Testing the link between the latitudinal gradient in species richness and rates of molecular evolution

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Abstract

Numerous hypotheses have been proposed to explain latitudinal gradients in species richness, but all are subject to ongoing debate. Here we examine Rohde's (1978, 1992) hypothesis, which proposes that climatic conditions at low latitudes lead to elevated rates of speciation. This hypothesis predicts that rates of molecular evolution should increase towards lower latitudes, but this prediction has never been tested. We discuss potential links between rates of molecular evolution and latitudinal diversity gradients, and present the first test of latitudinal variation in rates of molecular evolution. Using 45 phylogenetically independent, latitudinally separated pairs of bird species and higher taxa, we compare rates of evolution of two mitochondrial genes and DNA–DNA hybridization distances. We find no support for an effect of latitude on rate of molecular evolution. This result casts doubt on the generality of a key component of Rohde's hypothesis linking climate and speciation.

Introduction

The latitudinal diversity gradient – the increase in species richness from high latitudes towards the equator – is one of the most ubiquitous patterns in nature, having been demonstrated for a wide range of taxonomic groups (Fischer, 1960; Pianka, 1966; Rohde, 1992; Rosenzweig, 1995; Gaston, 2000; Willig, 2001). However, there is still much uncertainty and debate over the underlying causes of the latitudinal diversity gradient (e.g. Rohde, 1997; Rosenzweig & Sandlin, 1997; Willig, 2001). The major reason for this uncertainty is that although a large number of hypotheses have been put forward to explain the pattern, most remain inadequately tested.

One prominent hypothesis promoted by Rohde (1978, 1992), which we refer to here as the 'climate-speciation' hypothesis, proposes a link between climatic conditions in the tropics and higher speciation rates, via two causal pathways (Fig. 1). Both pathways lead to the prediction that rates of molecular evolution should increase from high latitudes towards the equator. The first path directly implicates rate of molecular evolution as a causal factor in the latitudinal diversity gradient. Rohde (1978, 1992) suggested that higher temperatures and increased levels of solar radiation at lower latitudes could have a direct mutagenic effect on DNA, raising the mutation rate of tropical species. Raised mutation rates could promote rapid speciation by speeding the accumulation of genetic differences, leading to more rapid reproductive isolation between populations (Rohde, 1978, 1992). Thus higher mutation rates at low latitudes could drive higher rates of speciation in the tropics.

The second pathway predicts an indirect link between molecular evolution rate and the latitudinal diversity gradient through variation in generation times. Higher mean temperatures at low latitudes could increase individual growth rates and shorten generation times, increasing the speed at which selection operates and elevating rates of speciation (Rohde, 1978, 1992: but see Fischer, 1960). As shorter generation times have been associated with faster molecular evolution (Sarich & Wilson, 1973; Gaut *et al.*, 1992; Martin & Palumbi, 1993; Mooers & Harvey, 1994; Bromham *et al.*, 1996; Muse & Gaut, 1997; Bromham, 2002), this pathway could also result in higher rates of molecular evolution towards lower latitudes.

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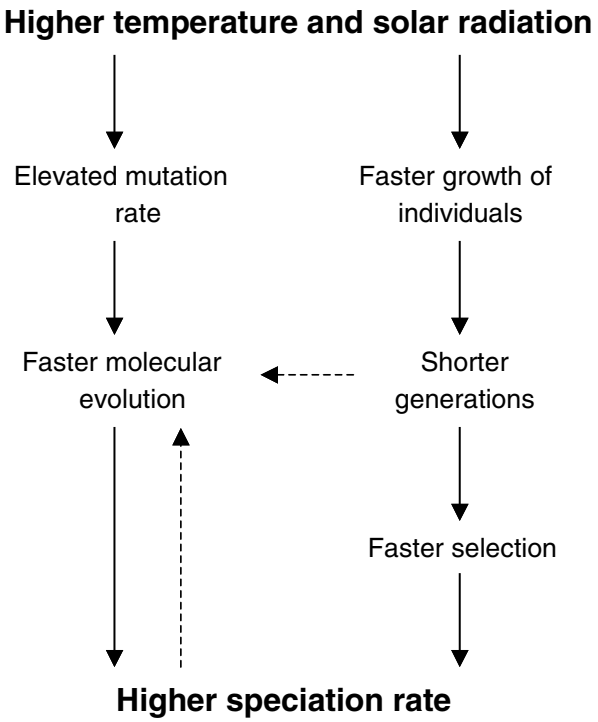

Fig. 1 The climate-speciation hypothesis (Rohde, 1978, 1992) links higher temperatures and solar radiation levels at low latitudes with faster speciation, via two causal pathways. First, higher temperatures may elevate mutation rates, and the resultant increase in rate of molecular evolution might increase rate of speciation. Secondly, higher temperatures and more solar radiation might increase growth rates, shortening generation turnover times and potentially speeding the process of speciation. Dashed lines indicate potential links between rates of molecular evolution, generation time and speciation rate which were not explicitly discussed by Rohde.

Therefore, the climate-speciation hypothesis generates the testable prediction that rates of molecular evolution should increase from high latitudes to the equator. Although Rohde did not explore the mechanisms that could cause this pattern, there are several potential links between latitude and rates of molecular evolution. Despite the widespread assumption of a 'molecular clock' (constant rate of molecular evolution in all lineages), it is clear that rates of molecular evolution can vary markedly between lineages. Furthermore, rate variation can be correlated with lineage traits such as life history or population processes (e.g. Martin & Palumbi, 1993; Barraclough *et al*., 1996). If these traits also vary with latitude, then we might expect rates of molecular evolution to vary latitudinally.

Body size is one trait which may be associated with both latitude and rates of molecular evolution. Average body size decreases from the poles to the equator for some taxa, consistent with Bergmann's Rule – the tendency for animals living in colder climates to have larger body size (e.g. Cushman *et al*., 1993; Blackburn & Gaston, 1996; Ashton *et al*. 2000). Body size is negatively associated with rate of molecular evolution in vertebrates: small species tend to have faster rates of molecular evolution than their larger bodied relatives (Martin & Palumbi, 1993; Mooers & Harvey, 1994; Bromham *et al*., 1996; Bromham, 2002). So if body size increases with latitude, and molecular evolution rate decreases with body size, it could generate a negative latitudinal gradient in the rate of molecular evolution.

Another potential link between latitude and the rate of molecular evolution is variation in diversification rate (the balance between speciation and extinction, measured as clade size), which has a central role in many explanations of the latitudinal diversity gradient (Rohde, 1992). Cardillo (1999) provided empirical evidence for an increase in diversification rate towards the equator in passerine birds and swallowtail butterflies. Evidence for a link between diversification rate and rate of molecular evolution has been demonstrated in studies which show an association between clade size and rate of molecular evolution for three genes in angiosperms (Barraclough *et al*., 1996; Barraclough & Savolainen, 2001) and for DNA hybridization data in passerine birds (Barraclough *et al*., 1998). If these results are indicative of a general relationship, then diversification rate could also provide a link between the latitudinal diversity gradient and rate of molecular evolution.

In this paper, we explore the potential links between rate of molecular evolution and the latitudinal diversity gradient. We also present the first comparative test of variation in rates of molecular evolution across latitudes, using both DNA sequences and DNA hybridization distances for phylogenetically independent pairs of bird species with latitudinally separated distributions. We use birds as a test group because they are a relatively large and geographically widespread taxon for which geographical distributions are well-known at the species level, and comparable DNA sequence data are available for a large number of species.

Methods

Phylogenetically independent contrasts

Our analysis was based on phylogenetically independent contrasts in molecular branch lengths between pairs of bird species inhabiting different latitudes. We used the pairs of species identified for previous study of latitudinal patterns (Cardillo, 2002) as the starting point for our analysis; this reduced the possibility of observer bias in selecting contrasts in which lower-latitude species have shorter branches. To generate these contrasts, published phylogenies and geographical range maps of species ranges were used to select the maximum possible number of phylogenetically independent pairs of species, where each pair met two criteria for latitudinal

separation (Cardillo, 2002). The criteria were: (1) the latitudinal midpoints of the two species' geographical ranges must be separated by at least 10°, regardless of hemisphere, and (2) the latitudinal overlap of geographical ranges of the two species must be no more than 25% of the latitudinal extent of either species range. Environmental conditions in a species' breeding range are commonly assumed to exert a stronger influence on a species than in its migratory range, so we follow previous studies (e.g. Blackburn & Gaston, 1996; Blackburn *et al.*, 1998) in defining the geographical range of a bird species as its breeding range.

Contrasts were checked against our reconstructed molecular phylogenies (see below) to confirm their phylogenetic independence, and omitted or re-chosen if necessary. We also analysed a set of contrasts based on DNA hybridization data, which included pairs of latitudinally separated species identified from published phylogenies and range maps, and higher-level contrasts (tribe and family level) taken from Cardillo (1999).

Estimation of branch lengths using sequence data

Starting with the set of contrasts identified in Cardillo (2002), we selected those for which DNA sequences were available on GenBank (www.ncbi.nlm.nih.gov) for both species in each pair, plus a close outgroup species. Sequences for two mitochondrial protein coding genes, cytochrome *b* (cyt*b*) and NADH subunit II (ND2), were available for a sufficient number of contrasts. Sequences were aligned by eye using the Se-Al sequence alignment editor (Rambaut, 1996). Molecular phylogenies were constructed from these alignments using maximum likelihood with an HKY + Γ substitution model (Hasegawa *et al.*, 1985; Yang, 1994), with parameters of the model (gamma shape parameter α and transition / transversion ratio ti / tv) estimated from the data. All phylogenetic analyses were conducted using PAUP* (Swofford, 1999). To reduce computational time, the phylogenies of nonpasserines and passerines were estimated separately for each of the two genes.

Branch lengths were estimated using two methods. First, branch lengths were estimated by maximum likelihood from the reconstructed phylogenies, using the HKY + Γ model with the values of ti / tv and α estimated for each phylogeny. To avoid bias because of node density effect, any nontarget taxa occurring in the ingroup were pruned from the tree, and branch length re-estimated by maximum likelihood (see Bromham, 2002). Secondly, branch lengths for each contrast were estimated using relative rates tests on HKY85 pairwise distance estimates. The relative rates test compares the distance between each pair of taxa and an outgroup in order to determine the relative amount of change in each lineage since their last common ancestor (Sarich & Wilson, 1973; Wu & Li, 1985; Li & Graur, 1991). Whereas low power for sequences typically used in molecular phylogenetics limits the usefulness of relative rates tests for establishing uniform rates for molecular clock studies (Scherer, 1989; Avise, 1994; Bromham *et al.*, 2000), relative rates tests provide a simple way of comparing relative branch lengths when seeking correlates of variation in the rate of molecular evolution (Mooers & Harvey, 1994; Bromham *et al.*, 1996).

We also tested for differences in the number of synonymous (silent) and nonsynonymous (replacement) substitutions for each contrast. These two classes of substitutions are influenced by different factors – for example, changes to mutation rate are expected to influence synonymous substitution rates, selection may influence nonsynonymous substitutions, and population size may influence both – and so can provide an informative comparison with analyses on total genetic branch lengths. Synonymous and nonsynonymous distances were estimated using phylogenetic analysis by maximum likelihood (PAML; Yang, 2001), and branch lengths calculated for each contrast using relative rates tests.

Many of the latitudinally separated contrasts from Cardillo (2002) were between very closely related pairs of bird species, and it is possible that deeper phylogenetic divergences and wider latitudinal separation are needed to detect significant differences in rates of molecular evolution. We therefore repeated the analysis on a new set of phylogenetically independent contrasts, chosen for wider latitudinal separation (20° between midpoints). In most cases this also resulted in contrasts with deeper phylogenetic divergences. We were unable to identify sufficient contrasts for ND2, so this analysis was carried out on a set of 16 contrasts for cyt*b* only (Table 2).

DNA hybridization data

In addition to the analyses based on sequence data, we examined rates of molecular evolution in 10 latitudinally separated contrasts with DNA hybridization data (Table 1). DNA hybridization distances represent the amount of difference in the entire single copy nuclear DNA of two genomes, and as such are a useful measure of genome-wide molecular change. Although the use of DNA-hybridization data to estimate phylogenies has been criticized, particularly the data from Sibley & Ahlquist (1990), this data has been profitably used to investigate lineage-specific rates of molecular evolution (Mooers & Harvey, 1994; Barraclough *et al.*, 1998), and in each of these cases, the results are consistent with similar studies using DNA sequence data (e.g. Bromham *et al.*, 1996; Barraclough & Savolainen, 2001; Bromham, 2002).

The 10 contrasts included five species-level contrasts identified from the primary literature (see Table 1), and five pairs of latitudinally separated higher taxa identified in a previous study (Cardillo, 1999). The higher-taxon contrasts were based on the phylogeny of Sibley & Ahlquist (1990), and identified by measuring the

Table 1 List of phylogenetically independent contrasts obtained from phylogenies reconstructed from cyt*b* and ND2 sequence data, and from published DNA hybridization matrices. The list includes DNA hybridization contrasts both at the species level and at tribe or family levels.

Lower latitude	Higher latitude	Outgroup	cyt*b*	ND2	DNA hyb.	Reference*
Synthliboramphus antiquus	*Synthliboramphus wumizusumi*	*Synthliboramphus hypoleucus*	*			1
Anas aucklandica	*Anas chlorotis*	*Anas sibilatrix*		*		2
Anas versicolor	*Anas puna*	*Anas querquedula*	*	*		2
Anas acuta	*Anas bahamensis*	*Anas castanea*	*	*		2
Anas rubripes	*Anas fulvigula*	*Anas acuta*	*	*		2
Anas castanea	*Anas bernieri*	*Anas bahamensis*	*	*		2
Anas penelope	*Anas sibilatrix*	*Anas bernieri*	*	*		2
Anas querquedula	*Anas hottentottus*	*Speculanas specularis*	*	*		2
Anas clypeata	*Anas smithii*	*Amazonetta brasiliensis*	*	*		2
Speculanas specularis	*Amazonetta brasiliensis*	*Anas clypeata*	*	*		2
Mycteria leucocephala	*Mycteria cinerea*	*Leptotilos javanicus*	*			3
Grus monachus	*Grus nigricollis*	*Grus rubicunda*	*			4
Grus vipio	*Grus rubicunda*	*Anthropoides virgo*	*			4
Anthropoides virgo	*Anthropoides paradisea*	*Buggeranus carunculatus*	*			4
Morus bassanus	*Papasula abbotti*	*Sula sula*	*			5
Metallura aeneocauda	*Metallura williami*	*Speculanas specularis*	*	*		6
Coccyzus americanus	*Coccyzus melacoryphus*	*Speculanas specularis*	*	*		7
Polioptila californica	*Polioptila albiloris*	*Toxostoma occelatum*	*	*		8
Euphagus carolinus	*Euphagus cyanocephalus*	*Agelaius tricolor*	*	*		9
Agelaius thilius	*Agelaius xanthopthalmus*	*Agelaius humeralis*	*	*		9
Pseudoleistes virescens	*Agelaius icterocephalus*	*Agelaius tricolor*	*	*		9
Agelaius tricolor	*Agelaius humeralis*	*Quiscalus major*	*	*		9
Quiscalus major	*Quiscalus lugubris*	*Agelaius tricolor*	*	*		9
Spizella arborea	*Spizella atrogularis*	*Pipilo ocai*	*			10
Pipilo chlororus	*Pipilo ocai*	*Pipilo albicollis*	*	*		11
Pipilo crissalis	*Pipilo albicollis*	*Agelaius tricolor*	*	*		11
Zonotrichia querula	*Zonotrichia capensis*	*Junco hyemalis*	*			12
Cranioleuca obsoleta	*Cranioleuca vulpina*	*Hellmayrea gularis*	*	*		13
Riparia riparia	*Riparia cincta*	*Psalidoprocne holomelas*	*			14
Toxostoma rufum	*Toxostoma longirostre*	*Oreoscopus montanus*	*	*		15
Toxostoma redivium	*Toxostoma occelatum*	*Polioptila albiloris*	*	*		15
Phylloscopus schwarzii	*Phylloscopus affinis*	*Phylloscopus humei*	*			16
Phylloscopus innornatus	*Phylloscopus humei*	*Phylloscopus affinis*	*			16
Phylloscopus borealis	*Phylloscopus magnirostris*	*Sylvia atricapilla*	*			16
Phylloscopus plumbeitarsus	*Phylloscopus trochiloides*	*Phylloscopus borealis*	*			16
Sylvia curruca	*Sylvia leucomelaena*	*Sylvia nisoria*			*	17
Eutoxeres aquila	*Lampornis clemenciae*	*Chaetura pelagica*			*	18
Mycteria leucocephala	*Mycteria cinerea*	*Mycteria americana*			*	3
Ciconia ciconia	*Ciconia maguari*	*Ciconia nigra*			*	3
Ardea herodias	*Tigrisoma lineatum*	*Plegadis falcinellus*			*	3
Ptilonorhynchidae	Menuridae	Maluridae			*	19
Oriolini	Artamini	Paradisaeini			*	19
Sturnini	Mimini	Muscicapinae			*	19
Carduelini + Drepanidini	Fringillini	Emberizini			*	19
Casuariini	Dromaiini	Apterygidae			*	19

*References: 1 (Friesen *et al*., 1996); 2 (Johnson & Sorenson, 1999); 3 (Slikas, 1997); 4 (Krajewski & Fetzner, 1994); 5 (Friesen & Anderson, 1997); 6 (Garcia-Moreno *et al*., 1999b); 7 (Johnson *et al*., 2000); 8 (Zink & Blackwell, 1998); 9 (Johnson & Lanyon, 1999); 10 (Zink & Dittmann, 1993); 11 (Zink *et al*., 1998); 12 (Zink *et al*., 1991); 13 (Garcia-Moreno *et al*., 1999a); 14 (Sheldon *et al*., 1999); 15 (Zink *et al*., 1999); 16 (Price *et al*., 1997); 17 (Blondel *et al*., 1996); 18 (Bleiweiss *et al*., 1997); 19 (Sibley & Ahlquist, 1990).

latitudinal midpoint of the geographical ranges of the species within each clade, and ensuring that the mean latitudinal midpoints of two clades were separated by at least 10° (Cardillo, 1999). We used relative rates tests on the ΔT_H distances provided in a number of sources (see Table 1) to measure rates of molecular evolution in each contrast.

Statistical analysis

We calculated contrasts in molecular branch lengths using the formula: 1 − (shorter branch length / longer branch length). The ratio was subtracted from one so that a larger value corresponds to a greater difference in branch length. We gave contrasts a positive sign if the

Lower latitude	Higher latitude	Outgroup
Synthliboramphus wumizusumi	*Synthliboramphus antiquus*	*Synthliboramphus hypoleucus*
Anas puna	*Anas querquedula*	*Amazonetta brasiliensis*
Anas fulvigula	*Anas penelope*	*Amazonetta brasiliensis*
Anas bernieri	*Anas acuta*	*Anas fulvigula*
Amazonetta brasiliensis	*Anas clypeata*	*Anas acuta*
Papasula abbotti	*Morus bassanus*	*Sula sula*
Anthropoides paradisea	*Grus vipio*	*Grus monachus*
Mycteria cinerea	*Grus monachus*	*Synthliboramphus wumizusumi*
Polioptila albiloris	*Agelaius tricolor*	*Cranioleuca vulpina*
Pipilo albicollis	*Pipilo chlororus*	*Agelaius tricolor*
Spizella atrogularis	*Zonotrichia querula*	*Pipilo chlororus*
Agelaius xanthopthalmus	*Pseudoleistes virescens*	*Euphagus carolinus*
Quiscalus lugubris	*Euphagus carolinus*	*Agelaius xanthopthalmus*
Toxostoma occelatum	*Phylloscopus schwarzii*	*Phylloscopus borealis*
Phylloscopus trochiloides	*Phylloscopus borealis*	*Phylloscopus magnirostris*
Phylloscopus magnirostris	*Phylloscopus innornatus*	*Riparia riparia*

Table 2 List of phylogenetically independent contrasts obtained from phylogenies reconstructed from cyt*b* sequence data, for which the latitudinal separation between species was increased to 20° between midpoints. References as listed for Table 1.

lower-latitude species had the longer branch, or a negative sign if the higher-latitude species had the longer branch. Under the null hypothesis of no association between latitude and rates of molecular evolution, we would expect the numbers of positive and negative contrasts, and their combined magnitudes, to be approximately equal. For each set of branch length contrasts given in Table 2, we tested this null hypothesis using two nonparametric tests: sign tests, which consider only the direction of the contrasts, and Wilcoxon signed rank tests, which consider both the ranked magnitudes and the direction of the contrasts.

Table 3 Tests for latitudinal variation in rates of molecular evolution in birds, using contrasts listed in Tables 1 and 2. For the DNA sequences cytochrome *b* (cyt*b*) and NADH2 (ND2), four measures of branch lengths were calculated: maximum likelihood (ML), and relative rates tests on total pairwise distances (Distance), synonymous substitutions (dS) and nonsynonymous substitutions (dN). For DNA–DNA hybridization distances, branch lengths were estimated using relative rates tests. Figures shown are the total number of contrasts available for each test, the number of positive contrasts (those in which the lower-latitude species had the longer branch length), and one-tailed *P*-values of sign tests and Wilcoxon signed rank tests for latitudinal bias in branch lengths.

Measure of branch lengths	Number of contrasts	Number of positive contrasts	Sign test	Wilcoxon signed rank test
cyt*b*				
ML	33	15	0.76	0.36
Distance	31	16	0.5	0.4
dS	28	13	0.71	0.38
dN	15	6	0.85	0.24
ND2				
ML	22	14	0.14	0.06
Distance	17	8	0.69	0.31
dS	18	7	0.88	0.22
dN	8	5	0.36	0.34
DNA hybridization				
Distance	10	7	0.17	0.64
cyt*b* (wider separation: Table 2)				
ML	16	9	0.41	0.44
Distance	15	10	0.15	0.29
dS	16	9	0.41	0.2
dN	11	4	0.89	0.11

Results

A total of 45 latitudinally separated, phylogenetically independent contrasts were identified (Tables 1 and 2). Each of the three genetic data sets (cyt*b*, ND2 and DNA hybridization) were analysed separately. For each of the two genes, the four ways of measuring branch length – maximum likelihood, relative rates, synonymous and nonsynonymous – were each tested separately. This gave a total of 13 tests, none of which indicated a significant association between latitude and rate of molecular evolution (Table 3).

Discussion

This study presents the first explicit test of the climate-speciation hypothesis for high tropical species richness (Rohde, 1978, 1992). A key prediction of this hypothesis is that rates of molecular evolution should increase towards lower latitudes, because higher ambient temperatures and solar radiation in the tropics will drive a higher mutation rate, directly through increased mutagenesis and indirectly through faster generation turnover. We found no evidence to support this prediction for birds, either for the two mitochondrial protein coding genes included in this study or for DNA hybridization data. This result suggests that the predicted association between latitude and rates of molecular evolution does not exist, is not very strong (unable to be detected without very large amounts of data) or is

not a general pattern (not a feature of all taxa or all genes).

The climate-speciation model may act at the level of primary producers, with secondary influences on species richness of other groups (Rohde, 1978, 1992). It has been suggested that plants, like vertebrates, show a generation time effect in the rate of molecular evolution (Gaut *et al*., 1992). If plants at lower latitudes have faster growth rates, then they may have faster generation turnover, and might therefore have more DNA replications per unit time and a higher rate of accumulation of DNA copy errors. A similar link can be made through an effect of light and temperature on the production of biomass: if a more productive plant produces more cells, and if reproductive cells (either sexual or vegetative) are produced after a series of somatic cell divisions, then it is possible that high productivity results in seeds produced via a higher average number of cell divisions, and therefore increased accumulation of DNA copy errors. So Rohde's hypothesis may hold for primary producers, if solar radiation and temperature in the tropics drive higher growth rates which increase substitution rate, and if faster molecular evolution drives speciation rate in primary producers with knock-on effects for species richness at higher trophic levels.

It may also be that the genes chosen – metabolic proteins coded in the mitochondrial genome – are not ideal for testing the climate-speciation model. Rapid speciation might be expected to be reflected in genes associated with adaptation, rather than 'housekeeping' genes that code for products associated with basic metabolism. However, an association between diversification rate and molecular evolution in angiosperms has been detected for three such housekeeping genes, *rbcL*, *atpB* and 18S (Barraclough & Savolainen, 2001). Similarly, although it might be predicted that mitochondrial genes would be less likely to show a generation time effect than nuclear genes (because they may replicate throughout the lifetime of a cell), the generation time effect in vertebrates has been reported for both mitochondrial and nuclear genes (Bromham *et al*., 1996; Bromham, 2002). So any general effect on mutation rates, such as the effect on faster generation turnover, should be detectable for the mitochondrial genes included in this study. Furthermore, there was no significant association between latitude and rate of molecular evolution for DNA hybridization data, which surveys the entire single-copy nuclear genome. This suggests that our failure to find a relationship between latitude and rate of molecular evolution is not because of an unrepresentative sample of genes.

The taxonomic level of the analysis may influence the test, as very closely related species may have had insufficient time to accumulate a sufficient number of substitutions to allow a latitudinal effect to be detected – although the set of cyt*b* contrasts from deeper in the phylogeny also showed no support for an association between rate and latitude. However, the five higher-level contrasts based on Sibley & Ahlquist's (1990) DNA hybridization data were all positive (the lower-latitude clade had the longer branch). This sample is too small to allow any conclusions to be drawn, so it would be helpful if a larger number of family level contrasts were available. Unfortunately, testing the hypothesis for deeper lineages is limited by the difficulty in obtaining a sufficient number of independent contrasts at higher taxonomic levels which are latitudinally separated.

If further tests confirm the findings reported here, that there is no observable gradient in rate of molecular evolution with latitude, then we can conclude that some of the predicted links of the climate-speciation hypothesis are not realized. The two major links predicted by the climate-speciation model are: (1) mutation rate increases with decreasing latitude; and (2) faster molecular evolution drives more rapid speciation. A direct connection between climate and germline mutation rates is somewhat speculative, and the extent to which higher temperatures and solar radiation have a mutagenic effect needs to be tested directly. In particular, it should be established whether mutation rates are sensitive enough to environmental influences to respond to the variation in mean temperature and solar radiation levels that occur across latitudes. A less direct link between climate and mutation rate could exist via the metabolic rate effect (Martin & Palumbi, 1993; Rand, 1994; Martin, 1995). If higher rates of metabolism produce more DNA-damaging metabolites which induce mutation, and if metabolic rates increase with ambient temperature, then species at lower latitudes might have higher mutation rates.

Another potential link between latitude and molecular evolution is the generation time effect, which is based on the assumption that shorter generation times drive more DNA replications per unit time and therefore more DNA copy errors. If the generation time effect is a general feature of molecular evolution, and if higher temperatures in the tropics do speed generation turnover, then this could generate a latitudinal gradient in rates of molecular evolution. However, although birds show a generation time effect, demonstrated for DNA hybridization data (Mooers & Harvey, 1994), and there is evidence that average body size for bird species decreases towards the equator (Blackburn & Gaston, 1996; Cardillo, 2002), we did not find any association between molecular rates and latitude for birds in this study. Although this does not disprove the climate-speciation hypothesis, it does suggest that it is not a general phenomenon.

Even if a link between climate, latitude and rates of molecular evolution can be demonstrated for other taxa, the climate-speciation hypothesis makes a further prediction that an elevated rate of molecular evolution drives a higher rate of speciation. Although an association between rates of molecular evolution and net diversification has been reported for three genes in

angiosperms (Barraclough *et al*., 1996; Barraclough & Savolainen, 2001), the direction of causation is not clear. It could be that faster speciation drives a high substitution rate, for example by repeated subdivision of the population, reducing the average effective population size, potentially accelerating the rate of fixation of neutral or nearly neutral substitutions (see Ohta, 1993). But the climate-speciation hypothesis requires that higher rates of molecular evolution promote faster speciation. There are two ways that faster molecular evolution could increase the rate of formation of new species. First, a higher mutation rate generates more genetic variation, which may then be selected in incipient species. This rests on the supposition that mutation rates are limiting for rate of adaptation (whereas this is to some extent true for bacteria, it is a less convincing proposal for complex multicellular plants and animals). Secondly, higher rates of molecular evolution may speed the rate of hybrid incompatibility by a more rapid evolution of incompatibility between the parent genomes, not necessarily at loci directly connected with adaptation to new niches (see Wu, 2001).

A large number of hypotheses exist to explain the latitudinal diversity gradient. Progress in this field now rests largely on the ability to test and compare these hypotheses. We have demonstrated that it is possible to test a central component of the hypothesis put forward by Rohde (1978, 1992) that links higher levels of solar radiation and higher temperatures in the tropics to a faster speciation rate. We find no evidence that rate of molecular evolution varies systematically with latitude for birds, and therefore that a key component of the climate-speciation model is not realized for this group. We have described here a comparative framework that makes it possible to extend this test to other taxa, given appropriate distribution data and DNA sequences. If further analyses confirm the findings of this study and establish that there is no systematic latitudinal variation in the rate of molecular evolution, this will not only cast doubt on an assumption of the climate-speciation model, it will also have practical implications for the use of molecular data in macroecology. If rates of molecular evolution are not influenced by latitude or its correlates, then this encourages the use of molecular phylogenies to examine variation in the tempo and mode of diversification across geographical regions (Nee *et al*., 1992; Purvis, 1996; Sanderson & Donoghue, 1996; Barraclough & Nee, 2001).

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