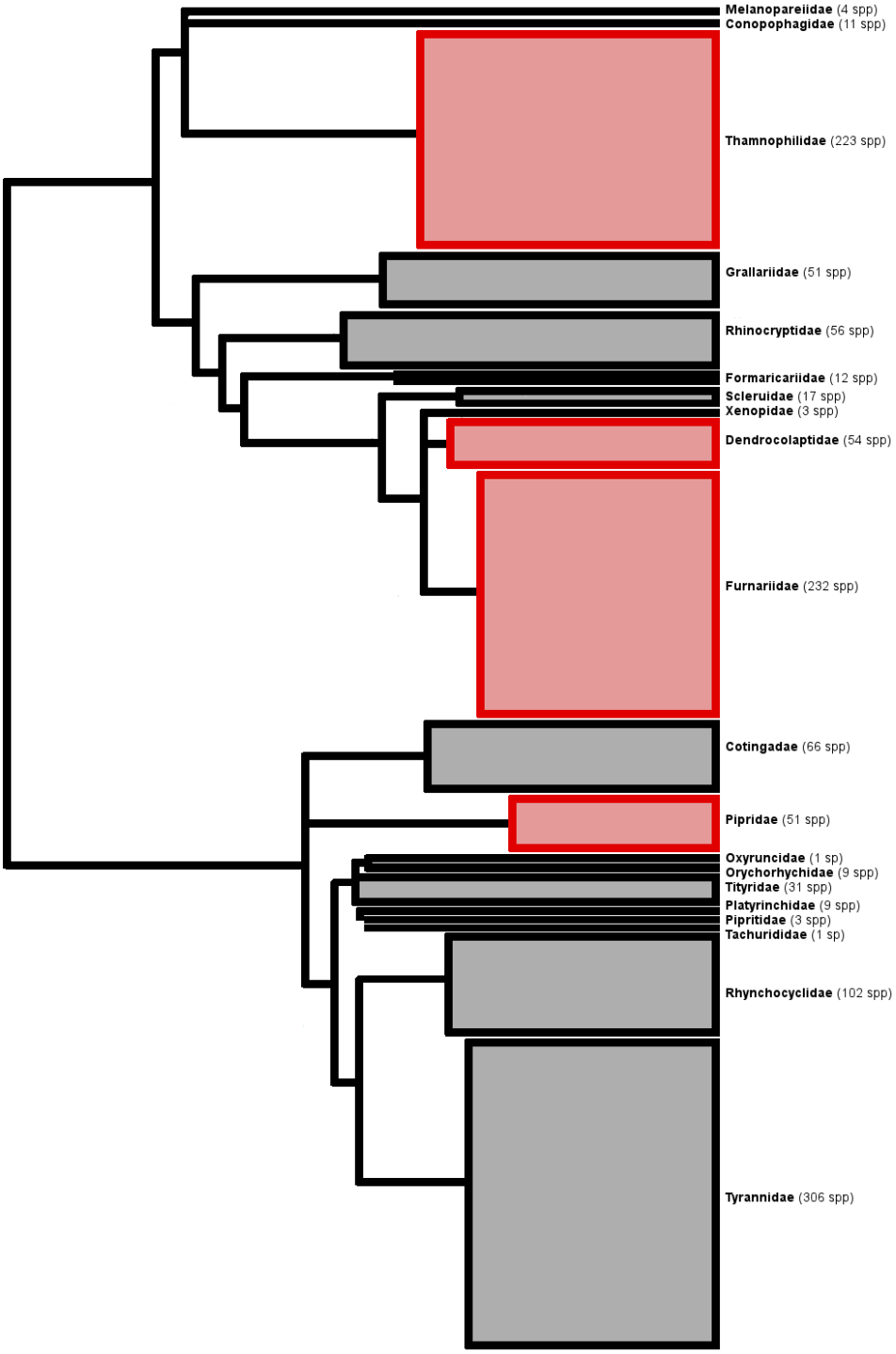

Figure 5. Relationships between families in the New World suboscines. Study families used in molecular analyses in this thesis are indicated in red. Height of boxes indicates family size. Adapted from Ohlson et al. (2013).

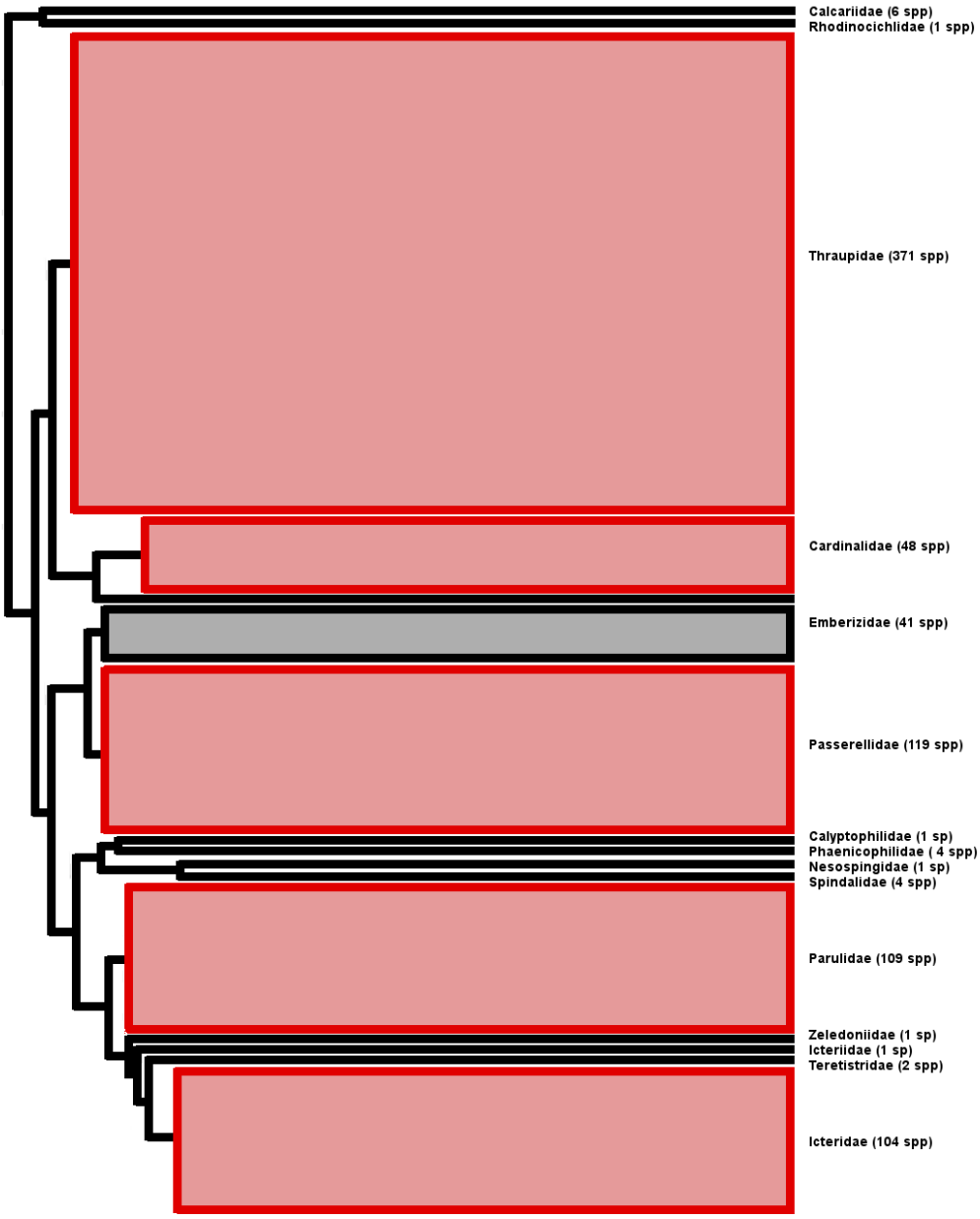

Figure 6. Relationships between families in the New World suboscines. Study families used in molecular analyses in this thesis indicated in red. Height of boxes indicates family size. Adapted from Barker et al. (2013).

3 Methods

3.1 Plant terrestrial ecoregion diversity patterns

Data acquisition

Terrestrial ecoregions (Olson et al., 2001) were used as the sampling unit, as they allow analysis of productivity–species richness relationships (PSRs) across a range of spatial scales spanning more than three orders of magnitude. Terrestrial ecoregions are biogeographic delineations of the earth's land surface intended for species conservation in the face of global change (Olson et al., 2001). The 867 described ecoregions separate distinctive biotas and regions of endemism by their natural boundaries. However, as Olsen et al. acknowledge, not all ecoregions are equally distinct, and expert subjective opinion was the basis of their division. All systems for dividing ecological regions are subjective as they require weighting of competing factors, and the use of arbitrary limits and cut-off values, causing a degree of controversy over their adequacy (Omernik, 2004), notably regarding their application to Indonesian conservation (Jepson & Whittaker, 2002). Despite any limitations, the ecoregion concept is appealing and can provide at least a first-order approximation of its intended outcome. Kier et al. (2005) compiled estimates of floristic species richness for each of the 867 recognised terrestrial ecoregions (Olson et al., 2001). GIS shapefiles for the terrestrial ecoregions were obtained from:

http://worldwildlife.org/publications/terrestrial-ecoregions-of-the-world (WWF, 2012).

The terrestrial ecoregions form a mosaic across the global landmass (Figure 7). They are subdivided into eight biogeographic realms, plus rock, ice and lakes (Figure 8). An example of one of these realms is the Neotropics, comprising 170 terrestrial ecoregions across the continental and insular Americas. In addition to realms, the terrestrial ecoregions are also separately divided into biomes the basis of shared, broad biological and physical/climatic attributes, including precipitation and seasonality (Figure 9). The biomes are noticeably less contiguous, with many being found across multiple realms. An example is the tropical and subtropical dry broadleaf forest biome, which is patchily

distributed through continental Central and South America, the Caribbean, continental Africa, Madagascar, India, south-east Asia, and the Pacific (depicted in dark purple, Figure 9). A full list of the biomes are presented below (Table 1).

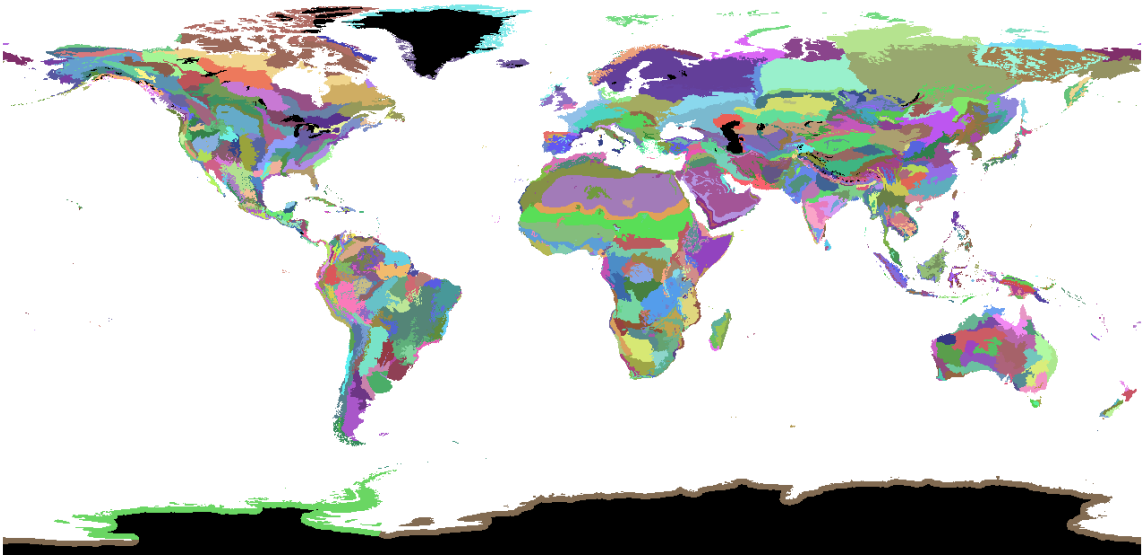

Figure 7. Map of WWF terrestrial ecoregions (WWF, 2012). Lakes, ice, and rock areas are depicted in black.

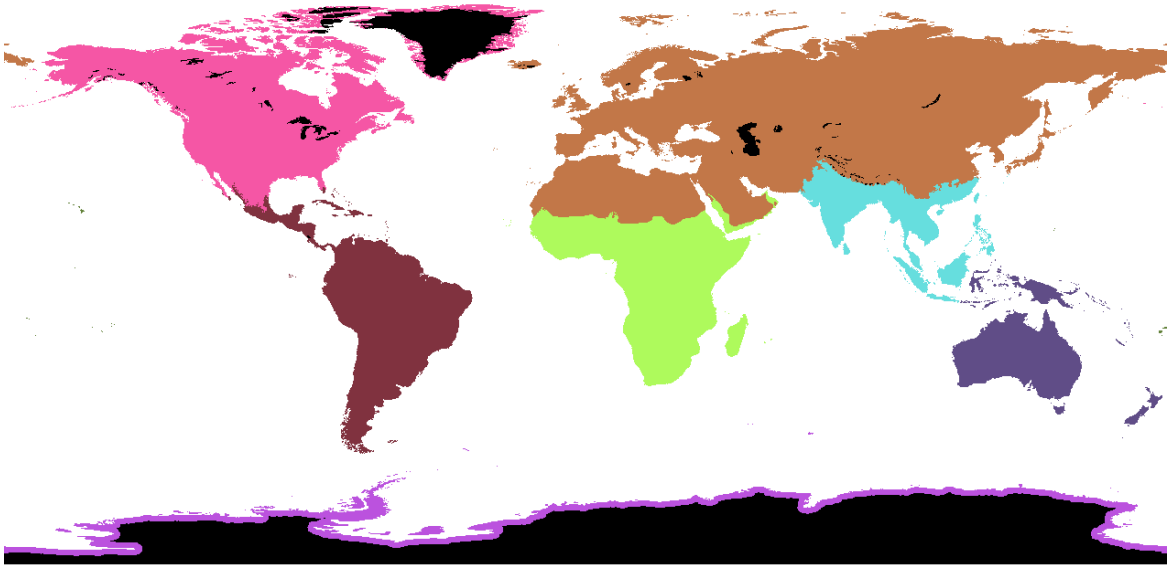

Figure 8. Map of the 8 WWF biogeographic realms (WWF, 2012). Lakes, ice, and rock areas are depicted in black.

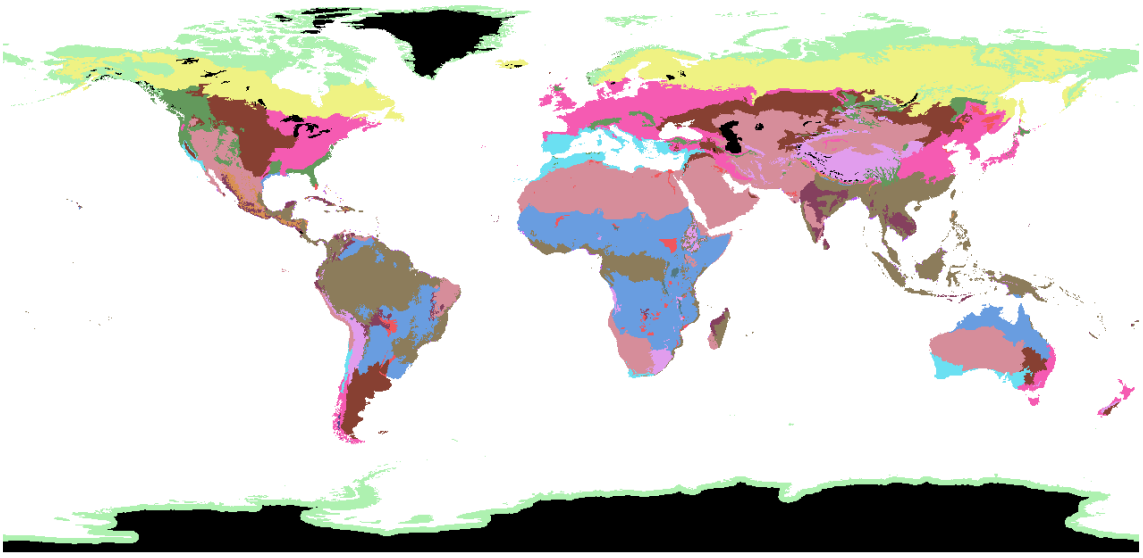

Figure 9. Map of the 14 WWF biogeographic biomes (WWF, 2012). Lakes, ice, and rock areas are depicted in black.

Table 1. List of the 14 WWF biogeographic biomes (WWF, 2012).

Desert and xeric shrubland
Tropical and subtropical moist broadleaf forest
Tropical and subtropical dry broadleaf forest
Tropical and suptropical coniferous forest
Temperate broadleaf and mixed forest
Temperate coniferous forest
Boreal forest / taiga
Tropical and subtropical grassland, savanna and shrubland
Temperate grassland, savanna and shrubland
Flooded grassland and savanna
Montane grassland and shrubland
Tundra
Mediterranean forest, woodland and scrub
Mangrove

Net primary productivity data are available as either sparsely scattered point estimates that have been measured using a range of techniques (Clark et al., 2001), or as modelled estimates that are available at resolutions as fine as 0.1 degrees. There has been controversy surrounding the use of modelled NPP that has ranged from issues regarding interannual consistency (e.g. NDVI, see Diallo, Diouf, Hanan, Ndiaye, & Prévost, 1991), through to assertions that, at a global scale, modelled NPP is fundamentally unrelated to true NPP because soil fertility is not considered and tropical NPP may be overestimated (Huston & Wolverton, 2009b). Some of the deficiencies in modelled NPP have been addressed by

more recent algorithms that account for a broader range of environmental factors (see Zhao, Heinsch, Nemani, & Running, 2005 and references therein). Indeed, without accounting for soil variation, current algorithms such as those used in NASA's MODerate resolution Imaging Spectroradiometer (MODIS) products perform well when compared with direct estimates of NPP (Zhao et al., 2005) and while such estimates contain error they do not overestimate tropical NPP (Gillman et al., 2015; Turner et al., 2006). Further, although typically more accurate, direct measures of productivity are too sparse to provide predictions of NPP in ecoregions. In particular, tropical estimates of NPP have only recently been improving in scope and quality (e.g., Malhi et al., 2009). Therefore, because of the general congruence between measured and modelled NPP and the lack of spatial coverage for more direct NPP measurements, modelled NPP was used in the present study. NASA's MODIS MOD17A2 estimates of net primary productivity for 2013 were downloaded from:

- http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MOD17A2_M_PSN (NASA Earth Observations, 2014).

These data were then combined in a GIS using ArcGIS 10.1 and extracted mean values of NPP for each ecoregion as zonal statistics.

3.1.1 Analysis

Overall patterns between plant species richness and NPP were examined using OLS regression in R 3.0.2 (R Core Team, 2014) for all ecoregions where estimates of NPP could be derived and species richness was above zero (N = 781). Although the terrestrial ecoregions vary from 6 km^2 to 4.6×10^6 km^2, most data fall between 1×10^3 km^2 and 1×10^6 km^2 (758 ecoregions, 93.7%). Within this range ecoregions were binned into orders of magnitude variation (e.g., 10^3 km^2 to 10^4 km^2) to produce relatively homogeneous size classes within which species–area relationships are substantially reduced. In addition, an extra class was added that included only the largest ecoregions: those with area greater than 10$^{5.5}$ km^2 (N = 115), which also includes the 16 ecoregions with areas greater than 10^6 km^2. While across the dataset, 17.6% of variation in species richness is explained by area on a log-log scale (8.8% on a semi-log scale with untransformed species richness), area did not explain more than 2% of variation in species richness in any of the defined size classes.

Several checks were performed to ensure comparisons across size classes were not confounded by other variables. First, Kier et al. (2005) provide data quality measures for their estimates, depending on what type of information was used to derive them. In the case of the 'poor' and 'very poor' estimates, species richness values are indirect and some have been partially derived with climate information. The distribution of the four quality categories was unequal across the size classes, with higher average quality in the large size class (10^5 km^2 to 10^6 km^2), and lower average quality in the medium size class. Therefore, analyses on the top two and bottom two quality categories were compared. The results across size classes were close to identical in both groups of data quality (Appendix 1). Second, the analysis would be weakened if a full range of productivity was not represented in each of the size classes. However, the range of productivity in all bins was similar (Figure 12). Third, realm and biome information were included in analyses to account for the possible confounding influence from these sources, whether through historical effects or from differences caused by sampling biases (i.e., more direct and accurate estimates of species richness in well-studied northern temperate ecoregions).

Lastly, ecoregion size classes were further subdivided into 12 bins based on log-transformed area to better determine the extent to which the strength of the PSR scales with ecoregion size. Bins contained between 9 and 110 ecoregions (median bin size: 61 ecoregions). For each bin, the PSR was determined, and then the relationships were examined across these bins. The relationship for productivity was compared with that for latitudinal midpoints of ecoregions.

3.2 Animal population and diversity patterns

3.2.1 Animal terrestrial ecoregion diversity

Animal diversity patterns in terrestrial ecoregions were also investigated. Data for estimated ecoregion species richness of two terrestrial vertebrate clades (mammals and birds), as compiled by the World Wildlife Fund (WWF) for the WWF Wildfinder database (WWF, 2006) were accessed under a CC3 non-commercial attribution licence through Databasin:

http://databasin.org/datasets/863c20b7776d41d68612fa181b50e10a (mammals)

http://databasin.org/datasets/798de47afddb48df9458338b04c995a4 (birds)

Procedures for initial analyses followed those for plants, using the same environmental data as described above (see 3.1 Plant terrestrial ecoregion diversity patterns, & 3.1.1 Analysis). All statistical analyses were performed in R 3.1.2.

3.2.2 Correlates of vertebrate diversity

The results of plant and animal PSRs in ecoregions above indicated that NPP explained substantially less variation in animal than plant species richness. Therefore, additional exploratory analyses were undertaken to investigate other climate-related factors that could account for these differences, to better explain patterns of vertebrate richness.

To explore additional patterns, a range of climate variables plausibly linked to net diversification patterns were considered: mean annual temperature, temperature seasonality, minimum temperature of coldest month, and annual precipitation. Data for these climate variables were obtained from the BIOCLIM dataset (Hijmans et al., 2005) as raster data in 2.5 arc-minute resolution:

- BIO1, BIO4, BIO6, and BIO12 from http://www.worldclim.org/current

For each, ecoregion averages were calculated as the mean values for pixels within the ecoregion, using the ecoregion boundaries defined in the shapefiles previously described (see 3.1

Data acquisition). Calculations were done using the *extract* function from the *raster* package in R 3.1.2 (R Core Team, 2014).

In addition to bird and mammal ecoregion diversity, total vertebrate phylogenetic diversity was also investigated. Data for phylogenetic diversity were also available from The Nature Conservancy (Hoekstra et al., 2010) using vertebrate species distribution data from WWF Wildfinder database (WWF, 2006), and were accessed under a CC3 non-commercial attribution licence through Databasin:

- http://databasin.org/datasets/4c0ab10592b14a7fb29588eda42a0d42

Phylogenetic diversity was calculated by Hoekstra et al. (2010) using the method described by Faith (1992), where phylogenetic diversity is the sum of branch lengths connecting all species in a phylogenetic tree. Data were also obtained for total vertebrate species richness, however there was a sufficiently strong correlation with phylogenetic diversity that the two diversity measures were considered equivalent and only results for phylogenetic diversity are presented here ($R^2 = 0.978$).

For Faith's phylogenetic diversity, bird species richness and mammal species richness, regressions were performed against the additional climate variables. Regressions were performed using the largest ecoregions subset (>$10^{5.5}$ km^2), as these ecoregions had the strongest PSRs (see 4.2.1 Animal terrestrial ecoregion diversity).

3.2.3 Range sizes and latitudinal ranges in animals

This section investigates Rapoport's rule in mammal and bird ranges using an approach designed to separate the effects of area and latitude. Despite a lack of general support for Rapoport's rule, there is some evidence that it holds in the Nearctic, and therefore this study was restricted to the northern hemisphere. Rapoport's rule predicts that latitudinal ranges limit the total geographical range sizes of tropical species because tropical species have narrow climate tolerances. Assuming no strong bias in range shapes occurs across latitudes, latitudinal range should also increase as range size increases. If causal of geographic range size variation rather than coincidental, then the harder latitudinal limits

on tropical species should result in ranges that are more ovate and latitudinally compressed than those of temperate species. Therefore, latitudinal range should have a steeper gradient with total range area in temperate than tropical species.

In the case of mammals, all terrestrial mammals were included in the analysis. Mammals vary considerably in body size and ecology, and broad groups can replace each other over space. In the case of birds, only non-migratory, lowland passerine birds were included. The body size range of passerines is considerably narrower than for mammals, and, by considering only non-migratory species, it is ensured that climate tolerances are linked to latitude. Lowland birds were defined as those recorded as having a minimum elevation below 500 metres above sea level (m.a.s.l.). Montane species that occur along ranges such as the Andes frequently have elongated ranges that are governed by habitat (Ruggiero, Lawton, & Blackburn, 1998 and references therein). These two taxa together allow several possible explanations for geographic variation in range size and/or latitudinal range to be separated.

All analyses of Rapoport's rule were investigated across three latitudinal bands in the northern hemisphere: 0–25° N, 25–50° N, and >50° N. The outcomes of previous studies have depended on whether species ranges are categorised by their latitudinal midpoints (Rohde, Heap, & Heap, 1993), or by their highest latitude, because of potential mid-domain effects (Luo et al., 2011). Therefore all analyses were performed with species categorised using both methods.

Data were acquired for species ranges, including the total geographical extents over which species occur (extent of occurrence; EOO), and latitudinal ranges, calculated as the difference between the northern and southern limits of the EOO. Mammal range data were accessed from the PanTHERIA database (Jones et al., 2009). Bird range data were derived from a GIS of bird distribution shapefiles (BirdLife International & NatureServe, 2011; Ridgely et al., 2011). The data release contained all species recognised by BirdLife International in 2011.

The shapefiles are available by request only through BirdLife International via:

- http://www.birdlife.org/datazone/info/spcdownload

Bird range sizes were calculated in ArcGIS 10.1. Distributions were projected in the cylindrical equal area projection, from which area, latitudinal centroid, and latitudinal maxima were calculated for all non-migratory passerines with latitudinal centroids in the northern hemisphere. All range sizes for birds and mammals are estimates of EOO. Therefore, wherever mentioned without qualification, range size refers to EOO. Land area for each of the three latitudinal bands (0–25° N, 25–50° N, and >50° N) was also calculated in ArcGIS 10.1, using Natural Earth land boundaries and the cylindrical equal area projection. The sum of ranges for all non-migratory passerine birds and all terrestrial mammals was also calculated for each latitudinal band.

To confirm a general range size pattern, median range sizes for birds and mammals were tabulated across northern hemisphere latitudes in the three latitudinal bands as above. The extent to which species ranges overlap at different latitudes was also estimated as the sum of geographic ranges. Land area, and the sum of range sizes were calculated for both mammals and non-migratory passerine birds in each latitudinal band using latitudinal midpoints of species ranges. Patterns in land area and the sum of range sizes for each taxon were then explored to determine the extent to which species overlap occurs, and thus whether higher species turnover is sufficient to explain the LDG as predicted under Rapoport's rule.

To determine how latitudinal range relates to range size at different latitudes, ordinary least squares regressions were performed on mammal and bird datasets. The square of latitudinal range will be proportional to range size in simple range shapes (i.e., latitudinal range is the diameter of an approximately circular geographic range). Therefore, to determine how range size and latitudinal range covary, range size was square-root transformed, allowing linear regressions for each of the latitudinal bands, forced through the origin.

3.2.4 Population size–range size covariation

This section examines the utility of range size, measured as extent of occurrence (EOO), as a surrogate measure of population size in birds. Although area of occupancy (AOO) is recognised as a good predictor of population size, the differences between EOO and AOO as range size estimates mean that EOO must be independently evaluated as a population size predictor before its use for this purpose (see 2.4.4 Range size as a surrogate measure of population size).

Bird species population size estimates were acquired from BirdLife International. Species trait data supplied by BirdLife International has been archived by them and is available upon request. BirdLife International population size estimates are more commonly made for the total number of individuals than for mature individuals. Because of this, the total number of individuals was used. Data quality was categorised by BirdLife International on the basis of the methods used in estimation. Because the purpose of this section is to evaluate EOO, species were only retained if they: 1) had "good" or "medium" quality estimates of population size, 2) have estimates of body mass, and 3) did not belong to the seabird orders: Procellariiformes, Pelecaniformes, Charadriiformes, or Sphenisciformes (Gaston & Fuller, 2009). Compared to terrestrial birds, seabirds may have radically different relationships between population size and geographic range, as they may cover large areas and in many cases have breeding ranges that are limited to dense colonies. Comparisons of seabirds to other birds is therefore difficult and requires additional consideration beyond the scope of this work. In addition, only species with body mass estimates were included, because body mass covaries with range size (Gaston & Blackburn, 1996b), and population density (Damuth, 1981; Enquist, Brown, & West, 1998). Body mass data were obtained from the CRC Handbook of Avian Body Masses (Dunning, 2007). On a log-log scale, it is predicted under the energetic equivalence rule that population density scales at a gradient of -0.75 × body mass, suggesting a straightforward approach for a first-order correction for the effect of body size on population density. The application of these three criteria resulted in a dataset of 381 bird species, spanning 80 families and 22 orders.

EOOs (herein, range sizes) were determined using GIS shapefiles for extents of occurrence held by BirdLife International/NatureServe. The data release was dated 2011, and contains all species recognised by BirdLife International at that time. This resource compiles spatial data from BirdLife International, Natureserve and Ridgely et al. (2011). The shapefiles are available by request only from BirdLife International:

- http://www.birdlife.org/datazone/info/spcdownload.

Range sizes were calculated in ArcGIS 10.1. To estimate the extents of breeding/resident ranges, shapefiles for all species were merged into a single feature class, and polygons for all records with 'Season' attributes other than 'Resident' or 'Breeding Season' were removed; all records with 'Origin' other than 'Native' or 'Reintroduced' were removed; and all records with 'Presence' other than 'Extant' or 'Probably Extant' were removed. The resulting feature class was dissolved on species name. Range sizes for each species were then calculated using a cylindrical equal area projection.

The bivariate relationship between population size and range size was regressed. In addition, body mass was added to the model, as a first-order correction of density variation. To determine clade-specific variation, order was also added to the model. The effect of IUCN Redlist conservation status was also examined to determine if category differences affect the relationship.

Apparent relationships can arise spuriously due to a lack of phylogenetic independence (Felsenstein, 1985). To account for this possibility, and simultaneously test the reproducibility of the pattern at lower taxonomic ranks, individual regressions were iterated for each family represented in the dataset by at least 10 genera. These regressions were initially run both on whole families, and using a single species from each genus (the median body mass species).

To evaluate the use of EOO as a population size estimator (i.e., with a body mass correction) in an ecologically and phylogenetically more restricted scenario, the initial analysis using only range size to predict population size was repeated on a dataset

restricted to passerine birds (N = 262 species). To investigate latitudinal variation, the data were subsetted into tropical (|latitudinal centroid| < 20°) and extratropical (|latitudinal centroid| > 20°) clades and the analysis repeated. Finally, analysis was also run using the subset of New World oscine and suboscine passerines. These taxa deserve particular focus here as they are used in molecular evolutionary rates studies later in this thesis. Because population size estimates have been prioritised for at-risk species, few species in the 'least concern' category have estimates available. In recognition of this the impact of IUCN category was analysed in the New World passerine subset.

3.2.5 Variation in population density with latitude and elevation

Species density varies spatially to a greater extent than do total numbers of individuals (see 2.3.5 Prominent hypotheses for the latitudinal diversity gradient: Species–energy theory). Given this disparity, we can expect there to be spatial covariation between density of individuals and species richness not accounted for by range size. Therefore, two additional tests were performed to investigate this effect across two major gradients of species richness: latitude and elevation. For these tests, population size data for passerine birds only was used (N = 262 species).

Firstly, residual variation in population size not explained by its relationship with range size was regressed against latitude using OLS regression. Data were initially subsetted into insular and mainland populations, although similar patterns were observed across both. Therefore, only total dataset regressions are shown. Both absolute and actual values of latitude were compared to determine if a latitudinal gradient in population density exists.

Secondly, the same residual variation was regressed against latitude for a subset of montane passerines. Passerines were considered montane if their minimum elevation was at least 1000 m.a.s.l.. This elevational cut-off is conservatively high to prevent the inclusion of species with lowland affinities, as several factors such as population connectivity might change between lowland and upland species. Because tropical montane regions are diversity hotspots, I subsetted the elevation dataset into tropical and temperate

comparisons (divided on the basis of species' latitudinal centroids being above or below |20°|).

Finally, patterns of genetic diversity for the most commonly sequenced avian gene, the mitochondrially encoded gene NADH dehyogenase subunit 2 (ND2), was investigated in all passerine birds with at least 20 sequences available (N = 102 species). Nucleotide diversity and maximum genetic divergence were calculated for each species using the *pegas* and *ape* packages in R 3.1.2. In addition to nucleotide diversity the ratio of diversity to divergence was calculated. Both were examined across latitude to determine if consistent patterns across a latitudinal gradient exist. The relationships were then re-examined using a subset of phylogenetically independent contrasts. First, for each genus with more than one species, a pair of species was selected on the basis of the greatest difference in absolute latitude. For this species pair, ND2 diversity and the divergence:diversity ratio was contrasted (value for higher absolute latitude species minus value for lower absolute latitude species) These contrasts were then regressed against contrasts for the difference in absolute latitude between these species pairs (higher absolute latitude minus lower absolute latitude).

3.3 Effect of range size on molecular evolutionary rates and patterns in New World birds

This study investigates the covariation of molecular evolutionary processes with extent of occurrence—a range size metric that serves as a first-order approximation of population size across ecologically similar species, as justified in the previous section. Two broad clades were selected for analysis: the New World nine-primaried oscines and the suboscines. Together, these clades represent approximately 20% of global avian diversity. The selection of these clades reflects a balance between sufficient ecological homogeneity to allow meaningful cross-taxon comparisons, and sufficient historical difference to prevent patterns spuriously generated by shared contingencies, as justified in the literature review (2.5.1 New World oscines and suboscines as a study system).

I will first describe the process of assembling the avian DNA sequence database that is used in this study, and the subsequent study. This database is available for download through Figshare (doi:10.6084/m9.figshare.1236602). I will then describe the process used to select representative sequences for analysis. I developed a phylogenetic pipeline in R to streamline these processes, which is also described, and for which the script is available upon request.

3.3.1 Sequence dataset assembly

All available mitochondrial sequence data on GenBank was collected for every avian species recognised by BirdLife International. Although the analysis here focuses on two clades of New World passerine birds, sequence data was collected for all birds to allow for other analyses in the future, and to generate a complete avian mt database for other researchers.

Sequence data was initially collected in February 2014 by downloading a flat-pack file of approximately 170,000 accessions from the GenBank nucleotide database using the search term: "birds[organism] AND (mitochondrial OR mitochondrion)". While it is possible to search for individual genes on GenBank, a complete set of gene name synonyms would be required to prevent possible omissions. For example, it is possible to find avian cytochrome *b* sequences with the gene name: "cytb", "cob", "cytochrome b", "cytochrome-

b", "cyt b", "cyb", "cyt-b", and "mtcyb". Similarly, for gene names that include numbers, Arabic or Roman numerals may be used in combinations with the different synonyms. For example, possible cytochrome *c* oxidase subunit 2 synonyms include: "cox2", "coxii", "coii", "co2"', "cytochrome c oxidase subunit ii", "cytochrome c oxidase subunit 2", "cytochrome oxidase subunit 2", "cytochrome oxidase subunit ii". Because of the wide range of possible synonyms across the set of mt protein-coding genes, the initial search was as broad as possible, with subsequent filtering to remove accessions that were not avian mtDNA genes.

By manually searching accessions, a list of synonyms was developed for each of the 13 protein-coding mitochondrial genes in the animal mitochondrial genome. Accessions that did not contain any of these synonyms were excluded. Additional exclusions were made for accessions that included in their definition: "predicted", "pseudo", or "-like", any of which indicate that the retrieved sequence is not believed to have been from a functioning mitochondrial gene. A number of other sequences were excluded on the basis of being from organisms outside of Aves.

Many accessions contain sequence data for more than one gene, either as concatenations of a set of genes, or as a whole or partial mitochondrial genome sequence. An R script was written to efficiently retrieve the sequence data for each gene by identifying the location of the sequence for each gene within the accession, and then extracting the sequence.

Sequence selection

While certain species are disproportionately highly sequenced—153 species have more than 100 available sequences across all mitochondrial coding genes—even many less commonly sequenced species still have multiple accessions available for one or more genes. For cytochrome *b*, 44% of species with sequences available had more than one cytochrome *b* accession. Therefore, it was necessary in these cases to select a representative sequence. Two criteria were applied to achieve this selection: sequence length and pairwise sequence distance to homologous sequences in other species. For each gene, all sequences for each species were collated by searching the avian gene database derived from GenBank

for gene sequences matching the species name or one of its recognised synonyms. Sequences were retained if they were of similar length to the longest available sequence (at least 90% of the longest sequence). The sequence length criterion allows for the largest number of DNA characters to be compared in phylogenetic analyses. After filtering for sequence length, if more than a single sequence remained then a multiple sequence alignment was constructed with the remaining sequences and the homologous gene sequences for all other species in the genus using the MAFFT alignment algorithm (Katoh & Standley, 2013). A distance matrix was calculated for each possible sequence against the sequences of congeners using the Tamura-Nei substitution model (Tamura & Nei, 1993). The sequence selected for analysis was that with the median pairwise distance to the congeneric homologues, on the basis that this was typical of the available sequences. The rationale for this decision is that atypically long distances may be the result of sequencing errors, Numts (nuclear, pseudogenic insertions of mitochondrial genes), or non-representative genetic clusters in the population (e.g., unrecognised subspecies with small N_e), while shorter-than-average sequences could indicate introgression, and could introduce biases in branch-length contrasts between sister species pairs. Also, in sister species pairs with different numbers of homologous sequences available, selecting the shortest distance biases towards shorter branch lengths in the species with a greater number of sequences, particularly if the other species has only a single sequence available. Finally, in cases where there was an even number of sequences for focal species such that two sequences are equally close to the median, the sequence with the shorter total distance of the two was selected. If more than one sequence met this criterion, the first of these (ordered by alphanumeric GenBank accession ID) was selected.

3.3.2 Phylogenetic and ecological data

To investigate patterns of molecular evolutionary rates on moderately large scales, a simple phylogenetic pipeline was developed in R. In its present form the pipeline is only suitable for use on a Macintosh system as three of its components are called via Terminal commands. However, the script could be modified to execute equivalent commands in a Windows command prompt, as the called software is available on both platforms. The pipeline facilitates: aggregation of the sequences for species, alignment of sequences for

each available gene, trimming of alignments, orientation of alignments to read in the 'forward' direction, trimming alignments to whole codons, concatenation of sequences across alignments, model-testing partitioning schemes and substitution models on the concatenated alignment, and construction of control and alignment files for phylogenetic analysis. Phylogenetic analysis can then run on the output files using GARLI and/or MrBayes. The pipeline has external software dependencies:

- MAFFT 7.164 (http://mafft.cbrc.jp/alignment/software/),
- TrimAl 1.2 (http://trimal.cgenomics.org/downloads), and
- PartitionFinder 1.1.1 (http://www.robertlanfear.com/partitionfinder/).

Software versions were the current, stable releases at the time the pipeline was developed.

MAFFT (Katoh & Standley, 2013) is called to align sequences using the 'auto' setting, which determines an appropriate algorithm for data. Because aligning closely related animal mitochondrial gene sequences is straightforward due to the lack of indels, this was considered sufficient. However, MAFFT's more computationally intense algorithms could be used if desired and include appropriate choices for evolutionarily more complex loci. The MAFFT 'adjustdirection' option is called to detect the direction of sequences as they are added to an initial alignment, and reverse them if needed. TrimAl (Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009) is then called to remove parts of the beginning or end of the alignment that are absent in some of the included species. A cut-off value of 70% was used (i.e., alignment ends were trimmed until at least 70% of species had sequence data), and this value is adjustable in the pipeline. The 70% value reflects the desire to produce compact alignments for the efficiency of later phylogenetic computation, while reflecting varying sequence availability across whole-family alignments. The orientation and position of the most likely reading frame is then determined from that with the fewest number of stop codons in the appropriate translation table. By doing so, each gene alignment begins at the first position of the first whole codon, and ends at the third position of the last whole codon, allowing later d_N/d_S analysis to be conducted on a concatenation of alignments for all available genes. However, the corollary is that the pipeline would require a number of modifications to incorporate non-coding sequences or coding sequences from genomes with different genetic codes. The sequences for each

species are then concatenated. A control file for PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012) is generated that divides the alignment into potential partitions by gene and codon position. A range of possible substitution models for partitions is set to include either the models available in GARLI or those available in Mr Bayes. The criterion for comparing the fit of different partition schemes and substitution models is set as Bayesian Information Criterion (BIC; Schwarz, 1978). There is no definitive basis for choosing BIC ahead of related penalised likelihood criteria, such as Akaike Information Criterion (AIC). Differences include the factors such as whether the range of model options includes the true model (Burnham & Anderson, 2004), which is unlikely that this is ever true for phylogenetics. On one hand, if the true model is unlikely to be included amongst the candidate models, then AIC should be preferred (Burnham & Anderson, 2004). At the same time, AIC often selects a more complex model than the true model, and is more suitable for predictive rather than confirmatory applications (Aho, Derryberry, & Peterson, 2014). The optimality criterion of BIC is based on consistency in a framework where overfitting of models represents the selection of a model more complex than the true model, which is a recognised problem in the accuracy of phylogenetic analyses (Li, Lu, & Ortí, 2008). Balancing these considerations and given the scale of opportunity for overparameterisation when data are partitioned by gene and codon position, BIC was the favoured information criterion. As with other components in the pipeline, this option could be readily changed if desired.

The output files (alignments and control files) are then available for use in GARLI or Mr Bayes. Phylogenetics software was excluded from the pipeline so that grid computing could be used to accelerate phylogenetic computation. For smaller projects, or for instances where bioinformatics jobs can be queued through command line calls, this step could be implemented in the pipepline as well. For the present work only GARLI was used, because the primary features of interest in the resulting phylogenetic trees were topology and branch length. The GARLI web service hosted at http://www.molecularevolution.org (Bazinet, Zwickl, & Cummings, 2014) produced all phylogenetic trees using GARLI 2.1 (Zwickl, 2006). GARLI performed best tree searches, as bootstrapped consensus trees do

not contain meaningful branch length estimates. An additional script recovers all sister species pairs from GARLI's output trees, along with their branch lengths.

The original alignments and GARLI output trees were then used to calculate d_N and d_S in HyPhy (Pond, Frost, & Muse, 2005). The HyPhy batch file AnalyzeCodonData was run using the codon evolution model MG94 F3x4 crossed with the GTR model. To estimate branch-specific d_N and d_S values, HyPhy runs exponentially faster than the widely used *codeml* from the PAML package (Yang, 2007), which was not able to execute analyses within a reasonable time period for the largest phylogenies. To ensure the HyPhy analysis was robust, a subset of smaller families were separately evaluated using *codeml* using a free-ratios model. Results were similar (Pearson's correlation coefficient = 0.917), and therefore the HyPhy analyses were used throughout.

Production of family-level trees

The phylogenetic pipeline was run on nine families of New World birds. These families were all of the families in the New World Emberizoidea and Tyrannides for which at least two mitochondrial genes were available for at least 80% of species in 80% of genera, and the total number of species with available sequences > 40. Relationships between the clades, and the families within them are provided above (Figures 5 & 6). Some of these values are arbitrary cut-offs that represent a balance between ensuring good taxon sampling, sufficient sequences for parameter estimation, and enough contrasts for a wide-ranging anaylsis of evolutionary rates. In practice, there was a distinction between large, well-sampled families, and other families. Because there is some uncertainty between family-level relationships, phylogenies were outgrouped by including species from each of the other families in the clade, and a more distant outgroup species on which the tree could be rooted.

All sister species pairs from maximum likelihood trees were extracted, along with their branch lengths, d_N, and d_S values. These values were log-transformed and comparisons made by subtracting the transformed value for the larger-ranged species from the smaller

ranged species (Freckleton, 2000). An example of a sister species pairing is shown below (Figure 10).

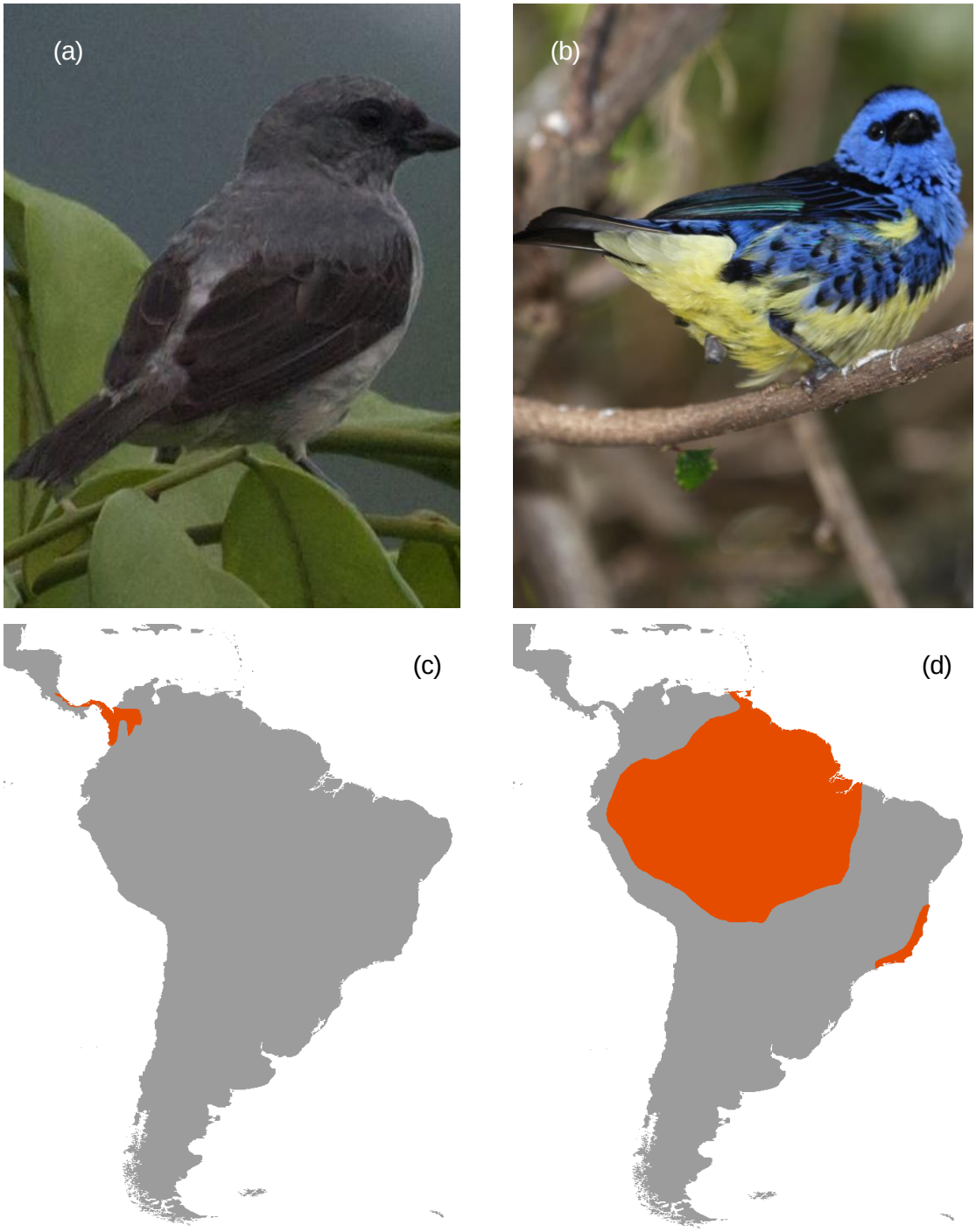

Figure 10. Example of a pair of sister species included in analyses: (a) *Tangara inornata* and (b) *Tangara Mexicana* from the family Thraupidae, in the nine-primaried oscines clade. Geographic extents of *T. inornata* and *T. mexicana* are depicted in (c) and (d) respectively. Images are licenced under the Creative Commons Attribution 2.0 Generic license. Brian Gratwicke is author of *T. inornata* photograph reproduced in (a). Sandysphotos2009 is author of *T. mexicana* photograph reproduced in (b).

Calculation of contrasts

For each sister species pair, transformed contrasts were used calculated for most variables as $\log_{10}V_L - \log_{10}V_S$ where, V is the variable of interest, and subscripts L and S refer to values for the larger and smaller ranged species in the contrast respectively. Transformed contrasts cannot be applied to variables that have negative or zero values without arbitrarily altering data values. Therefore, contrasts for mean annual temperature, and elevation variables were calculated as untransformed contrasts.

3.3.3 Dataset quality controls

All trees were examined to ensure no obvious sequencing errors affected sister species comparisons. In two trees (Thraupidae and Thamnophilidae), long branches, indicative of errant sequence inclusion, were identified. However, in neither case did these branches belong to sister species contrasts, and their removal did not affect the inferred topology. Therefore, these errors were assumed to be of minimal impact on the analyses.

A primary consideration in evolutionary rates analyses is that species are separated by a sufficient evolutionary distance that evolutionary metrics such as the substitution rate can be estimated with accuracy. Initially, all pairs for which d_N or d_S for at least one species was estimated as zero were excluded. However, while this removes pairs for which comparisons cannot be made, it does not resolve the wider problem. This problem can be considered in two parts: inflated variance in substitution rates between recently diverged species, and confidence in the reproductive isolation of contrasted sister species. Each of these considerations and their corrections are described.

High variance in substitution rates occurs over short time periods because substitution is a discrete stochastic process. Welch and Waxman (2008) propose a test to detect recently speciated pairs affected by elevated variances, because including such comparisons lowers power to detect relationships between species traits and evolutionary rates. Welch and Waxman state that high-variance contrasts can be identified by regressing standardised contrasts of substitution rates against $\sqrt{t}$, where t is time since divergence. Contrasts with

low $\sqrt{t}$ should be progressively removed until no negative trend is detected. Standardised contrasts were calculated as $\frac{|\ln(S_1)-\ln(S_2)|}{\sqrt{t}}$ (Welch & Waxman, 2008) where S_1 and S_2 are the substitution rates of the sister species. Lacking fossil data to independently date sister species divergences, t was estimated from the genetic distance between species as $\frac{100(S_1+S_2)}{1.6}$, assuming a 1.6% divergence MY^{-1} (Omland et al., 1999). This approach is not ideal, as t is not evaluated independently of the number of substitutions; therefore some high-variance pairs may avoid scrutiny precisely because the number of substitutions is elevated above the substitution rate. However, the paucity of the avian fossil record provides no straightforward alternative. An additional correction is made to the dataset as described below. Standardised contrasts were regressed for each of the nine family-level phylogenies, and contrasts were removed until no significant regression slope was detected.

Although recognised as species by BirdLife International, it is likely many species in the present study are not sufficiently reproductively isolated to exclude introgressive hybridisation, given the extended time over which bird species evolve hybrid infertility (Price & Bouvier, 2002). Hybridisation can weaken or eliminate the phylogenetic signal in the data if the mt genome of one species is replaced by that of the sister species. As the mt genome is effectively a single locus, such introgression would be detectable as short genetic distances between species. Given a 'rule' of 2% mtDNA divergence is applied to identify species across a wide range of organisms (e.g., BOLD; http://www.barcodinglife.org/), this provides a simple approach to exclude potentially problematic contrasts. Here, the 2% threshold was minimally modified to be a 1% threshold per species. This modification is intended to catch closely related pairs for which stochastic variation in substitution for one species causes high variance, but that might not otherwise be detected by the Welch and Waxman (2008) test above.

Outliers were analysed using Cook's distances to evaluate leverage, and studentised residuals to evaluate the magnitude of its deviation from model expectation. Outliers could be caused by unmeasured variables, a poorly fitted model, high-variance substitution rate pairs not detected using the Welch and Waxman test, or pairs where one

or both of the species have an atypical relationship between range size and population size. Two contrasts had elevated Cook's distances, although the first outlier more than four times greater. Of the two, only the first point was detected as an outlier by its studentised residual value (>2) (see Appendix 2: Data quality and outlier analyses for range size–population size study). This comparison was further investigated. The smaller-ranged species in this comparison (*Thripophaga cherriei*) is a species with a decreasing population trend occupying a range of 10km^2 along Rio Orinoco, Venezuela. It has historical records over a wider range, and may occupy, or have occupied, a wider range than is currently recognised (BirdLife International, 2015; Lentino et al., 2007). On these grounds, this outlier was removed to prevent its undue influence on the regression slope.

Finally, although clade selection focussed on selecting clades for which large amounts of sequence data were available, it was considered possible that the number of bases contrasted per sister species could still affect the power of the relationship. Therefore the number of bases (less ambiguities and missing data) that were contrasted for each sister species was compared (see Appendix 2: Data quality and outlier analyses for range size–population size study). No effect was evident, so all contrasts were retained.

3.3.4 Analysis

Regression analysis was performed on the relationship between range size and four evolutionary metrics: the total nucleotide substitution rate, d_N, d_S and ω. Regression analyses were ordinary least squares, performed in R 3.1.2. Because log-transformed contrasts are dimensionless, OLS regression is more appropriate for their analysis than other regression methods, even if ratios on both axes are estimated with error (Legendre & Legendre, 2012). While OLS regression is robust to the violation of some of its underlying assumptions, severe violations may cause model inferences to be unreliable. Regression models were checked for the global assumptions of linear models using the R package *gvlma*. All regression models presented in the Results meet these assumptions.

Initial analysis was performed on the dataset prior to applying quality controls (herein "raw dataset") to ensure that qualitatively similar patterns were observed. These results

were then compared with the dataset after removal (herein "final dataset"). Correlation analysis was performed to determine the relative contributions of d_N and d_S to the substitution rate and ω. To determine the consistency of the range size–evolutionary rates pattern, the analysis was repeated for each family separately.

Because there is a stronger relationship between range size and population size outside of the tropics than within, the analysis was also repeated on tropical and extratropical subsets. As with previous analyses, the final dataset was divided on the basis of species' latitudinal centroids being above or below |20°|. The maximum latitude of the each species pair was used.

3.3.5 Potentially confounding factors

Because many factors are proposed to affect molecular evolutionary rates it is possible that any correlation with range size is the spurious result of a range size covariate, or an otherwise unmeasured response in a covariate of the substitution rate. These were body mass, migratory status, and range-wide averages of net primary production, mean annual temperature, annual rainfall, elevation, and latitude. For climate variables, range-wide averages were calculated across resident ranges only, as the climate conditions experienced in resident ranges are experienced year-round, while the annual averages of conditions in seasonal ranges may not be representative of the period of the year in which migratory populations are present. For fully migratory species (i.e., those that do not have resident grounds; 26 species affecting 19 pairs) annual averages for both breeding and non-breeding ranges were calculated. Because correlations were stronger with values from breeding than non-breeding ranges, breeding range values were used.

Data

Range size: For each ingroup species, EOOs were determined using a GIS in R 3.1.2. BirdLife International and NatureServe (2014) species distribution maps were obtained as ESRI shapefiles by request from BirdLife International. Shapefiles for each species were imported in R using the *readOGR* function from the *rgdal* package. Data were projected in the cylindrical equal area projection, from which both breeding and non-breeding range

sizes were determined separately. Polygons were included for which species' presences were listed as "Extant" or "Probably extant", species' origins were "Native" and species' seasonal presences were recorded as either "Resident" or "Breeding season", or "Resident" or "Non-breeding season", for the breeding and non-breeding ranges respectively. Resident range data were calculated by retaining only those polygons for which season presence was recorded as "Resident". All data are presented in the electronic dataset, however, range size analyses in the thesis used resident/breeding ranges.

Elevation: Maximum and minimum species' elevations were obtained from BirdLife International as a part of their ecological dataset. These data are archived by BirdLife International, and are available upon request, but raw data cannot be supplied by a third party. Not all species had estimates for both elevational limits. In cases where lower limits were unavailable, the literature was searched for habitat descriptions. For species recorded as 'lowland', a value of 0 m.a.s.l. was used. This may introduce some inaccuracy in elevation estimates, although it allowed a considerable expansion of the elevational dataset. Average elevation was calculated as the average of minimum and maximum elevation.

Climate: The influence of a range of climate-related variables was considered as potential confounding factors: annual net primary productivity (NPP), mean annual temperature, annual precipitation, and temperature seasonality. In addition, latitudinal midpoint (range centroids) was also used as a dummy climate variable. NPP was derived from NASA MODIS data (see 3.1 Plant terrestrial ecoregion diversity patterns: Data acquisition). For one small-ranged insular species, NPP could not be estimated (*Nesospiza wilkinsi*), and therefore this contrast was excluded when NPP was used in analyses. Data for other climate variables were obtained from the BIOCLIM dataset in 2.5 arc-minute resolution from:

- http://www.worldclim.org/current

BIOCLIM and NPP data were imported as rasters into R 3.1.2. For each species, the range polygons compiled above to estimate range size were used to estimate species' climate

conditions, using resident and non-breeding ranges. Means for rasters were calculated using the *extract* function from the *raster* package.

Body size: Body size estimates were available for many species. Body mass data were obtained from the CRC Handbook of Avian Body Masses (Dunning, 2007). Mean body masses were accepted as representative of species; where more than one estimate of mean body mass was listed for a given species, these estimates were averaged.

Analysis

To evaluate the potential and actual effects of confounding variables, two analyses were undertaken. Firstly, a correlation matrix was constructed to determine if contrasts of any of the potentially confounding variables significantly correlate with either the range size contrast or contrasts of the evolutionary metrics, ω or the substitution rate. Secondly, for any potentially confounding variable that significantly covaries with either range size or an evolutionary metric, a multiple regression model was built to assess the impact on the relationship between range size and that evolutionary metric. Although the relationship should only be confounded if a correlation is found between the confounding variable and both range size and the focal evolutionary metric, all cases where only one correlation was detected were examined in case interactions with range size occurred.

Data generated for these analyses, including alignments, trees, and comparative ecological data are available through Figshare:

- http://figshare.com/s/9ef2e8dc785811e5994206ec4b8d1f61

3.4 Effect of climate and elevation on New World bird evolution

3.4.1 Climate

The effect of climate on rates of molecular evolution was assessed using a wide range of variables for precipitation (annual precipitation, precipitation seasonality, and precipitation in the warmest quarter), temperature (mean annual temperature, minimum monthly temperature, temperature seasonality, and temperature in the wettest quarter), and productivity (annual net primary productivity [NPP]).

As with previous climate variables, temperature and precipitation variables were derived from the BIOCLIM dataset (Hijmans et al., 2005), while NPP was derived from NASA's MODIS MOD17A2 estimates of net primary productivity for 2013 were downloaded from http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MOD17A2_M_PSN (NASA Earth Observations, 2014). Estimates for these variables were derived following the procedure described previously (see 3.3.5 Potentially confounding factors: Climate) within the resident portion of species' ranges. For fully migratory species, estimates were made separately for their breeding and their non-breeding ranges.

The climate variables were initially regressed against the four evolutionary metrics: the substitution rate, d_N, d_S, and ω. As with previous sections, all statistical analyses were performed in R 3.1.2. Because results of the ω–climate relationship indicated a substantial effect of temperature seasonality/isothermality, and because earlier results had indicated population densities decrease in low-seasonality environments, significant covariates of ω were also regressed in a model containing range size to ensure an orthogonal effect had been detected.

3.4.2 Elevation

The effect of elevation on rates of molecular evolution was assessed using the minimum and maximum elevational limits of species from BirdLife International (see 3.3.5 Potentially confounding factors: Data). In addition to these limits, average elevation was calculated as the midpoint between the minimum and maximum values, and elevational range was calculated as the difference between maximum and minimum limits. Calculated

in this way, average elevation may be unrepresentative of the typical elevation occupied by some species, if there is substantial skewness in their distribution across their elevational range. However, given a lack of detailed ecological data for many species, no straightforward alternative was possible. The measures of elevation were then regressed against the four evolutionary metrics: the substitution rate, d_N, d_S, and ω.

3.4.3 Apparent mutation rate variation

Given the lack of consistent mutation rate variation across population size, climate and elevation gradients, additional consideration was given to the effect of apparent mutation rate variation as a nuisance parameter, as such variation may be a source of noise in analyses. While analysing ω at least partially controls for mutation rate variation, there are many other potential consequences of mutation rate variation (see 2.4.2 Factors that affect the substitution rate: Mutation rate) that are unaccounted for, and that may introduce additional error in analyses.

Following Welch and Waxman's (2008) approach, a negative relationship between substitution rate variance and time since speciation reflects stochastic variation. We might extend this proposition to consider the relationship between non-synonymous substitution rate variation and time since speciation. By examining this relationship—for which there should be no strong time dependence (Subramanian & Lambert, 2011)—we can explore the stable variation in d_S between sister species. A LOESS curve was fitted to the relationship, with a 95% confidence interval to show the typical range of d_S variation, and identify outlying contrasts. An additional data subset was then generated that only included comparisons for which the d_S contrast fell within this range. Regression analyses were then repeated to determine if apparent mutation rate variation is a cause of additional noise. I refer to apparent mutation rate variation, as variation in d_S could also be caused by sequencing errors or the inflated variance in short branches due to stochastic variation caused by the discrete nature of the substitution process. However, both apparent and true mutation rate variation could introduce noise, and therefore distinguishing between them was not considered important.

4 Results

4.1 Plant terrestrial ecoregion diversity patterns

4.1.1 Plant diversity patterns

The first set of analyses presented here set out to establish the general patterns of species richness, and how those patterns vary with spatial scale. To investigate the relationship between NPP and plant species richness, regression analyses were performed across the entire set of terrestrial ecoregions. Overall, there is a weak, positive relationship between productivity and species richness across terrestrial ecoregions (Figure 11; adjusted R^2 = 0.193). While mean species richness is greater in regions where NPP is higher, species richness in highly productive ecoregions varies from hundreds of species to 10,000 species. Hence, while productivity correlates with maximum species richness, other factors determine actual richness at or below that level.

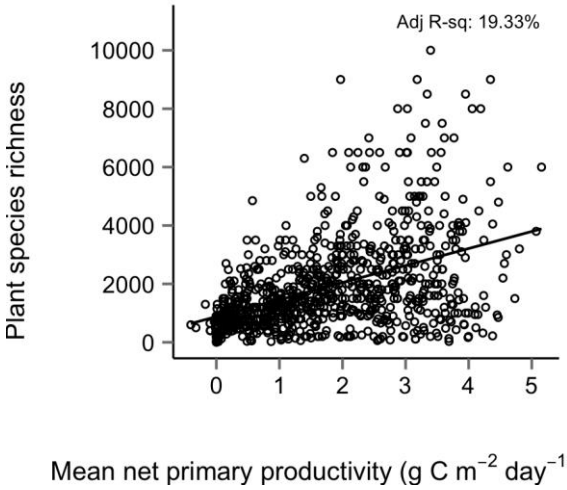

Figure 11. Relationship between modelled net primary productivity and plant species richness of global terrestrial ecoregions (N = 781). Plant species richness estimates for terrestrial ecoregions from Kier *et al.*, (2005). Net primary productivity is 2013 mean estimates for MOD17 modelled NPP (NASA 2014).

The NPP–species richness relationship was also investigated across subsets of ecoregions to determine if the relationship varies systematically with ecoregion size, as would be expected if drivers of species richness vary with scale. The strength of PSRs varies

systematically with ecoregion size. Amongst small ecoregions (10^3 to 10^4 km^2, N = 117), NPP is a poor predictor of species richness (Figure 12a; adjusted R^2 = 0.192). In addition, maximum species richness in these smaller ecoregions is 3800 species, indicative of a possible limit imposed by available area. Medium ecoregions (10^4 to 10^5 km^2, N = 339) display a PSR in which NPP has a modestly improved predictive ability (Figure 12b; adjusted R^2 = 0.239). Peak species richness is also substantially greater, at 90% of the global maximum ecoregion species richness. However, similar to the combined analysis of all ecoregions, at peak productivity the lower limit of species richness was indistinguishable from that found in low productivity ecoregions, indicative of unmeasured constraints that are not related to NPP. Amongst large ecoregions (10^5 to 10^6 km^2, N = 302), these unmeasured constraints on species richness were no longer evident, and the PSR was strong (Figure 12c; adjusted R^2 = 0.607). Finally, the analysis was repeated with only the largest ecoregions in the dataset ($\log_{10}$ area > 5.5, N = 115) and a strong, monotonic positive relationship was found (Figure 12d; adjusted R^2 = 0.723).

When binned into narrower size classes, the strength of PSRs scales closely with ecoregion size. In the smallest ecoregions, productivity and species richness do not appear to covary (3 > $\log_{10}$ area > 3.25, adjusted R^2 = 0.000), but the strength of the relationship increases monotonically as ecoregion size increases (adjusted R^2 = 0.864) (Figure 13a). When latitude replaced productivity as a predictor of species richness, the pattern was qualitatively similar, but the strength of relationship was weaker.

Because some lower-quality estimates of species richness made by Kier et al. (2005) were partially derived from species–area relationships (SARs), the effect of quality on the derived patterns was investigated. Relationships were almost identical qualitatively when the data were restricted to either only the upper or lower quality categories (Appendix 1: Effect of data quality on plant PSRs in terrestrial ecoregions). Relationships were stronger in upper quality categories where more direct estimates of species richness were made, indicating results are not dependent on estimates of species richness that may have partially derived from climate variables or SARs.

Historical contingencies could also affect species richness patterns, therefore the effect of biome and realm on PSRs was also investigated. Indeed, differences in species richness exist were found between realms and biome types, including variation not explained by PSRs. Biome type and realm predict ecoregion species richness within ecoregion size classes, and explanatory power increases in the larger ecoregion size classes (Table 2). Across all ecoregion size classes, the main biome type was tropical and subtropical moist broadleaf forests. However, biome proportions varied across size classes. To remove possible variation in strength of relationships across size classes resulting only from changes in biome proportions, PSRs were analysed in tropical and subtropical moist broadleaf forest ecoregions separately ($N = 225$). The overall predictive ability of the model was reduced, especially amongst small and medium ecoregions ($R^2 < 0.07$), while the qualitative pattern of increasing model strength with ecoregion size was upheld. The PSR in the largest ecoregions remained strong (Table 3, adjusted $R^2 = 0.576$).

Finally, habitat rarity has been invoked as a possible cause of low species richness as a potential definition of habitat harshness. Because of the physical limitations and isolations of rare habitats, few species can adapt to their conditions, and therefore immigration rates and species richness are low (Brown, 1981; Colinvaux, 1993). Therefore, if many low productivity ecoregions are small, then there may be an overarching confounding effect of available area. However, when dividing ecoregions into quartiles based on productivity, there was no evidence of an excess of large and highly productive ecoregions. Median ecoregion size declined between productivity quartiles, with an order of magnitude difference between the least and most productive quartiles (Figure 14).

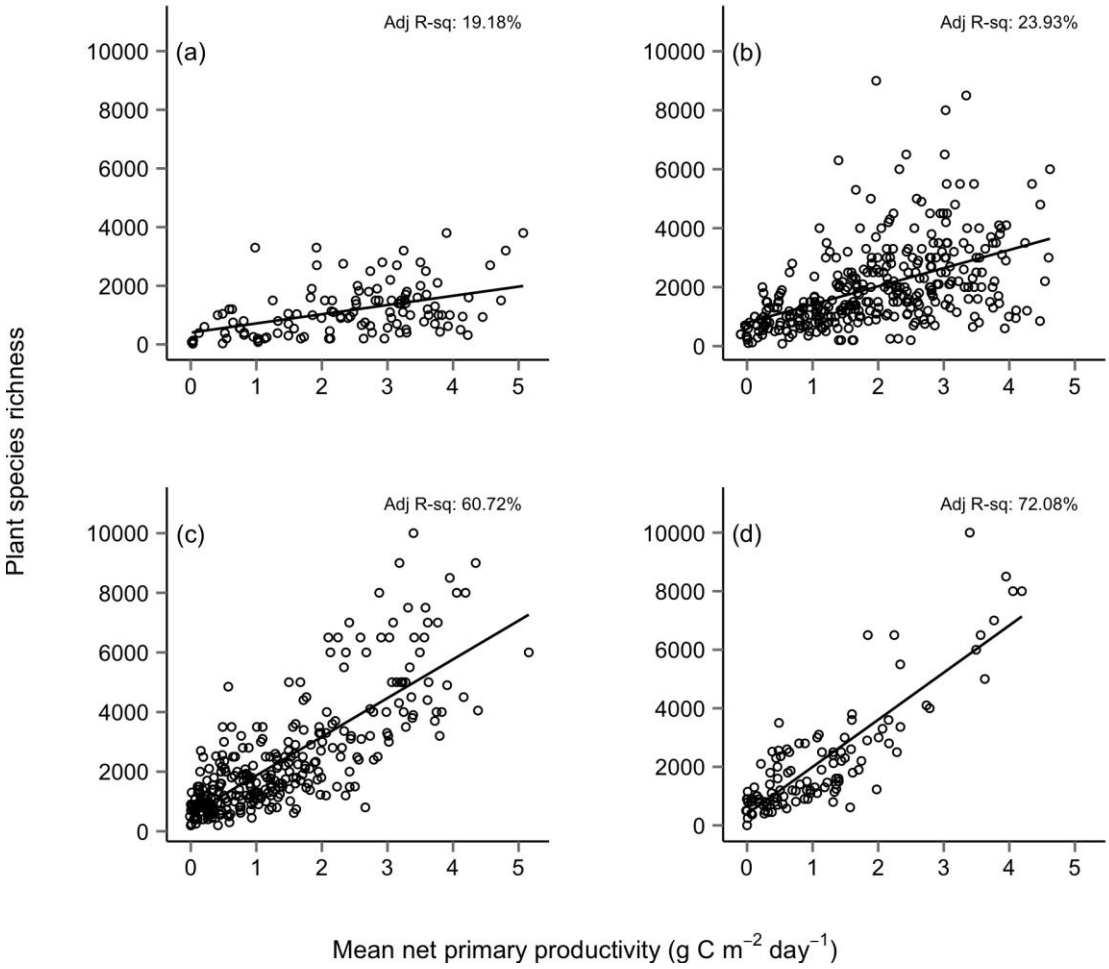

Figure 12. Relationship between modelled net primary productivity and plant species richness across sizes classes of global terrestrial ecoregions: (a) small ecoregions between 10^3 and 10^4 km^2 (N = 117); (b) medium ecoregions between 10^4 and 10^5 km^2 (N = 339); (c) large ecoregions between 10^5 and 10^6 km^2 (N = 302); and (d) the largest ecoregion subset (>$10^{5.5}$ km^2) (N = 115). Data sources as in Figure 11 caption.

Table 2. AIC-based model selection amongst linear regression models of the productivity-plant species richness relationships within terrestrial ecoregion size classes, controlling for $\log_{10}$area, realm and biome type. Evidence ratios are provided for the ratio of Akaike weights between the first and second models. Data sources as in Figure 11 caption. All tested models are included. Variables marked with an asterisk (*) were not significant in that model.

	Model d.f.	Adjusted R^2 (%)	Δ AICc	AICc weight	Evidence ratio
Small ecoregions 10^3–10^4 km^2					
NPP+realm+biome	93	48.91	0	0.53	3.61
Realm+biome	94	47.32	1.73	0.22	
NPP+realm+biome+area*	92	48.64	2.57	0.15	
Realm+biome+area*	93	47.42	3.38	0.01	
NPP+biome	100	38.82	8.99	<0.01	
NPP+biome+area*	99	39.64	9.01	<0.01	
NPP+realm	106	27.29	20.50	<0.01	
NPP+realm+area*	105	26.81	22.63	<0.01	
NPP	113	19.18	24.37	<0.01	
NPP+area*	112	19.20	25.46	<0.01	
Medium ecoregions 10^4–10^5 km^2					
NPP+realm+biome+area	317	53.26	0	>0.99	1.4×10^9
Realm+biome+area	318	50.75	16.44	<0.01	
NPP+realm+biome	318	47.60	37.49	<0.01	
NPP+biome+area	323	45.60	44.15	<0.01	
Realm+biome	319	45.78	47.86	<0.01	
NPP+realm+area	330	39.15	74.17	<0.01	
NPP+biome	324	38.57	84.22	<0.01	
NPP+area	336	32.51	102.87	<0.01	
NPP+realm	331	31.51	113.16	<0.01	
NPP	337	23.93	142.38	<0.01	
Large ecoregions 10^5–10^6 km^2					
NPP+biome+area	285	70.37	0	0.45	1.06
NPP+realm+biome+area	280	70.95	0.12	0.42	
NPP+realm+biome	281	70.49	3.62	0.07	
NPP+biome	286	69.85	4.10	0.06	
Realm+biome	282	64.53	57.96	<0.01	
Realm+biome+area	281	64.56	58.95	<0.01	
NPP+realm+area	292	62.42	63.81	<0.01	
NPP+realm	293	62.03	65.77	<0.01	
NPP+area	297	61.15	68.41	<0.01	
NPP	298	60.72	70.68	<0.01	
Largest ecoregions, > $10^{5.5}$ km^2					
NPP+biome+area	101	79.04	0	0.93	15.92
NPP+biome	102	77.73	5.54	0.06	
NPP+realm*+biome+area	96	79.23	8.72	0.01	
NPP+realm+biome	97	77.75	14.92	<0.01	
NPP+area	111	73.04	15.91	<0.01	
NPP	112	72.08	18.78	<0.01	
NPP+realm*+area	106	73.65	20.68	<0.01	
NPP+realm*	107	72.70	23.45	<0.01	
Realm+biome	98	67.77	55.84	<0.01	
Realm+biome+area	97	68.14	56.22	<0.01	

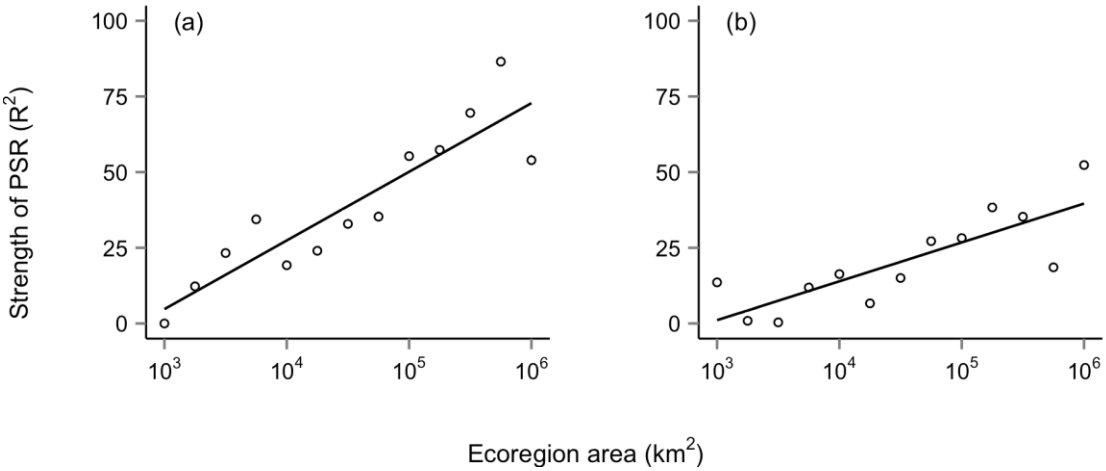

Figure 13. Strength of relationship (linear regression adjusted R^2) between species richness and: (a) modelled mean annual net primary productivity; and (b) ecoregion latitudinal midpoints, across ecoregions ranging from 10^3 to 10^6 km^2, binned by size into 0.25 increments on a logarithmic scale (i.e., bin 1: 10^3 to $10^{3.25}$ km^2). Data sources as in Figure 11 caption.

Table 3. Comparison of linear regression models of plant species richness and NPP for all terrestrial ecoregions (N = 781), and the subset of tropical and subtropical moist broadleaf forest ecoregions (N = 225) across four ecoregion size classes. Data sources as in Figure 11 caption.

	Adjusted R^2 (%)	
Ecoregion size	All biomes	Moist broadleaf forests
10^3–10^4 km^2	19.2	0
10^4–10^5 km^2	23.9	6.9
10^5–10^6 km^2	60.7	20.6
> $10^{5.5}$ km^2	72.3	57.6

Finally, several additional analyses were conducted to investigate the relationship between species richness and additional environmental variables (temperature, temperature seasonality, and precipitation) in a subset of the largest ecoregions (> $10^{5.5}$ km^2). These exploratory analyses dually seek to determine if these relationships vary in any systematic way between ecoregions that experience below-freezing winters, and those that do not (Figure 15).

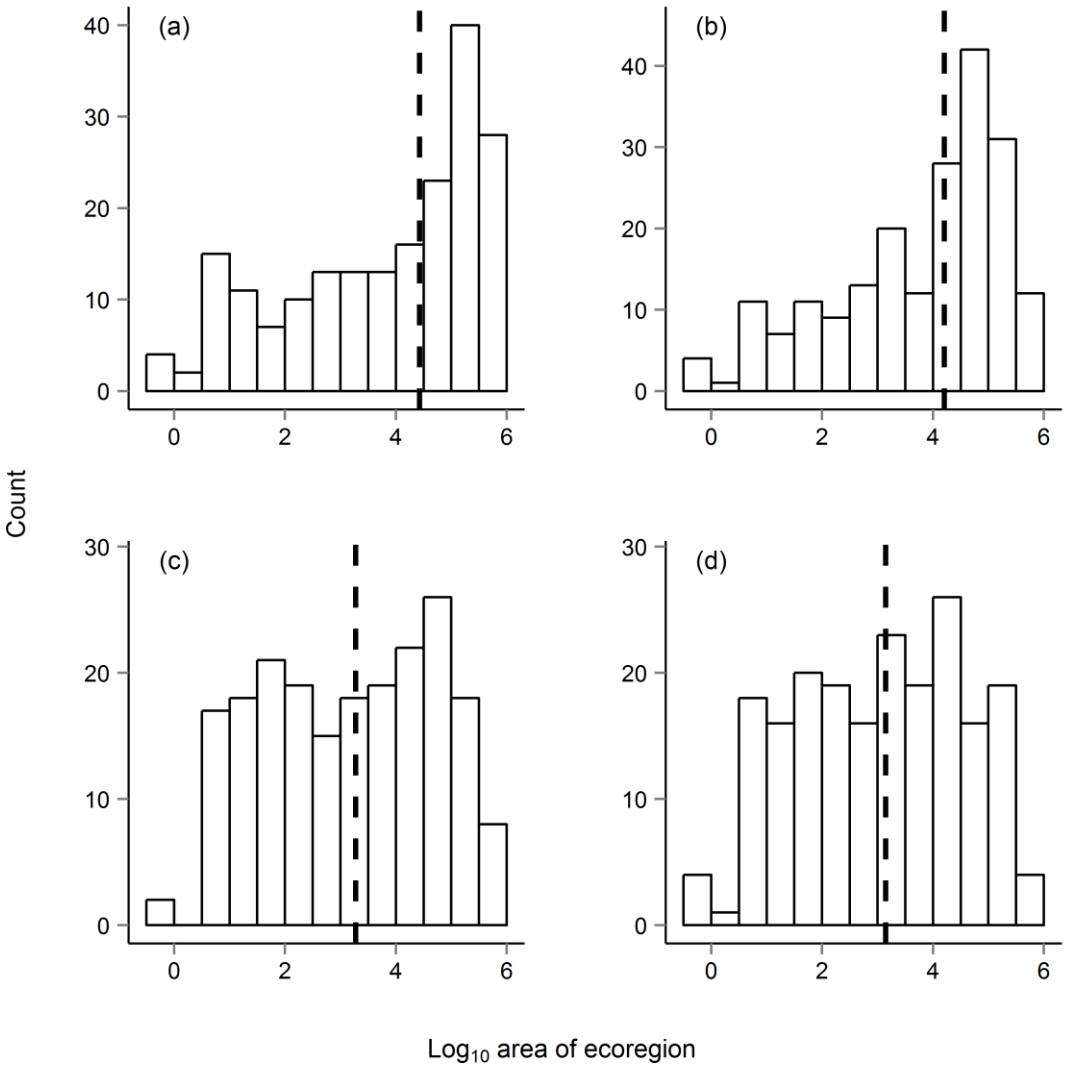

Figure 14. Area distribution of ecoregions across quartiles of net primary productivity, for (a) first/lower quartile (<0.77 g C m^{-2} day^{-1}), (b) second quartile (0.77 to 1.66 g C m^{-2} day^{-1}), (c) third quartile (1.66 to 2.79 g C m^{-2} day^{-1}), (d) fourth/upper quartile (> 2.79 g C m^{-2} day^{-1}). (*N* = 781 ecoregions). Dashed vertical lines are median areas for the productivity quartiles.

Species richness in plants appears to relate strongly to both NPP and precipitation (Figure 15a & c). Neither relationship shows evidence of substantial change in the transition between ecoregions that predominantly experience winter freezing and those that do not, although in both cases there is a deviation in the LOESS curve at this point. Mean annual temperature has a weak relationship with plant species richness (Figure 15b), with a shallow slope through ecoregions that experience winter freezing, and a wider range of species richness through those that do not. The relationship between plant species richness and temperature seasonality shows a substantial change between ecoregions that

experience freezing and those that do not, suggestive of high species turnover between these two floras.

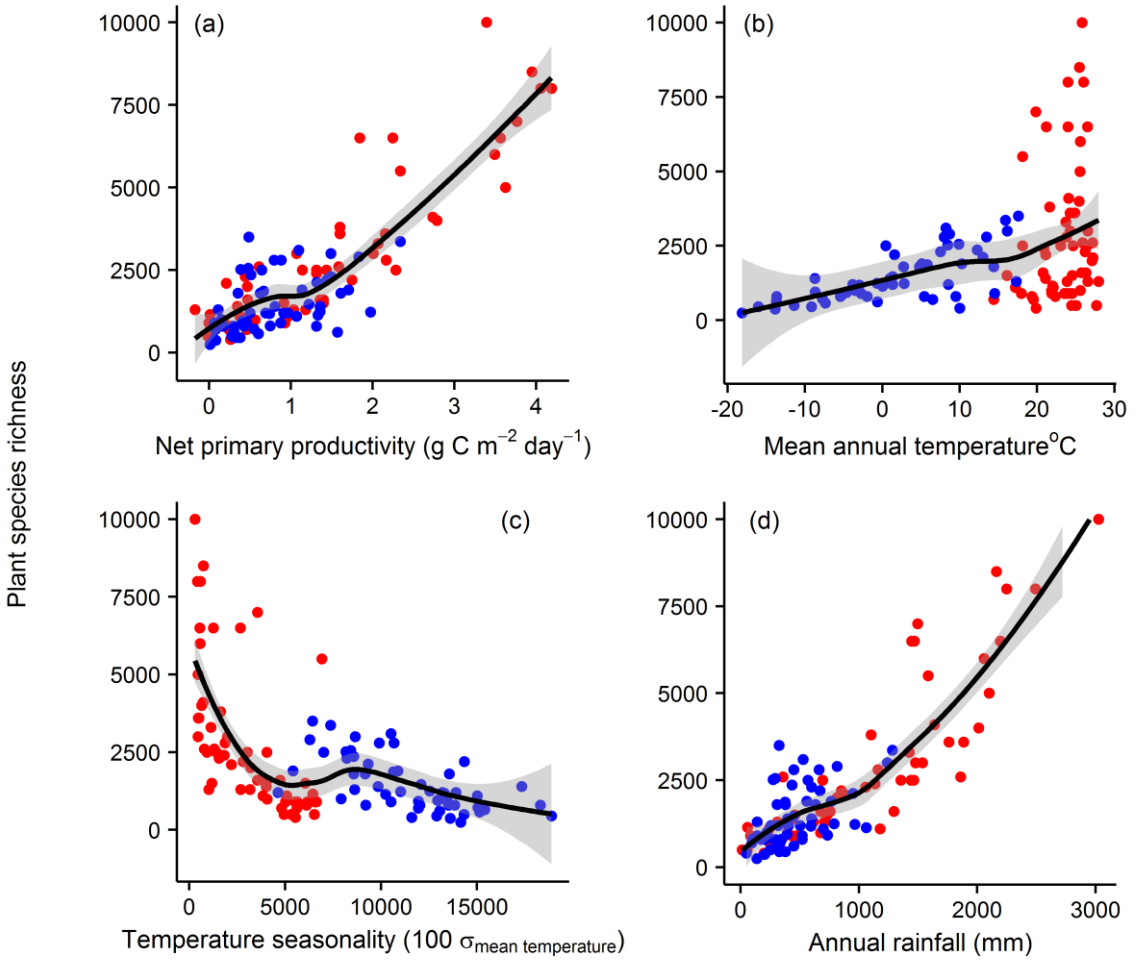

Figure 15. Relationship between plant species richness and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2 (N = 114 ecoregions). Red circles indicate ecoregions with average temperature in the coldest month above 0°C, and blue circles indicate ecoregions with average temperature in the coldest month below 0°C. Fits are LOESS curves; grey shading indicates 95% confidence intervals.

4.2 Animal population and diversity patterns

4.2.1 Animal terrestrial ecoregion diversity

The relationships between the species richness of two vertebrate classes (birds and mammals) and NPP was explored across ecoregion sizes classes. As with plants, the strength of regression model relationships increased in larger ecoregion classes for both birds (Table 4), and mammals (Table 5). Overall, NPP had lower explanatory power in both animal taxa than in plants, and the most complex model was favoured across most ecoregion size classes. Nonetheless, models preferred by AIC explained similar proportions of diversity in both animal taxa as in plants across all ecoregion size classes.

Biome is evidently of great importance in determining vertebrate richness in all but the smallest ecoregions (10^3 to 10^4 km^2) (Tables 4 & 5). To account for differences between biomes that might affect vertebrate PSRs, relationships were estimated using only ecoregions in the commonest biome: tropical and subtropical moist broadleaf forest (N = 225). However, after controlling for biome, the PSR is weaker in the large ecoregion size (10^5 to 10^6 km^2) class than the medium ecoregion size class (10^4 to 10^5 km^2). Nonetheless, the PSR in the largest ecoregions within this biome (>$10^{5.5}$ km^2) is substantially stronger than across all biomes (Table 6), and, for mammals, approaches the strength of the PSR in plants.

Table 4. AIC-based model selection amongst linear regression models of the productivity-species richness relationships for birds within terrestrial ecoregion size classes, controlling for area, realm and biome type. Evidence ratios are provided for the ratio of Akaike weights between the first and second models. Data sources as in Figure 1 caption. All tested models are included. Variables marked with an asterisk (*) were not significant in that model.

	Model d.f.	Adjusted R^2 (%)	Δ AICc	AICc weight	Evidence ratio
Small ecoregions 10^3–10^4 km^2					
NPP+realm+area	105	29.13	0	0.85	7.42
Biome+realm+area	93	38.12	4.01	0.11	
NPP+realm+biome+area	92	37.48	7.15	0.02	
NPP+realm	106	22.65	8.71	0.01	
Biome+realm	94	31.08	14.5	<0.01	
NPP+realm+biome	93	30.96	16.6	<0.01	
NPP+area	112	10.26	18.37	<0.01	
NPP+biome+area	99	18.81	24.62	<0.01	
NPP	113	2.63	26.63	<0.01	
NPP+biome*	100	9.59	35.39	<0.01	
Medium ecoregions 10^4–10^5 km^2					
NPP+realm+biome+area	317	54.61	0	0.99	170.09
Biome+realm+area	318	53.05	10.27	0.01	
NPP+realm+biome	318	46.77	52.78	<0.01	
NPP+realm+area	330	44.48	53.09	<0.01	
Biome+realm	319	45.84	57.47	<0.01	
NPP+biome+area	323	42.45	73.26	<0.01	
NPP+realm	331	36.21	99.1	<0.01	
NPP+biome	324	33.56	120.77	<0.01	
NPP+area	336	19.79	171.38	<0.01	
NPP	337	11.89	202.21	<0.01	
Large ecoregions 10^5–10^6 km^2					
NPP+realm+biome+area	280	66.13	0	>0.99	8010.67
NPP+realm+biome	281	63.89	17.98	<0.01	
Biome+realm+area	281	63.86	18.18	<0.01	
NPP+biome+area	285	62.33	25.75	<0.01	
Biome+realm	282	62.24	30.15	<0.01	
NPP+biome	286	59.66	45.12	<0.01	
NPP+realm+area	292	56.32	62.14	<0.01	
NPP+realm	293	53.48	79.94	<0.01	
NPP+area	297	36.74	167.87	<0.01	
NPP	298	32.97	184.22	<0.01	
Largest ecoregions, > $10^{5.5}$ km^2					
NPP+realm+biome+area	96	75.68	0	0.69	2.37
NPP+realm+biome	97	74.93	1.72	0.29	
NPP+biome+area	101	71.8	8.79	0.01	
Biome+realm	98	72.07	12.39	<0.01	
Biome+realm+area	97	72.32	13.01	<0.01	
NPP+realm	107	68.06	14.69	<0.01	
NPP+realm+area	106	68.26	15.28	<0.01	
NPP+biome	102	69.27	17.12	<0.01	
NPP+area	111	36.89	87.51	<0.01	
NPP	112	35.18	89.44	<0.01	

Table 5. AIC-based model selection amongst linear regression models of the productivity-species richness relationships for mammals within terrestrial ecoregion size classes, controlling for area, realm and biome type. Evidence ratios are provided for the ratio of Akaike weights between the first and second models. Data sources as in Figure 1 caption. All tested models are included. Variables marked with an asterisk (*) were not significant in that model.

	Model d.f.	Adjusted R^2 (%)	Δ AICc	AICc weight	Evidence ratio
Small ecoregions 10^3–10^4 km^2					
NPP+realm+area	105	35.92	0	0.84	5.3
NPP+realm	106	33.25	3.34	0.16	
Biome+realm+area	93	34.3	22.48	<0.01	
NPP+realm+biome+area	92	34.68	23.77	<0.01	
NPP+realm+biome	93	32.27	25.98	<0.01	
Biome+realm	94	30.87	26.42	<0.01	
NPP*+area	112	5.42	35.99	<0.01	
NPP*	113	2.17	38.74	<0.01	
NPP+biome*+area	99	12.95	26.63	<0.01	
NPP*+biome*	100	9.23	35.39	<0.01	
Medium ecoregions 10^4–10^5 km^2					
NPP+realm+biome+area	317	60.24	0	> 0.99	1448.87
Biome+realm+area	318	58.35	14.56	<0.01	
NPP+realm+area	330	56.26	17.19	<0.01	
NPP+realm+biome	318	55.06	40.29	<0.01	
Biome+realm	319	53.73	48.96	<0.01	
NPP+realm	331	50.63	57.1	<0.01	
NPP+biome+area	323	36.6	150.99	<0.01	
NPP+biome	324	29.57	185.47	<0.01	
NPP+area	336	16.84	228.54	<0.01	
NPP	337	10.02	254.23	<0.01	
Large ecoregions 10^5–10^6 km^2					
NPP+realm+biome+area	280	75.48	0	>0.99	27111.3
NPP+realm+biome	281	73.64	20.42	<0.01	
Biome+realm+area	281	72.67	31.24	<0.01	
Biome+realm	282	71.45	43.17	<0.01	
NPP+biome+area	285	67.8	75.65	<0.01	
NPP+biome	286	65.46	95.5	<0.01	
NPP+realm+area	292	59.85	133.77	<0.01	
NPP+realm	293	57.43	150.26	<0.01	
NPP+area	297	40.68	245.49	<0.01	
NPP	298	37.45	260.36	<0.01	
Largest ecoregions, > $10^{5.5}$ km^2					
NPP+realm+biome+area	96	79.61	0	0.86	6.32
NPP+realm+biome	97	78.63	3.69	0.14	
NPP+biome+area	101	75.14	14.54	<0.01	
Biome+realm	98	73.91	24.78	<0.01	
Biome+realm+area	97	74.23	25.03	<0.01	
NPP+biome	102	71.54	28.51	<0.01	
NPP+realm+area	106	67.57	37.86	<0.01	
NPP+realm	107	67.09	38.27	<0.01	
NPP+area	111	42.02	97.98	<0.01	
NPP	112	39.52	89.44	<0.01	

Table 6. OLS multiple regression model explanatory power for NPP and area in predicting species richness in moist broadleaf forest ecoregions only (N = 225 ecoregions).

	Adjusted R^2 (%)	
Ecoregion size	Birds	Mammals
10^3–10^4 km^2	9.46	9.35
10^4–10^5 km^2	26.32	17.7
10^5–10^6 km^2	18.75	21.04
> $10^{5.5}$ km^2	57.29	67.6

4.2.2 Correlates of vertebrate diversity

Net primary productivity

There is a distinct pattern in the relationship between NPP and animal diversity. The low explanatory power for NPP is at least in part explained by different responses in ecoregions that have temperatures in their coldest month above and below freezing. The pattern was most distinct for phylogenetic diversity (Figure 16a), although similar patterns are evident separately for bird (Figure 17a) and mammal (Figure 18a) species richness. Scatter around the LOESS curve falls distinctly into two categories based on minimum temperature, indicating that residuals are almost twice as large as they would be if each category was treated separately. Further, the response of diversity to NPP in the two categories is different: for ecoregions that experience freezing temperatures in their coldest month, there is a very weak, and marginally non-significant relationship between NPP and phylogenetic diversity (R^2 = 0.066, P = 0.059). However, for ecoregions that do not experience freezing temperatures during their coldest month, there is a positive relationship between NPP and phylogenetic diversity (R^2 = 0.537, P = 4.2×10^{-11}). An ANCOVA model that includes a binary dummy variable to code ecoregions as above and below freezing during their coldest month indicates that phylogenetic diversity is significantly lower at the same NPP in ecoregions that experience freezing (freezing point dummy variable coefficient = -0.016, $P < 2 \times 10^{-16}$).

Mean annual temperature

Although there is a general pattern of increasing diversity with mean temperature, for ecoregions that experience below-freezing winters there is evidence of substantially

constrained diversity (Figure 16b). Scatter around the LOESS is considerably lower for these ecoregions. In the subset where minimum temperature in the coldest month is above freezing, there is a rapid increase in average diversity, and substantially more variation around the LOESS line, indicating other factors than mean annual temperature play the primary role in determining diversity in these ecoregions. There is also a visual indication that the rate of increase in diversity slows in the subset of ecoregions that experience winter freezing as mean annual temperature increases. This pattern is particularly apparent in the relationships between NPP, and bird (Figure 17b) and mammal (Figure 18b) species richness.

Temperature seasonality

Of the climate variables investigated, temperature seasonality had the strongest linear relationship with vertebrate phylogenetic diversity (Figure 16c) (OLS regression, $R^2 = 0.640$, $P = 2.2\times10^{-16}$). As seasonality increases, phylogenetic diversity decreases, as do both bird and mammal species richness (Figures 17c & 18c). However, the LOESS curves for all three diversity metrics indicated a distinct gradient change occurred at the point transitioning between ecoregions with above and below freezing temperatures in their coldest month. A segmented regression model on the relationship with phylogenetic diversity indicated a breakpoint temperature seasonality value of 5,517, which approximately coincides with the breakpoint indicated by LOESS. The segmented regression model had increased explanatory power over OLS regression ($R^2 = 0.715$, $P = 1.12\times10\text{-}^{10}$).

Annual precipitation

A positive relationship between annual precipitation and vertebrate phylogenetic diversity exists (Figure 16d). The relationship is either monotonic or a decreasing function of precipitation, although the latter interpretation is strongly influenced by a single point where annual precipitation is 3026mm.

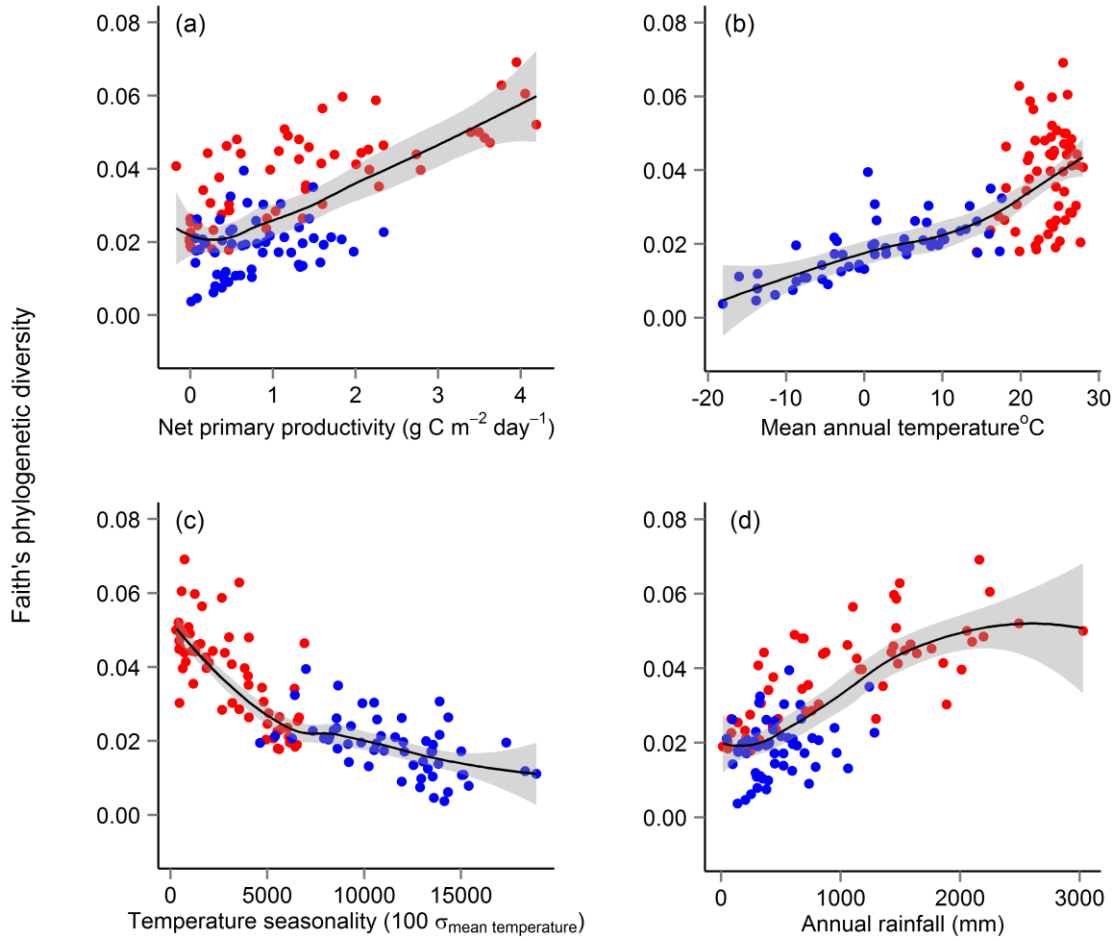

Figure 16. Relationship between total vertebrate phylogenetic diversity and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2 (N = 114 ecoregions). Red circles indicate ecoregions with average temperature in the coldest month above 0°C, and blue circles indicate ecoregions with average temperature in the coldest month below 0°C. Fits are LOESS curves; grey shading indicates 95% confidence intervals.

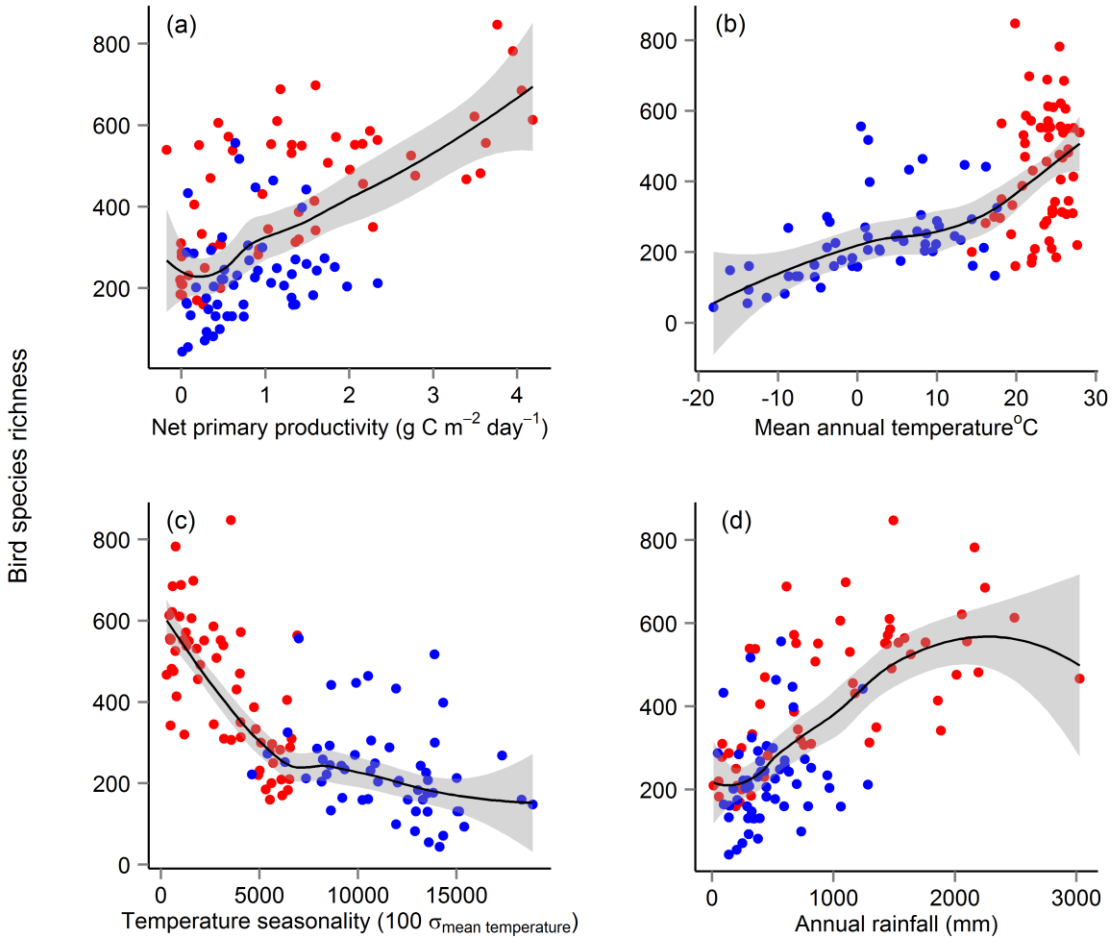

Figure 17. Relationship between bird species richness and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2 (N = 114 ecoregions). Red circles indicate ecoregions with average temperature in the coldest month above 0°C, and blue circles indicate ecoregions with average temperature in the coldest month below 0°C. Fits are LOESS curves; grey shading indicates 95% confidence intervals.

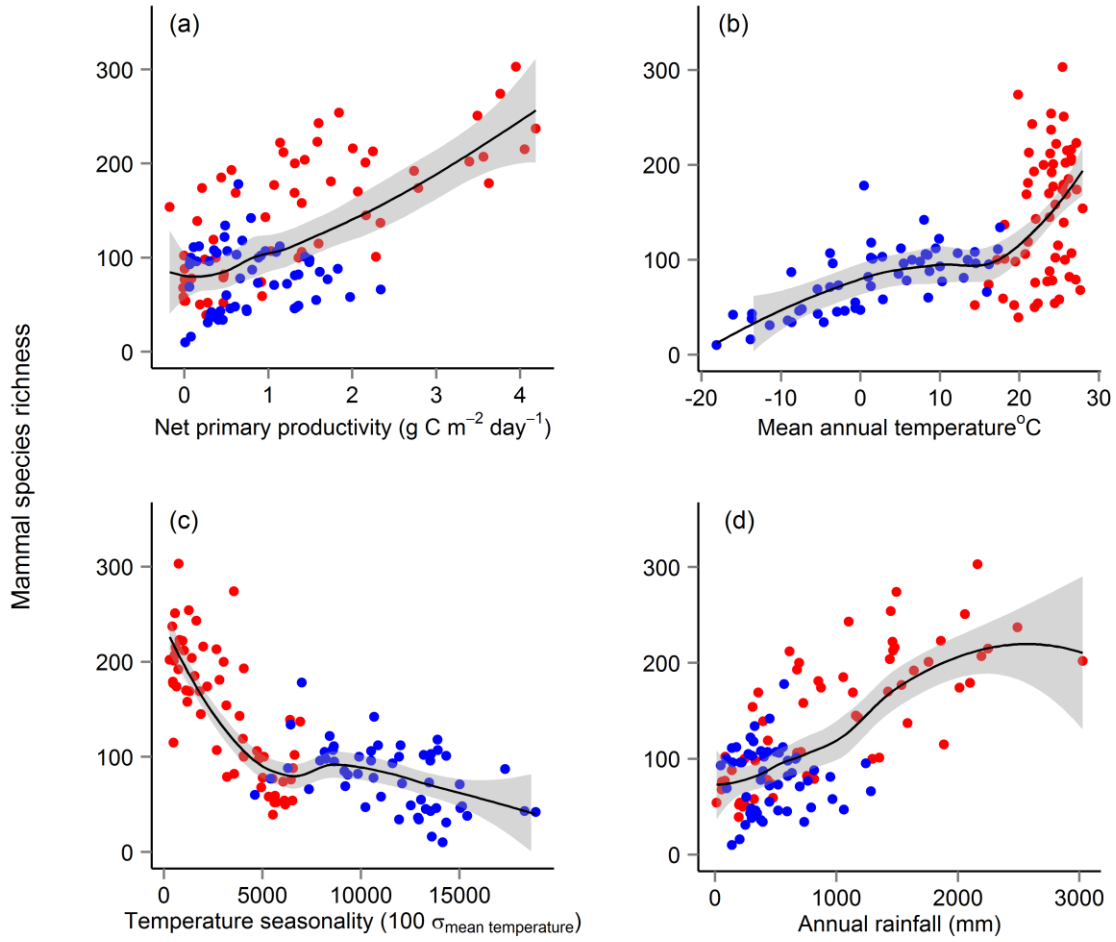

Figure 18. Relationship between mammal species richness and (a) net primary productivity, (b) mean annual temperature, (c) temperature seasonality, and (d) annual precipitation in all terrestrial ecoregions larger than $10^{5.5}$ km^2 (N = 114 ecoregions). Red circles indicate ecoregions with average temperature in the coldest month above 0°C, and blue circles indicate ecoregions with average temperature in the coldest month below 0°C. Fits are LOESS curves; grey shading indicates 95% confidence intervals.

Plant species richness

Plant ecoregion species richness has a strong, decelerating positive relationship with vertebrate phylogenetic diversity that is linearised by $\log_{10}$-transforming plant species richness (Figure 19). There is a distinct effect of ecoregion temperature, with ecoregions that experience freezing conditions in their coldest month having significantly lower phylogenetic diversity than those that do not. An ANCOVA model predicting phylogenetic diversity that included above and below freezing temperatures in the coldest month as a categorical variable was better fit than the simpler model that excluded it (ΔAIC=- 83.53, evidence ratio 1.3×10^{18}), and explained most of the variation in vertebrate diversity (multiple $R^2 = 0.8194$, $P = 2.2\times10^{-16}$).

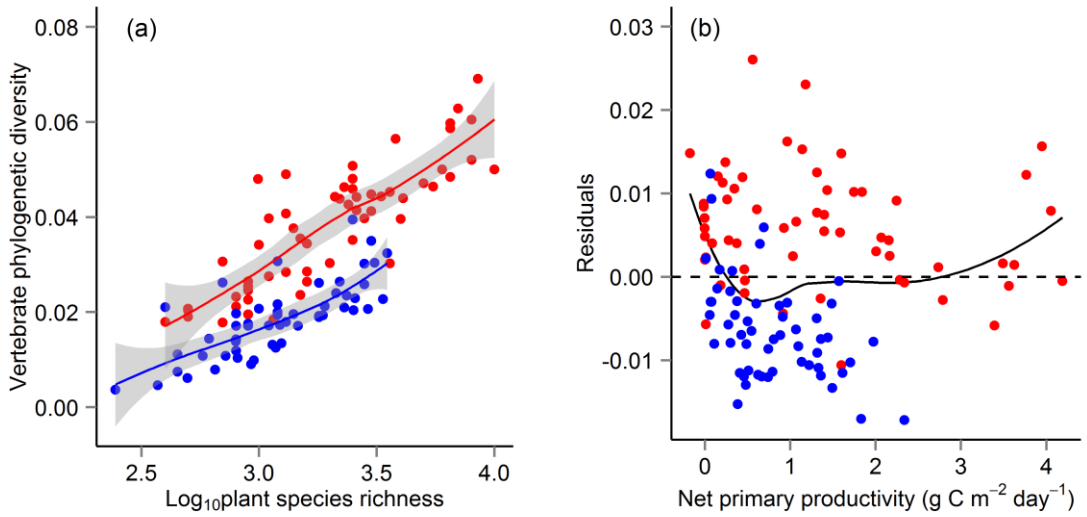

Figure 19. Relationship between (a) plant species richness and Faith's vertebrate phylogenetic diversity; and (b) the relationship between the residuals of the relationship in (a) and net primary productivity. Data points represent individual terrestrial ecoregions larger than $10^{5.5}$ km^2 (N = 114 ecoregions). Red circles indicate ecoregions with average temperature in the coldest month above 0°C, and blue circles indicate ecoregions with average temperature in the coldest month below 0°C. Fits are LOESS curves; grey shading indicates 95% confidence intervals.

4.2.3 *Range sizes and latitudinal ranges in animals*

Having established what some of the broad patterns of species richness are across the terrestrial ecoregions dataset, attention was given to possible explanations for these patterns. Rapoport's rule, is one such explanation that states that latitudinal range sizes vary systematically with latitude because of reduced tolerances of climate variation in tropical species. However, while evidence has been presented demonstrating that this pattern of smaller latitudinal range exists (e.g. Stevens, 1989), it has not been demonstrated that this pattern necessarily relates to climate tolerances. A reasonable null hypothesis is that latitudinal range variation would also exist if range sizes themselves co-varied with latitude. Therefore, the relationship between geographic range size and latitudinal range was investigated to determine if there are systematic biases in low- and high-latitude bands. On average, range sizes of both mammals and non-migratory passerine birds increase outside of the tropics in the northern hemisphere (Table 7). However, across the three latitudinal bands studied (0–25° N, 25–50° N, and > 50° N) there is no evidence that broader latitudinal tolerances cause increased range sizes. Latitudinal ranges increase approximately proportionately to range size in both taxonomic groups (Figures 20 & 21). At large range sizes, some differences in latitudinal range are evident between latitudinal bands. Differences between the bands were tested by including the latitudinal bands as a dummy variable in the model with an interaction term to determine if the slopes differed significantly. When latitudinal bands were defined by species' latitudinal midpoints, latitudinal ranges increased with range size more rapidly for species in the tropical band than in the extratropical bands (Figure 20a). The slopes of all three of these latitudinal bands were significantly different (interaction terms, $P < 2\times10^{-16}$). When latitudinal bands were instead defined by maximum latitude, latitudinal ranges of mammals increased with range size more rapidly in the mid-latitudinal band (Figure 20b), and the differences in the slopes between all three of these latitudinal bands were also significant (interaction terms, $P < 2\times10^{-16}$). For non-migratory passerine birds, when latitudinal ranges were defined by midpoints, patterns were qualitatively identical to those for mammals (Figure 21a). The slope of the 25-50° N was significantly shallower than the other bands (interaction term, $P = 0.011$), while the slopes of those other bands did not significantly differ (interaction term,

$P = 0.416$). When latitudinal bands were instead defined by maximum latitude, the highest latitudinal band remained the band with the shallowest slope (Figure 21b), and the slopes of all three bands were significantly different (interaction terms, $P < 2\times10^{-4}$). Thus, in both taxa and using both approaches to defining latitudinal ranges, the highest latitudinal band (> 50° N) had significantly smaller latitudinal ranges than at least either the mid or low latitude bands at equivalent range sizes.

Table 7. Median range sizes of non-migratory passerine birds and mammals distributed across three latitudinal bands in the northern hemisphere.

	Median range size (km^2)					
	Midpoint method			Maximum latitude method		
Latitudinal band	0–25° N	25–50° N	>50° N	0–25° N	25–50° N	>50° N
Land area	2.85×10^7	3.96×10^7	3.17×10^7	2.85×10^7	3.96×10^7	3.17×10^7
Mammals	209,659 (*N* = 1644)	400,422 (*N* = 892)	3,219,745 (*N* = 104)	242,998 (*N* = 1866)	437,500 (*N* = 1019)	3,545,669 (*N* = 309)
Birds	240,000 (*N* = 1987)	755,000 (*N* = 363)	7,100,000 (*N* = 25)	251,500 (*N* = 2142)	710,000 (*N* = 426)	7,100,000 (*N* = 43)

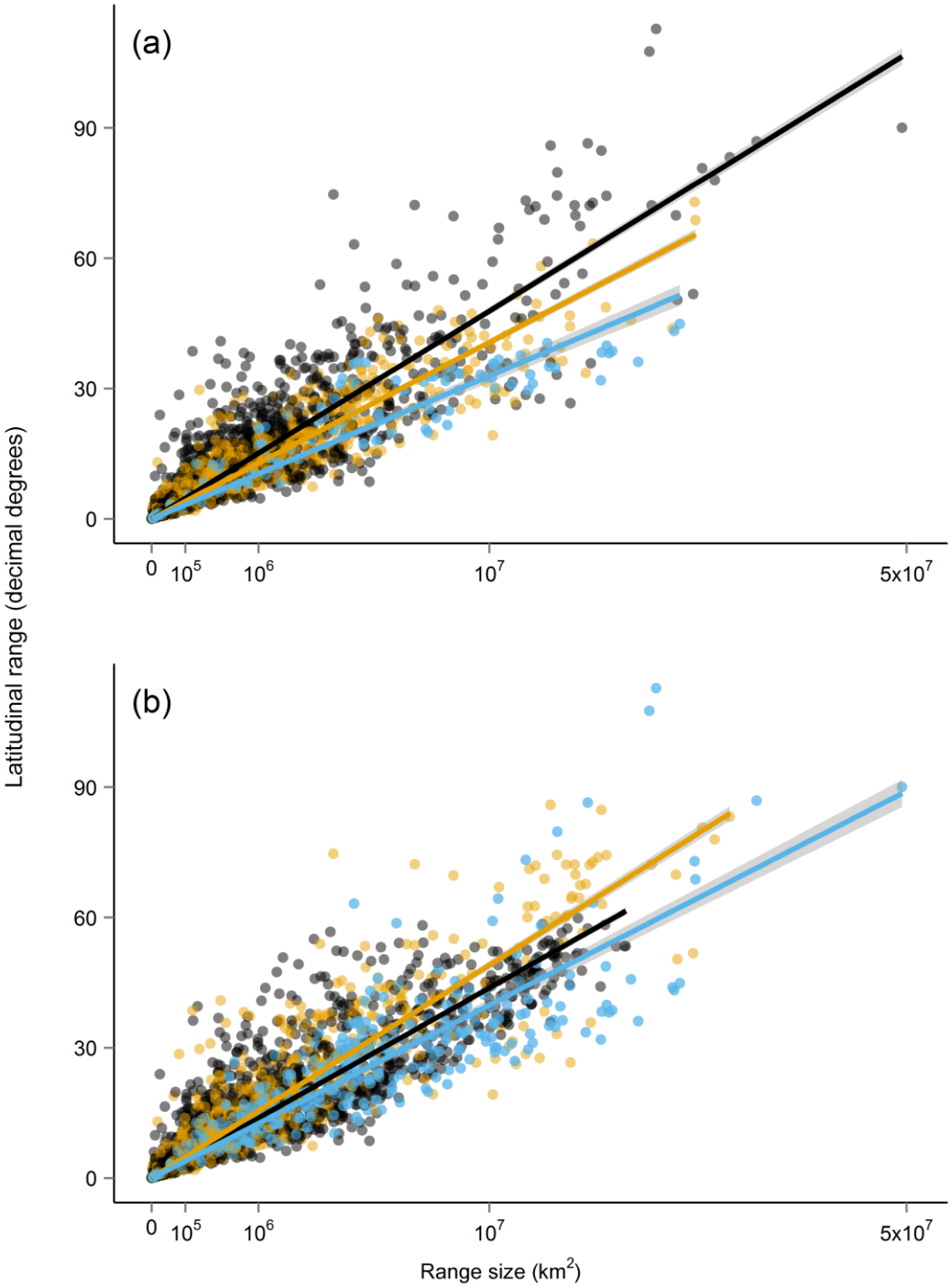

Figure 20. Scaling of latitudinal range with range size for mammal species in three latitudinal bands in the northern hemisphere. Species were assigned to latitudinal bands by: (a) the latitudinal midpoint of their range, or (b) their maximum latitude. Black points indicate species with ranges in the 0–25° N band, orange points indicate those in the 25–50° N band, and blue points indicate those in the >50° N band. Fits are linear regressions forced through the origin, with 95% confidence intervals indicated by grey shading.

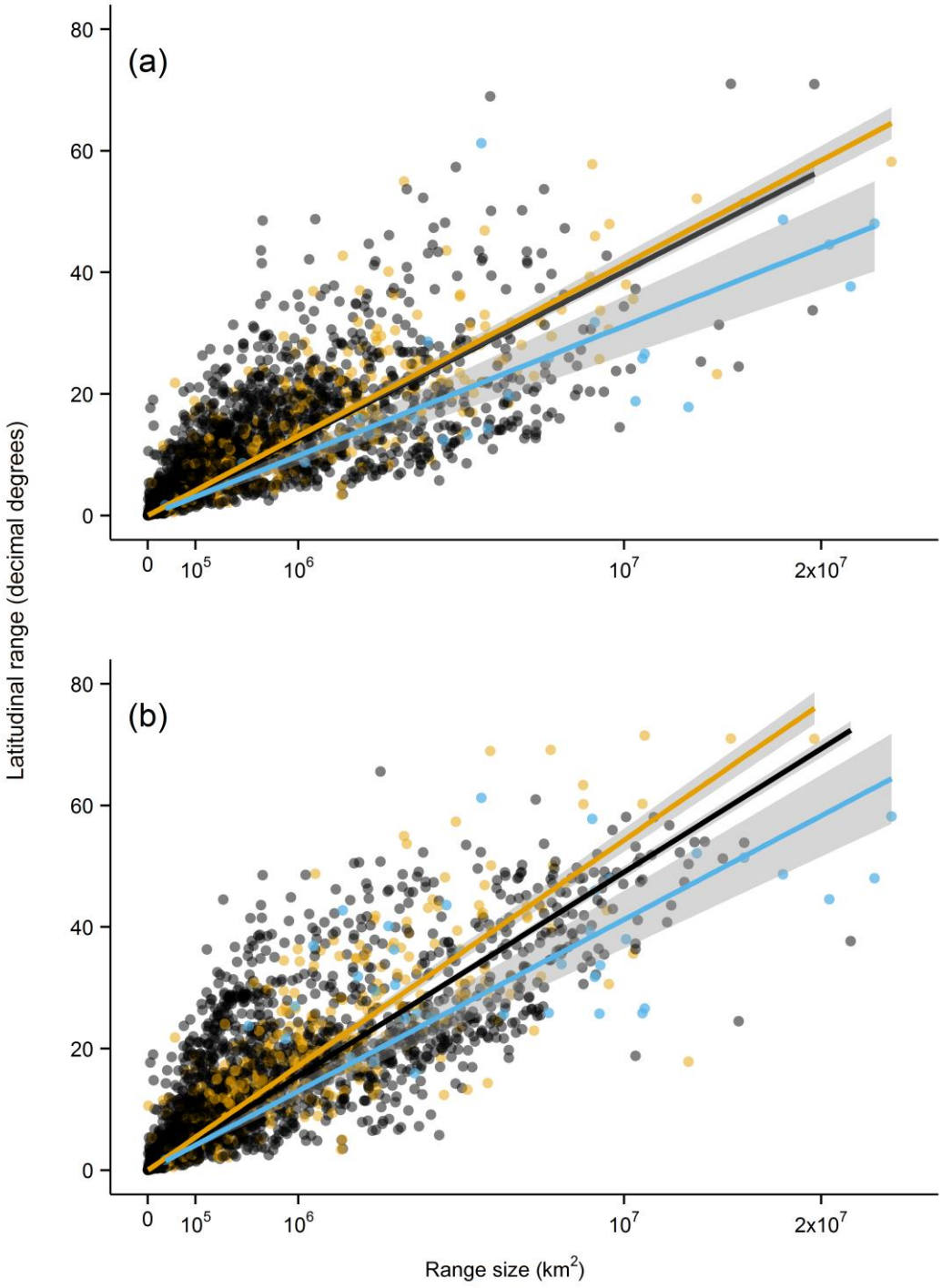

Figure 21. Scaling of latitudinal range with range size for non-migratory passerine bird species in three latitudinal bands in the northern hemisphere. Species were assigned to latitudinal bands by: (a) the latitudinal midpoint of their range, or (b) their maximum latitude. Black points indicate species with ranges in the 0–25° N band, orange points indicate those in the 25–50° N band, and blue points indicate those in the >50° N band. Fits are linear regressions forced through the origin, with 95% confidence intervals indicated by grey shading.

4.2.4 Population–range size correlations

The previous sections establish links between broad plant/animal species richness patterns and NPP, and a range of environmental variables. As the overarching goal in this thesis is to investigate if causal or incidental links between these patterns could be mediated by molecular evolutionary processes. As such it is important to separate climate-derived correlations from those driven by demongraphic factors such as population size. However, as direct population size estimates are not available for the majority of species, this section seeks to estalish if range size can be used as a surrogate measure.

Across 381 species from 22 bird orders, there is a positive relationship between range size, as extent of occurrence, and population size ($R^2 = 0.371$, $P < 2.2 \times 10^{-16}$). The relationship is strengthened and linearised by the inclusion of body mass with range size as predictors of population size in a multiple regression ($R^2 = 0.482$, $P < 2.2 \times 10^{-16}$) (Figure 22). Because of the ecological variation that occurs between different orders of birds in terms of generation time, longevity, and trophic level, taxonomic order was also used as a dummy variable in the multiple regression, which also modestly increased the model's explanatory power ($R^2 = 0.539$, $P < 2.2 \times 10^{-16}$).

The birds for which population size estimates are available are far more likely to be small-ranged and at heightened conservation risk than typical bird species. About 70% of birds have ranges greater than 10^5 km^2, although this is true of only 30% of birds with population and body mass estimates. While three quarters of bird species are listed as 'least concern' in the IUCN Redlist, 42% of birds with population size and body mass estimates available are either endangered or critically endangered (Table 8).

Although body mass is a significant predictor of population size in a multiple regression with range size, the bivariate relationship between population size and body mass is non-significant (Figure 22, $P = 0.11$). It is worth noting that the relationship between range size and body mass is heteroskedastic. In small ranges, body masses are typically small; large-bodied species tend to occupy large ranges. Variation in body mass is higher for species occupying larger ranges, and variation in range size is higher for species with smaller body

sizes (Figure 22). Because of these patterns, and the substantially stronger bivariate relationship between range size and population size, it appears that the body size and range size covariation is obscured. To alternatively investigate the body mass–population size relationship, the dataset was limited to the subset of species with a range size greater than 10^6 km^2. Within this subset (N = 58), where there is no longer a significant range size–population size relationship (R^2 = 0.036, P = 0.152), body mass is a signficant predictor of population size (R^2 = 0.378, $P = 2.8 \times 10^{-7}$).

IUCN conservation status of species also affects the relationship between range size and population size. For species listed as critically endangered, the slope of the relationship is significantly shallower than for other conservation statuses ($P < 0.02$), and is not signficantly different from zero (95% confidence interval, -0.043 to 0.192, P = 0.225). While the slope for species listed as least concern is the steeper than for other statuses, the difference is not statistically significant (Figure 23). However, power was low as only 3.4% of species with good or medium quality population size estimates fall into the least concern category.

The relationship between range size and population size was examined as a restricted subset including only the passerine birds (N = 262 species) without applying a body mass correction. In this subset, the strength of the relationship is similar to that found for the full dataset after including mass in the regression model (R^2 = 0.449 and 0.482 respectively). However, when tropical and extratropical subsets of passerines were considered, the extratropical subset had a distinctly stronger relationship than the tropical subset (R^2 = 0.505 and 0.302 respectively), and this difference was not substantially reduced by the inclusion of body mass (R^2 = 0.535 and 0.358 respectively).

Single family analyses were also performed to check the generality of the pattern. Within each bird family with species from at least ten genera, population size is significantly positively correlated with EOO, with approximately 40 to 60% of population size variation explained on a log-log scale (Table 9). To reduce any potential impact of phylogenetic non-independence of data points, the same test was repeated with a single representative for

each genus. In five of the six families, the relationship was stronger than when all species were included. The exception was Rallidae, which no longer had a significant relationship when using one species per genus (Table 9).

Analysis was also performed when restricted to New World passerine birds in the oscine and suboscine clades, using only EOO as a predictor of population size. In this limited dataset (N = 39 species), there is an apparent trend of shallower slopes for endangered and critically endangered species, indicating range size comparisons may be poor indicators of population size in these conservation categories when range size is large (e.g., above 10^4 km^2) (Figure 24). Once these conservation categories were excluded, there is a strong, positive linear, log-log relationship between range and population sizes (R^2 = 0.697, P = 1.6 × 10^{-5}). The relationship is particularly strong when only endangered/critically endangered species with nominally large ranges (>10^4 km^2) are excluded (R^2 = 0.742, P = 6.4 × 10^{-11}).

Table 8. Distribution of bird range sizes and IUCN conservation categories for the dataset of species for which population size and body mass were available, and for all birds.

Range size (km^2)	% (dataset)	% (all birds)	IUCN category	% (dataset)	% (all birds)
10^0–10^1	4.72	0.48	Least concern	3.41	76.28
10^1–10^2	11.02	1.42	Near threatened	21.78	8.83
10^2–10^3	15.22	3.54	Vulnerable	32.55	7.10
10^3–10^4	18.11	7.36	Endangered	29.40	3.96
10^4–10^5	19.16	16.40	Critically endangered	12.86	1.83
10^5–10^6	16.53	31.43			
> 10^6	15.22	39.06			

Table 9. Within-family tests of the population size–range size relationship for all families with at least 10 genera. Comparisons are made using a limited dataset where one species per genus is included, selected as the species closest to the median mass for its genus. These comparisons are contrasted with the full dataset, where multiple species per genus may be present. Both variables are $\log_{10}$-transformed. Taxonomic grouping follows that used by BirdLife International.

	One species per genus				All species			
Family	N	Slope	R^2	P	N	Slope	R^2	P
Accipitridae	17	0.628	0.648	9.8×10^{-5}	26	0.630	0.591	4.5×10^{-6}
Anatidae	15	0.569	0.446	0.007	30	0.362	0.436	7.1×10^{-5}
Emberizidae	13	0.539	0.635	0.001	17	0.523	0.585	3.4×10^{-4}
Fringillidae	14	0.474	0.504	0.004	18	0.422	0.411	0.004
Psittacidae	20	0.644	0.595	6.9×10^{-5}	35	0.482	0.412	3.2×10^{-5}
Rallidae	10	0.316	0.332	0.081	14	0.341	0.413	0.013

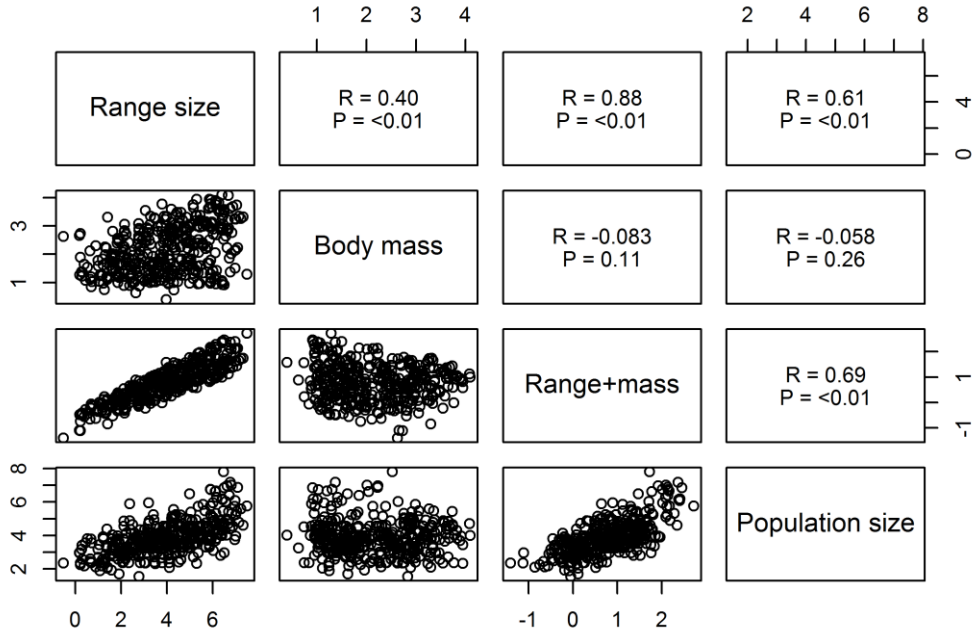

Figure 22. Matrix of regression variable relationships in predicting population size from range size (extent of occurrence) and body mass (N = 382 bird species). All variables are log-transformed. Range + mass represents the regression model equation of 0.44686 × $\log_{10}$ range size - 0.45584 × $\log_{10}$ body mass.

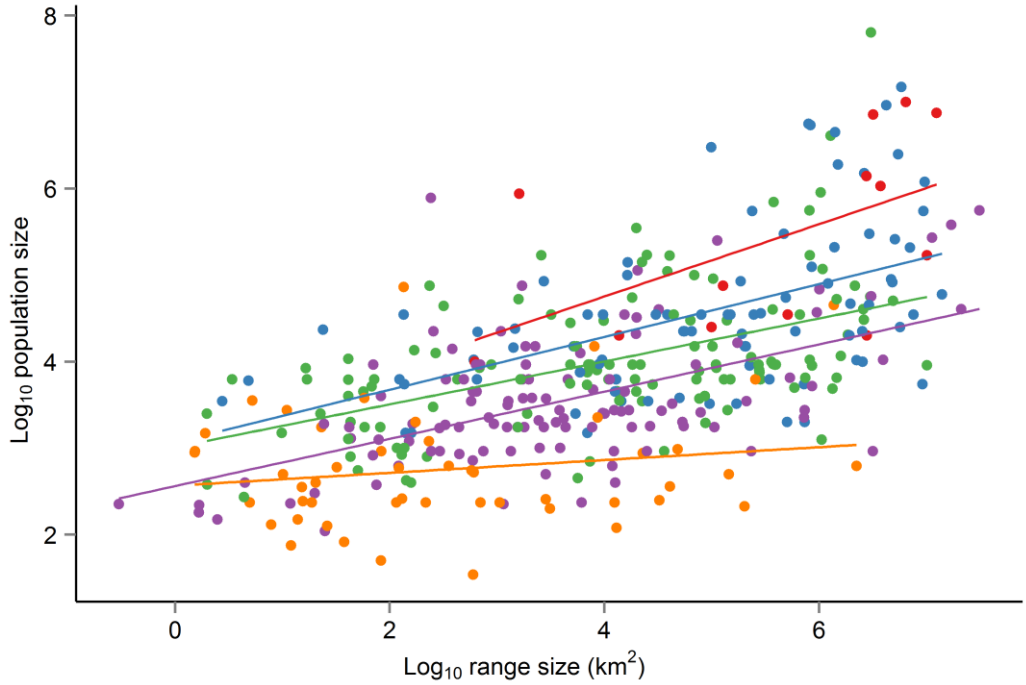

Figure 23. Relationship between range size (extent of occurrence) and population size across the IUCN Redlist categories for all birds with population size and body mass estimates available (N = 381 species). Colour denotes IUCN category: least concern (●); near threatened (●); vulnerable (●); endangered (●); critically endangered (●).

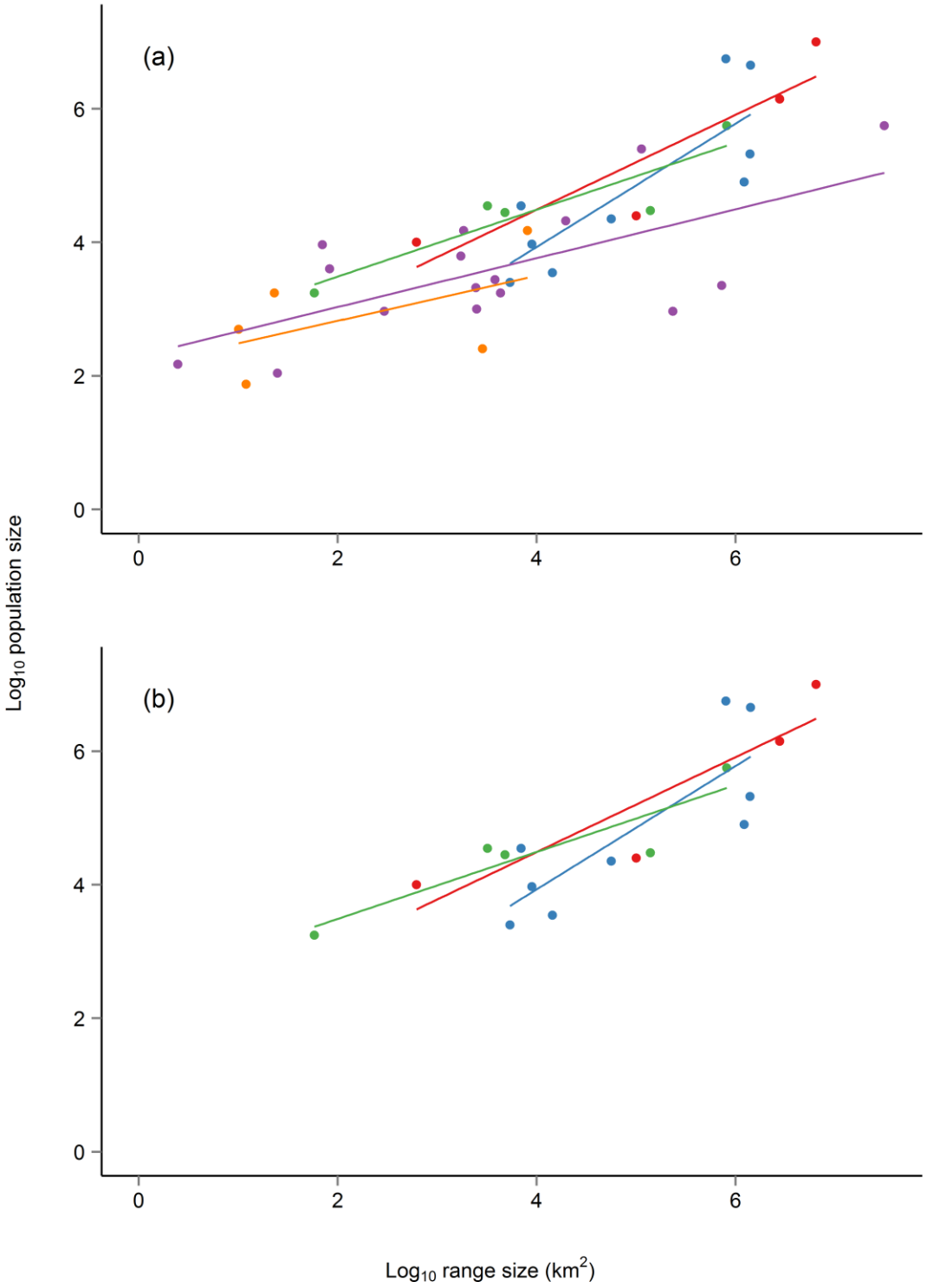

Figure 24. Relationship between range size (extent of occurrence) and population size across the IUCN Redlist categories for oscine and suboscine passerine birds of the New World (N = 39 species), where: (a) all species are included; and (b) endangered/critically endangered species are excluded. Colour denotes IUCN category: least concern (●); near threatened (●); vulnerable (●); endangered (●); critically endangered (●).

4.2.5 Variation in population density with latitude and elevation

To further determine if the use of geographic range size as a population size surrogate is appropriate, it is important to determine if the relationship between them is biased by factors that might affect population density. Although the strongest effect on population size was from range size in the above analyses, other factors that can affect population size include latitude and elevation. In the case of latitude, only a minor additional effect was detected. Regressed against the residuals of the population size–range size relationship, absolute latitude explains a modest additional part of variation, although this is marginally non-significant (R^2 = 0.014, N = 262, P = 0.052). This appears to be influenced by different responses in the northern and southern hemispheres (Figure 26), as when actual, rather than absolute, latitude is used, patterns in the residuals arise that appear related to available land area (negative residuals in southern hemisphere, positive in northern hemisphere, with reduced residual values around the Isthmus of Panama and Sahara Desert; Figure 26b).

A substantial effect of elevation on population densities was detected. Restricting the dataset to montane birds, defined as those species with lower elevational limits above 1000 m.a.s.l., average elevation has a significant, negative relationship with the residuals of the population size–range size relationship (R^2 = 0.141, N = 54, P = 0.005). While this effect is apparent across the dataset as a whole, there is substantial noise at lower average elevations. This is an apparent effect of different responses with latitude: the response can be divided into a significant and non-significant fraction on the basis of latitude, with different relationships apparent. For a subset bounded within the tropics (defined as within 20° of the equator), there is a strong, negative relationship (R^2 = 0.423, N = 37, P = 1.2×10^{-5}) (Figure 27b), with population densities declining distinctly above 2000 m.a.s.l.. A positive, but non-significant relationship is recorded in the extratropics (R^2 = 0.163, N = 17, P = 0.108) (Figure 27b).

Genetic diversity

Latitudinal variation in population sizes could cause latitudinal gradients in N_e, which could be detectable at a population-genetic level as a parallel gradient in genetic diversity

(e.g., nucleotide diversity or heterozygosity). While it might be expected that tropical species to have lower genetic diversity, this is complicated by two additional factors. Background selection also reduces genetic diversity, and is posited to be stronger in temperate than tropical regions. Pleistocene glaciations caused range shifts and bottlenecks in many temperate species, reducing their diversity (Hewitt, 2004; Melo-Ferreira et al., 2007). Tropical regions also experienced substantial changes in habitat boundaries, sea level and climate, including reduced rainfall through the Pleistocene, with refugial habitats playing a potentially important role for many taxa (Haffer & Prance, 2001). Therefore, I examined the relationship between genetic diversity and latitude. Both nucleotide diversity and maximum (within-species) distance were investigated for passerine birds across latitude, using the most widely sequenced avian gene (ND2). Nucleotide diversity and maximum distance are themselves strongly correlated (Pearson correlation, R^2 = 0.654), their ratio was also correlated with latitude to understand changes in controlled levels of nucleotide diversity. This is potentially important when considering a wide latitudinal range because not only might the average nucleotide diversity of temperate species been reduced by Pleistocene glaciations, but divergence and diversity are also both correlated with population size, which also varies on average with latitude.

Nucleotide diversity was elevated on average in the tropics, peaking close the equator, although there was no distinct pattern above 20°N. However, the ratio of diversity:distance (nucleotide diversity:maximum genetic distance) followed a distinct latitudinal gradient across the latitudinal range of all included species (Figure 25). When the diversity: distance ratio was regressed against absolute latitude, a substantial proportion of variation was explained (OLS regression, N = 101, R^2 = 0.299, P = 3.4×10^{-9}). While the upper limit on nucleotide diversity increases with range size, resulting in a significantly positive correlation (Pearson's r = 0.293, P = 0.003), there was no relationship between range size and the diversity: distance ratio (Pearson's r = 0.020, P = 0.838). Because these contrasts are not phylogenetically independent, they were repeated using congeneric species pairs. One pair was selected for each genus for which two or more species were available on the basis that the pair had the largest latitudinal distance. The species were then contrasted on the basis of largest absolute latitude minus smallest absolute latitude. The 17 resulting

independent contrasts show similar patterns to those for ND2 diversity and the diversity:divergence ratio. Although there is a negative trend between the ratio of ND2 diversity for the independent contrasts and latitudinal contrasts, the relationship is not significant (Pearson's r = 0.438, P = 0.069). There is a significant, negative relationship between contrasts of the divergence:diversity ratio, and contrasts of absolute latitude (Pearson's r = 0.497, P = 0.036). Because of the small number of independent contrasts, these relationships were also investigated using Wilcoxon signed-ranks tests. Both ND2 diversity and the diversity:divergence ratio were significantly higher in the lower latitude species (Wilcoxon signed rank test, V = 30, P = 0.027 for both relationships).

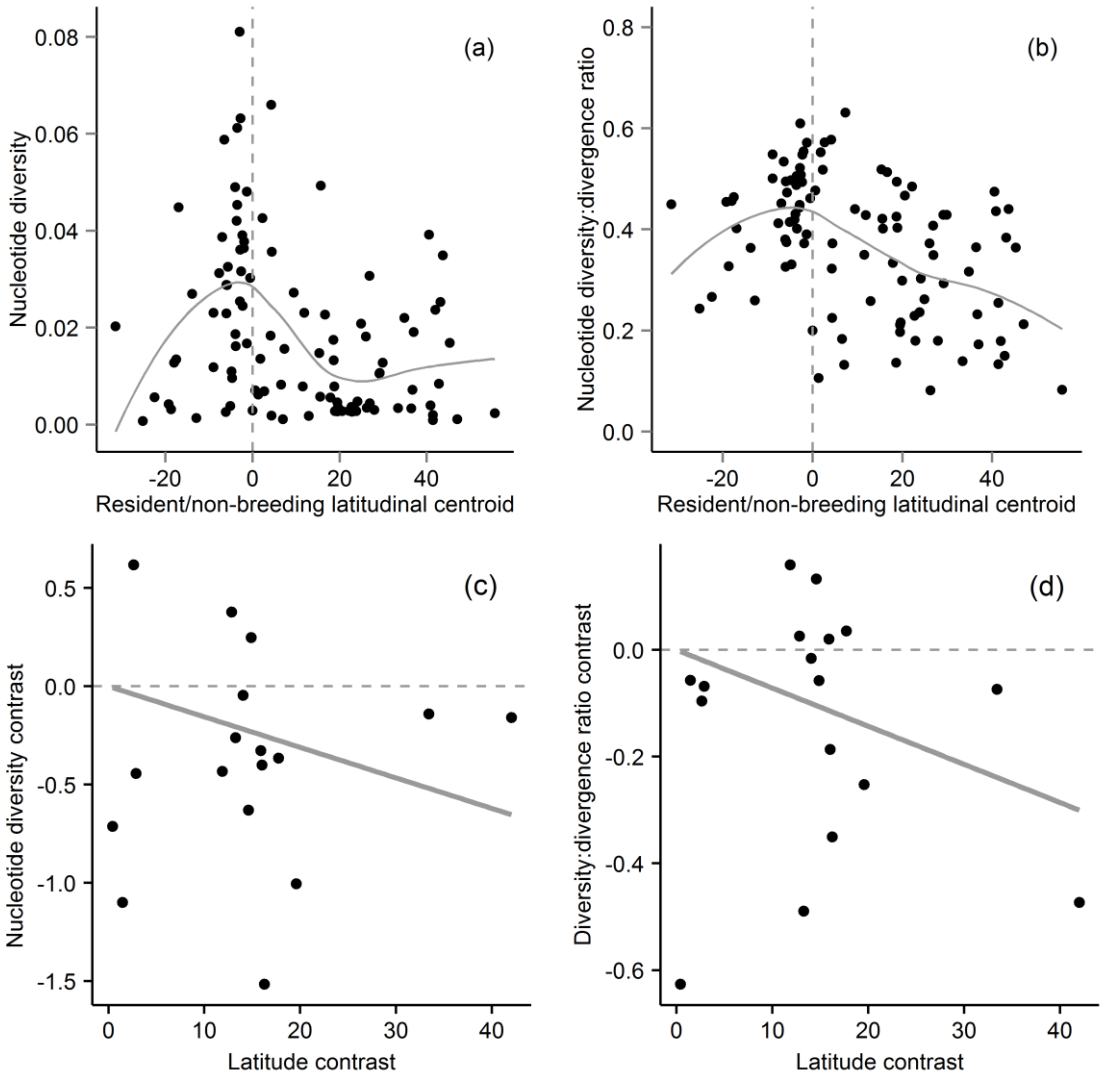

Figure 25. Latitudinal variation in (a) nucleotide diversity and (b) the ratio of nucleotide diversity to nucleotide divergence in a global dataset of passerine birds for all species with 10 or more ND2 sequences available (N = 101 species). Fitted line is a LOESS curve. Horizontal dashed lines denote the equator. Phylogenetically independent contrasts for the same relationships are also shown in (c) and (d) respectively (N = 17 contrasts) (see text for methods).

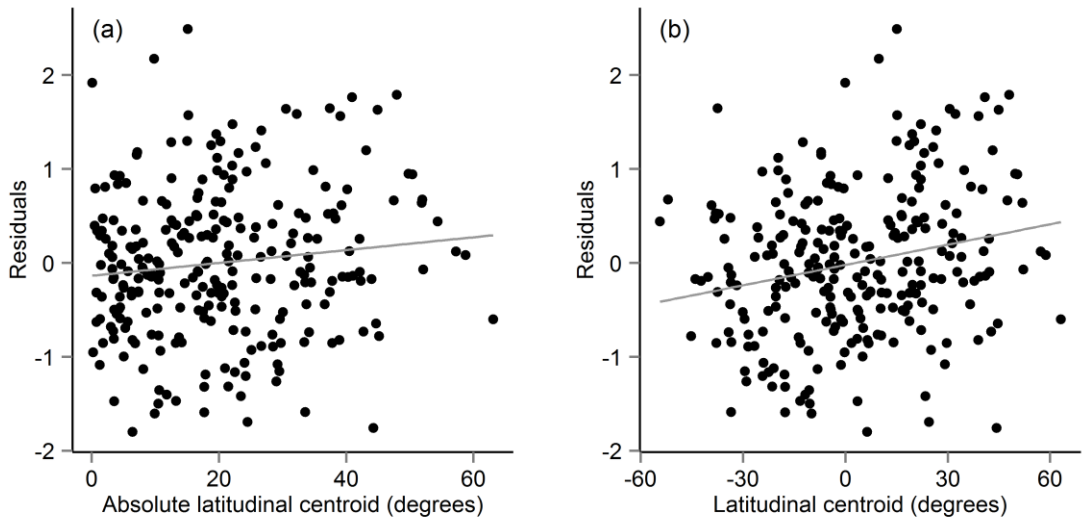

Figure 26. Relationship between the residuals of the population–range size relationship and (a) species' absolute latitudinal centroid, and (b) species' latitudinal centroid for passerine birds. Grey lines are regression fits. (N = 262 species).

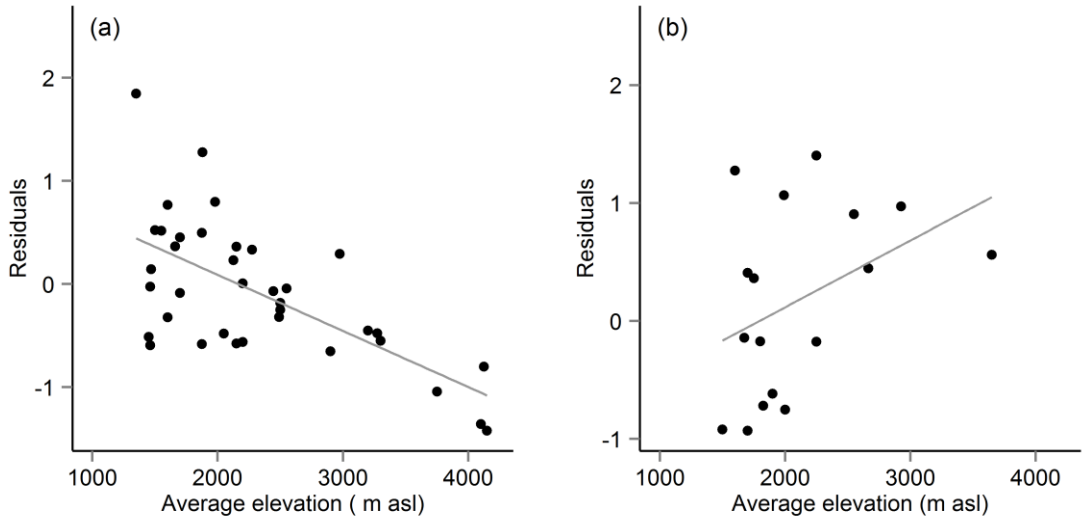

Figure 27. Relationship between the residuals of the population–range size relationship and average elevation, for montane passerine bird species (a) within 20° of the equator and (b) outside of 20° from the equator. Grey lines are regression fits. (N = 37 and 17 species respectively).

4.3 Effect of range size on molecular evolutionary rates and patterns in New World birds

4.3.1 Sequence dataset assembly

Having established the patterns of species richness with a range of environmental correlates, and provided a basis for using geographic range size as a population size surrogate, the next aim is to establish the relationships between molecular evolutionary rates and patterns, and the factors that might influence it. Such relationships could provide plausible empirical links between population-genetic processes and macro-scale emergent patterns of species richness. To improve upon past work seeking such relationships, emphasis in design was given to investigating combinations of taxa and loci for which taxon sampling was complete or nearly complete to increase the chance of reliably detecting relationships. The first section presented here is the taxon and sequence selection process.

Across 170,000 GenBank accessions initially collected, 90,421 accessions were identified as containing sequences for one or more avian mitochondrial protein-coding gene. After individual genes were extracted, a total of 97,701 mtDNA gene sequences were collated. Of the 10,064 species recognised by BirdLife International at the time of access, sequences were retrieved for 7,373 species (73.3%), either listed under a matching name or a recognised synonym. Synonyms were listed in the database following the names recognised by BirdLife International.

The majority of sequences were for three genes: cytochrome *b*, NADH dehydrogenase subunit 2, or cytochrome *c* oxidase subunit 1, as these genes are widely used for molecular systematics and as population-genetic markers (Table 10). However, because the accessions for the other genes tend to be distributed non-randomly in taxonomic clusters they were retained for analyses, despite relatively low overall coverage.t The complete database of mtDNA sequences is available online, and is accessible through Figshare:

- http://dx.doi.org/10.6084/m9.figshare.1236602

Table 10. Sequence availability for mitochondrially encoded protein-coding genes across all bird species. Genes prefixed 'ND' are NADH dehydrogenase subunits; genes prefixed 'CO' are cytochrome *c* oxidase subunits; genes prefixed 'ATP' are ATP synthase subunits; CYTB is cytochrome *b*; where present, suffixes refer to subunit number.

Gene	Sequences	Percent of total	Gene (cont.)	Sequences (cont.)	Percent of total (cont.)
ND1	910	0.93	CO1	21000	21.49
ND2	29002	29.68	CO2	2190	2.24
ND3	5732	5.87	CO3	1136	1.16
ND4	575	0.59	ATP6	2342	2.40
ND4L	138	0.14	ATP8	1727	1.77
ND5	1506	1.54	CYTB	28563	29.24
ND6	2880	2.95			

4.3.2 Phylogenetic and ecological data

From the sequences collated as above, two clades of New World passerine birds were identified as containing numerous families with both large numbers of species and near-complete taxon sampling: the nine-primaried oscines and the subsoscines. Nine family-level phylogenies were produced from all species for which molecular data was available, and from these phyologenies, 365 sister species pairs were produced. Of these, 65 pairs had at least one zero value for d_N or d_S. Because ω ratios are indeterminate with such zero values, these pairs were excluded from all analysis, leaving 300 sister species pairs. These 300 pairs and their corresponding ecological and evolutionary metrics are referred to herein as the "raw dataset".

The median number of base pairs of mtDNA across the raw dataset was 2281. In 10 pairs (3.3% of pairs) fewer than 1,000 base pairs were available for comparison. However, there is no evidence that this led to inflated substitution rate variance for these pairs (Appendix 2: Data quality and outlier analyses for range size–population size study), therefore no contrasts were excluded on this basis. Medians for each family and clade are included below (Table 11).

The oscine and suboscine clades in the raw dataset contained similar numbers of contrasts, and had similar range size ratio distributions (Table 11). Contrasts were less evenly distributed between families and there was also more variation in the magnitude of range size contrasts. Families contained between 12 and 74 sister species pairs, and while the largest range size contrast overall was six orders of magnitude between sister species,

contrasts in some families were restricted to below two orders of magnitude (e.g., Cardinalidae, and Dendrocolaptidae; Table 11). Similarly, median range size contrasts in families ranges from approximately two-fold to eight-fold differences. These patterns suggest the power to detect patterns within families is variable.

4.3.3 Dataset quality controls

Additional quality controls were then applied to produce an alternative dataset that was expected to have an increased sensitivity to detect patterns when signal:noise ratios were low. Accordingly, excluding low-power pairs detected with the Welch and Waxman (2008) test, the raw dataset was reduced from 300 sister species pairs to 214 pairs. The number of comparisons was then reduced to 208 pairs by applying the minimum genetic distance cut-off of 0.01 substitutions site^{-1} in both sisters. Finally, one outlier was removed, producing 207 contrasts. This subset is referred to herein as the "final dataset", in contrast to the "raw dataset" containing all 300 species. There was a significant, negative relationship between ω and range size in the subset of contrasts that were excluded from the final dataset (OLS regression forced through origin, $R^2 = 0.042$, $P = 0.047$; Figure 28a), which was weaker than that in remaining comparisons (OLS regression forced through origin, $R^2 = 0.077$, $P = 5.1\times10^{-5}$; Figure 28b), although the difference was not statistically significant (Fisher r-to-z transformation, $P = 0.54$). Analyses for both datasets are presented.

There was no basis to assume that contrasts with less molecular data (fewer base pairs) had lower power, and therefore no contrasts were removed on this criterion directly. However, the median number of base pairs per species in the final dataset was higher than in the raw dataset (2436 bp and 2281 bp respectively), and the proportion of comparisons with fewer than 1000 base pairs declined (1.9% and 3.3% respectively). Overall, 85% of comparisons in the final dataset used more 2000 bp of sequence data per species.

Table 11. Distribution of species ranges in the raw dataset for sister species comparisons across nine families of passerine birds. Range size contrasts are calculated from the range size of the larger-ranged species divided by the range size of the smaller-ranged species, using combined resident and breeding ranges. Numbers in brackets are log-transformed contrasts.

Clade	Family	N (pairs)	Median range size contrast	Maximum range size contrast	Median base pairs per comparison
Passerines	(All)	300	4.29 (0.6)	1.2×10^6 (6.1)	2281
Oscines	(All oscines)	161	4.06 (0.6)	1.2×10^6 (6.1)	2184
	Cardinalidae	13	6.11 (0.8)	83.7 (1.9)	2082
	Icteridae	22	8.32 (0.9)	5.3×10^2 (2.7)	2263
	Parulidae	31	4.06 (0.6)	1.2×10^6 (6.1)	5240
	Passerellidae	21	2.67 (0.4)	1.3×10^5 (5.1)	2181
	Thraupidae	74	4.18 (0.6)	8.5×10^3 (3.9)	2183
Subocines	(All suboscines)	139	4.45 (0.6)	4.2×10^5 (5.6)	2401
	Dendrocolaptidae	17	2.34 (0.4)	56.4 (1.8)	3068
	Furnariidae	61	5.43 (0.7)	4.2×10^5 (5.6)	2073
	Pipridae	12	2.89 (0.5)	2.5×10^2 (2.4)	1691
	Thamnophilidae	49	4.56 (0.7)	5.3×10^4 (4.7)	2436

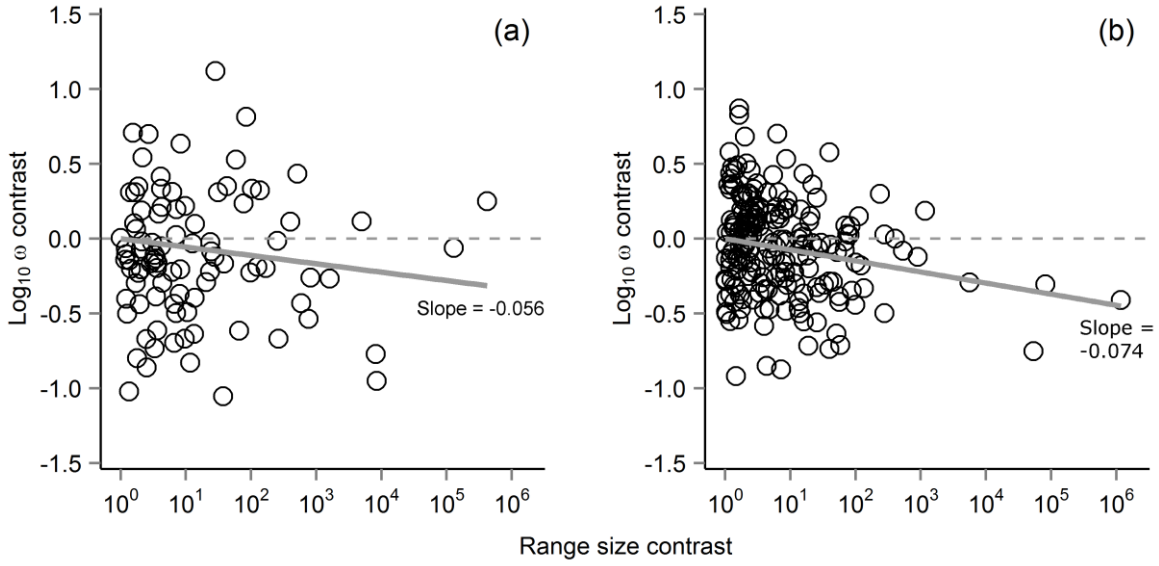

Figure 28. Relationship between transformed contrasts of resident/breed range size and ω for a) contrasts that were excluded from the final dataset as low-power (93 pairs), and b) contrasts included in the final dataset (N = 207 pairs). Solid grey lines are regression fits forced through the origin.

4.3.4 Analysis

With the molecular datasets assembled, and phylogenetic analyses completed, data analyses on the rate of molecular evolution could then be conducted. Initial analyses used

the raw dataset (N = 300 contrasts). To compare with previous studies, Wilcoxon signed-ranks tests were performed to test if positive (larger populations evolving faster), or negative (smaller populations evolving faster) relationships predominated, with the latter being a prominent prediction of nearly neutral theory (Ohta, 1972b). Smaller populations evolved faster when evolutionary rates were measured by ω (median log-transformed contrast -0.062, V = 17549, $P = 8.3\times10^{-4}$). The same pattern was evident for the substitution rate, although the median contrast was smaller, and the difference was non-significant (median log-transformed contrast -0.019, V = 20090, P = 0.098).

A regression analysis shows a significant, negative relationship exists between contrasts of the non-synonymous substitution rate (d_N) and range size (OLS regression forced through the origin, R^2 = 0.036, $P = 9.9\times10^{-5}$). No such pattern was detected between ratios of the synonymous substitution rate (d_S) and range size (OLS regression, R^2 = 0.004, P = 0.536). Corresponding patterns were recorded for the contrasts of range size with ω ratios and substitution rate ratios respectively (Table 12; Figure 29). Not only were regression slopes were similar between d_N and ω regressions, and between d_S and substitution rate regressions, similar residual scatter around the regression line was evident (Figure 29).

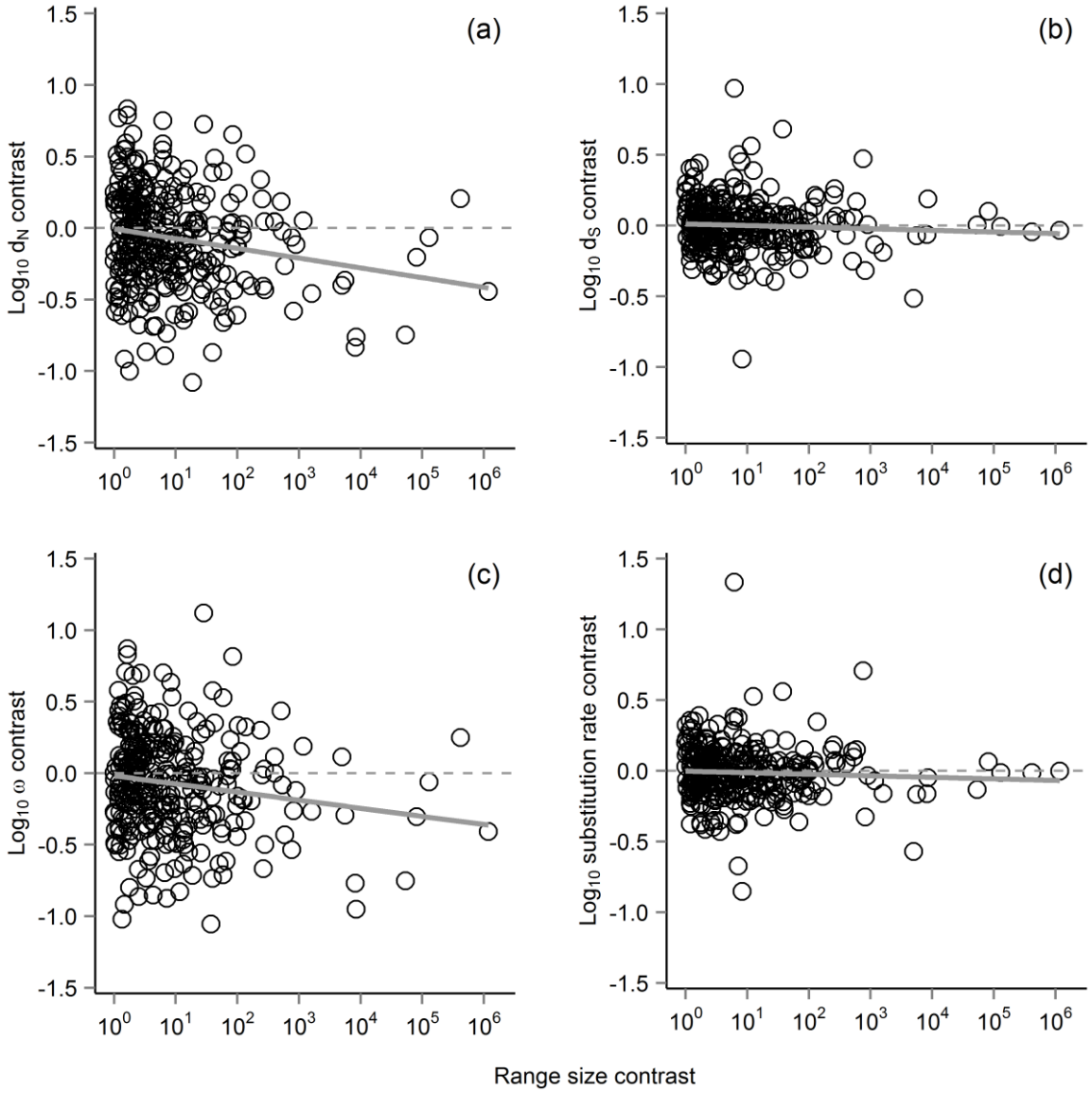

Figure 29. Relationships between relative substitution rates and relative resident/breeding range size for all sister species pairs of New World passerine birds in the raw dataset (N = 300 pairs). Relative rates of (a) non-synonymous substitutions, (b) synonymous substitutions, (c) ω, and (d) the substitution rate are shown. Both axes are on log scales. Solid grey lines are regression fits forced through the origin. Coefficients in Table 12.

Repeating the analysis with the final dataset (N = 207 contrasts), the effect of applying dataset filtering for contrasts that were a priori expected to be low-power was evident. The relationships between range size and both d_N and ω were stronger, and a slight, but significantly negative relationship was also detected between range size and the substitution rate (Table 12; Figure 30). The non-significant relationship between range size and d_S observed for the raw dataset remained non-significant in the final dataset.

To determine the consistency of the patterns, range size and ω ratios were regressed for each of the nine bird families separately, and also for the two broad containing clades to which these families belong (nine-primaried oscines and suboscines). Both clades had significantly negative slopes (Table 13; Figure 31). The slopes for seven of the nine families were negative, with the exceptions being Furnariidae, which had a flat regression slope (coefficient = 0.00, $P = 0.908$) and Cardinalidae, which had a non-significant, positive slope (coefficient = 0.09, $P = 0.259$). Both families had restricted range size variation across their contrasts (Table 13), although this was also true of several other families that had negative slopes. Across the seven families with negative regression slopes, four of the slopes were significantly negative (Table 13). The lack of significance appears related to power: in each clade, the family with the largest range size contrast had a significantly negative relationship (Parulidae within the nine-primaried oscines, and Thamnophilidae within the suboscines, Table 13).

Table 12. Ordinary least squares results for the regression of range size contrasts against the contrasts of four evolutionary metrics for sister species of New World passerine birds. All contrasts are the $\log_{10}$-transformed differences of values for the larger-ranged species less the smaller-ranged species. Regressions are forced through the origin.

	Raw dataset (N = 300 pairs)			Final dataset (N = 207 pairs)		
	Slope	R^2	P	Slope	R^2	P
Substitution rate	-0.012	0.007	0.151	-0.019	0.026	0.019
d_N	-0.068	0.036	9.9×10^{-5}	-0.085	0.095	5.6×10^{-6}
d_S	-0.005	0.001	0.536	-0.010	0.010	0.152
ω	-0.066	0.060	1.8×10^{-5}	-0.074	0.077	5.1×10^{-5}

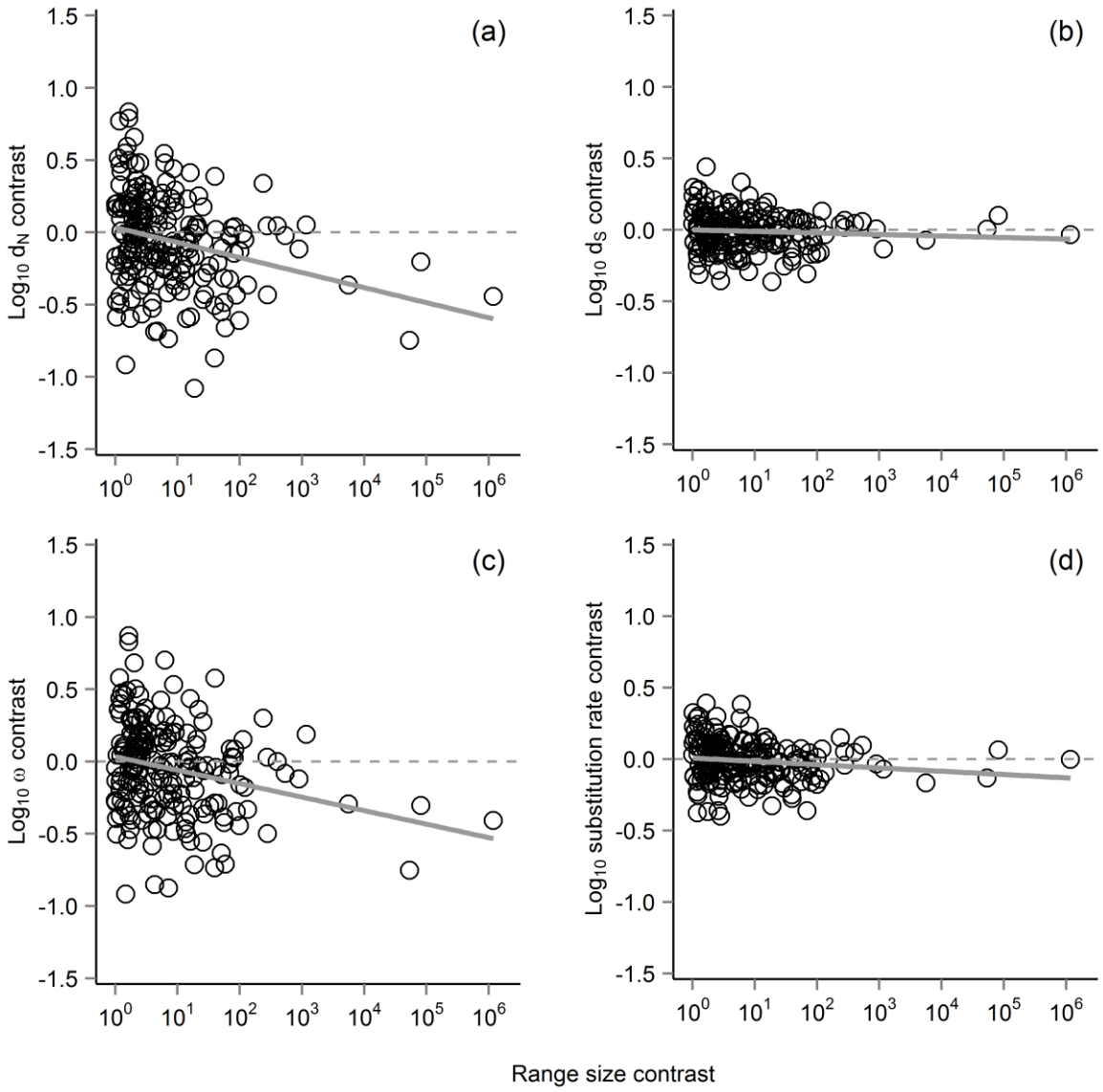

Figure 30. Relationships between relative substitution rates and relative range size for all sister species pairs of New World passerine birds in the final dataset (N = 207 pairs). Relative rates of (a) non-synonymous substitutions, (b) synonymous substitutions, (c) ω, and (d) the substitution rate are shown. Both axes are log-scaled. Solid grey lines are regression fits forced through the origin. Regression coefficients are reported in Table 12.

Table 13. Regression slopes of the ω –range size relationship for nine families of passerine birds. For pairs of sister species, ratios of range size for the larger-ranged species to the smaller-ranged species were contrasted with ratios of ω.

Order	Family	N (pairs)	Maximum range size contrast	Slope	P
Passserines	(All)	207	1.2×10^{6}	-0.07	5.1×10^{-5}
Nine-primaried oscines	(All oscines)	109	1.2×10^{6}	-0.06	0.003
	Cardinalidae	13	84	0.09	0.259
	Icteridae	18	536.2	-0.06	0.248
	Parulidae	22	1.2×10^{6}	-0.06	0.030
	Passerellidae	13	39	-0.23	0.011
	Thraupidae	43	897	-0.08	0.133
Subocines	(All suboscines)	98	5.3×10^{4}	-0.09	4.6×10^{-3}
	Dendrocolaptidae	16	56	-0.22	0.038
	Furnariidae	35	277	0.00	0.908
	Pipridae	8	52	-0.20	0.353
	Thamnophilidae	39	5.3×10^{4}	-0.11	2.5×10^{-3}

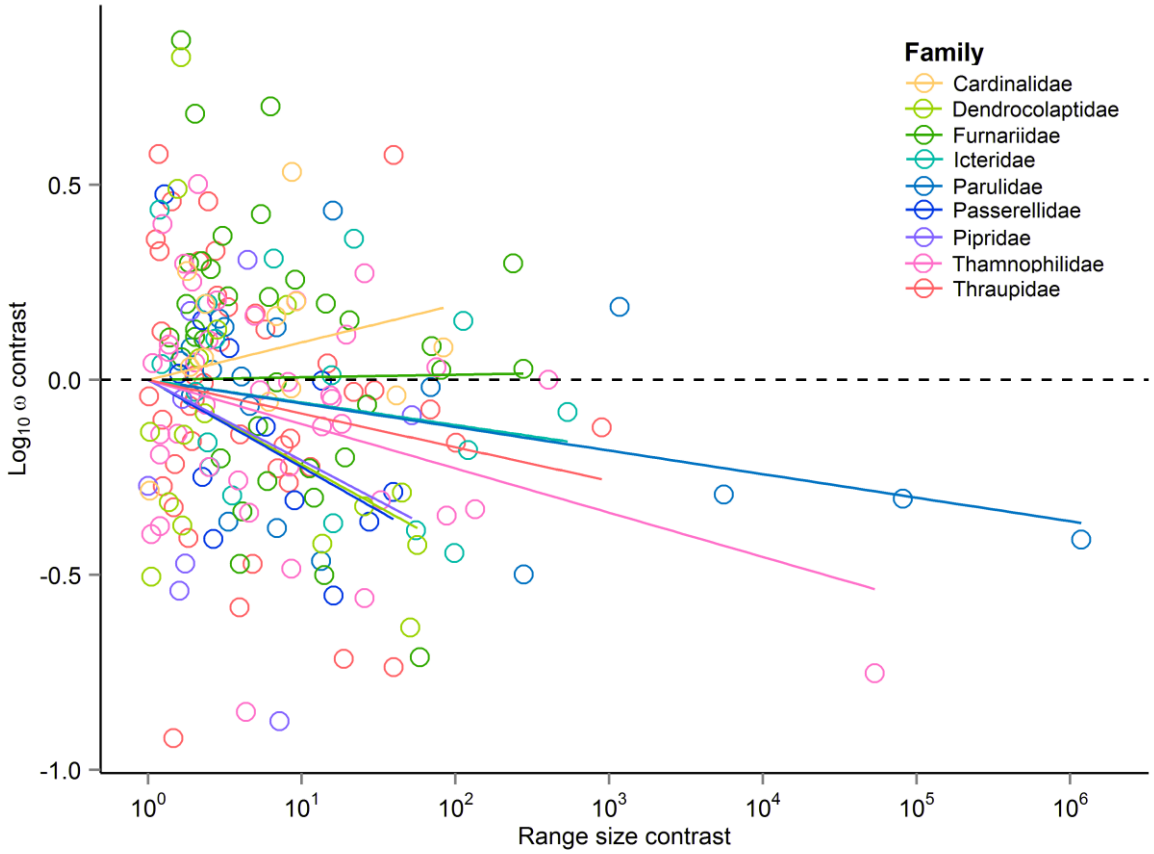

Figure 31. Relationships between ω and range size contrasts for sister species pairs from each of nine families of New World passerine birds in the final dataset (N = 207 pairs). Lines are regression fits forced through the origin. Regression coefficients are reported in Table 13.

Supporting the observation of increased ω in species for smaller ranges, the pairwise increase in d_N is significantly weighted towards more rapid accumulation in smaller-ranged species (95% confidence interval of standardised major axis regression slope, 0.898 ± 0.064) even without accounting for the magnitude of differences in range size. For d_S, there is no significant difference from the expected slope of 1 where substitutions accumulate at the same rate in both groups (95% confidence interval of standardised major axis regression slope, 1.019±0.034) (Figure 32).

Finally, given that there are differences in the responses of ω and the substitution rate to range size variation, it is important to consider the drivers of these evolutionary metrics. The ω ratio is more strongly driven by changes in d_N than d_S (Pearson's r = 0.924 and -0.148 respectively), while the reverse is true of the substitution rate albeit to a lesser extent (R = 0.583 and 0.839 respectively) (Table 14).

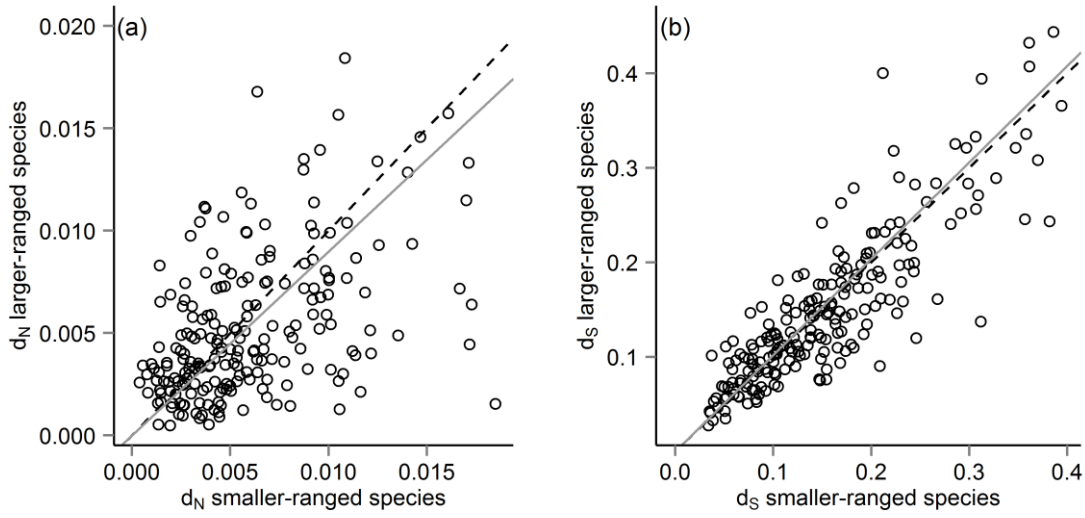

Figure 32. Accumulation of (a) non-synonymous and (b) synonymous mutations in sister species, contrasted between the smaller-ranged and larger-ranged species in each pair (N = 207 pairs). The dashed line shows the expected fit if there is no difference in the substitution rate between larger- and smaller-ranged species. The solid grey line shows a standardised major axis regression fit, forced through the origin.

Table 14. Correlation matrix of evolutionary metrics and range size, showing Pearson's *r* for the final dataset ($N = 207$ sister species pairs).

	Range ratio	d_N ratio	d_S ratio	ω ratio	Substitution rate ratio
Range ratio	-				
d_N ratio	-0.263	-			
d_S ratio	-0.077	0.241	-		
ω ratio	-0.237	0.924	-0.148	-	
Substitution rate ratio	-0.138	0.583	0.839	0.263	-

4.3.5 Potential confounding factors

Because several factors have previously been identified that affect or potentially affect rates of molecular evolution, it is necessary to determine if any of these have influenced the relationship detected between range size and the rate of molecular evolution. The factors that were considered were body mass, migratory status, and range-wide averages of net primary production (NPP), temperature, rainfall, elevation, latitude. Results of the relationships with these variables are presented below (Table 15), with the exception of migration, as this was treated as a binary variable (migratory or non-migratory) rather than a continuous variable (e.g., mean distance migrated). While a mean distance of this sort could be estimated using the difference in centroids of species' breeding and non-breeding ranges, such distances might not be representative of true distances migrated for individuals given that they might occupy distinct parts of the total breeding and non-breeding range interannually, nor for species that also have resident ranges.

Environmental/ecological factors

All bivariate relationships were visually inspected prior to correlating with range size and evolutionary metrics. No significant correlation was found between the substitution rate, and any of the potential confounding factors (Table 15). However, several variables correlated significantly with range size and/or ω.

Three variables correlated with both range size and ω. These were: latitude, NPP, and temperature seasonality. A multiple regression model was used to predict ω from range size and all three of the potentially confounding variables. In this model, range size was

the only significant predictor. Stepwise elimination of non-significant variables produced a model in which both range size and temperature seasonality were significant predictors of ω. The slope for range size in the multiple regression (-0.077) was shallower than that found in the bivariate ω–range size relationship (-0.097), although the difference in slopes between regressions was not significant ($Z = -0.559$, $P = 0.576$).

For each of the remaining potentially confounding variables that correlated significantly with range size but not with ω, an additional analysis was performed. First, contrasts for each variable were regressed against range size contrasts using OLS regression. Residual variation in range size that was not explained by its relationship with each variable was then used to predict ω to ensure that the ω–range size relationship remained significant. The ω–residual relationship was significant for all three variables (mean annual temperature, $P = 5.3\times10^{-4}$; annual precipitation, $P = 3.2\times10^{-4}$; and minimum elevation, $P = 4.4\times10^{-4}$).

Table 15. Test for confounding effects from factors that could influence rates of molecular evolution. Bivariate Pearson correlation coefficients are shows for two evolutionary metrics, and range size. Significant relationships are bolded. Symbols after R^2 values where present indicate significance of relationships: ~ P < 0.1, * P < 0.05; ** P < 0.005; *** P < 0.0005.

Variable	Variable contrast	Range size correlation (R)	ω correlation (R)	Substitution rate correlation (R)
Body mass	Log_{10} transformed	-0.064	0.113	0.017
Latitude	Difference (absolute degrees)	**0.207****	**-0.219****	-0.004
Net primary production	Log_{10} transformed	**-0.225****	**0.191***	0.012
Mean annual temperature	Log_{10} transformed	**-0.237****	0.135~	0.01
Temperature seasonality	Log_{10} transformed	**0.271*****	**-0.255*****	-0.044
Annual precipitation	Log_{10} transformed	**-0.236****	0.098	0.068
Precipitation seasonality	Log_{10} transformed	0.021	-0.255	-0.044
Mean elevation	Difference (m.a.s.l.)	-0.044	0.012	-0.091
Minimum elevation	Difference (m.a.s.l.)	**-0.217****	0.125~	-0.015
Maximum elevation	Difference (m.a.s.l.)	0.061	-0.053	-0.113

Migration

Because migration is weakly evolutionarily conserved, some sister species pairs vary in their migratory status. Given that the energetic demands of migration could elevate the strength of negative selection, migratory status of species could affect the ω–range size relationship. For 28 comparisons in the final dataset, sister species differed in their migratory status. In three cases, the migratory species was an altitudinal migrant, which were not considered in this analysis. For 20 of the 25 remaining pairs, the larger-ranged species was migratory. These contrasts had a negative ω–range size relationship, with an apparent negative intercept, indicating a separate effect of migration. Similarly, for the five pairs in which the smaller-ranged species was migratory, a negative ω–range size relationship was evident, although there was an apparent, positive intercept. However, there were too few data points to be certain of these apparent migration-induced shifts. Excluding all species with incongruent migratory statuses, did not substantially change the overall negative ω–range size relationship ($N = 179$, $R^2 = 0.053$, $P = 0.002$).

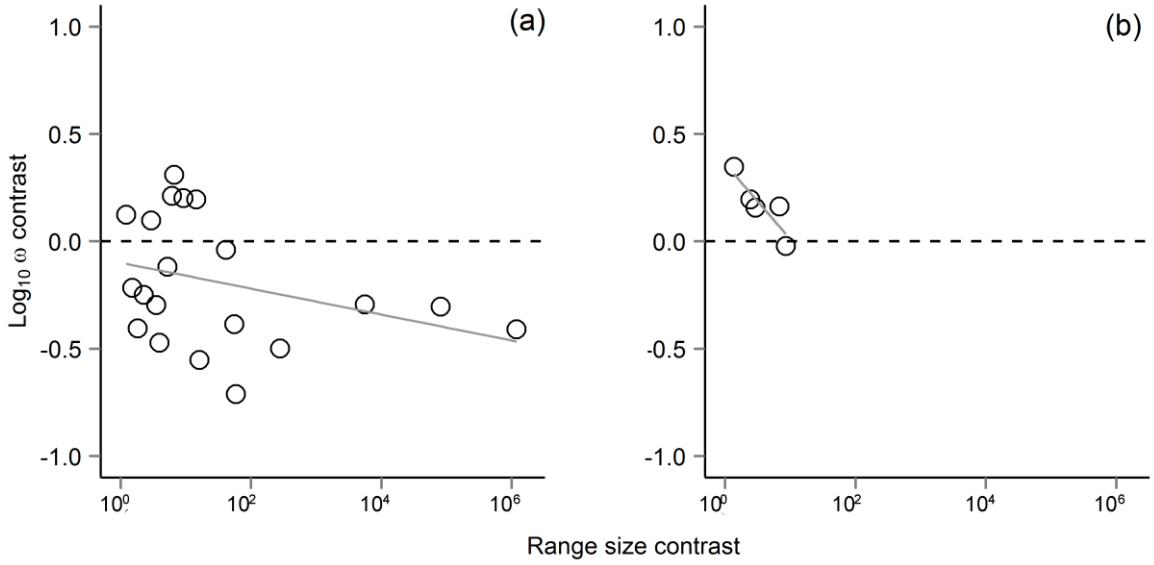

Figure 33. Effects of migration on the ω–range size relationship, shown on sister species pairs of New World passerine birds where (a) the larger-ranged species is migratory and the smaller-ranged species is not (N = 20 pairs), and (b) the smaller-ranged species is migratory and the larger-ranged species is not (N = 5 pairs). Solid grey lines are regression fits.

Latitude

Although no confounding effects of latitude on the ω–range size relationship were detected using the above method, there remains considerable variation in avian range size that occurs with latitude across the New World (Figure 34). Also, the population size–range size relationship estimated previously (see 4.2.5 Variation in population density with latitude and elevation) indicated that range size is more strongly correlated with population outside of tropics than inside. Investigating the effect of latitude as a categorical (tropical/extratropical) factor may be of interest. Therefore, the ω–range size relationship was separately regressed for contrasts located inside and outside of the tropics. A cut-off value of 20 degrees of absolute latitude was used, such that pairs were considered tropical if both species had their latitudinal centroids within 20 degrees of the equator. Within the tropical subset, range size is a weaker predictor of ω than it was across the entire final dataset (R^2 = 0.037) (Figure 35a). Outside of the tropics, the ω–range size relationship appears stronger (R^2 = 0.125) (Figure 35b), although the difference in correlations is not significant (Fisher r-to-z transformation, P = 0.24).

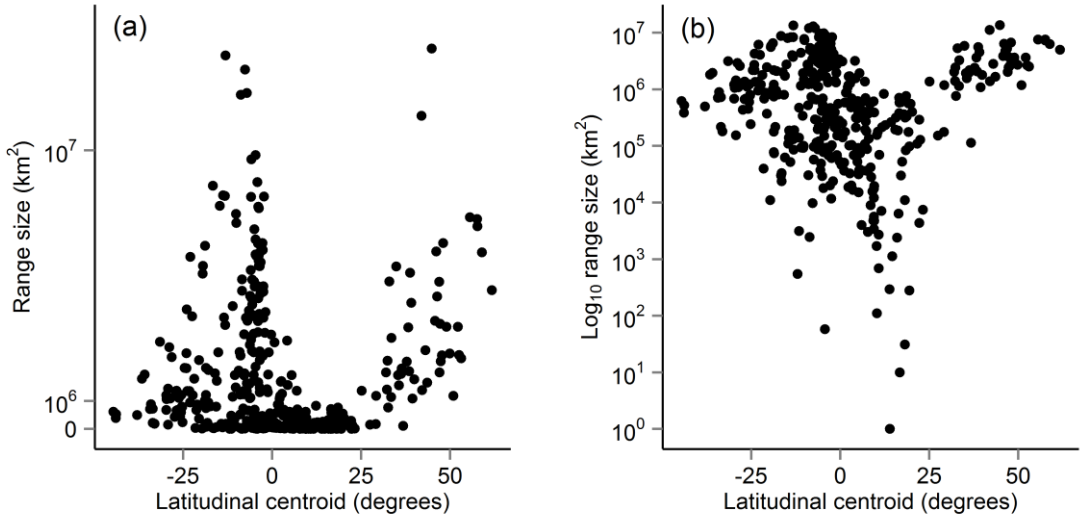

Figure 34. Variation in range sizes across latitude for 414 species of New World passerine bird in the final dataset, presented using (a) untransformed range sizes, and (b) log-transformed range sizes. Latitude represents the latitudinal midpoint of each species, calculated as the centroid of its breeding/resident range polygon.

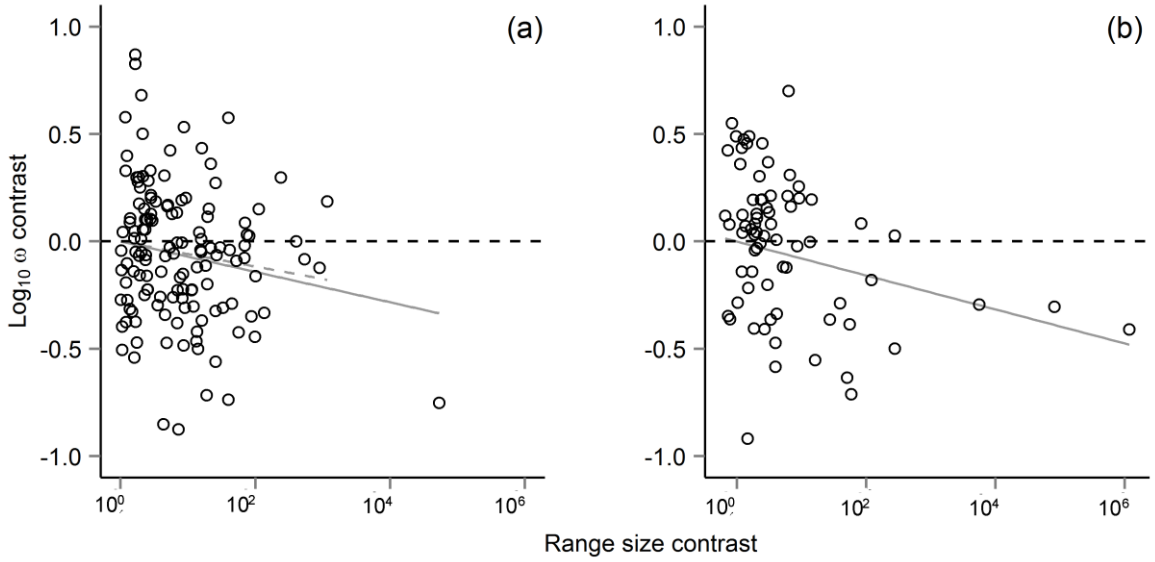

Figure 35. The ω–range size relationship for sister species contrasts of New World passerine birds (a) inside 20° of the equator (tropical) (N = 135 pairs), and (b) outside of 20° of the equator (extratropical) (N = 72 pairs). Tropical contrasts were defined as those for which both species had absolute latitudinal centroids < 20°. Solid grey lines are OLS regression fits forced through the origin. The dashed grey line in (a) shows the regression fit if the high-leverage outlier (range size contrast > 4) is excluded.

4.4 Effects of climate, energy, and elevation on New World bird evolution

4.4.1 Climate

Following the investigation of the effect of population size on rates of molecular evolution, the final major analyses to plausibly link population-genetic processes to species richness are those that correlate molecular evolutionary rates to environmental factors, including latitude, NPP, and climate variables relating to temperature and precipitation. In terms of linking to causal processes, an important part of these analyses is to separate variation due to mutation rate from variation due to the strength of selection.

Several environmental variables produced significant relationships with ω. These variables were latitude, isothermality, temperature seasonality, and NPP (Table 16; Figure 36). These correlations were similar or stronger when only resident ranges were considered, or when resident and breeding ranges were considered, rather than when resident and non-breeding ranges were considered. Each relationship is examined in more detailed below.

Latitude

The effect of latitude on rates of molecular evolution was evaluated using both resident/breeding and resident/non-breeding ranges of birds, both of which correlated significantly with ω (Table 16). However, given that latitude is a dummy variable, it was included in a series of regression models with the significant climate variables that are expected to be more directly linked to molecular evolutionary rate variation. These models indicate that the effect of latitude on ω is sufficiently accounted for by the effect of either isothermality or temperature seasonality (Table 17). Of these two variables, temperature seasonality seems to account for all variation in ω explained by latitude. The third variable—NPP—did not account for this variation. The regression slope of latitude was only minimally altered by its inclusion, and latitude remained a significant predictor of ω in the model. However, NPP was not significant in this model (P = 0.117). Given that latitude is a stronger predictor of ω than NPP, it appears that the correlation between ω and NPP is the spurious result of collinearity with latitude. It is worth noting that there is modest support for retaining NPP in the multiple regression model predicting ω (ΔAIC = 0.868, evidence ratio 1.5), although when temperature seasonality replaces latitude in the

model, there is similarly modest support for excluding NPP from the model (ΔAIC = 0.49, evidence ratio 1.27).

Table 16. Bivariate correlations between independent contrasts of six climate variables (plus latitude), and three evolutionary metrics across sister species contrasts of New World passerines. For each combination, the climate variable is estimated first for pairs of birds that both have resident ranges (i.e., excluding fully migratory species; N = 188 pairs). These pairs are also combined with pairs for which at least one species is fully migratory (total N = 207 pairs), using either breeding or non-breeding ranges as shown. Regression coefficient (slope) and explanatory power (R^2) for each relationship is given. Bold indicates significant relationships, and level of significance is indicated next to the slope: ~ P < 0.1, * P < 0.05; ** P < 0.005; *** P < 0.0005.

	Range used*	ω		d_S		Substitution rate	
		Slope	R^2	Slope	R^2	Slope	R^2
Absolute latitude	R/NB	**-0.006***	**0.032**	0.001	0.003	-0.001	0.002
	R/B	**-0.007****	**0.048**	0.001	0.011	0	0
Isothermality	R	**1.307*****	**0.075**	-0.173	0.008	0.112	0.003
	R/NB	**1.203*****	**0.064**	-0.137	0.005	0.126	0.004
	R/B	**0.9*****	**0.064**	-0.184	0.017	-0.025	0
Mean annual temperature	R	0.001~	0.019	0	0	0	0.008
	R/NB	0.001	0.013	0	0	0	0.008
	R/B	0.001~	0.018	0	0.005	0	0
Temperature seasonality	R	**-0.248****	**0.059**	0.024	0.004	-0.032	0.005
	R/NB	**-0.211****	**0.043**	0.011	0.001	-0.037	0.007
	R/B	**-0.251*****	**0.065**	0.031	0.007	-0.019	0.002
Annual precipitation	R	0.135	0.007	0.035	0.003	0.061	0.007
	R/NB	0.107	0.004	0.046	0.005	0.065	0.009
	R/B	0.152	0.01	0.022	0.001	0.046	0.005
Precipitation seasonality	R	-0.068	0.002	0.016	0.001	0.013	0
	R/NB	-0.048	0.001	0.002	0	0.006	0
	R/B	-0.063	0.002	0.002	0	-0.004	0
Net primary productivity	R	**0.339****	**0.036**	-0.018	0.001	0.022	0.001
	R/NB	**0.337****	**0.035**	-0.014	0	0.021	0.001
	R/B	**0.33****	**0.036**	-0.025	0.001	0.009	0

*R=Resident, R/NB=Resident/non-breeding, R/B=Resident/Breeding

Table 17. Effects on absolute latitude as a predictor of ω and substitution rate from the inclusion of isothermality, temperature seasonality, and NPP in OLS regression models.

Model	Latitude coefficient	Model P	Latitude P
Latitude	-0.007	8.4×10^{-4}	8.4×10^{-4}
Latitude + isothermality	0.002	3.7×10^{-4}	0.659
Latitude + temperature seasonality	-0.000	1.0×10^{-3}	0.998
Latitude + NPP	-0.005	1.9×10^{-3}	0.027

Temperature

Temperature variation appears to affect evolutionary metrics more strongly than does mean annual temperature. While no evidence of an effect of mean annual temperature could be detected on any evolutionary metric, two related measures of temperature variation—isothermality (Figure 36b), and temperature seasonality (Figure 36c)—are significantly co rrelated with ω. As noted above, temperature seasonality appears to account for all of the variation observed in ω that can be attributed to latitude, while isothermality appears to have a marginally stronger bivariate relationship with ω. Hence, the two metrics appear to perform similarly. Although relationships with ω were detected, no temperature-related metric appeared to have any effect on d_S, nor the total substitution rate.

When assessed in regression models predicting ω that also included range size as a predictor, both isothermality and temperature seasonality remained significant predictors, indicating their relationship with ω is not spuriously caused by collinearity with range size ($P < 0.006$).

Precipitation

Neither annual precipitation nor precipitation seasonality are significant predictors of any of the three evolutionary metrics. There were non-significant trends for a positive relationship between ω and annual precipitation and a negative relationship between ω and precipitation seasonality. However, the explanatory power of these relationships was almost zero ($R^2 < 0.01$; Table 16).

Net primary productivity

As discussed above, NPP was only a significant predictor for ω when other climate variables were excluded from the regression model. Given the strong relationship between precipitation and NPP (Pearson's r, 0.644, $P < 2.2\times10^{-16}$), this is unsurprising, as such collinearity violates the assumptions underlying the linear model. While it is unclear if the relationship between ω and NPP is the result of collinearity with another variable that has

a more direct relationship with ω, the ω–NPP relationship itself was stronger than relationships between ω and other variables that might be expected to show collinearity, such as mean annual temperature (Table 16).

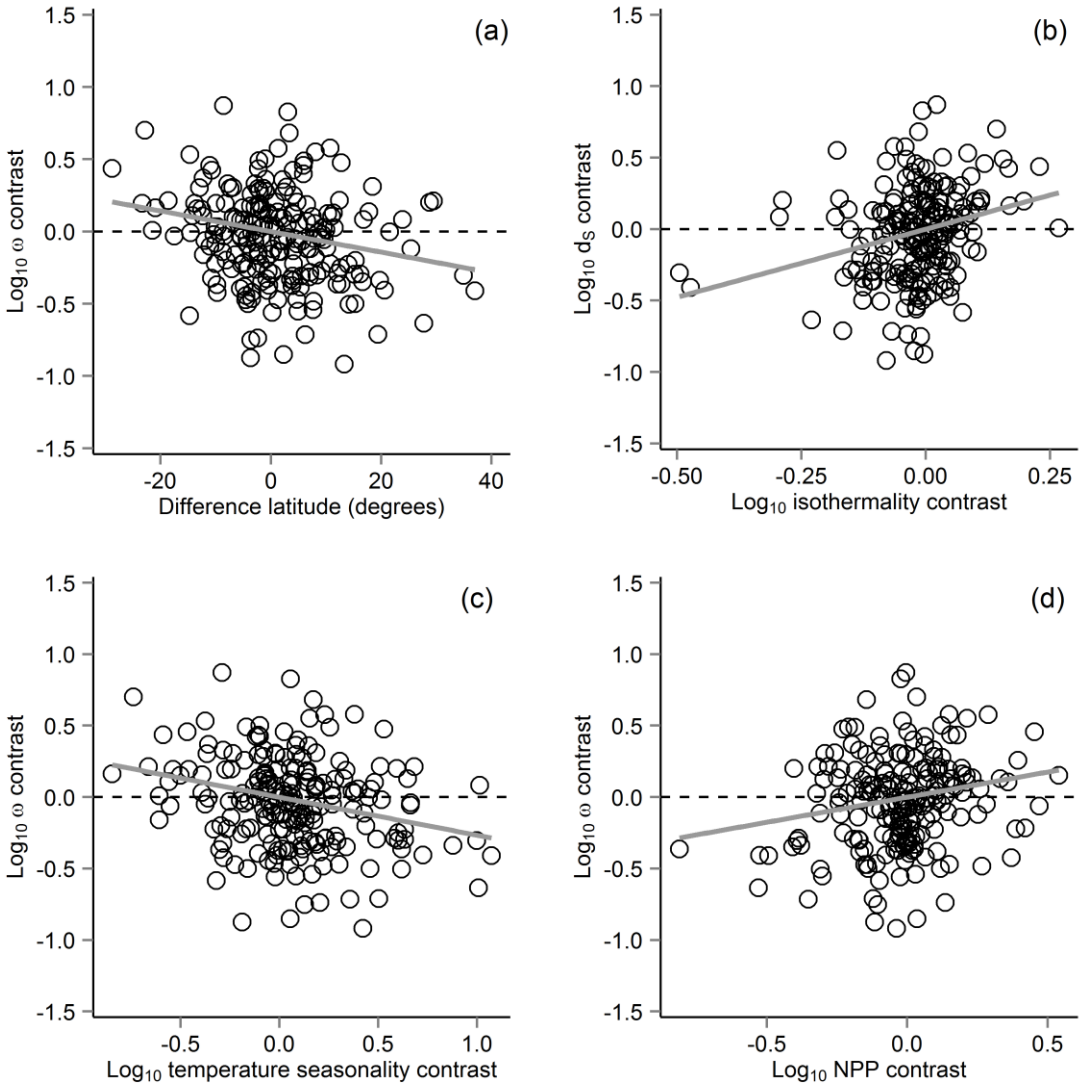

Figure 36. Relationships between log-transformed ω contrasts and four significant climate predictors for sister species of New World passerine birds: (a) latitude, measured as the difference in absolute latitudes of resident/breeding range centroids, (b) log-transformed isothermality contrasts, (c) log-transformed temperature seasonality contrasts, and (d) log-transformed contrasts of net primary productivity (N = 207 sister species pairs).

4.4.2 Elevation

The influence of several measures of elevation on evolutionary metrics was also assessed. Because patterns of diversity along elevational gradients appears to differ with latitude,

analyses were run on the entire final dataset (Figure 37a & b), and then repeated using a tropical subset where species pairs where only included if the maximum latitudes of both species were within 20 degrees of the equator (Figure 37c & d).

When all species pairs were considered, two measures of elevation (minimum elevation and elevational range) significantly correlated with evolutionary metrics. Both maximum elevation and elevational range were negatively correlated with ω. No significant correlations occurred with other evolutionary metrics, although both maximum elevation and elevational range were marginal non-significant predictors of the substitution rate (Table 18). For the tropical data subset, the relationship between elevational range and ω was stronger than when all pairs were considered, and both of the marginally non-significant relationships with the substitution rate that occurred across all pairs became significant, negative relationships. Further, elevational range was a significant, negative predictor of d_S in the tropical subset (Table 19).

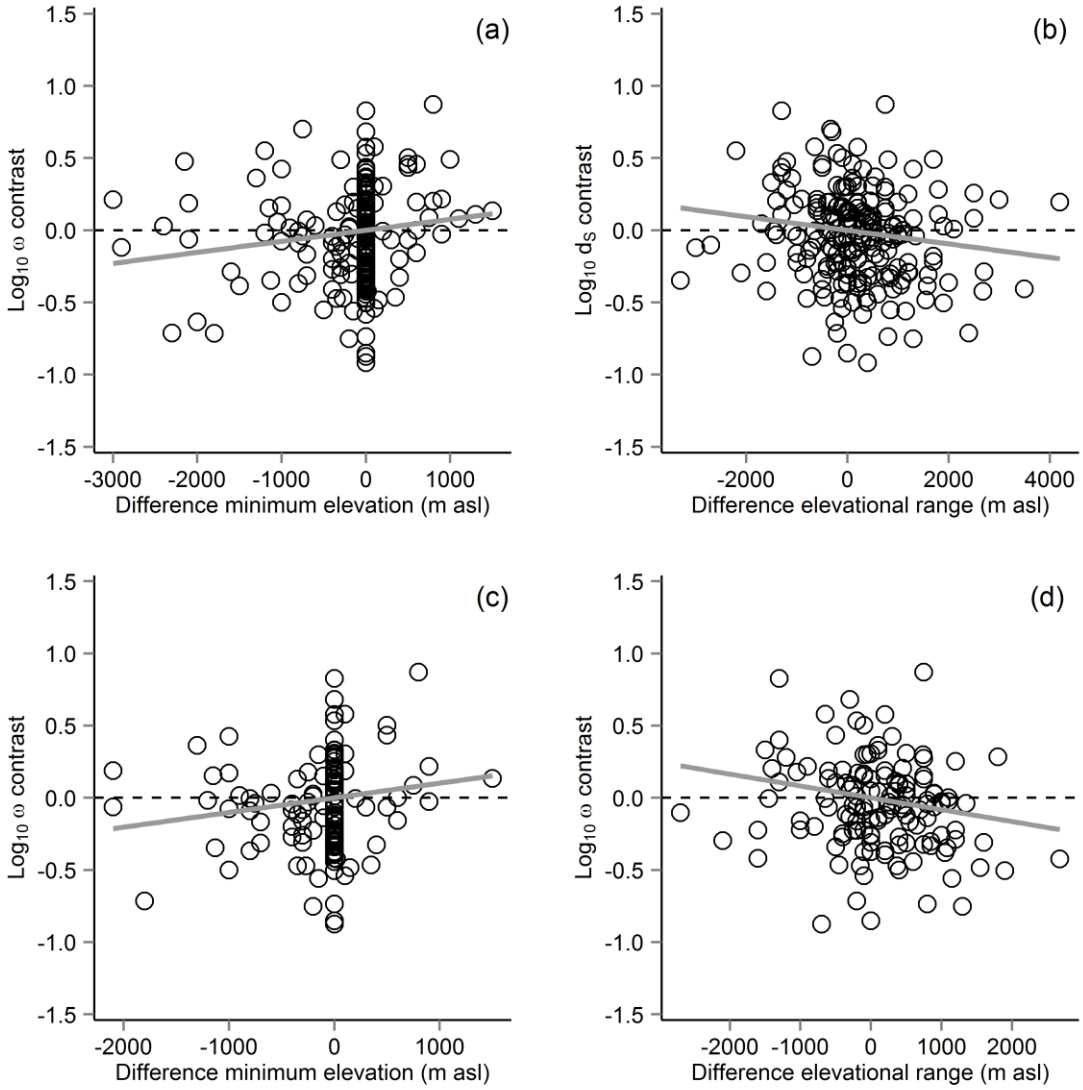

Figure 37. Relationship between ω and two measures of elevation for sister species of New World passerine birds. For (a) and (c) differences in minimum elevation are used, and (b) and (d) differences in elevational range are used. Top row are for all sister species contrasts (N = 207 pairs), bottom row are the same relationships for tropical-only species pairs (both species within 20° of equator, N = 135 pairs).

Table 18. Results of regression models predicting three evolutionary metrics (ω, d_S, substitution rate) from elevational contrasts of sister species of New World passerine birds (N = 207 pairs). Bold indicates significant relationships, and level of significance is indicated next to the slope: ~ P < 0.1, * P < 0.05; ** P < 0.005; *** P < 0.0005.

	ω		d_S		Substitution rate	
	Slope	R^2	Slope	R^2	Slope	R^2
Minimum elevation	**7.6×1^{-5} ***	**0.023**	-1.5×1^{-6}	0.000	1.3×1^{-6}	0.000
Average elevation	9.7×1^{-6}	0.001	-1.3×1^{-5}	0.006	-1.5×1^{-5}	0.007
Maximum elevation	-1.6×1^{-5}	0.003	-1.1×1^{-5}	0.010	-1.4×1^{-5} ~	0.013
Elevational range	**-4.7×1^{-5} ***	**0.024**	-1.2×1^{-5}	0.011	-1.7×1^{-5} ~	0.016

Table 19. Results of regression models predicting three evolutionary metrics (ω, d_S, substitution rate) from elevational contrasts of sister species of tropical New World passerine birds that occur within 20° of the equator (N = 135 pairs). Bold indicates significant relationships, and level of significance is indicated next to the slope: ~ P < 0.1, * P < 0.05; ** P < 0.005; *** P < 0.0005.

	ω		d_S		Substitution rate	
	Slope	R^2	Slope	R^2	Slope	R^2
Minimum elevation	1.0×1^{-4} ~	0.026	1.8×1^{-5}	0005	1.9×1^{-5}	0005
Average elevation	-2.6×1^{-6}	0	-2.0×1^{-5}	0.008	-2.7×1^{-5}	0.012
Maximum elevation	-3.7×1^{-5}	0.010	-2.4×1^{-5} ~	0.027	**-3.1×1^{-5} ***	**0.036**
Elevation range	**-8.2×1^{-5} ***	**0.044**	**-3.5×1^{-5} ***	**0.049**	**-4.3×1^{-5} ****	**0.060**

4.4.3 Apparent mutation rate variation

With the exception of a tropical effect detected for elevation, no climate or ecological variables show evidence of influencing d_S. However, d_S variation is substantial across the dataset (Figure 30b), and, because of low ω values in coding mtDNA, is the main factor influencing the substitution rate even after applying quality controls that reduce data variance (Figure 30d). Given this, it is of interest if d_S variation—whether purely stochastic, or biologically determined by mutation rate shifts—affects the ability to detect signal in the analysis.

Three approaches were used to detect a suitable cut-off value for d_S. When d_S contrast variance was inspected visually, some inflated time-dependent variance over short genetic distances was seen that was not removed by the Welch and Waxman test (Figure 38).

There are outliers scattered outside the distribution of the majority of the data, beyond a break in the distribution that occurs at $|d_S$ contrast$|= 0.1$, above the 95% confidence interval line for the stable d_S LOESS regression fit. For contrasts with genetic distances greater than approximately 0.1, where the inflated short-term variance has subsided, there is a distinct break between contrasts where $|d_S$ contrast$|< 0.1$, and higher variance contrasts. There is a breakpoint in a histogram of $|d_S$ contrast$|$ values that occurs at $|d_S$ contrast$|= 0.1$ (Figure 39a). Above this value is evidence of a bimodal distribution, the cause of which is unknown. Also, when $|d_S$ contrast$|$ values are displayed in rank order, there is a linear, continuous phase where values appear to fall into a stable, continuous range, and then increase exponentially through the additional values above $|d_S$ contrast$| > 0.1$. As all three analyses converge on a normal range limit of 0.1, this cut-off was used for several additional exploratory analyses.

Re-analysing the ω–range size relationship with a d_S cut-off produces a result that is comparable to the previous exclusion of tropical comparisons (see 4.3.5 Potential confounding factors: Latitude). The slope of the two subsets is effectively identical (Figure 40), and model explanatory power is similar (limited d_S model $R^2 = 0.115$; extratropical model, $R^2 = 0.125$). However, the dataset reduction is substantially less severe when limiting d_S variation than when limiting to tropical species pairs ($N = 131$ pairs and $N = 72$ pairs respectively).

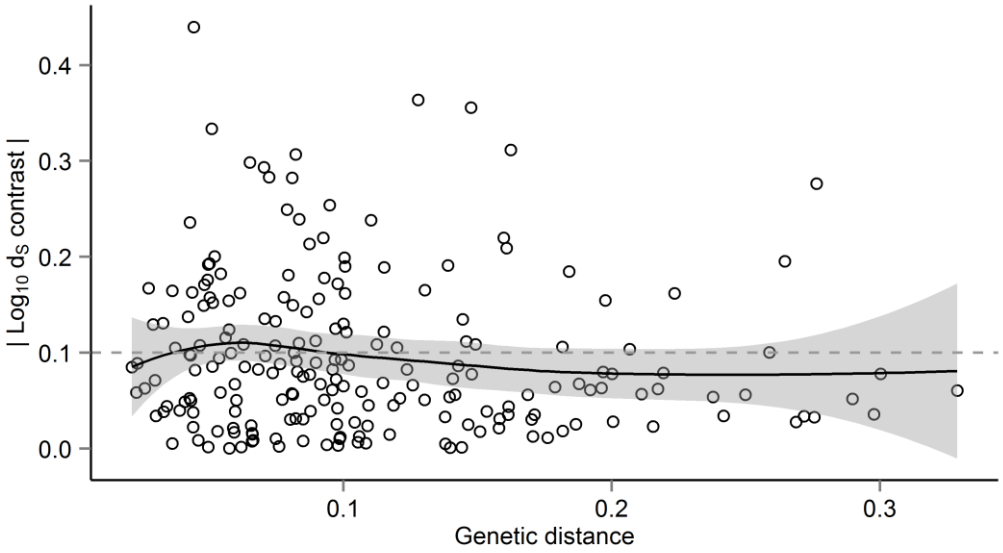

Figure 38. The relationship between d_S variance and time and genetic distance between sister species pairs of New World passerine birds. The solid line is a LOESS fit, with a 95% confidence interval. The dashed grey line shows the approximately stable upper limit on the confidence interval (excluding the flaring due to data paucity at greatest divergences).

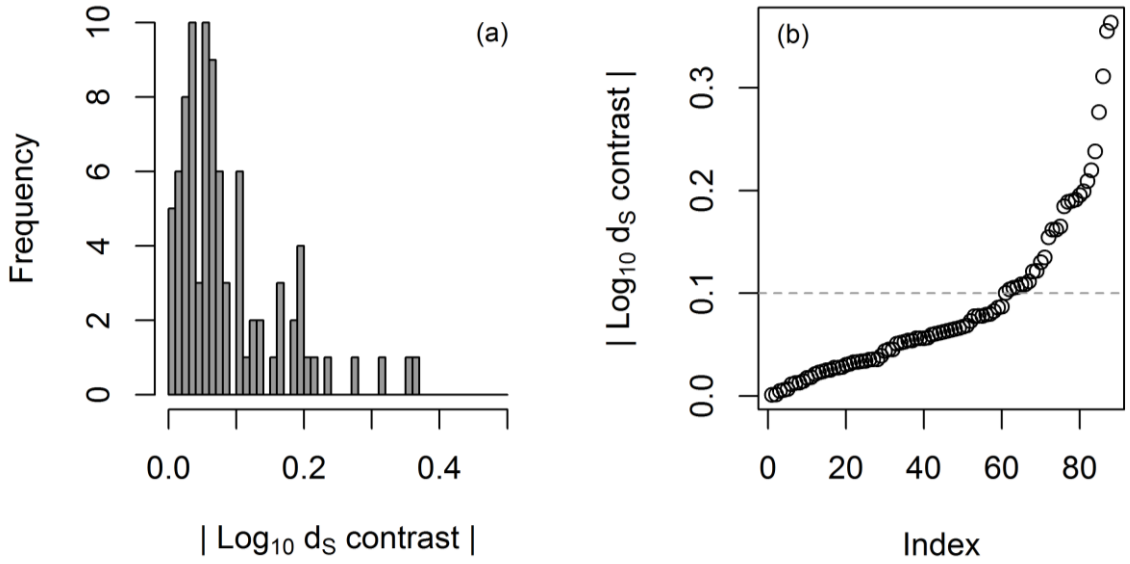

Figure 39. Diagnostics of d_S variation. (a) Histogram of the distribution of $|\ d_S$ contrasts$|$, showing a biomodal distribution and a break at a value of 0.1 (b) Rank-order $|\ d_S$ contrasts$|$ showing a linear aggregation of values up to 0.1 and then a subsequent non-linear increase.

When the same process was repeated for climate, where temperature seasonality was the major predictor of ω variation, applying a d_S cut-off produced a similar pattern, and model explanatory power was improved (OLS regression forced through the origin, $R^2 = 0.125$, P

= 3.3×10^{-5}) (Figure 41a). However, in the case of temperature seasonality, the extratropical subset had improved explanatory power (OLS regression forced through the origin, R^2 = 0.207, P = 5.3×10^{-5}) (Figure 41b). This appears to be caused by the inclusion of a large number of low-power tropical data points caused by the limited range of tropical seasonality (Figure 42).

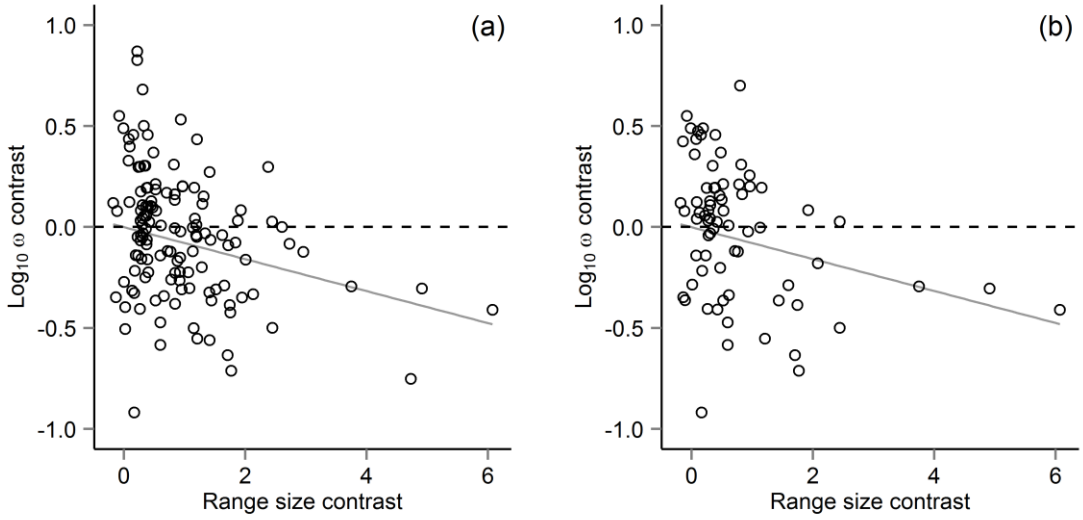

Figure 40. A comparison of ω–range size relationships in two subsets of the final dataset for sister species pairs of New World passerine birds. (a) Data exclude comparisons with large |ds contrast| values (N = 131 pairs), and (b) data exclude comparisons where both species are within 20° of the equator (N = 72 pairs).

Although variation in the putative mutation rate seems inevitably limited by setting an upper limit on pairwise d_S variance, it is interesting to note that the substitution rate—which is largely driven by synonymous mutations—is still significantly predicted by altitudinal range after applying the $|d_S$ contrast$|<0.1$ limit. The strength of the relationship is unchanged by applying this limitation (R^2 = 0.060 and 0.060 before and after respectively).

Given the observed asymmetry between range size and temperature seasonality responses to these two data subsets, a final pair of explanatory models was considered to determine if these effects are indeed different. Firstly, a multiple regression model for ω in the $|d_S$ contrast$| < 0.1$ subset was built using stepwise regression using AIC, initially starting with

all of the significant predictors of ω: range size, isothermality, temperature seasonality, NPP, minimum elevation, and elevational range. The final model selected by AIC included: range size, temperature seasonality, elevational range, and minimum elevation. However, given a modest evidence ratio (1.29) between this model and a model excluding elevational range, which is not a significant predictor in the model ($P = 0.108$), I will present the results of the simpler model. In this model the slope for range size was less steep than when range size was the sole predictor of ω (-0.046 and -0.074 respectively). The slope for temperature seasonality was also slightly less steep in the model than as a single predictor (-0.251 and -0.199 respectively), while the slope for minimum elevation was almost unchanged (1.0×10^{-4} in both cases). The model had similar explanatory power to the extratropical subset ω–temperature seasonality relationship, albeit with three predictors ($R^2 = 0.212$, $P = 1.0\times10^{-6}$).

Secondly, both subset criteria were applied to see if there was an additive effect on model explanatory power. Using the same starting model, the final model selected by AIC included only temperature seasonality and minimum elevation. The slope for temperature seasonality was steeper in this model than that for the final dataset in its entirety (-0.328 and -0.251 respectively), while the slope for minimum elevation was marginally shallower (0.9×10^{-4} and 1.0×10^{-4} respectively). This model had greater explanatory power than any of the previously examined models ($R^2 = 0.303$, $P = 1.7\times10^{-4}$).

The exclusion of range size in this final model is evidently the result of collinearity between the two measures in North America but not in Amazonia (Pearson's r for pairs outside and inside of 20° from equator, R=0.607 and -0.084 respectively). Although this may suggest that range size does not have a direct effect on ω, both variables were significant across a full latitudinal range, including when controlling for d_S variation (OLS regression, both predictors $P<0.006$).

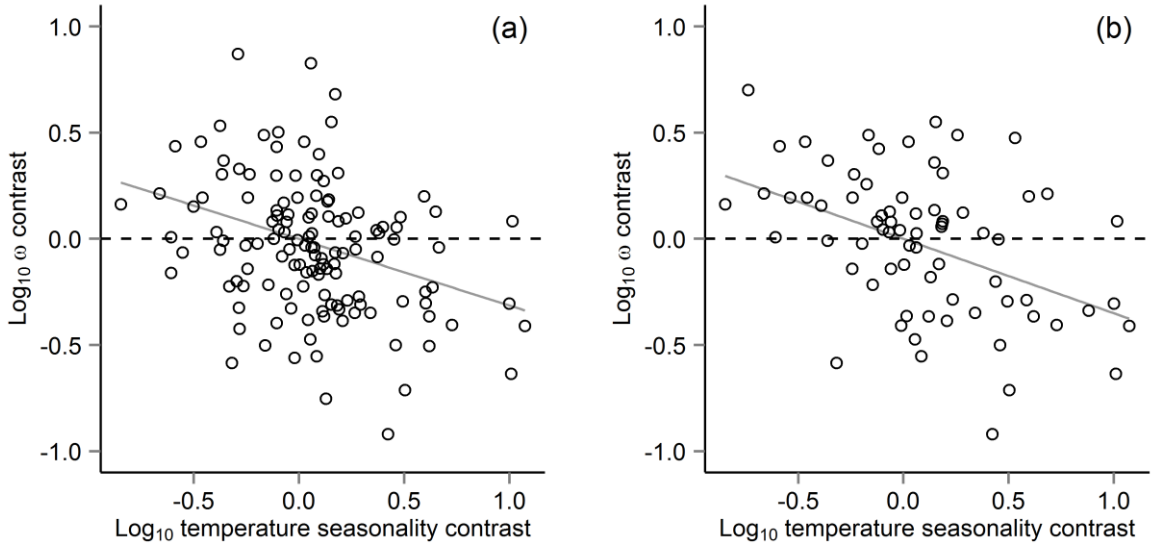

Figure 41. A comparison of ω–temperature seasonality relationships in two subsets of the final dataset for sister species pairs of New World passerine birds. (a) Data exclude comparisons with large |ds contrast| values (N = 131 pairs), and (b) data exclude comparisons where both species are within 20° of the equator (N = 72 pairs).

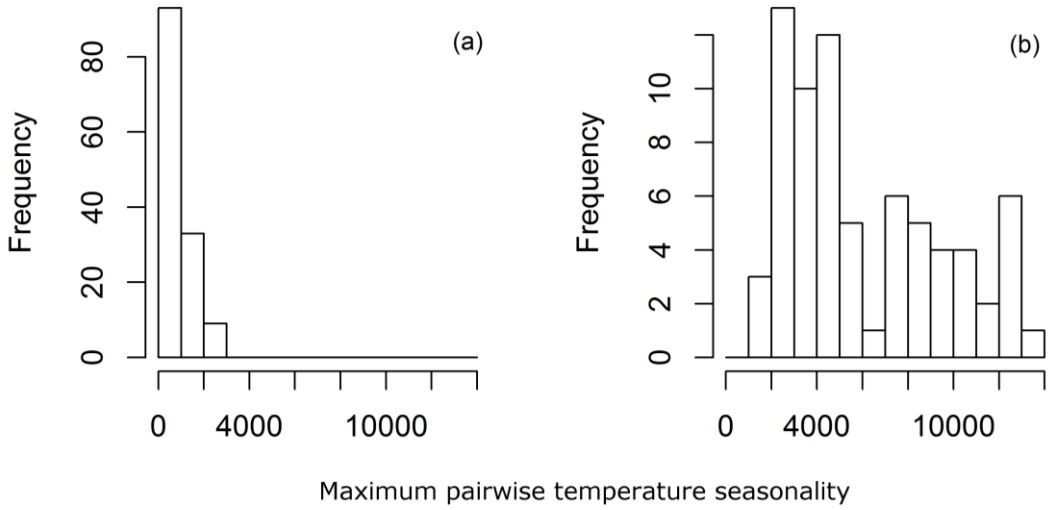

Figure 42. Maximum value for temperature seasonality in sister species contrasts of New World passerine birds in (a) both species in pairs within 20° of the equator (N = 72 pairs), and (b) with one or both species within pairs outside of 20° of the equator (N = 131 pairs). Temperature seasonality is 100σ of mean monthly temperatures.

5 Discussion

5.1 Plant terrestrial ecoregion diversity patterns

5.1.1 Plant diversity patterns

Terrestrial ecoregions provide a set of floristically distinguishable vegetation patches across the world that range in size by several orders of magnitude. Because their species compositions differ from their neighbours, when treated as sampling units they have greater independence than would large-grain samples across landscapes falling within the same ecoregion. As such, ecoregions can provide insights into processes that occur at different spatial scales. Indeed, the strength of productivity–species richness relationships (PSRs) are dependent on ecoregion size, demonstrating the importance of scaling factors in the determinants of species richness patterns, and the centrality of scale in the long-running debate about the nature of macro-scale PSRs.

While the overall PSR across all terrestrial ecoregions is poor, which could imply a generally weak relationship exists between species richness and productivity, analysis of ecoregions binned into size classes reveal a distinct scaling effect of area. In small and medium ecoregions (10^3 to 10^5 km^2), NPP has a weak relationship with plant species richness, while in large ecoregions (>10^5 km^2), NPP predicts a substantial amount of the variation in plant species richness. When binned into narrower size classes, the strength of the PSR scales linearly with ecoregion size (Figure 13a), indicating a central role for area in determining the importance of factors that affect species richness. Because there is an interaction between ecoregion size and the strength of the PSR, including ecoregion size as a covariate is not sufficient to account for the influence of ecoregion size on PSRs. Doing so improves the model fit across the whole dataset (OLS regression; species richness ~ NPP + $\log_{10}$area, adjusted R^2 = 0.430; ΔR^2 = 0.237) but fails to capture the heterogeneity in the relationship where productivity accounts for almost no variation in species richness in small ecoregions to three-quarters in the largest ecoregions (Figure 13a).

It is worth noting that the relationship can be linearised by transformation. While a log:log species richness–NPP relationship performs similarly to the untransformed relationship in terms of being heteroskedastic, when NPP is instead square-root transformed the relationship linearises, and shifts in intercept are evident between the different area groups are apparent (Figure 43). Similar to the untransformed model, the relationship remains stronger in the largest ecoregions (Adjusted $R^2 = 0.571$) than in medium or small ecoregions (Adjusted $R^2 = 0.296$ and 0.303 respectively) and is substantially reduced in the smallest ecoregions (Adjusted $R^2 = 0.131$), while still remaining significant ($P = 0.02$).

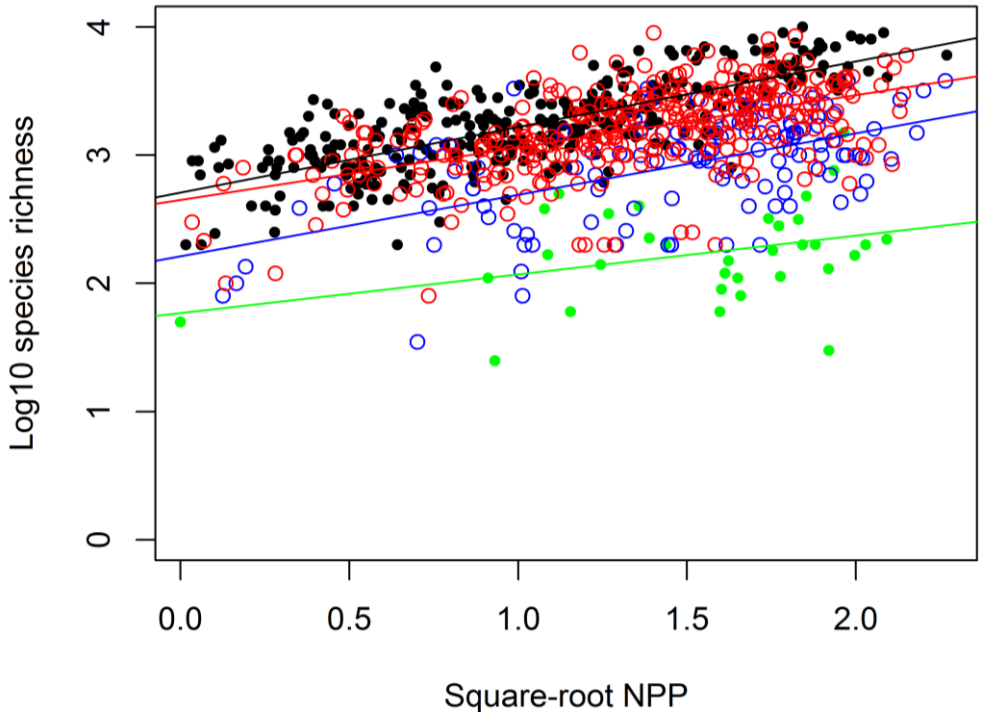

Figure 43. Relationship between square-root-transformed modelled net primary productivity and log-transformed plant species richness across sizes classes of global terrestrial ecoregions: green points, smallest ecoregions (<10^3 km^2); blue points, small ecoregions between 10^3 and 10^4 km^2; red points, medium ecoregions between 10^4 and 10^5 km^2; black points, large ecoregions (>10^5 km^2). Data sources as in Figure 11 caption.

Reconciling the varying nature of PSRs across scales requires consideration of the ecological and evolutionary dynamics that occur at these different scales. Small ecoregions—from 10^3 to 10^4 km^2—are equivalent to circular areas with radiuses from 17.8 km to 56.4 km. As such, it is unlikely that many existing species in regions of this size would be the result of an accumulation of lineages diversifying within the ecoregion, although areas sufficiently isolated by distance or environment (e.g., islands and montane

regions) are likely to contain endemics that have diverged from species in the regional pool. The species richness dynamics of small ecoregions are likely to be controlled by the constraints of their size and ecological factors such as regional species pools, aspects of site history such as disturbance, and dispersal rates from neighbouring regions with compatible floras. In other words, their PSRs are likely to be weak for the same reasons that PSRs have been found to be weak in other small-scale studies—the confluence of myriad ecological factors, many of which are unrelated to productivity. Area was non-significant in models predicting small ecoregion species richness (Table 2). The preferred model included NPP, biogeographic realm and biome type as predictors, although this model was only moderately preferred over the latter two predictors excluding NPP. The apparent upper limit on species richness in these ecoregions (Figure 12a) could be a result of their size—given similar alpha diversity and turnover, smaller ecoregions will have fewer species.

Compared to smaller ecoregions, the species composition of large ecoregions (>10^5 km^2) is likely to more strongly reflect diversification processes occurring within the ecoregion, as indicated by the increasingly strong relationship with NPP. There is a long-recognised correlation between environmental/biologically available energy, of which productivity is a measure, and global-scale species richness in both plants and animals (Cusens et al., 2012; Evans, Warren, et al., 2005; Gillman & Wright, 2006; Hawkins et al., 2003). Unlike small and medium ecoregions where realm is an important predictor of species richness, only biome type, NPP and ecoregion area were included in the preferred model predicting large ecoregion species richness. The unimportance of biogeographic realm in predicting species richness is suggestive of a reduced role for history and contingency in large ecoregions. Further, in the largest ecoregions (>$10^{5.5}$ km^2), NPP alone predicts more than 70% of variation in species richness (Table 2), suggesting a close relationship with net diversification. No inference can be made about whether productivity or the climatic correlates of productivity are most closely associated with changes in net diversification, as causal pathways are yet to be determined. However, it has recently been shown that climate variables (e.g., temperature and rainfall) outperform productivity as predictors of species richness in forests at the global scale (Šímová et al., 2011).

Medium-sized ecoregions differ from small ecoregions by having a less clear upper limit on diversity: species richness in these ecoregions peaks at levels similar to that of large ecoregions. Unlike small ecoregions, the inclusion of NPP in the model predicting species richness was substantially more strongly favoured in medium ecoregions (evidence ratio 1.4×10^9, Table 2). Also different from the other ecoregion size classes, the most complex model predicting species richness was strongly favoured in medium ecoregions (Table 2). NPP's predictive ability for species richness was low (bivariate R^2 = 23%, Table 2); at moderate and high productivities substantial variation is observable in the species richness of ecoregions (Figure 12b).

The role of scale in the study of diversity patterns has been emphasised before, in particular relating to PSRs (e.g., Chalcraft, Williams, Smith, & Willig, 2004; Chase & Leibold, 2002; Gillman & Wright, 2006; Whittaker, 2010; Whittaker et al., 2001). Nonetheless, there remains a tendency to conflate small- and large-scale mechanisms that shape diversity patterns. For example, Huston (1999) argues that diversity patterns ranging from scales of a few metres to global diversity gradients can be explained by the intensity of competition and competitive exclusion, a mechanism that is theorised to operate on alpha diversity amongst interacting individuals (Grime, 1973; Huston, 1979), and predicts a unimodal PSR. More recently, Huston and Wolverton (2009a) have suggested that NPP may be lower in tropical than temperate zones, placing the latitudinal species richness gradient in a framework implying a global unimodal PSR. Their model places primacy on soil fertility and points out that many measures of NPP are indirect. However, directly measured annual Neotropical productivity is substantially higher than annual temperate NPP (e.g., Aragão et al., 2009) and is in generally good agreement with modelled NPP derived from remotely sensed data (Zhao et al., 2005). Correspondingly, a recent study of latitudinal patterns of directly measured NPP found a monotonic decline in NPP with increasing latitude (Gillman et al., 2015). In any case, such a universal mechanism as Huston (1999) proposed is unlikely to exist, as these results suggest the nature and strength of PSRs change with grain size and extent (see also Gillman & Wright, 2006; Whittaker & Heegaard, 2003; Whittaker et al., 2001). Indeed, the importance that

climate and productivity have in predicting species richness decreases at smaller grains and extents whereas factors such as habitat heterogeneity, and edaphics/nutrients become more important at smaller grains and extents (Field et al., 2009).

Recent literature on the productivity–species richness relationship has emphasised the importance of exploring relationships based on causal mechanisms through techniques such as structural equation modelling, rather than identifying bivariate correlations (Adler et al., 2011; Grace et al., 2014). Such approaches are critical to moving forward in our understanding of PSRs, and integrating scale into such investigations could further develop our understanding of these relationships. Work on fine-scale patterns in grasslands has greatly improved our knowledge of PSRs. However, with such a strong reliance on a single biome there is the risk of overgeneralising results. For example, while Adler et al. (2011) points out that productivity is a poor predictor of species richness in small-grain, temperate grassland plots, we cannot generalise from this that productivity is a poor predictor of species richness wholesale. Instead, as Adler et al. suggest, we must look more deeply for causal mechanisms in species richness patterns.

Finally, PSRs are ecologically important in the context of a changing climate. While small-grain studies reveal complex PSRs occurring on short time scales, at large grains species richness patterns appear to obey more predictable laws over longer time scales. The implications of species loss from global change can thus be considered on two levels: the direct effects of species loss on ecosystem functioning, occurring on short time scales and local spatial scales; and the long-term prospects for recovery of global diversity. On the first level, the short-term, local effects of losing species may be variable—perhaps on average resulting in lower productivity (Hooper et al., 2012) and loss of ecosystem services (Cardinale et al., 2012). On the second level, it appears that the prospects for recovery of global diversity may rely on the species pools harboured in large ecoregions. Isolated, small or low-productivity ecoregions not only contain fewer species, but their patterns of species richness appear more closely tied to the those of surrounding large ecoregions rather than the climate correlates that have previously linked to diversification rates.

Productivity and species richness have a complex relationship that varies in strength with spatial scale. While it has previously been shown that scale is important in PSRs at the local-to-landscape level, the present work demonstrates that even with much larger sampling units, scaling effects are not only detectable but strong. These results suggest caution when investigating species richness patterns across ecoregions if assuming a homogeneous relationship exists with causal factors—for example, if studying species richness along a productivity gradient. Moving forward in understanding species richness patterns will require clearer thinking about the multitude of causes of diversification patterns, and the scales on which they act and interact. Moving forward will also require more sophisticated causal modelling at large scales, in line with the recent direction at small scales. Long-term protection of global biodiversity requires a better understanding of the flow of species and heterogeneity in diversification across ecoregions, as well as predictive modelling of the global changes that will affect the climates and boundaries of ecoregions.

5.2 Animal population and diversity patterns

5.2.1 Animal diversity patterns

Although mammal and avian PSRs follow a similar pattern to that of plants, where NPP explains greater proportions of species richness variation in larger ecoregion size classes, the degree of that explanatory power is markedly different. Approximately 40% of animal diversity is explained by NPP when controlling for area. Model explanatory power was increased by including additional variables for realm and biome, indicating that factors such as habitat structure and complexity, and history may play more important roles than NPP in determining animal species richness. NPP offered little additional explanatory power to a model already including realm, biome and area. This pattern suggests that NPP, which indicates the total resource supply to consumers in the ecoregion, is not a direct and major determinant of mammalian or avian species richness despite the general, positive relationship between NPP and species richness that has been previously observed (Cusens et al., 2012).

Because NPP relates to the biologically available energy for consumers, this result has implications for species–energy theory. Species–energy theory predicts a positive relationship exists between the total number of individuals and species richness. However, while the number of individuals in regions may set a theoretical upper limit on diversity, a causal relationship between the number of individuals and species richness assumes species richness is at net diversification equilibrium (Hurlbert & Stegen, 2014). In addition to the doubt that this is generally true on continental landmasses (Harmon & Harrison, 2015), it may be long term stability of energy availability, rather than annual average energy, that is important for diversity processes (Duncan, Chauvenet, Brown, & Pettorelli, 2015). Stability of energy supply has long been thought important for diversity by increasing the length of stable trophic chains amongst consumers (Brown, 1981; Hutchinson, 1959).

Plant species richness appears to affect animal species richness more directly than does NPP. When including both ecoregion plant species richness and NPP in a model predicting animal diversity, there is no evidence that NPP adds additional explanatory

power (P = 0.706). This is consistent with previous studies that have found plant species richness is a strong predictor of mammal species richness at least in some feeding guilds (Andrews & O'Brien, 2000), and plant food diversity predicts the species richness of avian frugivores (Kissling, Rahbek, & Böhning-Gaese, 2007).

5.2.2 Correlates of vertebrate diversity in large ecoregions

Additional exploratory analyses were undertaken to determine the cause of the difference between animal and plant PSRs. At least part of the greater variance found in animal PSRs is the result of a shift in the animal PSR curve in regions with below-freezing winters (Figure 16a). This shift appears to explain much of the difference in plant and animal PSRs, as the same shift also occurs when plant diversity replaces NPP as the predictor or animal diversity. When minimum monthly temperature is included with NPP in a regression model predicting animal phylogenetic diversity in the largest ecoregions (>$10^{5.5}$ km^2), the model is able to explain almost three-quarters of the variation, comparable to the plant PSR in the same ecoregions (multiple regression R^2 = 0.723 and 0.721 respectively). Further, in the subset of ecoregions for which below-freezing winters occur, there is effectively no relationship between NPP and animal phylogenetic diversity (R^2 = 0.066, P = 0.059), indicating that different driving or limiting factors are important in these ecoregions.

Of interest is the comparative relationships between plant and animal species richness, and temperature seasonality. Temperature seasonality appears to have a more important relationship with animal (Figure 16C) than plant diversity (Figure 15C). Noticeably, the effect of winter freezing has a stronger limiting effect on animal than plant diversity. These disparate relationships suggest different factors limit diversity for these broad taxa. This point warrants greater future exploration.

While climate is an important contributor to shaping diversity patterns, its exact role has been widely debated (Allen et al., 2002; Brown, 2014; Currie et al., 2004; Hurlbert & Haskell, 2003; Stevens, 1989; Storch, 2003; Wiens & Donoghue, 2004). As a single, climate-based predictor, temperature seasonality has a strong relationship with animal phylogenetic diversity (Figure 16d). If temperature seasonality, rather than average annual

temperature, is important as a large-scale predictor of species richness, it implies that climate-mediated physiological limitations might be important in net diversification. This inference is supported over long timescales by the strong affinity between species richness and seasonality, rather than mean annual temperature, in the fossil record (Archibald et al., 2010; Mannion et al., 2014). The gradient of the phylogenetic diversity–temperature seasonality relationship was shallower in ecoregions with below-freezing winters, implying that seasonal coldness is of particular importance. The shallower gradient could be caused by historical area effects and niche conservatism (Wiens & Donoghue, 2004), or, consistent with the continued lower extratropical net diversification, a climatic gradient in speciation or extinction.

The climate harshness hypothesis claims that seasonal, temperate regions can generally be considered abiotically harsher environments than the tropics. Thus, this hypothesis differs from the tropical niche conservatism hypothesis by emphasising that not only are ancestral climate niches retained, adaptation to a new environment cannot be understood as occurring within a finite period. Any physiological adaptations to seasonally cold environments with a genetic basis that enable survival must be maintained by negative selection (Schemske, 2002). Further, the ability to tolerate seasonally cold environments, such that populations persist interannually, does not necessarily equate to equal fitness in tropical-adapted and temperate-adapted species. In particular, periodic extremes in temperature can cause mass mortality events (e.g., Bumpus, 1899), and because seasonal environments by definition are more variable in temperature, they should experience more such events. While this could be offset by climate tolerances being narrower in the tropics (Janzen, 1967), greater abiotic selection has been widely recognised as a feature of species in temperate regions (Dobzhansky, 1950; Schemske, 2009; Schemske et al., 2009).

Not all previous studies have found a strong link between temperature seasonality and contemporary species richness. For example, Kerr (1999) found the difference between winter and summer temperatures was only weakly correlated with species richness of North American mammals. Similarly, Ruggiero and Kitzberger (2004) found AET and habitat heterogeneity explained species richness patterns in more groups of South

American mammals than did temperature seasonality. A range of other studies have found support for seasonality playing an important role in shaping animal species richness patterns (Carrara & Vázquez, 2010; Chen, Mao, Zhang, Zhou, & Gao, 2014; Tello & Stevens, 2010). Tello and Stevens (2010) show within-taxon effects are important in explaining New World bat species richness: in large-ranged species, both energy (annual precipitation and NPP, and mean annual temperature) and seasonality of energy are important determinants of species richness, while for small-ranged species, environmental heterogeneity was a more important species richness predictor. Thus, the outcomes of studies may depend on the selected study clades, often in ways that are not measured or considered. For example, because species richness patterns in birds are heavily influenced by large-ranged species (Jetz & Rahbek, 2002), studies including many highly species-rich sites may indicate that species richness has a strong seasonality dependence, if the results of Tello and Stevens (2010) generalise to birds.

Finally, the relationships between climates variables such as NPP and temperature seasonality were stronger with vertebrate phylogenetic diversity than for bird diversity alone (Figures 16 & 17). This different strength of relationships might truly reflect differences between these groups, although other explanations are also possible. For example, as the bird species richness estimates from the WWF Wildfinder database do not exclude migrant birds, estimates of species richness may be inflated in some cases. Specifically, the strength of relationship with a climate variable such as temperature seasonality will be affected if all species are included, as the breeding ranges of many temperate-tropical migrants have a wider range of temperature seasonality than those species ever experience by migrating to a non-breeding range. This issue could be addressed in future work by estimating species richness using non-breeding ranges, or by excluding migrant species.

5.2.3 Range sizes and latitudinal ranges in animals

It has been argued that Rapoport's rule—the tendency for species to have larger latitudinal ranges at high latitudes—could be a cause of the LDG. The original formulation specifies that there is a latitudinal gradient in species range size caused by wider climate tolerances

in more seasonal environments (Stevens, 1989), or as a result of longer-term climate fluctuations (Dynesius & Jansson, 2000). Correspondingly, Stevens argued that the latitudinal ranges of species should increase towards the poles. However, a number of additional factors, both related and unrelated to climate, could cause latitudinal differences in range size. For example, in mammals, body sizes tend to be larger at higher latitude (i.e., Bergmann's rule; Ashton, Tracy, & Queiroz, 2000; Blackburn & Hawkins, 2004; Freckleton, Harvey, & Pagel, 2003), and larger-bodied species tend to occupy larger ranges, although the pattern is not necessarily simple (Gaston & Blackburn, 1996b). Also, across the ranges of large-ranged species, local adaptation may reduce or negate the need for individuals to be adapted to range-wide conditions (Joshi et al., 2001). Therefore, to control for such influences, I examined the relationship between range size and latitudinal range across three latitudinal bands in the northern hemisphere. Under Rapoport's rule, latitudinal ranges should be larger in the extratropics than the tropics because of greater climate tolerances (Stevens, 1989). However, once geographic range sizes are controlled, latitudinal ranges for tropical species were equal to or larger than latitudinal ranges of extratropical species in both birds and mammals, regardless of the method used to categorise species to latitudinal bands.

A number of factors can influence geographic range sizes (Gaston & Fuller, 2009; Orme et al., 2006). Dispersal ability has long been linked to range size (Lester, Ruttenberg, Gaines, & Kinlan, 2007). This general pattern is supported by the consistently larger median range sizes of non-migratory passerine birds than mammals in the present work (Table 7). Latitudinal gradients in dispersal ability have been linked to competition dispersal trade-offs (Jocque et al., 2010; Salisbury et al., 2012), creating an additional explanation for latitudinal variation in range sizes. Land area at different latitudes also varies substantially (Table 7). The distribution of oscine and suboscine bird range sizes across the New World examined in a subsequent section of this thesis (Figure 34) suggest a complex pattern occurs latitudinally, with land area playing a clear role in setting upper range size limits, and broader distributions of tropical than temperate range sizes. A substantial proportion of species in low-latitude, northern hemisphere bands are likely to have area-constrained ranges because the two major landmasses—the Americas and Africa—have landmasses

with severe viable land area limitations between the equator and 25° N—the Isthmus of Panama, and the Sahara Desert. Given the lack of evidence for disproportionate increases in latitudinal ranges of extratropical species presented here, and the lack of general support for Rapoport's rule in other studies (Gaston, Blackburn, & Spicer, 1998; Orme et al., 2006; Rohde, 1999; Rohde et al., 1993; Roy, Jablonski, & Valentine, 1994), Rapoport's rule is unlikely to explain latitudinal variation in range sizes. Species at high latitudes must indeed survive a wider range of conditions than tropical species. However, it does not follow that because such species experience a wider range of climate conditions than those of tropical species, this will result in larger latitudinal ranges.

5.2.4 Population–range size correlations

Extent of occurrence (EOO) and area of occupancy (AOO) are two conceptually distinct approaches to estimating range size. The perceived appropriateness of each depends on if they are seen as serving different purposes (Gaston & Fuller, 2009), or if EOO is perceived as a relatively deficient measure because species are only present in part of the EOO (Jetz, Şekercioğlu, & Watson, 2008). Estimates of AOO are available for far fewer species than estimates of EOO, and the former are usually conducted for smaller-ranged species (Harris & Pimm, 2008). As EOO is generally recognised as being less strongly correlated with population size than is AOO (Gaston & Fuller, 2009), the lack of AOO estimates is potentially problematic for range size estimates of N_e in global studies.

However, EOO covaries with contemporary population size on a log-log scale to perhaps a greater extent than has previously been appreciated (Figure 22). Additional variation that exists in the broad relationship across all birds (excluding seabirds) can be accounted for by including coarse measures that account for density variation such as body size. The EOO–population size relationship was stronger within families, presumably as various ecological and life history traits are less variable than across all birds. The relationship is not driven by unequal sampling of particular genera, as including only single representatives of each genus does not worsen the strength of relationship. This finding suggests that EOO can provide a first-order approximation of population size on a log-

scale, provided either compensations for range-wide densities can be made, or EOOs are used for a phylogenetically restricted dataset.

Population size estimates graded as being medium or good quality were available for 381 species that also had body size estimates. However, these species are not a representative subsample of the global avifauna. Many of the species with available population size estimates are at an elevated extinction risk, as predicted under IUCN Redlist criteria, and only 3% of these species are in the 'least concern' category. By contrast, across the New World oscines and suboscines that are the study taxa for the molecular evolutionary component of this thesis, 85% fall into the 'least concern' category. In these passerine clades there is limited evidence that the population size–range size relationship for endangered or critically endangered species has a different gradient than other IUCN categories, which could potentially bias the use of range size as a population size estimator above range sizes of 10^4 km^2. However, this only affects 4 of 300 species, and all of them are the smaller-ranged species in sister species contrasts of larger- and smaller-ranged species.

Within the passerines, there was evidence that the EOO–population size relationship was weaker for tropical than extratropical species, with EOO explaining 30.2% and 50.5% of population size variation respectively. Body mass did not reduce the difference between the tropical and temperate EOO–population size relationships. Instead, the increased variance is likely to be caused by the wider range of population densities in the tropics, where rare species at low densities make up substantially larger proportions of communities (Terborgh et al., 1990; Thiollay, 1994). Because of this tropical–temperate disparity, some caution is needed when assuming EOO–population size relationships in species-dense tropical communities. By its nature, AOO is likely to be an improved measure of population size in the tropics, although I am not aware of this having been explicitly tested.

5.2.5 Variation in population density with latitude and elevation

Although communities at low latitudes frequently harbour greater densities of individuals than high latitude communities, they also contain many more species. Because the

gradient of species–energy relationships is steeper than individuals–energy relationships for many taxa including birds (Storch, 2003), median population sizes inevitably decrease in high-energy regions. Despite this, latitude had only a minor effect on population size, and it was marginally non-significant when absolute latitude was used because the relationship was flat or negative in the southern hemisphere and positive in the northern hemisphere. Whether this is a genuine population density effect is unclear: a possibility for different population size effects in the northern and southern hemispheres is based on geometric constraints of smaller southern hemisphere landmasses. This interpretation is further supported by the substantial change in the relationship for species with range centroids close to areas of restricted land area (Figure 26). If this is the case, there may be complex interactions between species densities, land area, climate, and resource availability that shape the distribution of population densities, making it difficult to generalise about population density and latitude. For example, in areas where the longitudinal extent of a landmass reduces, the ranges of many species suited to the climate and habitat at that latitude may be compressed, forcing a greater degree of competition. Under such a scenario the expected outcome would be an average reduction in population density. An additional possible reason for a minimal latitudinal effect is that the majority of species with population size estimates are listed as threatened to some extent on the IUCN Redlist. A stronger latitudinal signal in population densities may occur if more "Least concern" species had reliable population size estimates for inclusion.

The effect of latitude on population densities might also be obscured by small-ranged tropical species. Population densities, when estimated by EOO rather than AOO, are elevated in small ranges because there is a non-zero intercept between EOO and population size (Figure 23). This relationship might be expected, given that EOO and AOO have a non-linear relationship, with small EOO ranges generally having high AOO (see Figure 3, Gaston & Fuller, 2009). The EOO–population size relationship is log-linear, but has a positive intercept ($\log_{10}N \approx 2$), indicating that even in small ranges, populations number approximately 100 (Figure 23). It is likely that the positive intercept in the EOO–population size relationship reflects the elevated extinction risk in such populations (Lynch et al., 1995), illustrating minimum viable population sizes.

Species' average elevations had a stronger, negative effect than did latitude on population densities. However, this effect was only evident for montane tropical species. Residuals for this group show a strong negative trend above 2000 m.a.s.l., indicating tropical densities decline in high altitudes. No similar pattern was noted for temperate species, for which all species above 2000m had positive residuals. However, only one species above 3000 m.a.s.l. had a population size estimate.

The mid-slopes of tropical mountains are well-recognised zones of high avian diversity (Orme et al., 2005). Patterns of lower population densities could reflect this high diversity. However, such an interpretation faces two challenges. First, a similarly strong pattern was not evident in tropical lowland species (Figure 26). Second, the pattern continues to above 4000 m.a.s.l., where species richness is no longer elevated, and yet similarly species-poor regions in the temperate zone do not show comparable declines (Figure 27b). A consistent explanation for this apparent pattern in not evident.

Finally, genetic diversity patterns were investigated across latitude. The nucleotide diversity:divergence ratio was a useful measure to test the generality of patterns, as it controls for range size variation, and, to some extent, age of species. There was a distinct latitudinal pattern in the diversity:divergence ratio, which peaked in the tropics (Figure 25b). This indicates that genetic diversity accumulates more rapidly in tropical populations than in temperate populations. There are three possible causes for this pattern. Firstly, background selection reduces temperate genetic diversity through effects on N_e. This is consistent with the climate harshness hypothesis. Secondly, Pleistocene glaciations isolated and reduced in size many temperature populations (Weir & Schluter, 2007). For subspecies that have neither fully split from their parent species nor gone extinct, such reductions could lead to high divergence and low diversity across latitude. A similar pattern of low temperate nucleotide diversity was attributed to effects of glaciations in a previous study (Hughes & Hughes, 2007). Such an effect is clearly consistent with climate harshness as well, although it could only be a major cause of species richness variation during glacial periods. However, it is worth noting on this point that divergence is typically greater in

tropical, and not temperate populations (Martin & McKay, 2004). Thirdly, positive selection is more common in extratropical than tropical species. Under any of these scenarios, elevated tropical diversity is indicative that factors beyond population size influence N_e, with some evidence that this influence is strong. On average, tropical populations should have lower N_e on account of having smaller N_c. However, the combination of historical effects, and natural selection contrive to produce a genetic diversity pattern reversed from the expectation of N_c alone.

5.3 Effect of range size on molecular evolutionary rates and patterns in New World birds

5.3.1 Sequence dataset assembly

When data were initially accessed from GenBank in February 2014, there were 170,000 accessions retrieved under the search "birds[organism] AND (mitochondrial OR mitochondrion)". The same search in September 2015 retrieved 247,000 accessions. Some accessions are spurious (not avian or not mitochondrial), including some shotgun genomic sequences from avian nuclear genomes, but there is no reason to believe the proportion of these accessions has changed. This continued expansion of the data available is an exciting prospect for the future of projects such as this where dense taxon sampling is important.

It is possible that if taxon selection for analyses performed in this and the subsequent section were repeated now, additional families within the two existing clades, or possibly new clades, would be able to be included. Even if this were not the case, the data available for species included here would almost certainly be more complete. However, it remains currently infeasible to expand analyses of this type to include nuclear genes, as taxon sampling remains sparse relative to mt genes.

5.3.2 Analysis

For the 300 pairs of New World nine-primaried oscines and suboscines where non-zero values for d_N and d_S were estimated for both species, there is a significant, negative correlation between contrasts of range size and ω. Although the strength of this correlation is weak, it increased when controls were applied to remove comparisons that were expected to be subject to greater levels of noise. A negative slope for the ω–range size relationship was also separately found in both bird orders and in seven of the nine families. Only Cardinalidae had a non-significant positive slope, which may have resulted from low power, as range size contrasts for this family fell into a narrow interval. However, if the Cardinalidae regression is not forced through the origin, it also has a negative slope (-0.05), albeit with an unexplained positive intercept. The regression slopes for both orders, and four of the seven families with negative slopes were significantly different from zero. This provides strong, overall evidence that a negative relationship exists between range size and rates of molecular evolution in avian coding mtDNA, as

measured by ω. Although ω is a ratio, rather than a rate, it serves as an indicator of a relative rate of molecular evolution by controlling for mutation rate variation.

The slope of the regression overall for $\log_{10}$-transformed ω contrasts and $\log_{10}$-transformed range size contrasts was -0.074. Thus, across range sizes up to 10^7 km^2, for every order of magnitude increase in population size ω declines on average by approximately 15.7%. If we consider the comparison of a 2000bp sequence of mtDNA between recently speciated sister species (e.g., genetic distance = 0.02 substitutions site^{-1}) the substitution rate of the larger population (≈ 0.01 substitutions site^{-1}) would lead to an expected difference of 2.6 non-synonymous substitutions across the sequence caused by the population size difference, given an approximate 70% of sites being non-synonymous. As a total of 40 substitutions would be expected between the species, the stochastic nature of the discrete substitution process provides an explanation for the noisy ω–range size relationship, before accounting for other issues such as the translation of EOO into N_e.

Range size, as estimated by EOO, provides a first-order estimate of population size, and this relationship is stronger when used to estimate population sizes between closely related species (4.2.4 Population–range size correlations). Therefore, it can be inferred that ω correlates negatively with population size within avian coding mtDNA. The negative slope for ω is closely paralleled by the pattern for the non-synonymous substitution rate, while no similar pattern is detectable for synonymous substitutions (Figure 30). Because many more non-synonymous than synonymous substitutions are believed to be subject to purifying selection, this pattern is indicative of more permissive molecular evolution in small populations—a pattern predicted by nearly neutral theory for loci evolving under purifying selection.

If the ω–range size relationship were driven by changes in the mutation rate, a correlation between d_S and range size would have been expected. There was a minor trend of a negative relationship between d_S and range size, but it was not significant in either the raw or final datasets (Table 11). However, because ω is low in avian mtDNA, even a small shift in the mutation rate could have a substantial effect on ω if neutral and non-neutral

substitution rates are affected differently by this shift (e.g., Nei, 2013), or if the mutation rate affects the strength of negative selection against some mutations (e.g., because deleterious epistatic interactions with other mutations occur more frequently under an elevated mutation rate; Lynch et al., 2011). One approach to ruling out a mutation rate effect is to investigate the influence of those comparisons with elevated mutation rates. Three approaches were applied to delineate typical from atypical apparent mutation rate variation (i.e., elevated d_S contrasts; 4.4.3 Apparent mutation rate variation), which converged on a cut-off of $|\log_{10} d_S \text{ contrast}|=0.1$. When relationships were reanalysed, not only was there no evidence that the ω–range size relationship was caused by shifts in the mutation rate, but the relationship became substantially stronger when d_S variation was limited.

In the raw dataset, the substitution rate showed a qualitatively similar, but non-significant, negative relationship with range size as that observed for ω. This relationship became significantly negative in the final dataset, where power was increased. The different strengths of response for the substitution rate and ω to range size variation reflects the relative importance of d_S in each (Table 14). Mutation rate variation in avian mtDNA is high, can occur over short timescales, and is poorly understood (Galtier, Jobson, et al., 2009; Galtier, Nabholz, et al., 2009; Nabholz, Glémin, & Galtier, 2009). The effect of such variation is uncontrolled when rates of molecular evolution consider only the substitution rate.

Differences in d_S between sister species can arise from the inherently stochastic nature of nucleotide substitution (Weir & Schluter, 2008), or alternatively could represent differences in the mutation rate. Mutation rate variation can have many subtle and overlapping causes, relating to aspects of life history and biology. For mtDNA in particular, reactive oxygen species (ROS) originating as by-products of metabolism have been thought to induce mutations (Cooke, Evans, Dizdaroglu, & Lunec, 2003), suggesting that metabolic rate may be linked to mutation rate (Baer, Miyamoto, & Denver, 2007; Martin & Palumbi, 1993). However, some studies of the mitochondrial mutational spectrum have suggested replication error, rather than ROS damage, is the main source of mitochondrial mutations

(Kauppila & Stewart, 2015; Khrapko et al., 1997). Also, the longevity of birds compared to similarly sized mammals has been linked to lower ROS production despite higher metabolic rates (Barja, 2007), and the same pattern occurs in volant versus non-volant mammals: bats live three times as long as predicted by their mass, despite high metabolic rates (Brunet-Rossinni & Austad, 2004). Further, fundamental life history differences can also alter the neutral substitution rate (Lehtonen & Lanfear, 2014), and differences in DNA repair efficiency, generation time, male/female ratios, and cell division rate can all affect the mutation rate (Thomas & Hahn, 2014).

Synonymous substitutions should reflect the mutation rate more closely than non-synonymous substitutions because of the relative absence of direct purifying and positive selection. Non-synonymous mutations make up a small fraction of coding mtDNA substitutions in avian mtDNA. As ω for branches with >0.1 substitutions per site is <0.05 (median ω in final dataset = 0.031), and synonymous sites comprise 15–20% of sites across mtDNA genes, synonymous substitutions comprise more than 80% of fixed mtDNA differences. Because of this, although there is greater proportional variation in non-synonymous than synonymous substitutions between sister species (see Figure 30a and b), smaller variations in the synonymous substitution rate can have major influences on the nucleotide substitution rate. The importance of synonymous mutations in determining the nucleotide substitution rate has implications for studies where substitution rate variation in loci under strong purifying selection is taken as a primary indicator of an ecologically significant evolutionary effect without commensurate consideration of d_N and d_S variation. In addition to not accounting for mutation rate variation, the total nucleotide substitution rate also does not account for other minor variations that might occur between contrasted sequences, such as the proportion of synonymous sites in a sequence. Because of the genetic code's degeneracy, amino acid substitutions can alter the number of synonymous sites in a sequence (e.g., CAG Gln → CUG Leu results in the twofold-degenerate third position becoming a fourfold-degenerate site, and the non-degenerate first position becoming twofold degenerate). For the mtDNA exome, where synonymous substitutions at synonymous sites are 20 times more likely than non-synonymous substitutions at non-synonymous sites, the ratio of synonymous to non-synonymous sites in a sequence can

therefore alter the substitution rate by affecting the proportion of effectively neutral sites, without a biologically meaningful cause. Such an effect is likely to be minor, particularly when compared to mutation rate variation.

5.3.3 Potentially confounding factors

Temperature seasonality was the only potentially confounding variable that remained significant with range size in a multiple regression predicting ω. Rather than confounding the range size relationship, temperature seasonality produced a largely additive contribution to the multiple regression model. Therefore, although range sizes tend to be larger at higher elevation (Table 7), and should therefore be larger in more seasonal environments, there is sufficient latitudinal variation in range sizes, particularly in the tropics (Figure 34), to isolate distinct effects of range size and seasonality. Because temperature seasonality increases away from the tropics as species densities decrease and population densities increase, the negative relationship between temperature seasonality and ω might represent increased population sizes relative to range sizes. It is also possible that differences in selective pressures relating to seasonality shape ω differently in tropical and temperate regions.

A small number of comparisons allowed the effect of migration to be investigated (25 pairs in which one species was fully migratory and one species was not). It has previously been suggested that migratory species will have lower ω because of greater metabolic demands causing stronger, purifying selection (Sun et al., 2011). Although migrants did indeed have lower ω on average than non-migrants (72% of pairs where migratory status varied), this is confounded by most migrants having larger ranges (80% of pairs where migratory status varied). However, in 4 of the 5 pairs in which migrants had smaller ranges, the migratory species still had lower ω. Further supporting the inference that migrants do have lower average ω, there was a negative ω–range size relationship in both migratory contrast groups (i.e., migrants with larger ranges than non-migrants, and the reverse). In the case where the migratory sister species had the larger range there was a negative intercept, whereas when migrants had smaller ranges there was a positive intercept. Because the sister species contrasts were arranged as larger-to-smaller range size contrasts, in both

cases, the curve shift indicates a categorical difference that correlates with migration, consistent with migrants having lower ω than non-migrants.

It is possible that migration causes stronger selection to meet metabolic demands. If this is the cause of the observed ω difference, then those higher metabolic demands more than offset the countering relaxed selection from overwintering in a seasonally cold habitat (i.e., the temperature seasonality effect noted above). However, at least one additional possibility is that densities of migratory bird species could be different from densities of resident species, creating a bias when estimating population size from range size. Given that proportionately more high-latitude species are fully migratory, and at high latitudes species densities are lower and population densities likely to be higher (e.g., Storch, 2003), this additional possibility cannot be ruled out without further investigation.

5.3.4 Comparisons with prior studies

While two previous studies of sister taxon contrasts have reported higher ω in smaller populations (Johnson & Seger, 2001; Woolfit & Bromham, 2005), only one of these found a statistically significant difference (Johnson & Seger, 2001), and no studies to date have demonstrated a significant correlation between population size and ω. There are several reasons why the present study may have increased power to detect such patterns. This study uses range size as a proxy for population size for sister species contrasts of the ω–population size and substitution rate–population size relationship, rather than using less direct proxies such as land area, which introduce additional noise. One previous study used range sizes as a proxy for population sizes to test for a confounding population size effect on independent contrasts of birds (Gillman et al., 2012). They found no effect of population size, although their contrasts were selected on the basis of differing in body size, which is a covariant of population size. This is the first investigation of the ω–population size relationship to use predominantly mainland–mainland sister species pairs, rather than mainland–island comparisons. The use of mainland–mainland comparisons avoids the potential confounding effects that might be expected when contrasted species necessarily occupy different environments, including adaptation to novel environments, different background selection, and island density compensation effects that bias range

size–population size relationships (see 2.4.3 Empirical tests of the N_e—molecular-rates relationship).

Only one of the three studies investigated if island size impacted the magnitude of effects on molecular evolution (Woolfit & Bromham, 2005), while another provided no criteria regarding land mass size and included, for example, Madagascar as an island (Johnson & Seger, 2001). Nonetheless, both of these studies identified a pattern of increased ω in island taxa. However, the third study found no difference in ω (Wright et al., 2009). In addition to ω, both Woolfit and Bromham (2005) and Wright et al. (2009) investigated the effect of population size on the nucleotide substitution rate, and found differing patterns, with the former study finding no relationship and the latter finding a positive relationship.

The present study supports the conclusion of both Johnson and Seger (2001) and Woolfit and Bromham (2005): the mitochondrial exomes of smaller populations experience weaker purifying selection, as predicted under nearly neutral theory. Woolfit and Bromham (2005) detected a weak increase in ω within island lineages, although no relationship was apparent with island size when tested using Spearman rank correlation. In such a situation, the lack of a correlation with the predicted causal factor makes the result difficult to interpret. Although a lack of power to detect a pattern is a possible cause, as is genuinely no pattern existing, the same pattern (equivalent to a flat regressed slope with an unexplained intercept) is can also be caused by a bias from an unmeasured variable. In this case, such a variable could relate to the island–mainland differences described above. Interpreting Woolfit and Bromham's results in the context of the present study, it would appear that the taxonomic breadth, use only of island size, and the small number of comparisons may have reduced power to detect a pattern that may be present. Woolfit and Bromham made 44 ω comparisons across vertebrates and invertebrates. The only population size proxy used was island size, yet effective population sizes of vertebrates and invertebrates are unlikely to be comparable within the same area. Power might have been higher in the present study because only relative area was considered, and that area was derived from species ranges rather than the size of the land masses on which they occur. Woolfit and Bromham did not detect a significant difference in substitution rate

between island and mainland lineages. The results from the present study suggest that the substitution rate in mtDNA is only minimally affected by population size because a majority of substitutions are effectively neutral. Minor variations in the neutral substitution rate may cause a substantial proportion of the variation in the total substitution rate, and obscure effects on the non-neutral component. Therefore, power is lower than when ω is used. This interpretation is consistent with the significant ω variation detected by Woolfit and Bromham.

Unlike Woolfit and Bromham (2005), the findings of Wright et al. (2009) are substantially different to those of the present study. Wright et al. found no difference in ω between island and mainland lineages, and an increased substitution rate in larger, mainland populations. It is possible that the difference is caused by the use of island–mainland comparisons by Wright et al., although, this seems unlikely to be a major cause of the differences, because of the similarity of the present study with the studies of Woolfit and Bromham (2005), and Johnson and Seger (2001), which both used island–mainland comparisons as a proxy for population size differences. Instead, several differences of method could underpin these differences. Firstly, it is unclear how often the contrasts included by Wright et al. are between true sister species. Some comparisons in their study (e.g., *Acrocephalus* spp.) contrast members of island clades with members of mainland clades where both clades contain a number of species. In these cases, multiple speciation events have occurred in the contrasted lineages since their divergence, and number of speciation events may vary between linages. The potential effects of speciation on rates of molecular evolution could be problematic in these cases (Venditti & Pagel, 2010). Secondly, several species in each clade were available to contrast. Wright et al. used minimum patristic distance to choose both their ingroup species and the GenBank accessions used to represent them. Because the number of accessions available for different species varies, this approach biases towards low substitution rates for well-studied species that have many more accessions than others. The choice of median patristic distance for sequence selection in the present study means that additional sequences for a species improve the chosen sequence's representativeness, rather than worsen it. The present study was also able to select true phylogenetic sister species in most cases, as the only limitation for inclusion was

that sequences were available, and the study clades were selected specifically because they have complete or near-complete taxon sampling. Therefore only relatively rare exceptions from true sister species contrasts should exist: when a known species has not been sampled, when species are undersplit, or when phylogenies had topological errors.

Wright et al. (2009) made phylogenetic inferences for ω and substitution rate from four-species phylogenies, using complete or partial accessions for a single gene, cytochrome *b*, ranging in length from 639bp to 1140bp. These comparisons may not have provided sufficient data in all cases to accurately estimate ω or the substitution rate. These were estimated using the free-ratios model in *codeml* in the PAML package, and a GTR+I+Γ nucleotide substitution model in PAUP* respectively, both of which are parameter-rich, and require more data than simpler models to produce stable parameter estimates. While the use of 3- or 4-species phylogenies for studies of evolutionary patterns has been common historically, it has been recognised that these do not provide sufficient data to estimate model parameters (Sullivan et al., 1999; Weir & Schluter, 2011). Notably, in 14 of Wright et al.'s 48 pairs (29.2%), ω varied between species by more than two orders of magnitude. Under the maximum likelihood inference of *codeml*, such values are symptomatic of closely related species that lack sufficient data to produce a reliable estimate of ω, and occur in pairs separated by one non-synonymous change ($\omega \approx 0$), or pairs where one species lacks any synonymous changes ($\omega \approx \infty$). In the remaining two thirds of pairs, less than an order of magnitude variation is recorded. Similarly, in 65 of 365 pairs in the present study (17.8% of pairs, slightly more than half the rate of occurrence in Wright et al., 2009) a zero value for d_N was recorded in at least one sister species. These pairs were excluded from analysis on the basis that it is not possible to produce stable evolutionary estimates from these pairs. Most of these comparisons (72.3%) would have also been excluded from the filtered dataset on the genetic distance criterion.

Despite contrary results on the surface, it is possible to reconcile the present study with Wright et al. (2009). Excluding the 14 pairs with unreliable ω ratios results in a small dataset that is broadly consistent with the results of the present study: for a majority of comparisons (19 of 30 pairs with non-zero ω differences) ω is higher in the island species,

although the difference is not statistically significant (Wilcoxon signed-ranks test, $V = 155$, $P = 0.113$), and the comparisons do no produce a significant correlation between the island–mainland size ratio and ω ($R^2 = 0.035$, $P = 0.291$). Also consistent with the present study, the previously significant positive relationship between area and the substitution rate is no longer detected (Wilcoxon signed-ranks test, $V = 360$, $P = 0.2891$). There may be a lack of power to detect significant relationships in either ω or the substitution rate because only a small sample size of 30 pairs remains, because island–mainland comparisons are affected by island density compensation, or because only island and mainland areas were used, rather than range sizes. Nevertheless, by including only pairs from which stable estimates can be produced, the results of all four sister-taxon studies of population size–evolutionary rates can be reconciled: a negative, ω–population size relationship is consistently found.

5.3.5 Use of range size as an N_e estimator

To establish the usefulness of EOO as an estimate of N_e, EOO was used to estimate N_c. A strong log-log relationship between EOO and N_c was recovered, indicating that EOO is sufficient to estimate the evolutionarily relevant metric N_e in sister species comparisons, provided N_c and N_e are themselves correlated. However, N_c is itself only an approximation of N_e. Even if we assume N_e and N_c are strongly correlated (which may be broadly reasonable given similar ecologies, life histories, and demographies), population sizes fluctuate over the timescales relevant to substitution processes, and many factors affect how closely contemporary estimates reflect the evolutionarily relevant long-term N_e. Species are affected by climate variation differently: species-specific population size changes during glaciations will depend on factors including habitat availability, latitude, migratory status, and dietary and habitat generalism (Both et al., 2010; Clavel, Julliard, & Devictor, 2010; Jiguet, Gadot, Julliard, Newson, & Couvet, 2007). Large-scale human activities have also impacted the contemporary population sizes of most or all terrestrial species to varying extents. Habitat types and locations are differentially subject to human exploitation and appropriation (Haberl, Erb, & Krausmann, 2014), and species' abilities to respond to these changes are varied (Schwitzer, Glatt, Nekaris, & Ganzhorn, 2011).

The ω–range size relationship was weaker in the tropics than in temperate regions. This may be due to the weaker EOO–population size relationship detected in the tropics (4.2.4 Population–range size correlations). A weaker relationship between EOO and population size in the tropics is indicative of a wider range of population densities occurring in the tropics. It has been well established that tropical communities have a wide range of densities and a preponderance of rare species (Terborgh et al., 1990). As tropical communities are also the more species rich, the inclusion of all species in the present study weighs towards many tropical comparisons, where power to detect relationships is evidently lower (Figure 35). Future studies that might estimate tropical population sizes using EOO should consider this limitation, and whether alternative methods of population estimation are available.

5.4 Effect of latitude, elevation, climate and migratory status on New World bird evolution

5.4.1 Latitude

Species' latitudinal centroids significantly covary with ω, although no latitudinal effect was detected for d_S or substitution rate. Under the assumption that a large proportion of synonymous substitutions are effectively neutral, and that these proportions are similar between sister species, it appears that an increase in non-synonymous substitutions occurs on average in species closer to the equator, and this increase is not detectably driven by an increased mutation rate. As with the negative ω–range size relationship, the negative ω–latitude relationship is consistent with a gradient in purifying selection.

Latitude *per se* cannot be the cause of gradients in evolutionary metrics such as ω. Two measures of temperature variation—temperature seasonality and isothermality—appear to account for the effect of latitude on ω. When either metric is included with latitude in a regression model predicting ω, latitude becomes a non-significant predictor of ω, with negligible regression coefficients (Table 17). The effect of these climate variables is discussed below.

As the contemporary and historical climate variables that relate to diversity patterns covary imperfectly with latitude, causal covariates of climate should correlate more strongly than latitude with those diversity patterns. Further, if molecular evolution is implicated as a causal intermediate between climate and diversity, there should be direct correlations between climate and evolutionary metrics such as ω, d_S or the substitution rate (Davies et al., 2004). Theories that invoke molecular evolution as causally linking climate and diversity must explain why the relationship between latitude and molecular evolution is weak, while the relationship between latitude and diversity is strong.

5.4.2 Climate

Mean annual temperature

Mean annual temperature had a weak, positive correlation with ω, which was non-significant. No relationship was detected between mean annual temperature and d_S. As many synonymous mutations should be neutral, d_S is commonly linked to the mutation rate (e.g., Lanfear et al., 2010). Gradients in ambient temperature have been linked to the kinetics of important metabolic processes, and in turn to mutation rates and diversification processes in ectotherms (Allen et al., 2002). Mutation rates have also been linked to diversification in birds (Lanfear et al., 2010), although ambient temperature is unlikely to play a direct role in mutation rates for endotherms (Allen et al., 2002). Indeed, I find no evidence that mutation rates are systematically affected by mean annual temperature. If mutation-rate-linked diversification rates play a role in the LDG, it is through the proliferation of particular lineages, rather than any direct effects of ambient temperature.

The lack of a relationship between mean annual temperature, or latitude, and d_S differs from the results from a previous study in mammals (Gillman et al., 2009). Gillman et al. found faster substitution rates in tropical than temperate mammals that were not driven by significantly elevated ω. From the present study, this pattern indicates a predominant mutation rate effect, although this effect was not formally tested for by Gillman et al. Although a direct ambient temperature–mutation rate link is not predicted for endotherms by the metabolic theory (Allen et al., 2002), such an effect could be plausibly induced by a Red Queen mechanism (Gillman et al., 2009). No elevated mutation rate effect was found in the present study for birds. It is unclear whether the difference is a taxon-specific effect, or the result of differences in methods. Taxon-specific differences are possible—in a study of mammalian diversification, no link was found to rates of molecular evolution (Goldie, Lanfear, & Bromham, 2011), unlike the results from a previous investigation of birds (Lanfear et al., 2010). Such contrasting results indicate that even amongst endotherms, fundamental diversification processes may differ, highlighting the need for urgent, and detailed investigation of these processes.

Productivity

A weak link between productive energy and rates of molecular evolution (ω) was detected. However, this link was not sustained in a multiple regression model that included latitude as an additional predictor of ω. As a broad measure of resource supply to consumers, NPP should correlate approximately with the number of individuals in a community, and, given interannual stability in NPP, the depth of the food chain (Brown, 1981; Hutchinson, 1959). A link between NPP and rates of molecular evolution is predicted under the integrated evolutionary speed hypothesis (Gillman & Wright, 2014), and the lack of a link here must be considered to be evidence against the generality of this hypothesis.

Temperature variation

Isothermality and temperature seasonality—both measures of intra-annual climate stability—are significant correlates of ω and the overall nucleotide substitution rate, but not of d_S. Thus, an accelerated rate of non-synonymous substitution appears to occur in birds found in climates with high intra-annual temperature stability, and—as with latitude and range size—there is no evidence that this acceleration is caused by a mutation rate effect. There are at least three distinct, potential causes of this pattern: 1) reduced severity and frequency of extreme climate events in temperature-stable climates increases N_e through reduced mass mortality (Maruyama & Kimura, 1980), resulting in a greater proportion of positively selected variants being fixed; 2) reduced climate harshness in temperature-stable environments results in a greater proportion of non-synonymous mutations being effectively neutral and able to go to fixation; or, 3) greater species density and elevated net diversification in temperature-stable environments causing more frequent population bottlenecks and smaller average population sizes, such that elevated ω results from rather than causes higher speciation rates (e.g., Venditti & Pagel, 2010).

The three above scenarios can be explained using population-genetic mechanisms that link the relationship between temperature seasonality and ω to other predictions. Under the first scenario, increased ω is the result of increased N_e. The assumed reduction in frequency and severity of population size fluctuations (Vucetich, Waite, & Nunney, 1997), increases the ratio of N_e:N_c in the tropics. In turn, this increases ω by increasing the rate of input of

positive variation available to selection. However, increased N_e should also increase the effectiveness of purifying selection. To increase ω, this scenario therefore requires adaptation to be mutation-limited, and for a greater number of adaptive mutations to be fixed than slightly deleterious mutations are removed by selection. Because the fixation of positive mutations reduces genetic diversity, increased ω is not expected to be accompanied by increased nucleotide diversity. In the second scenario, N_e:N_c is increased in the tropics because of reduced, climate-induced background selection. Therefore, the N_e:N_c ratio is the result, rather than the cause, of a shift in ω. Because the shift in ω results from different tropical and temperate distributions of fitness effects (DFEs) under which there are more effectively neutral tropical mutations, it reflects non-adaptive processes, with no immediate role for positive or negative selection. A consequence of this gradient in purifying selection is that increased tropical ω should be accompanied by increased nucleotide diversity. More direct evidence could be provided with examinations of DFEs at different latitudes. In the third scenario, no direct climate effects are responsible for tropical–temperate ω disparity. Because tropical species densities are higher without commensurate increases in densities of individuals, average population sizes are smaller in the tropics. This could resolve either as lower population densities in the tropics, or smaller tropical range sizes. Both appear to be true on average (Figure 44), although the effect of latitude on density in relationship is weak, and the difference was marginally non-significant when latitude was regressed against the residuals of the range size–population size relationship (OLS regression, $P = 0.052$). The estimate of this effect is limited to species with population size estimates, which are typically at-risk species, introducing additional uncertainty about its generality. Nevertheless, the effect of temperature seasonality on ω could represent a density-induced, tropical/temperate disparity in population size not accounted for by range size. As with the first scenario, increased tropical ω is not expected be accompanied by increased nucleotide diversity.

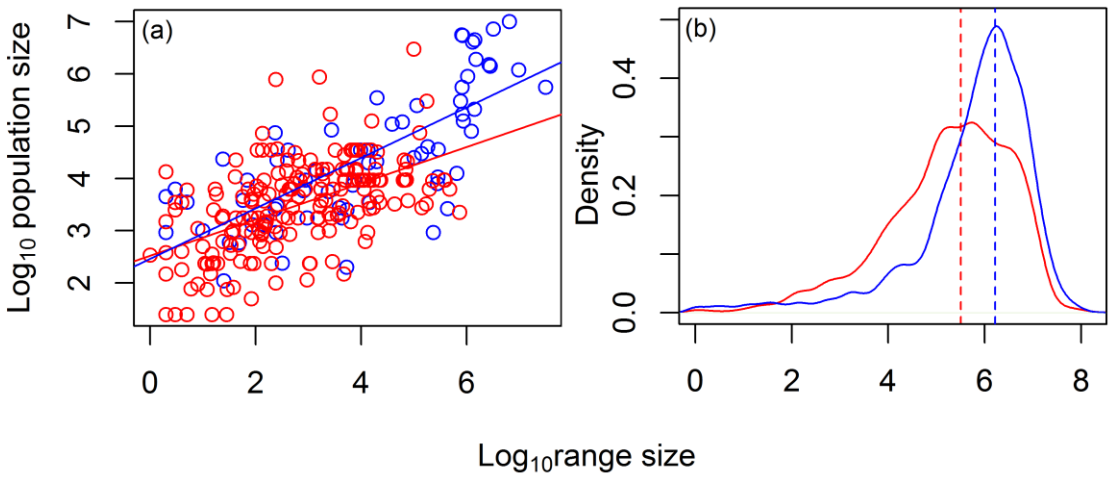

Figure 44. Tropical and temperate species differences in (a) the range size–population size relationship of passerine birds (N = 262 species), and (b) the distribution of range sizes for almost all recognised bird species (N = 9965 species). In (a) blue points represent temperate species (|latitude|>20°) and red points represent tropical species (|latitude|<20°). Lines are OLS regression fits. In (b) solid lines represent the kernel density of species ranges for, and dashed lines represent median values for tropical and temperate groups, with colours as described for (a).

Framing the three above explanations for a negative ω–temperature seasonality relationship in the context of expectations around within-species genetic diversity provides an approach to evaluate their importance. Firstly, results presented earlier (Figure 25) show that for a given level of genetic divergence, nucleotide diversity is higher on average in tropical than temperate populations. Similarly, across a global dataset of 72 vertebrate species, Adams and Hadly (2013) found greater nucleotide diversity in low-latitude than high-latitude populations. The overall pattern detected by Adams and Hadly was separately significant for birds and mammals, and glacial and non-glacial species. A non-significant trend in the same direction was found for ectothermic taxa (fish, amphibians, reptiles), which may have lacked power, as sample size was small in these species (< 10). Other studies have found greater genetic diversity at the centre of species' ranges (rather than at lowest latitude), but have either considered restricted latitudinal ranges (Miller, Bermingham, Klicka, Escalante, & Winker, 2010), or have not factored the effect of latitude in the study design (Eckert, Samis, & Lougheed, 2008). Miller et al. (2010) investigated nine species that span the narrow land bridge between the North and South American continents between southern Mexico and Ecuador. Almost all of their samples were taken

within 10 degrees of the equator, which is unlikely to provide sufficient climatic variation to detect patterns that may exist in more broadly distributed species. Thus, where it has been investigated broadly, there is support for a latitudinal gradient in genetic diversity. Greater genetic diversity in tropical populations is indicative of higher N_e in those populations. This is consistent with the main effect of temperature seasonality on ω being to shift the DFE of non-synonymous mutations to include a greater proportion of effectively neutral mutations. As noted above, sufficient variation occurs between mammal and avian net diversification patterns that any such effect needs to be separately investigated before generalising about vertebrate endotherms, let alone ectotherms.

5.4.3 Elevation

Several elevation-related effects on molecular evolution were detected in the present study. For most of these relationships, stronger relationships were detected within the tropics than across the whole dataset where both tropical and temperate pairs were considered. Firstly, minimum elevation correlates positively with ω. An increase in ω at higher minimum elevations could occur if population connectivity or population size declined with elevation and connection to lowlands. There is evidence that populations are more strongly structured at high elevation, in diverse groups including plants (Hensen et al., 2012) and vertebrates (Giordano, Ridenhour, & Storfer, 2007), because of increased landscape heterogeneity. There is also evidence that population densities (Smith et al., 2000) and population sizes (Patterson, Meserve, & Lang, 1989) decline with increasing elevation in some species. These factors could also reduce N_e in species with minimum elevations that exclude more connected, lower-elevation landscapes. In this way montane Andean landscapes can be viewed as analogous to oceanic islands (Hughes & Eastwood, 2006).

The other major effect detected for ω related to elevational range. As elevational range increased, ω declined. Two effects could be responsible for this pattern. Firstly, similar to the above—but in reverse—larger elevational ranges could, on average, relate to larger population sizes. Secondly, a wider elevational range implies surviving a wider range of climates, which could result in stronger purifying selection. On the first point, while this

should only be feasibly true for montane species, as lowland species can cover large ranges with minimum elevational ranges, including both range size and elevational range in a model predicting ω results in a non-significant coefficient for elevational range ($P = 0.217$). On the second point, including both temperature seasonality and elevational range in a model predicting ω results in model where both predictors are significant ($P < 0.04$). Therefore, the effect of elevational range appears to be a population size effect that is not wholly distinct from the effect of range size on population size.

In the present study there is also evidence of an elevation-related mutation rate shift in the studied bird species. Both d_S and substitution rate shifts were negatively correlated with maximum elevation and elevational range, although the relationships were only significant within a tropical subset. A recent study found no basal metabolic rate difference along an elevational gradient in tropical birds (Londoño, Chappell, Castañeda, Jankowski, & Robinson, 2015), sugesting that this effect is related to another factor that influences the mutation rate. A reduced mutation rate at high elevation is consistent with the findings of two previous investigations. Bleiweiss (1998) attributed rate differences in hummingbirds to decreased mutation rates that might result from lower metabolic rates at high elevation. However, this study uses no phylogenetic controls, and there are a number of other concerns that limit its validity. Average elevations for 26 hummingbird species were regressed against genetic distances calculated by DNA-DNA hybridisation. These genetic distances were the reciprocal median melting temperatures between each hummingbird species and a swift outgroup. Given the deep divergence between hummingbirds and swifts (approximately 60 MY; Jetz, Thomas, Joy, Hartmann, & Mooers, 2012), the extent to which these genetic distances are influenced by contemporary mean elevation of a given species must be minimal. Although selection of a single species from each clade would reduce the potential effect of phylogenetic non-independence, an appropriate estimation of altitude would require a clade average over its evolutionary history. However, this result was supported by a subsequent study on the effect of elevation on molecular evolutionary rates in mammals that addressed the lack of phylogenetic independence by using a dataset of 29 mammalian sister species (Gillman et al., 2009). They found a pattern consistent with that identified by Bleiweiss (1998)—a slower substitution rate at higher elevation, with no

difference detectable in ω, consistent with a mutation-rate effect. While the results presented here further support this pattern, it should also be noted that the pattern is weak and the reason for its emergence only in tropical contrasts remains unclear.

5.4.4 Apparent mutation rate variation

Given the lack of significant relationships between a suite of predictors and d_S, attention was shifted to the potentially nuisance role of d_S variation obscuring subtle patterns. There is strong evidence that outlying values of d_S contrasts, representing large apparent mutation rate variation between sister species, introduces noise into measured relationships with other evolutionary metrics. I refer to apparent mutation rate variation because although between-species mutation rate differences could be responsible, d_S variation between sister species represents the average substitution rate in the lineages since coalescence, which may not reflect current mutation rates. It is also possible that sequencing errors have contributed to these differences, in which case high d_S variance reflects poor data quality.

Substantial variation in d_S has been raised previously as an issue related to the use of mtDNA as a population-genetic marker. Mutational hotspots in mammalian mtDNA, for example, have been invoked as causes of widespread homoplasy (Galtier, Enard, Radondy, Bazin, & Belkhir, 2006). Mutation rates across both birds and mammals appear to be remarkably variable (Nabholz et al., 2008), and can result from a wide range of ecological factors that can readily evolve within species' lifetimes (Thomas & Hahn, 2014). Life history traits such as longevity, generation time, and fecundity are linked to mutation rates and may be causal in the present study. However, there is not currently enough data for these life history traits to be evaluated at the sister species level (longevity estimated for <10% passerine species in the AnAge database: http://genomics.senescence.info/species/).

While the cause of the majority of d_S variation was not formally determined, controlling for d_S allowed for substantially stronger patterns to be detected in ω. For example, temperature seasonality and minimum elevation explained 30% of the variation in ω for temperate sister species once d_S was controlled. This strongly suggests that the increase in

d_S did not simply result in an increased substitution rate with proportional effects on all categories of mutation. If d_S variation reflects genuine biases in the mutation rate, causes of this non-proportional change should be considered. Nei (1975, 2013) has suggested that the non-deleterious mutation rate is constant per unit time, while the deleterious mutation rate is constant per generation. The mutation rate is linked to the fundamental properties of populations—life history traits such as longevity and fecundity. Also, changes to the mutation rate affect genetic load, and therefore N_e (see 2.4.2 Factors that affect the substitution rate: Mutation rate). As such, a complex relationship between the mutation rate and ω might exist. More detailed explorations of the mutation rate between closely related species are needed to better understand the causes and consequences of pairwise d_S variation between sister species.

In the context of apparent mutation rate variation as a nuisance variable, it is notable that d_S variance was also higher in the tropics. Variance of log-transformed dS contrasts in pairs with average absolute midpoints below 20° was lower than for remaining pairs (0.018 and 0.011 respectively). Relationships for ω with range size and with temperature seasonality were both stronger outside than inside of the tropics. It is difficult to establish with certainty whether more variable mutation rates, or the weaker EOO–population size relationship, caused the reduced explanatory power of the ω–range size in the tropics. Similarly, the range of temperature seasonality is limited in the tropics, which could also explain a tropical–temperate relationship disparity. However, it is possible that more variable tropical mutation rates are a cause. It remains to be established whether more variable mutation rates are the result of being more finely tuned in the tropics, linked to more variable life history traits, or sloppier because of reduced selection.

While applying a control on d_S variation allows for the detection of underlying patterns such as the effect of population size on rates of molecular evolution, it must be acknowledged that simply applying such a control discounts the importance of mutation rate variation to empirical patterns of molecular evolution. The variation seen in d_S shows that non-adaptive variation is the major factor determining variance in sister species contrasts of molecular rates. This explains the poor resolution of the substitution rate in

phylogenetically proximate comparisons examining rates of molecular evolution (e.g., Woolfit & Bromham, 2005), and why in studies where substitution rate patterns have been identified in sister species, it is often mutation rate effects that have been observed (e.g., Wright et al., 2009).

5.5 Implications for understanding diversity, diversification, and evolution

5.5.1 Using mtDNA as a molecular marker

The use of mtDNA as a population-genetic and phylogenetic marker has been a perennial controversy. The enthusiasm of early assessments of mtDNA as a population genetic marker (Avise et al., 1987) were followed by many caveats and criticisms of the same uses (Ballard & Kreitman, 1995; Ballard & Whitlock, 2004; Bazin et al., 2006; Galtier, Nabholz, et al., 2009). Much of the issue has revolved around inappropriately treating mtDNA as a strictly neutral marker, an inference based on misunderstandings of the neutral theory (Ballard & Kreitman, 1995). Instead of evolving strictly neutrally, animal mtDNA evolves under strong purifying selection, with some evidence of positive selection (Foote et al., 2011; Meiklejohn et al., 2007; Stewart et al., 2008).

The action of selection in relation to neutral theory must be one of the commonest misunderstandings in molecular evolution. Neutral theory does not assume any loci evolve strictly neutrally, only that the majority of substitutions and polymorphisms are neutral. While there are predicted levels of neutral polymorphism at equilibrium, neutral theory does not assume the circumstances under which equilibria occur are common, and non-equilibrium genetic diversity does not reflect on the validity of neutral theory. The evolution of mtDNA as it is currently understood is wholly consistent with neutral theory, despite claims to the contrary (Stoeckle & Thaler, 2014). Given the persistent misunderstandings, and conflation of neutral theory and strictly neutral evolution, it may be better to adopt the language of Lynch (2007b), who refers to adaptive and non-adaptive forces that are influenced by population-genetic processes, and imply no dichotomy.

The balance of adaptive and non-adaptive forces acting on the archetypal, uniparentally inherited animal mt genome results in a small genome that lacks introns (Lynch et al., 2006), evolves rapidly (Brown, George, & Wilson, 1979), has shallow coalescence and low diversity within well-mixed populations (Stoeckle & Thaler, 2014), and has a limited range of genetic diversity across organisms, which cannot be assumed to correlate with N_e (Bazin et al., 2006; Mulligan, Kitchen, & Miyamoto, 2006). While species-specific N_e estimates from mtDNA diversity are unreliable, when there is generalised differences in N_e, these

differences can manifest as measurable variation in mtDNA diversity (Hughes & Hughes, 2007; Mulligan et al., 2006). Supporting this, I find similar evidence for a detectable latitudinal gradient in genetic diversity for avian mtDNA in the present work. Within a restricted taxon, such as passerine birds, such patterns are detectable, presumably because they are relative. However, it would be inappropriate to expect absolute values to show similar patterns across taxa with distinct mt selection regimes—for example, by comparing avian and mammal mt diversity (Stanley & Harrison, 1999).

Given the evidence for the combination of both high substitution rates and strong purifying selection on the housekeeping genes of the animal mt genome, many effectively neutral mutations must fix in the genome, or function would be rapidly eroded. Therefore, the substitution rate must largely comprise non-adaptive change. Where there is evidence of occasional positive selection (Balloux et al., 2009), it is not clear that such selection should be strongly mutation-limited, given the high copy number and mutation rate in the selection phase of mt transmission (Stewart & Larsson, 2014; Wai et al., 2008). With these factors in mind, the mt genome is a locus at which nearly neutral evolution is likely to be detected, if it is indeed a common feature of evolution.

Nearly neutral, slightly deleterious, substitutions have been implicated as the cause of evolutionary differences between taxa in a number of studies (see 2.4.3 Empirical tests of the N_e—molecular-rates relationship). A range of surrogates for N_e of varying adequacy have been used across these studies, and they have been conducted at a range of taxonomic scales. I present the first study that demonstrates a significant correlation between the N_e surrogate used and a measure of the rates of molecular evolution across sister species. It is also the only study of its type to demonstrate a correlation between the surrogate and N_c. The results provide the strongest evidence to date that slightly deleterious substitutions accumulate more rapidly in smaller populations at the sister species scale of comparison. However, the results also show the overwhelming influence of the mutation rate in determining the substitution rate in avian mtDNA. Thus, of the non-adaptive component of mt genomic evolution, the majority of substitutions are effectively, rather than nearly, neutral.

The strong influence of the mutation rate agrees with previous studies that have found strong substitution rate variation. For example, Nabholz et al. (2008) found the mt mutation rate varied by up to 2 orders of magnitude across mammals, and this variation was only partially explained by body size and longevity. Such variation is sufficient to question the use of a mt molecular clock. The broad correlation with body size and longevity across mammals is well established—a low mt mutation rate is required to prevent the accumulation of deleterious somatic mutations through a long-lived or many-celled organism's lifetime (Bromham, 2011), although the proximate direction of causality is not necessarily established. Low mt mutation rates might facilitate longevity, or selection for longevity could drive selection for reduced mt mutability. Because strong selection acting on multiple copies of mtDNA occurs during transmission between generations, it remains possible that, rather than being fine-tuned, the highly variable mutation rate is the result of weaker selection similar to highly variably genome size in many eukaryotic lineages when genome size it is not under direct selection (Lynch, 2007b; Lynch et al., 2011).

Animal mtDNA recombination rates are low, giving rise to situations under which population-structure inferences are difficult or misleading. Because even occasional interbreeding between subspecies can result in loss of haplotypes, mtDNA may not provide useful markers for such inferences. For example, Zink (2004) finds that a majority of continental avian subspecies lack a population-genetic structure that would justify this taxonomic ranking. He concludes that subspecies rankings are misleading, although recognising there can be conflicts between conclusions based on molecular and morphological evolution, although not specific issues that are associated with mtDNA. However, because introgression between subspecific populations should only be impossible if they are separated by complete geographic barriers, the lack of structure could be interpreted more simply as the results of occasional migrants. Given that the rapidly evolving mt genome can be degraded by slightly deleterious mutations in a small subspecific population, then the input from another subspecies could sweep through the population without affecting the morphological differences that have resulted in

subspecific assignment. Hence, the strength of Zink's (2004) conclusion should be tempered by a lack of corresponding nuclear markers. The lack of substructure seems to suggest that rapid fitness differences can accumulate between mitochondrial genomes of isolated populations, and that selective sweeps to restore fitness may be common. Selective sweeps within populations have previously been identified as a possible cause for restricted diversity in the DNA barcode region of North American birds (Kerr et al., 2007).

Although important caveats around the use of mtDNA remain, as a rapidly evolving marker with shallow coalescence, it is able to play a role that nuclear DNA is not. While, in the present study, it was not possible to compare nuclear and mt genomes as markers, the shallow coalescence of mtDNA and high mutation rate was almost certainly an advantage for the assessment of the ω–range size relationship. This is because range sizes do not evolve under Brownian motion (Diniz-Filho & Tôrres, 2002), and are subject to contingent effects of climate and geography. Thus, over time molecular evolutionary variance caused by population size differences between sister species will represent average population size since divergence, which will be increasingly poorly estimated by contemporary EOO. This suggests a window of time over which a reliable signal can be detected, which will be negatively affected by incomplete lineage sorting, and low mutation rates.

5.5.2 *The evolutionary speed hypothesis*

The evolutionary speed hypothesis (ESH), like the more-individuals hypothesis, has an intuitive logic that appeals to the notion of wet tropical rainforests teeming with organisms living a fast-paced life. ESH states that the LDG results from shorter generation times, elevated UV exposure, and higher metabolic rates. Under ESH, these factors elevate tropical mutation rates, driving faster positive selection, and leading to more rapid divergence in tropical species (Rohde, 1992). ESH therefore leads to a prediction of a faster rate of molecular evolution in the tropics, as further explored in the expansion of ESH referred to as the integrated evolutionary speed hypothesis (IESH; Gillman & Wright, 2013; Gillman & Wright, 2014). While not all of ESH's explanatory factors are as readily applicable to endotherms as they are to ectotherms, it has been proposed that the Red Queen hypothesis (Van Valen, 1973) may explain a parallel LDG in endotherms (Gillman et al., 2009). Indeed, Gillman et al. (2009) found evidence of accelerated tropical mammalian substitution rates without concurrently elevated ω. These results are consistent with a higher real-time mutation rate in tropical mammals being responsible for the detected substitution rate effect. Although this provides no direct evidence of faster, selection-driven tropical speciation, a gradient in positive selection could be enabled by a higher mutation rate if selection is mutation-limited (Gossmann, Woolfit, & Eyre-Walker, 2011). A correlation between mutation rate and net diversification rates has been detected for mt but not nuclear genes in a radiating clade of amphibians (Dugo-Cota, Castroviejo-Fisher, Vilà, & Gonzalez-Voyer, 2015), and for nuclear genes between families of birds (Lanfear et al., 2010), but not for nuclear or mt genes in families of mammals (Goldie et al., 2011).

IESH predicts that a gradient in molecular evolutionary rates is caused by a temperature gradient, or energy-related latitudinal gradient, such as NPP. A recent test of IESH found support for a temperature-related gradient in molecular evolutionary rates in a radiation of tropical amphibians (Dugo-Cota et al., 2015). However, there is little evidence that this pattern also occurs in endotherms. Such a gradient should be detectable in sister species contrasts. While Gillman et al. (2009) found an association in their dataset for small mammals, Weir and Schluter (2011) point out that within this dataset there is no evidence

that substitution rate differences scale with the latitudinal differences between contrasted sister species. Instead there is only a categorical difference between high- and low-latitude species, such that no rate difference is apparent regardless of whether species were separated by a few or 50 degrees of latitude. In the present work using birds, I was unable to detect any molecular rates patterns with any evolutionary metric and mean annual temperature. A relationship between NPP and ω existed, but was evidently the result of collinearity with latitude, rather than being directly related to NPP (Table 17). Instead the variables that correlated significantly with ω related to temperature variation, with elevated ω in less seasonal, and more isothermal environments. While several mechanisms, including more tropical positive selection could potentially explain this pattern, when combined with results for nucleotide diversity within species the most likely explanation is relaxed purifying selection in the tropics (see 5.4.2 Climate: Temperature variation). This explanation is inconsistent with IESH.

In the IESH framework, the LDG is driven by the speciation rate through elevated rates of molecular evolution. However, within mammals, clade-specific patterns of molecular evolutionary rates found between low-latitude and high-latitude sister species are more consistent than speciation patterns, or indeed spatial diversity patterns for their clades. For example, Gillman et al. (2009) detected the same pattern of increased substitution rate for tropical Lagomorpha as for other mammalian orders. However, Lagomorpha has a distinct, north-temperate peak in diversity, and low tropical diversity (Rolland et al., 2014). Indeed, the effect size (mean tropical–temperate substitution rate contrast) estimated by Gillman et al. for Lagomorpha was greater than for Chiroptera, Primates, and Carnivora, all of which have distinct LDGs with diversity peaks in the tropics. Further, Rolland et al. (2014) found that speciation rates in Lagomorpha and Primates do not differ between temperate and tropical regions, and temperate speciation rates are higher than tropical speciation rates in Carnivora, although Gillman et al. detected elevated tropical substitution rates in all three. It is possible that other factors concurrently affect net diversification, causing such variable patterns. However, this leaves little scope to evaluate the contributions of mechanisms that might operate under IESH. In addition to this, several studies have found evidence for stronger recent selection for population

differentiation in temperate zones in birds (Lawson & Weir, 2014; Martin, Montgomerie, & Lougheed, 2010; Weir & Wheatcroft, 2011), as well as rapid boreal speciation due to ice sheet formation during Pleistocene glaciations (Weir & Schluter, 2004). Collectively, these lines of evidence suggest that population divergence and speciation rates alone are insufficient to explain net diversification and diversity patterns. The role of extinction in net diversification patterns is at least important (Rolland et al., 2014), as elevated speciation rates do not lead to high speciation richness if extinction rates are similarly elevated (Weir & Schluter, 2007).

IESH is notable amongst LDG explanations for making testable predictions. However, multiple lines of evidence now contradict several of its premises, at least as a major and general explanation for the LDG. Taxon-specific support exists, but IESH cannot explain the molecular evolutionary patterns observed in birds, nor the diversification patterns inferred in mammals. Attention for primary mechanisms should be given to alternative explanations for the LDG that can accommodate the wide range of observations that are available.

5.5.3 Towards a framework for understanding spatial diversity patterns

There is insufficient evidence that positive selection and speciation alone drive global diversity patterns, such as the LDG. However, given the evidence that net diversification rates have historically been—and continue to be—elevated in the tropics (see 2.3.4 Causes of species richness variation: Net diversification rates), there remains impetus to understand if links exist between population-genetic-level phenomena, climate, and diversity patterns. To this end, I will review in brief some of the general observations regarding spatial diversity patterns and outline the implications for an LDG framework. I will then develop some of these ideas, and show that many of these observations fit together cohesively, suggesting a unifying framework might be possible to explain why the tropics harbour disproportionately high biodiversity. I outline the limitations in current knowledge that prevent a fully specified framework being developed.

1) High tropical diversity occurs at multiple taxonomic scales

Biological diversity is almost invariably higher in the tropics, regardless of how we define diversity. Genetic diversity is elevated in tropical populations (Adams & Hadly, 2013), as is divergence between tropical populations (Martin & McKay, 2004); tropical species harbour more recognised subspecies than temperate species (Martin & Tewksbury, 2008); not only are tropical communities more species-rich than temperate communities, but most taxa separately follow a latitudinal gradient in species richness from the equator to the poles (Hillebrand, 2004); and, the species richness gradient is repeated at genus and family level (Currie et al., 2004), and higher taxonomic levels (Kerkhoff, Moriarty, & Weiser, 2014). Any explanatory framework for the LDG should be able to explain why these multiple levels of biological diversity are consistently higher in the tropics or, alternatively, why these levels of diversity are unconnected.

2) Tropical communities harbour many more rare species than temperate communities

The major accounts of tropical diversity emphasise the impressive array of species that comprise tropical communities, with observations of high turnover and the occurrence of many rare species. However, it is poorly understood how rare and common species contribute to species richness at different spatial scales. At macro scales, there is good evidence that common species shape species richness patterns (Evans, Greenwood, & Gaston, 2005; Jetz & Rahbek, 2002), while at smaller spatial scales, rare species appear to play an important role in local richness (Heegaard, Gjerde, & Sætersdal, 2013), and comprise large components of tropical community richness (Terborgh et al., 1990). While the contribution of rare species to diversity patterns may be scale-dependent, it is evident from the latitudinal distribution of population sizes that low-abundance species persist for longer periods of time in the tropics than the extratropics. An explanatory LDG framework that includes net diversification differences should be able to explain the relative prevalence of rare species in the tropical communities.

3) Species richness is weakly correlated to numbers of individuals

While some groups, such as ants and herbs, have a strong individuals–richness correlation (Kaspari et al., 2000; Stevens & Carson, 1999), many other broad groups, including trees and birds, do not show a similar relationship (Currie et al., 2004; Enquist & Niklas, 2001; Terborgh et al., 1990). A weak positive relationship between the numbers of individuals and species in a community would be expected if they both covary with climate-linked variables, such as NPP. Currie et al. (2004) argue that for an energy → individuals → species richness relationship, the individual links must be stronger than for a direct energy → species richness link. However, as Currie et al. demonstrate, this expectation is not generally true at large spatial scales. While such relationships might hold at times for small spatial scales, a further issue is that the empirical slope of the energy–species richness relationship is substantially steeper than predicted by for relationship if mediated by the number individuals (Currie et al., 2004; Preston, 1962). In other words, observed patterns in high-density samples reflect expanded species pools rather than a simple sampling relationship. Generally, the number of species increase more rapidly than the number of individuals, indicating low average population densities in high richness communities, which cannot be the result of a species–energy effect. Therefore, an LDG framework should be able to explain the differential accumulation of tropical and temperate species richness in communities with comparable total numbers of individuals. An interesting consideration is the fate of energy in tropical communities if it is not proportional to numbers of individuals.

4) Contemporary and fossil record species richness patterns in animal taxa have stronger links to temperature variation than to mean temperature

The present work demonstrates that temperature seasonality is a strong predictor of contemporary vertebrate species richness. The palaeontological record shows this has also been true over long periods of time (Archibald et al., 2010; Mannion et al., 2012; Mannion et al., 2014). Also, both contemporary and past records show diversity is relatively poorly correlated with temperature averages. The range of temperatures experienced by organisms in a community appears instead to be an important determinant of richness. Secondly, minimum temperature is important, and there is evidence of a threshold in the

seasonality–richness relationship in regions that experience below-freezing winters. Therefore, an LDG framework should be able to explain the shape, strength, and sub-patterns evident in the relationship between animal species richness and temperature seasonality.

5) Niche conservatism results in strong biases affecting which broad taxa are represented in communities, based on their climate niches

The disjointed seasonality–richness relationship in mammals at the threshold of above- and below-freezing ecoregions (Figure 18c) indicates fundamental differences in the faunas—such as the meeting of two faunas with substantially distinct component species. Indeed, climate niche conservatism has been widely observed in many taxa (Wiens et al., 2010), such as tree frogs (e.g., Wiens, Graham, Moen, Smith, & Reeder, 2006), but also extensively in plants (Kerkhoff et al., 2014; Ricklefs & Renner, 1994), for which biome is highly phylogenetically conserved during speciation (Crisp et al., 2009). Time and area have been hypothesised to be important causes of the LDG as the tropics have been historically larger (Fine & Ree, 2006; Fine et al., 2008; Wiens & Donoghue, 2004), and because the degree of temperate seasonality has varied over geological time (see Archibald et al., 2010 and references therein). Because climate niche conservatism almost certainly plays a role in shaping the strength of the LDG, any framework attempting to explain the LDG should reference, and be consistent with, a role for this phenomenon.

6) Mutation rates are not accelerated in tropical endotherms, but ratios of non-synonymous to synonymous substitutions are latitudinally biased

Mutation rates appear to show latitudinal biases in some taxa, consistent with the metabolic theory of ecology (Brown, 2014). While climate-induced mutation rate variation is theoretically sound for ectotherms, endotherms would need secondary mechanisms for similar effects to occur (Gillman et al., 2009). In the present work, I find no evidence for a latitudinal gradient in avian mutation rates. Further, there is inconsistent evidence of a link between mutation rates and diversity in endotherms (e.g., Goldie et al., 2011), which may reflect dual effects of mutation rates on both speciation and extinction (Lancaster, 2010). However, ω is higher at low latitudes (Table 16), and has a particularly strong relationship

with temperature seasonality (4.4.3 Apparent mutation rate variation). A framework for the LDG that considers within- and between-species patterns should give regard to the common factor of temperature seasonality, which correlates with both molecular evolutionary rates and species richness.

7) Net diversification rates are higher in the tropics

Separating extinction and speciation rates to understand the processes of net diversification is an unresolved challenge. Fossil and phylogenetic studies produce varying results, and neither necessarily produces robust estimates of extinction (Mittelbach et al., 2007; Rabosky, 2010; Rabosky et al., 2015), although substantial progress has been made on improving phylogenetic estimates (Pyron, 2014; Pyron & Burbrink, 2013; Rolland et al., 2014). The evidence on average supports both higher origination and lower extinction rates at low latitudes, in the past and currently, contributing to the LDG (see 2.3.4 Causes of species richness variation: Net diversification rates). An LDG framework should be able to explain the causes of latitudinal extinction and speciation biases, and their contribution to spatial diversity patterns.

Synthesis of principles

I will first discuss several aspects of net diversification broadly, to highlight some of the complexities in understanding net diversification, and how net diversification processes contribute to the LDG. Many aspects of net diversification remain poorly understood, and even the broadest aspects of continental species richness patterns, such as the limits of diversity, and the importance of diversity-dependence on net diversification remain contested (e.g., Harmon & Harrison, 2015; Rabosky & Hurlbert, 2015). The importance of adaptive and non-adaptive processes in diversification rates and patterns also remains uncertain (Hedges, Marin, Suleski, Paymer, & Kumar, 2015; Moen & Morlon, 2014).

Let us consider what we mean when we refer to net diversification rates, and how those rates relate to diversity patterns. The rate of change in species richness for an entire clade is one measure of net diversification that can test for latitudinal biases when examined in sister clades (Cardillo, 1999; Cardillo et al., 2005). These tests have recovered a pattern that

equal-aged clades have diversified more rapidly in the tropics, although an overall net diversification rate does not allow detailed investigation of the underlying mechanisms, which could relate to differences in speciation or extinction. The origination rate of species or genera is sometimes used as a measure of the speciation rate within a region, especially when considering the fossil record (Jablonski et al., 2006). In the case of marine bivalves, Jablonski et al. show that origination rates continue to be higher in the tropics than elsewhere during the last 11 MY, although such approaches also suffer from the limitation of not allowing any distinction between the influence of time on the accumulation of lineages, and the propensity for lineages to diverge and speciate. Because of this limitation, models of net diversification are frequently presented as the difference between per-lineage averages rates of speciation and extinction (e.g., Rabosky et al., 2015; Sepkoski, 1998). For non-equilibrium species richness, this approach should remove the influence of time. However, if we consider per-lineage rates in the context of LDG, such averages may not adequately capture net diversification dynamics. For example, given the latitudinal distribution in range sizes (Table 7), latitudinal variation in extinction risk may bias the retention of small-ranged species in the tropics. While these lineages contribute somewhat to species richness (Jetz & Rahbek, 2002), most probably have little bearing on future speciation unless they expand their ranges, as range size is an important determinant of the rate at which new lineages arise (Dynesius & Jansson, 2014). Vicariant speciation is more likely in a large population (Moen & Morlon, 2014), and mixing will be reduced as range size increases in limited dispersers, increasing population structure with all other things being equal. Therefore, small-ranged species, although they may have recently speciated, will have low future per-lineage speciation rates. In principle, this could lower tropical per-lineage speciation rates, even in circumstances where large-ranged tropical species have a higher speciation rate than temperate species with the same ranges. Unfortunately, this is difficult to test, as it requires the reconstruction of ancestral species' ranges. The issue of determining an appropriate measure of net diversification rate is highlighted when considering the contemporary speciation rate for New World birds, for which all regions and clades contain a wide range of rates (Rabosky et al., 2015). Essentially the same finding has been previously made for palaeontological estimates of speciation rates across a wide range of taxa (Sepkoski, 1998). Rabosky et al. conclude that

recent speciation rates are similar across latitudes, which could be caused by diversity-dependent effects, or tropical niche conservatism, although they consider the latter unlikely. This result is also consistent with tropical taxon undersplitting (Tobias et al., 2008), because this specifically biases against young species rather than species richness patterns in general (contra Rabosky et al., 2015), or protracted tropical speciation (Moen & Morlon, 2014), both of which slow down recent apparent speciation rates. Whether artefactual or genuinely reflectively of contemporary speciation rates, Rabosky et al.'s finding highlights the need to more fully understand the distribution of speciation rates, and how these contribute to overall net diversification.

There is also a second issue with our understanding of net diversification. Net diversification is usually compartmentalised into separate speciation and extinction processes. In doing so, speciation and extinction are sometimes viewed as competing explanations for spatial diversity patterns. This is epitomised by the long-running dichotomy between the tropics as a 'cradle' or a 'museum' of diversity (Stebbins, 1974). Within the cradle view of tropical diversity, emphasis has been placed on the importance of biotic interactions in the tropics, which could increase speciation rates through greater opportunities for specialisation. Within the museum view of tropical diversity, abiotic factors relating to climate could increase extinction rates in temperate zones (Dobzhansky, 1950; Schemske, 2002, 2009). While these views point to ways in which speciation and extinction are distinct, I will argue below that they are not wholly separate processes, a point which has been underappreciated in the literature.

Despite having their own contributing factors, speciation and extinction are linked because they share fundamental processes. To understand this perspective, it is necessary to consider the process of speciation. Speciation does not occur without the formation of genetic or behavioural barriers to reproduction. While speciation can be an instantaneous event such as by polyploidy, this occurs only in a minority of cases, and is considerably rarer in animals than in plants (Orr, 1990; Wood et al., 2009). Instead, the evolution of reproductive barriers typically relies on the survival of distinct lineages with sufficient geographic isolation in allopatry (Coyne & Orr, 2004), or ecological isolation in other

geographic arrangements of populations (Nosil, 2012). For speciation to occur, isolated lineages must not be extirpated by demographic fluctuations (i.e., the death of all individuals, herein 'population death'), or gene flow (i.e., genetic homogenisation). The risk of population death reduces with migration from the source population; however, migration also increases the risk of extirpation by genetic homogenisation. If we consider a simple example with a single locus and two alleles, in the absence of selection, the chance of homogenisation from migration at a diverged locus is proportional to the migration rate, because the migration rate controls the proportion of the source population allele in the divergent population, and following fundamental population genetics, the chance of extirpation by gene flow is equal to the proportion of the source population allele, because fixation probability and frequency are predicted to be equal (Kimura, 1983). Therefore the rescue affect from migration against one extirpation risk is approximately balanced on a first order approximation by an increase in another extirpation risk, although true patterns would be complicated by selection and recombination.

Given the situation of a completely isolated lineage with no migration from its source population, the sole extirpation risk is from population death. In the absence of migration, the population-genetic behaviour of this lineage would be indistinguishable from that of an equivalent lineage with full species status, assuming there are not average fitness differences between newly forming and already established lineages. Critically, the chance of survival over a given time period is identical for both lineages if they have equal population sizes, as it is underpinned by a population size-dependent extirpation/extinction risk (Harris & Pimm, 2008). From this, it is apparent that speciation and extinction rates are linked through sharing identical determinants of lineage persistence. Another way to consider this is to reject the existence of a hard boundary between subspecific lineages and species, which is an imprecise, if valuable, human construct (Harrison & Larson, 2014; Mallet, Beltran, Neukirchen, & Linares, 2007). Then, all lineages simply share a size-dependent extinction risk. Many of the newly arising lineages will still have a higher absolute extirpation rate than the rate of extinction for full species, but this is because they are small (Rosenblum et al., 2012). If the persistence of diverged lineages is an important and underappreciated component of speciation as has been

proposed (Allmon, 1992; Dynesius & Jansson, 2014; Rosenblum et al., 2012), then speciation and extinction rates are also strongly linked.

The perspective I am developing here can be clarified by taking a system approach to the processes of net diversification. Much emphasis in the speciation literature has been on the importance of within-species lineage splitting because this controls the rate at which lineages that may eventually speciate arise. This rate of lineage formation—which we could consider to be lineage flux—is important for the speciation rate, provided that speciation is limited by the rate of arising lineages (but see Rosenblum et al., 2012). However, regardless of whether speciation is limited in this way, we can expect that both fluxes and pools are basic considerations necessary for understanding the dynamics of such systems, in the same way that fluxes and pools are both needed to understand the dynamics of the carbon cycle.

What I denote as lineage flux here is affected by a range of species traits and environmental factors. For example, biogeographic phenomena such as orogeny and river formation through population ranges are likely to cause new lineages to arise, while species abilities to disperse will control propensity for vicariance under such circumstances, and subsequent lineage formation across those barriers. A key consideration is that lineage flux might not be strongly diversity dependent, particularly if there is a relationship between diversity and average range size, as seen across latitudinal gradients. Biogeographic barrier formation can cause multifurcation in widespread species (Ribas et al., 2012), while such barriers are less likely to cause any lineage splitting in small-ranged species (Moen & Morlon, 2014). Thus, in addition to the limited circumstances under which lineage flux might affect speciation, there is little reason to believe that lineage flux should be generally biased towards a higher tropical rate. Therefore, there is little reason to believe this component of the wider net diversification process is a major contributor to the LDG. In practice, true lineage flux would be difficult to measure if most newly arising lineages were quickly extirpated (Rosenblum et al., 2012), as those lineages would lack obvious genetic structure, or behavioural differences from their source populations. For lineages arising through small founding populations, extirpation is likely

unless they rapidly expand, given the expected fitness reductions in very small populations (Lynch et al., 1995). The risk of extinction for vertebrates over 40 generations only falls below 1% with approximately 7000 adults (Reed, O'Grady, Brook, Ballou, & Frankham, 2003), while populations of 100 individuals face an almost 50% extirpation risk over 10 years (Traill, Brook, Frankham, & Bradshaw, 2010). The distribution of resident times in the lineage pool—again, therefore lineage persistence—influences the apparent lineage flux.

The factors that control the resident time in the lineage pool are those that affect either lineage persistence—in terms of survival (Allmon, 1992; Dynesius & Jansson, 2014) or continued isolation (Behm, Ives, & Boughman, 2010; Jocque et al., 2010; Salisbury et al., 2012)—or speciation duration, the time from initial lineage splitting until speciation is complete (Orr & Orr, 1996). These two factors interact to create the speciation rate (i.e., per newly arising lineage). The product of the lineage flux and speciation rate is the rate at which new species arise in the species pool, and can therefore also be considered species flux. Changes in either speciation duration or lineage persistence can affect the size of the lineage pool, and the speciation rate. However, when comparing regions for which both variables could vary considerably, it is noteworthy that the comparisons of a single variable may be uninformative about the speciation rate, because the speciation rate depends on both speciation duration and lineage persistence. The evidence that these two factors that control lineage pool resident time vary across latitude has been discussed sporadically throughout this thesis, and will be revisited below.

A decrease in speciation duration increases species flux, and depletes the lineage pool. There is some evidence that recent speciation has been more rapid in temperate regions through several mechanisms (Weir & Schluter, 2004, 2007), and evidence of more rapidly diverging traits in contemporary temperate lineages (Lawson & Weir, 2014; Martin et al., 2010; Weir & Wheatcroft, 2011), although this may be restricted to the Pleistocene. Dynesius and Jansson (2014) outline a number of factors that would affect speciation speed in divergent lineages, which can be summarised as: dispersal, sexual selection, biotic interactions, specialisation, population size and size variability, environmental gradients,

geographic barriers, mutation rate, and climate variability. Some of these factors vary on average with latitude (e.g., population size, biotic interactions, and climate variability), although they do not collectively indicate a singular bias towards shorter absolute speciation duration in the tropics. Others are more varied, or inconsistent between lineages (e.g., mutation rate, geographic barriers).

An increase in lineage persistence increases species flux, and increases the lineage pool. We would expect to see greater lineage persistence in stable climates because an invariant environment should induce less hard abiotic selection than an environment that fluctuates close to species' climate tolerances (Schemske, 2002). Lineages can evolve strategies that reduce the impact of long-term climate instability, such as increased dispersal (Dynesius & Jansson, 2000). However, such strategies cannot eliminate all climate-induced death, because even a perfectly adapted species must still experience purifying selection to maintain its adaptations. In any case, there is evidence that species are often affected negatively by climate instability. Lineages in stable climates exhibit deeper coalescence, and have greater genetic diversity, indicative of more persistent lineages (Carnaval, Hickerson, Haddad, Rodrigues, & Moritz, 2009). While Carnaval et al. observed this for frogs in tropical refugial lineages, there is evidence that similar patterns occur across a wide range of taxa when tropical and temperate taxa are compared. Larger tropical lineage pools and longer subpopulation persistence in the tropics are supported by several lines of evidence discussed through this thesis. Compared to temperate lineages, tropical lineages on average have: a) higher intraspecific genetic diversity (Adams & Hadly, 2013), b) greater intraspecific genetic divergence (Eo, Wares, & Carroll, 2008; Martin & McKay, 2004), c) more subspecies (Martin & Tewksbury, 2008), and d) a wider range of apparent species ages (Chek, Austin, & Lougheed, 2003). One way that increased lineage persistence should manifest is as smaller minimum viable population sizes (MVPs). A latitudinal gradient in MVP has been poorly explored, although one study that investigated the relationship between latitude and MVP found no relationship (Reed et al., 2003). However, this may be caused by the short-term definition of MVP used by Reed et al. for the purposes of conservation (99% survival over 40 generations), which is unlikely to have bearing on long-term diversity patterns. Given that many ecological factors might affect

MVPs, phylogenetically independent contrasts of MVP size would be a more robust approach to understanding latitudinal MVP variation. As such, MVPs relevant to net diversification processes remain unexplored. Nevertheless, the characteristics of tropical lineages that are better understood are generally consistent with increased lineage persistence. Lineage persistence, in turn, is correlated with longer-term climate stability.

Of the three components to speciation—the rate of lineage splitting, lineage persistence, and speciation duration—lineage persistence is the only the component with a latitudinal pattern that is consistent with greater tropical net diversification. There has been emerging evidence that lineage persistence might generally be key in understanding speciation (Dynesius & Jansson, 2014; Rosenblum et al., 2012). In addition to speciation, I have outlined a case for linking lineage persistence to both detectable lineage flux, and also to extinction rates. As such, several lines of evidence suggest that lineage persistence might be a critical component of spatial diversity patterns through its effect on net diversification.

As outlined above, lineage persistence is mechanistically linked to climate stability. Climate stability has not been widely accepted as a cause of tropical and temperate species richness disparities, with role of refugia debated heavily, and the importance of such refugia probably varying with latitude (Willis & Whittaker, 2000). Climate stability and climate harshness both relate to the physiological tolerances of organisms (2.3.5 Prominent hypotheses for the latitudinal diversity gradient: Climate tolerance). They are conceptually distinct because unstable climates induce periodic, perhaps unpredictable variation in climate that induces selection, while harsh climates either consistently induce selection, or do so on regular cycles. However, in practice, climate harshness is difficult to separate from climate stability because they co-occur at the large scales that affect net diversification. Environments with strong temperature seasonality (harshness) also experience the strong effects of long-term and short-term climate extremes (instability), such as Milankovitch cycles (Dynesius & Jansson, 2000), and severe, mortality-inducing weather events (Bumpus, 1899). Temperature seasonality is linked to the strength of the LDG in the fossil record (Archibald et al., 2010; Mannion et al., 2012; Mannion et al., 2014),

and in the present study to molecular evolutionary patterns (Table 16), indicating a wide range of temporal scales at which seasonality is linked to diversity processes.

I propose that the limits imposed by climate tolerances cause the conditions that ultimately result in the LDG. The accumulation of diversity requires long periods of time in which lineages remain unbroken. To summarise the above, climate tolerances play several direct and indirect roles in this. Firstly, adaptations to seasonality must be maintained by selection, thus reducing the ratio of census to effective population sizes, which are further reduced by extreme mortality inducing events. With lowered effective population sizes, lineages therefore are more vulnerable to extirpation, and less capable of future adaptation. Lowered lineage persistence reduces the rate at which new species are formed, and increases extinction rates. Selection for increased dispersal improves survival in seasonal climates, which increases population mixing, decreasing population structure, and reducing the chance that newly arising lineages remain isolated. Seasonal disruptions to energy supply increase the reliance on short growing seasons, while energy rich regions are buffered against similar effects by year-round effects (also see 2.3.5 Prominent hypotheses for the latitudinal diversity gradient: Species–energy theory). These influences are summarised above (Figure 1).

The difference between climate tolerances and niche conservatism as explanations for the LDG lies in whether species can ever fully adapt to seasonal, temperate environments. Although caution must be applied when invoking the concept of climate harshness, there is a clear case for viewing climate harshness as more than a circular concept as has been suggested in the past (e.g., Rohde, 1992). All life has a requirement of liquid-phase water and energy, and the periodic absence of these has consequences for life. While adaptation may reduce the effects of such periodic absences, there are limits on such adaptation on two accounts. Firstly, while adaptation can reduce the negative population impact of climate harshness, it does not follow that evolved strategies eliminate all of the selective costs of climate harshness, such that well-adapted temperate and tropical individuals experience the same climate-induced risk of death in their own environment. Evolved strategies such as hibernation in mammals are not simply acquired traits, but involve

substantial trade-offs in terms of physiological costs from metabolic depression (Humphries, Thomas, & Kramer, 2003). Seasonal energy limitations are clearly the cause of such trade-offs, and therefore temperature seasonality is a cause of climate harshness through both physiological tolerance ranges and resource supply. Secondly, even if organisms were hypothetically able to adapt equally well to temperate as tropical conditions such that seasonal coldness and resource limitations did not induce additional mortality in the individuals bearing those adaptations, the adaptations themselves must be maintained by purifying selection. An additional consideration is that because all species interact with and rely on other species, the adaptation of any given species is limited by the co-adaptation of other species. The population stability of a consumer is reliant on the biotic resources available to it, and poorly adapted prey will not provide a consumer with a long-term resource, affecting the stability of food chains (Hutchinson, 1959). Therefore, there are always climate harshness costs that must be borne by populations. These limitations should result in reduced nucleotide diversity at the same level of genetic divergence, and lowered ω, which is precisely what is observed for New World birds (Figures 25 & 30c respectively).

5.6 Limitations of study

With limited resources, time, and existing knowledge, most studies require trade-offs that compromise one or more aspects of the work, and are not able to be conducted on the scale that researchers would ideally like. While consideration has gone into minimising the impacts of compromises, there are a number of resulting limitations in the present work that should be acknowledged. I will discuss these limitations, with rationales for the decisions made.

5.6.1 Effective population size approximation

Avian range sizes are used to approximate N_e, but they are removed from the relevant population-genetic quantity they estimate by two degrees. Because range sizes can evolve rapidly (see 2.4.5 Factors affecting species' range sizes), and differently from many traits that have direct genetic contributors, its contemporary size is an estimate of N_c. The estimate of an absolute N_c requires a range-wide average population density, while a relative population size (i.e., for as a contrast between sister species) assumes equivalent range-wide population densities. Secondly, N_e is almost always lower than N_c, although the ratio is not predictable without knowledge of population size fluctuations. However, the time to coalescence and expected diversity at a neutral locus can be estimated as the long-term harmonic mean of the population size (Charlesworth, 2009). The effects of selection and other factors that cause deviations from an ideal Fisher-Wright population, will have further effects on estimates on N_e.

Possible approaches to addressing adequate N_e estimation included a range size approach, as used here, estimating N_e independently from population-genetic expectations, using species for which direct estimates of population size are available, or using other indirect population size estimators (see 2.4.3 Empirical tests of the *Ne*—molecular-rates relationship). For practical reasons (see below 5.6.2 Restriction to mitochondrial housekeeping genes) population-genetic estimators could not be used in a study using large numbers of species for which each would need N_e estimation. If feasible, however, such an approach would have benefits over or alongside the current approach. Restricting to species that have direct estimates of population size would address the additional error

introduced from estimating population size from range size, on the seemingly reasonable assumption that N_c is a better estimator of N_e than is range size. However, any such benefit is only valid for sister species, or for species in which it can otherwise be established that current N_c differences are representative of N_c differences since divergence. Further few sister species comparisons in a dataset restricted to species with population size estimates are possible. In the final dataset in the present work (N = 207 sister species) there are 29 sister species for which both species have population size estimates. However, for none of these comparisons is the data quality for both species better than "poor". BirdLife International defines poor quality population size estimates as those made with qualitative information, and any quantitative data used is considered to be unreliable or unrepresentative (see http://www.birdlife.org/datazone/info/spcquality). Such estimates are not better than range size as relative population size estimators between sister species (OLS regression of ω–population size relationship, N = 29, slope = -0.124, P = 0.08), although the negative slope of the relationship demonstrates a common pattern. A range of other indirect estimates of N_e have been used in the past, such as landmass area, life habit, and body size. As such, studies have compared island and mainland taxa (Johnson & Seger, 2001), free-living versus endosymbiotic taxa (Woolfit & Bromham, 2003), and large and small taxa (Popadin et al., 2013). All of these approaches almost certainly do contrast taxa of different N_e, however all of them are potentially confounded by other factors that concurrently affect the strength of natural selection. A different scale of study would allow robust N_e estimates from patterns of linkage disequilibrium (Waples & Do, 2010), or over the longer term by using silent site diversity (Charlesworth, 2009). However, given the drawbacks with the other approaches to estimating N_e, I used range size, as a relative estimator of population size. This allows for the greatest number of comparisons to be made between true sister species. Range size was only used to approximate N_e after establishing a strong relationship between range size and population size exists.

5.6.2 Restriction to mitochondrial housekeeping genes

Because of the limitations of public sequence databases, and the infeasibility of multi-locus nuclear sequencing of the 1200 species analysed in the present project, it was only possible to estimate molecular evolutionary rates using mtDNA for a large number of sister species

sampled from phylogenies with a high density of taxon sampling. Animal nuclear and mitochondrial genomes evolve with different mutation rates (Brown et al., 1979), are transmitted differently between generations (Hill et al., 2014), are subject to vastly different levels of recombination (Macaulay, Richards, & Sykes, 1999), coalesce on different timescales (Charlesworth, 2009), and have different average N_e (Cooper, Burrus, Ji, Hahn, & Montooth, 2015). While coding mtDNA evolves under strong purifying selection, with evidence of occasional positive selection (Meiklejohn et al., 2007), patterns of selection are more varied across the nuclear genome. Therefore, we cannot generalise about molecular evolution from mitochondrial evolution, and a combination of mt and nuclear genes would have been preferred in the current study if feasible.

The high degree of taxonomic resolution across short timescales achieved with mtDNA between species in the absence of introgression is a convenience to researchers in the face of limited data. However, the higher density in the mt genome of informative sites at short genetic distances can be offset by sequencing more of the nuclear genome. Similarly, given a limited amount of sequence for comparison, the shallow coalescence of mtDNA is advantageous for these types of comparative studies. The further that the time to gene coalescence pre-dates speciation in sister species, the greater the proportion of observable divergence that relates to their usually unknown, pre-speciation ecology. However, any problems of this nature can be resolved in the future with sufficient data. Modern genomics permits detailed re-creation of N_e through time using Markovian coalescent models (Li & Durbin, 2011), which, in principle, would enable molecular rates–N_e correlations on multiple temporal scales through time in a lineage, although not using ω (Kryazhimskiy & Plotkin, 2008).

5.6.3 Taxonomic breadth

It is widely recognised that while vertebrate evolution is of substantial interest to us as vertebrates, and is facilitated by the relatively extensive knowledge of vertebrate ecology, the evolutionary patterns inferred in vertebrates, and perhaps multicellular eukaryotes in general, is of limited value to the wider tree of life. The complexity of eukaryotic genomes is theorised to result from different selection thresholds for purging slightly deleterious

mutations from populations, allowing fundamentally different genomic structures (Lynch, 2007b; Lynch et al., 2011). As bird and mammal diversification appear to differ in their relationship to substitution rates (Goldie et al., 2011; Lanfear et al., 2010), results presented here should not be extrapolated to mammals, nor wider beyond the vertebrates without further corroboration.

5.6.4 Sister species comparisons

The use of sister taxa in contrasts of molecular evolutionary rates and ecological traits controls for phylogenetic non-independence (Felsenstein, 1985), thereby removing autocorrelation from phylogenetic imbalances. Trait differences between sister taxa reflect changes since lineage divergence, removing the effect of their shared history that is present in non-independent comparisons. Provided genes and lineages coalesce similarly, then correspondence can be sought between genetic and other changes between sister taxa. These characteristics of independent sister-taxon contrasts mean that both type 1 and type 2 errors should be reduced in such analyses.

In the case where contrasted taxa are sister species, some accuracy is sacrificed because of two factors. Firstly, amongst close comparisons, variance in contrasts is higher because genetic distances are shorter and, for contrasted traits evolving under Brownian motion, trait differences are smaller (Welch & Waxman, 2008). Secondly, the error introduced by incongruences between gene and species histories has a larger influence in sister species contrasts because the size of the error proportionately decreases as genetic distance increases.

Because most species have persistence times that fall within hundreds of thousands to a few million years, this timeframe places a window on the ecological phenomena experienced by diverging sister species lineages. In particular, some species may be disproportionately shaped by Pleistocene glaciations if they occur at high latitudes. It has been argued that the Pleistocene has shaped recent diversification patterns in birds and mammals, and—most importantly—that those present patterns may differ from those that shaped global diversity over deeper time (Weir & Schluter, 2007).

North temperate species have been the focus of intense, long-term scientific scrutiny, and indeed our concept of species derives from these well-studied groups. Sexual selection appears to have driven rapid morphological evolution in north temperate species (Martin et al., 2010; Weir & Wheatcroft, 2011), creating divergences that allow for distinctions between even closely related species. It has become increasingly apparent that tropical bird species can lack similar degrees of morphological distinctiveness. Combined with study bias, there is evidently substantial cryptic tropical diversity yet to be formally recognised (Naka, 2010; Tobias et al., 2008). If cryptic tropical diversity had a major effect on the results presented here, then we could expect there to be a correlation between branch length and latitude, with longer tropical branches. However, there is no evidence of a strong correlation of this nature (Figure 45), indicating this effect has not caused the patterns presented in this thesis.

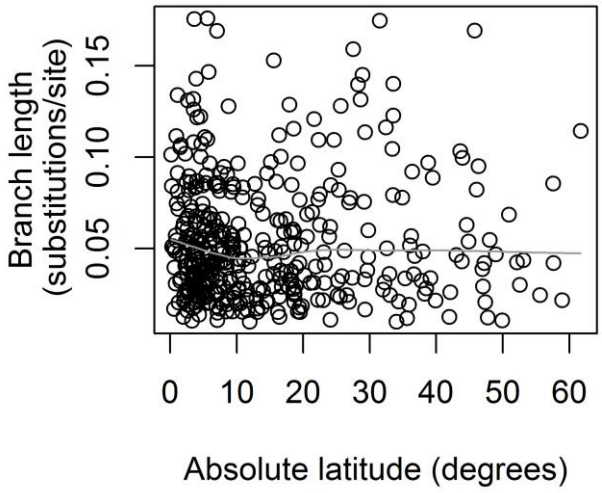

Figure 45. Relationship between the number of substitutions per site and absolute latitude for all species in the final dataset ($N = 404$). Grey line is a LOESS fit.

Ultimately, a major aim in the present work was to establish the effect of N_e on rates of molecular evolution as determined by population size variation. While acknowledging the limitations of sister species comparisons, the design decisions that led to the present study and its use of range size as a population size estimator necessitated sister species contrasts, rather than greater distance independent contrasts.

5.7 Directions for future study

Studies of molecular rate variation have historically provided inconsistent results. The present study offers a range of methods considerations to improve power to detect patterns, some of which are exploratory and need further investigation. In addition to the need for investigation with a wider range of taxa and genes, a range of potentially valuable future study directions have arisen from this work, and are highlighted below.

5.7.1 Understanding mt mutation rate variation

The large variance in mt mutation rates between sister species was not strongly linked to any of the studied variables in the present study. The mutation rate is known to be linked to several variables that were not a part of the present work, although it is as yet unestablished how much variation can be explained by these traits. This presents a problem: is mt mutation rate variation a nuisance parameter in studies such as the present work, or is it simply poorly understood? At least one possibility is that mutation rates are high and more variable in the mt than the nuclear genome because of weaker selection, paralleling the larger and more variable genome sizes in multicellular versus unicellular organisms, the bulk of which is intronic or intergenic (e.g., Lynch, 2006). It is worth noting that nuclear DNA also has variable mutation rates, including considerable variation across the genome (Hodgkinson & Eyre-Walker, 2011).

5.7.2 Better approximations of N_e

As species become more intensively sequenced, it will also become possible to make molecular estimates of N_e on comparably sized datasets, rather than relying on N_e surrogates. Analyses based on such estimates would benefit from being substantially more direct, and less susceptible to distortions of habitat, range, and population that have occurred evolutionarily recently in the Anthropocene. Methods for estimating N_e through time include Bayesian skyline plots (Drummond, Rambaut, Shapiro, & Pybus, 2005) Multiple genome sequences across a range of human populations have allowed detailed re-creations of past human N_e in different lineages (Li & Durbin, 2011). Because sufficient data allows the concurrent estimation of lineage-specific N_e and the timing of divergence

between populations through recombination, it may be possible to correlate evolutionary rates through time with N_e in natural populations.

5.7.3 Investigate more explicit links between multiple diversity levels

The LDG encompasses greater genetic, to subspecific, and species diversity, and I have argued that these levels of diversity are causally connected in the generation of the LDG. Formally establishing these connections requires additional evidence than is currently unavailable. Patterns in newly radiating lineages may be one avenue to understand these connections, particularly in comparison to other clades, such as clades in which past radiations have happened. The radiations of speciose clades should show genetic signatures in diversity and divergence consistent with lineage persistence. Spatial patterns may also be evident in clades than span a wide latitudinal range. Further, clades that do not exhibit a typical LDG should show spatially contrasting patterns.

5.7.4 Latitudinal variation in minimum viable populations

There is currently very little understanding of the extent to which minimum viable population sizes (MVPs) contribute to the LDG. In theory, differences in MVPs between regions would lead to differences in lineage persistence because of shifts in extinction risk across population size classes. Better understanding of MVPs will be difficult to gain empirically. However, some experimental work with bacteria could be undertaken to determine the effects of different conditions on MVPs. This area of investigation would also benefit from modelling that explicitly incorporates latitudinal variations in climate. This would improve the applicability of population viability analyses to the LDG.

5.7.5 Latitudinal variation in population structure and dynamics

Despite the trend of increased genetic diversity and subspecies numbers, tropical population densities must be lower on average than extratropical ones—a factor that should result in lower genetic diversity, and reduced chances of subspecies formation, with all else being equal. Hence, all else is not equal, and we must look to explanations consistent with all of these observations. If MVPs genuinely do not vary with latitude as has been suggested (Reed et al., 2003), then we need to develop a better understanding of

lineage persistence across latitudes, particularly given the relatively poor dispersal abilities of tropical species (Jocque et al., 2010; Salisbury et al., 2012), which should reduce population viability.

6 Conclusion

Both plant and animal diversity is related to NPP, although the relationship is only strong at large scales, and is stronger in plants than in animals. Instead, temperature seasonality appears a stronger driver of animal diversity. While being a less important driver of plant diversity, there is evidently turnover in floras in regions where winter freezing occurs. Both plant and animal diversity are themselves strongly correlated, although the relationship is different between regions where winter freezing occurs, and regions where it does not.

Temperature seasonality appears to play an important role in determining an array of the features of avian diversity. At the population-genetic scale, seasonality correlates with ω in mtDNA, indicating that purifying selection is weaker, and genetic diversity is greater, in species found in thermally consistent environments. Temperature seasonality also correlates with current phylogenetic diversity and species richness of all vertebrates, matching long-term patterns of diversity in the fossil record.

While the relaxation of purifying selection is relatively easy to detect in mtDNA, patterns in the overall substitution rate are weaker, and are obscured by substantial mutation rate variation. This problem may be especially acute for close comparisons, such as sister species, for which there may have been a short-term acceleration of mutation in one lineage. For some questions, the use of more distant comparisons may be an appropriate solution, while for other questions, other measures may be needed to address noise from such variation. Because ω controls for this effect to some extent, it may provide a better indication of molecular rate variation when it is desirable to remove nuisance mutational variation.

With the rapid expansion of molecular data, there will be excellent scope to expand beyond mtDNA comparisons even for proximate comparisons such as those undertaken

here. With genome sequencing planned for all recognised bird species, large-scale reappraisal of these patterns will become possible without sacrificing phylogenetic proximity in contrasts. Re-examining these questions using nuclear DNA will vastly improve our understanding of the processes that shape global diversity.

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Appendices

Appendix 1: Effect of data quality on plant PSRs in terrestrial ecoregions

The impact of data quality on the plant PSRs was evaluated to determine if all of the four tiers of data quality in Kier et al. (2005) were comparable. Across the ecoregion size classes described in text, PSRs were explored first in the lower two quality categories (Figure 46) and then the analysis was repeated in the upper two quality categories (Figure 47). It is evident that there is a minor difference between the upper and lower qualities categories, as PSRs are universally stronger in the upper quality category. However, the difference reduces from smaller to larger ecoregion size classes. In the largest ecoregions, the difference is minimal (ΔR^2 = 5.2%). Given that the largest ecoregions also contain the strongest PSRs, the cost in loss of dataset size was considered greater than the minimal reduction in explanatory power, and all categories were retained.

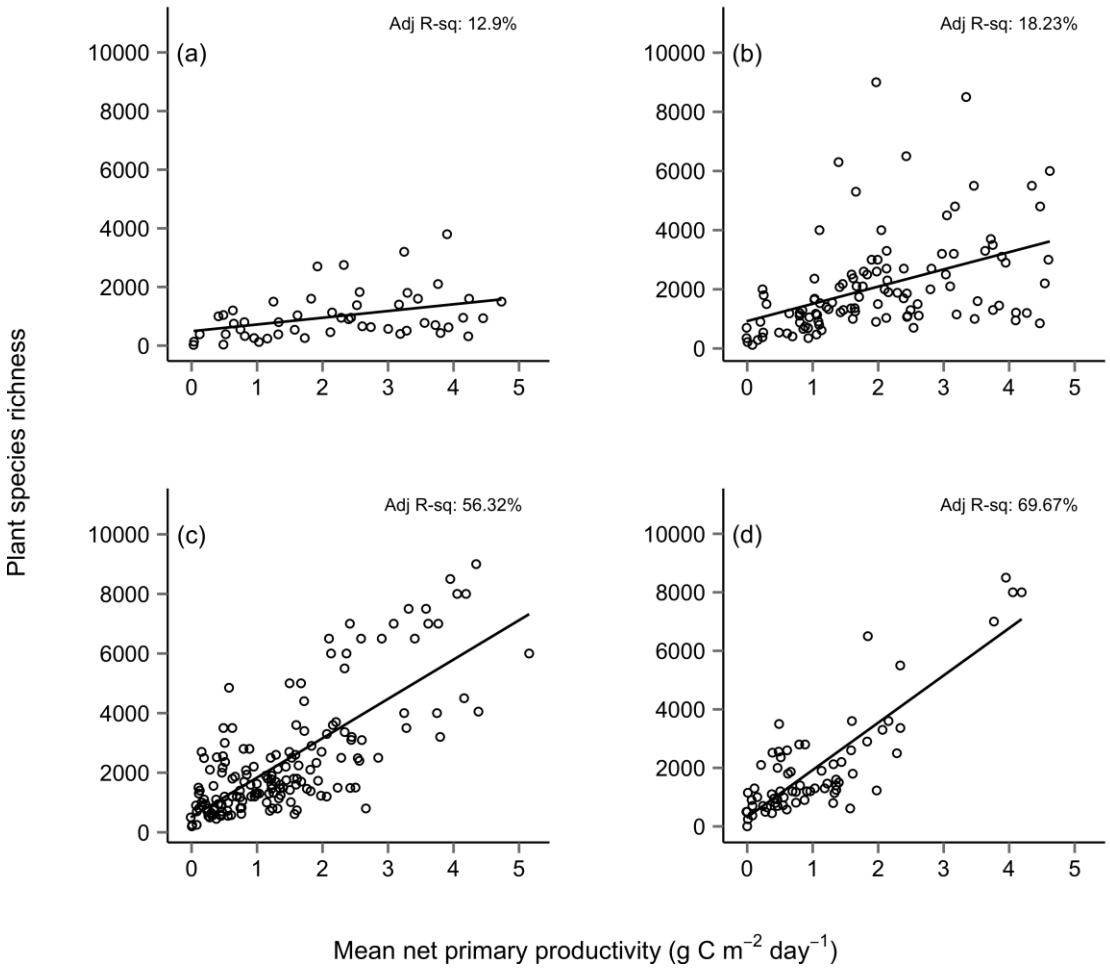

Figure 46. Relationship between modelled net primary productivity and plant species richness across sizes classes of global terrestrial ecoregions for ecoregions with upper quality estimates (quality categories 1 and 2 in Kier *et al*. 2005): (a) small ecoregions between 10^3 and 10^4 km^2 (N = 51); (b) medium ecoregions between 10^4 and 10^5 km^2 (N = 108); (c) large ecoregions between 10^5 and 10^6 km^2 (N = 160); and (d) the largest ecoregion subset (>$10^{5.5}$ km^2) (N = 70). Net primary productivity is 2013 mean estimates for MOD17 modelled NPP (NASA 2014).

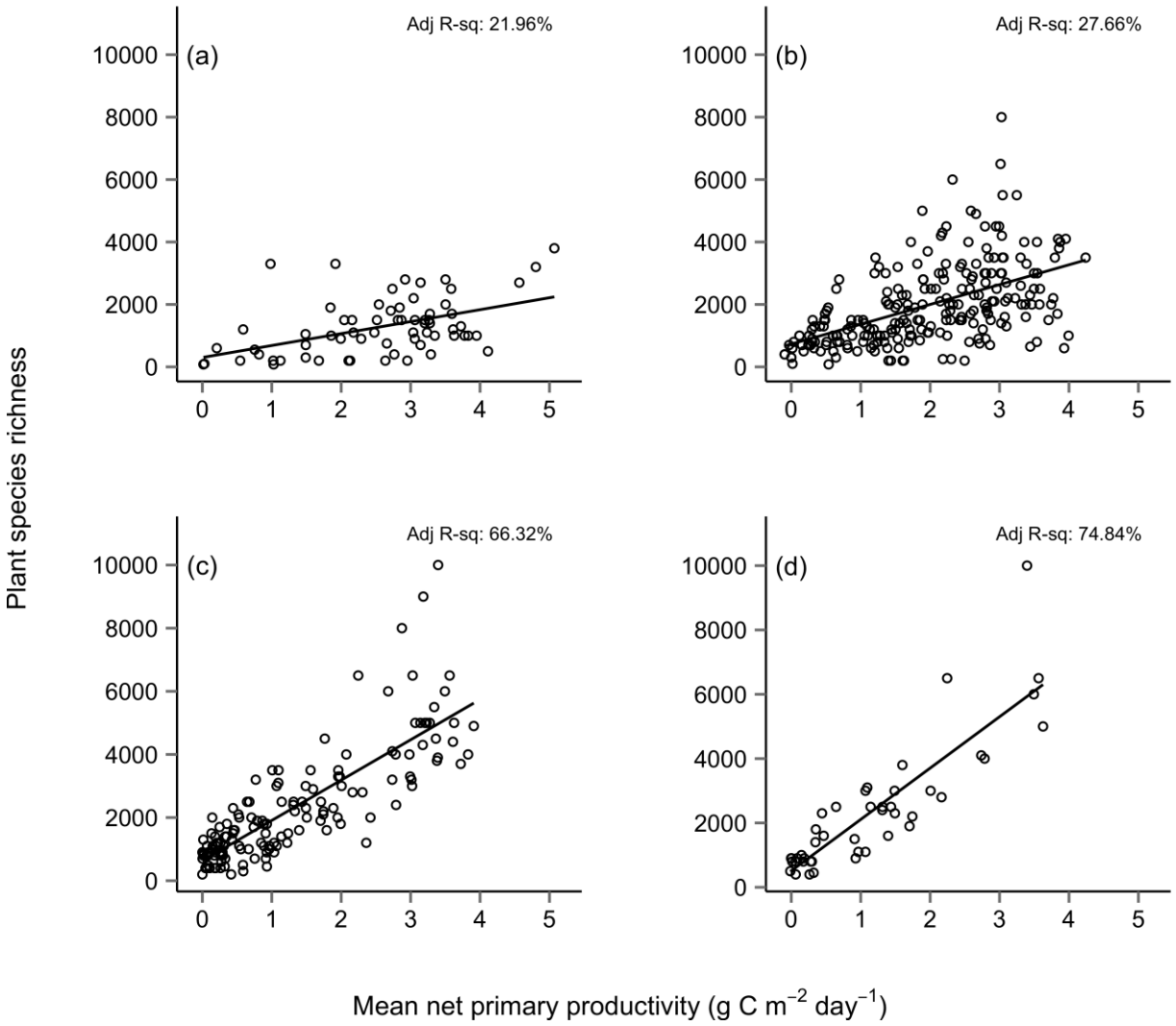

Figure 47. Relationship between modelled net primary productivity and plant species richness across sizes classes of global terrestrial ecoregions for ecoregions with lower quality estimates (quality categories 3 and 4 in Kier *et al*. 2005): (a) small ecoregions between 10^3 and 10^4 km^2 (N = 66); (b) medium ecoregions between 10^4 and 10^5 km^2 (N = 231); (c) large ecoregions between 10^5 and 10^6 km^2 (N = 142); and (d) the largest ecoregion subset (>$10^{5.5}$ km^2) (N = 45). Net primary productivity is 2013 mean estimates for MOD17 modelled NPP (NASA 2014).

Appendix 2: Data quality and outlier analyses for range size–population size study

While several outliers had large studentised residuals (>2), only one of these outliers also had large leverage. Overall, two points show noticeably elevated leverage, however the magnitude of this leverage is substantially greater for the data point with a large residual value (row 86; *Cranioleuca gutturata–Thripophaga cherriei*).

After applying filtering to the dataset on the basis of genetic distance (see 3.3.3 Dataset quality controls) an additional check was performed to determine if there was a lack of data in any comparisons—measured as the number of non-ambiguous bases compared in each sister species contrast—to stably estimate the substitution rate contrast. There is no 0rate, and this was similarly the case in the raw dataset before filtering was performed (Figure 49).

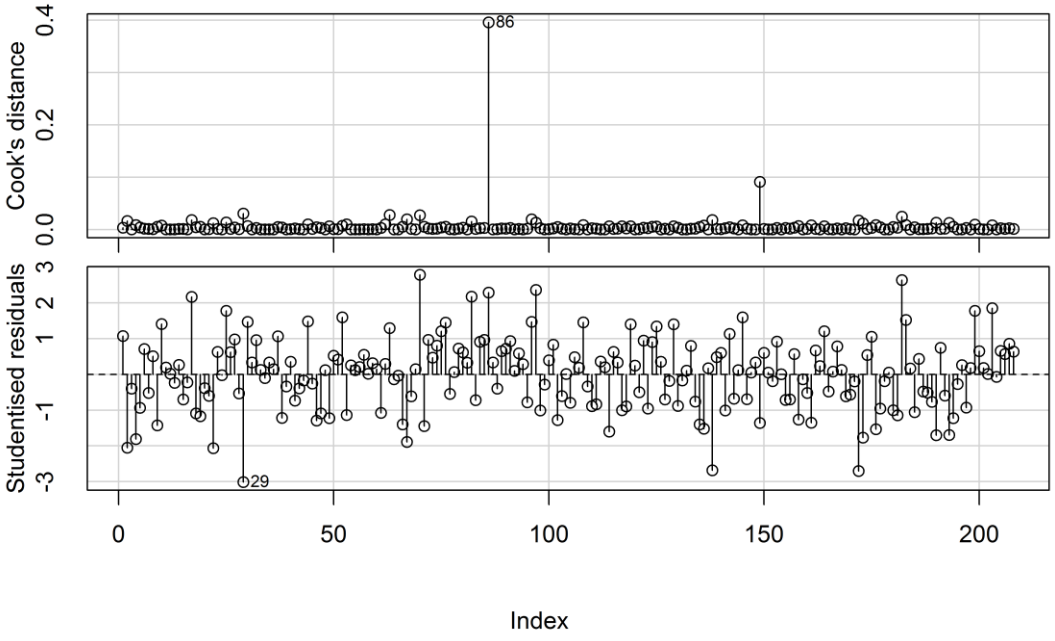

Figure 48. Two analyses for outliers. Cook's distance detects leverage of data points (above), while studentised residuals show the standardised magnitude of deviation from predicted values.

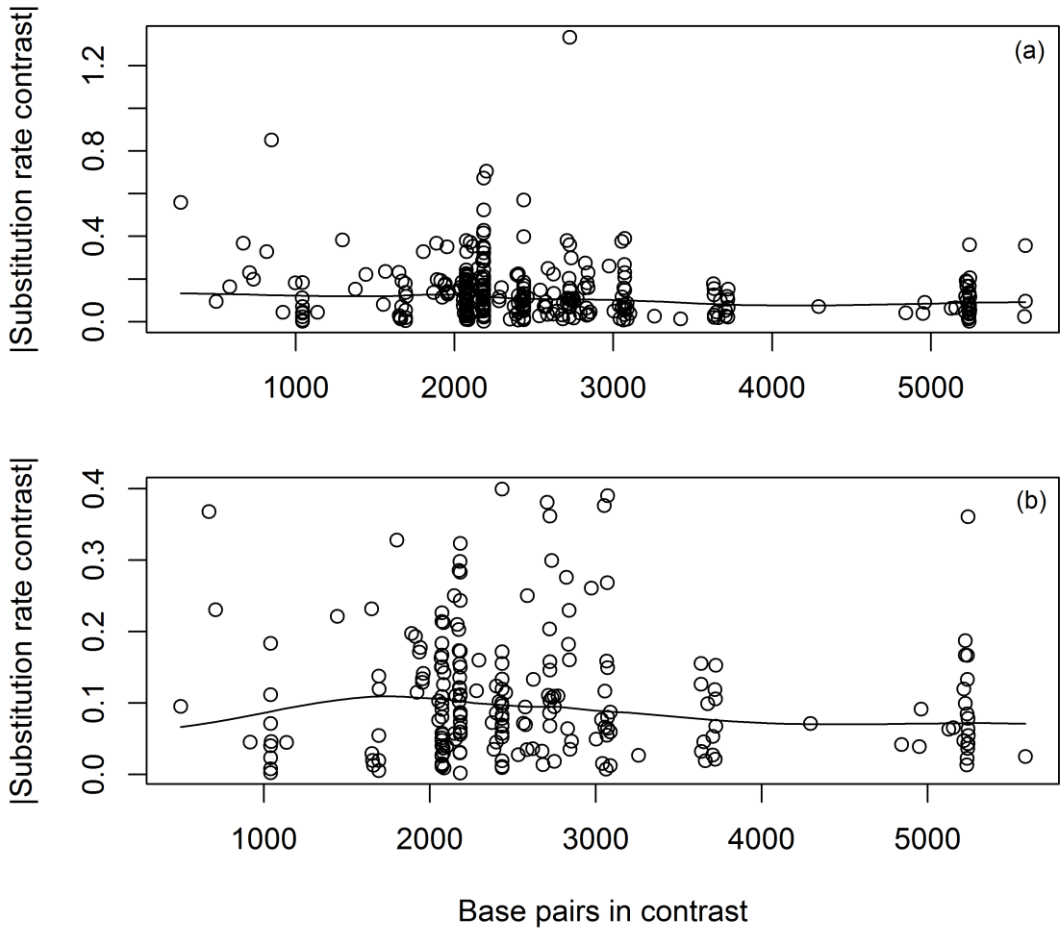

Figure 49. Influence of number of bases on variance in the substitution rate in (a) the raw dataset ($N = 300$ contrasts) and (b) the final dataset ($N = 207$ contrasts).