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Testing historical explanations for gradients in species richness in heliconiine butterflies of tropical America

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We compiled a large database of 58 059 point locality records for 70 species and 434 subspecies of heliconiine butterflies and used these data to test evolutionary hypotheses for their diversification. To study geographical patterns of diversity and contact zones, we mapped: (1) species richness; (2) mean molecular phylogenetic terminal branch length; (3) subspecies richness and the proportion of specimens that were subspecific hybrids, and (4) museum sampling effort. Heliconiine species richness is high throughout the Amazon region and peaks near the equator in the foothills and middle elevations of the eastern Andes. Mean phylogenetic terminal branch length is lowest in the eastern Andes and tends to be low in species-rich areas. By contrast, areas of high subspecies richness, where subspecies overlap in range and/or hybridize, are concentrated along the course of the Amazon River, with the eastern Andes slopes and foothills relatively depauperate in terms of local intraspecific phenotypic diversity. Spatial gradients in heliconiine species richness in the Neotropics are consistent with the hypothesis that species richness gradients are driven at least in part by variation in speciation and/or extinction rates, resulting in observed gradients in mean phylogenetic branch length, rather than via evolutionary age or niche conservatism alone. The data obtained in the present study, coupled with individual case studies of recently evolved *Heliconius* species, suggest that the radiation of heliconiine butterflies occurred predominantly on the eastern slopes of the Andes in Colombia, Ecuador, and Peru, as well as in the upper/middle Amazon basin. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **105**, 479–497.

ADDITIONAL KEYWORDS: conservatism – evolutionary age – extinction – range maps – speciation – subspecies richness – suture zones – terminal phylogenetic branch length.

INTRODUCTION

Understanding the processes responsible for spatial variation in species richness is a central goal in ecology and evolution (Rosenzweig, 1995; Ricklefs & Miller, 1999; Gaston, 2000; Hawkins *et al*., 2003). It is also a vital prerequisite to conservation of the earth's living resources in the face of rapid environmental change (Myers *et al*., 2000; Lamoreux *et al*., 2006). However, despite broad agreement that, in general, community species richness increases from the poles to the tropics, there remain many conflicting hypotheses to explain this pattern (Willig, Kaufman & Stevens, 2003; Mittelbach *et al*., 2007). These hypotheses are often grouped into three major classes: null, ecological or evolutionary. Proponents of the geometric constraints model argue that a tropical peak in species richness can result from stochastic placement of species ranges within a bounded domain (Colwell & Hurtt, 1994; Rosenzweig, 1995; Willig & Lyons, 1998; Colwell & Lees, 2000). Such models assume nothing about the ecology or evolution of species, such as the importance of competition, or variation in rates of speciation or extinction. Ecological models suggest

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*Corresponding author. E-mail: neil.rosser@ucl.ac.uk that species richness is a result of the number of niches available, which are in turn affected by primary productivity and ultimately climate. However, whether or not communities present at different latitudes are at regional dynamic species richness equilibria is questionable (Rees *et al*., 2001). By contrast, evolutionary models suggest that tropical richness is a result of higher diversification rates or a longer time for diversification in more tropical regions; in these models, community species richness does not need to be at equilibrium (Mittelbach *et al*., 2007). Ecological and evolutionary explanations need not be mutually exclusive; for example, it is possible that ecological limits lead to a negative feedback of diversity on speciation rates (Rabosky, 2009).

Among ecological and evolutionary hypotheses for high diversity of species in the tropics, perhaps the oldest is 'the evolutionary age' hypothesis: that tropical regions have been more stable over geological time, permitting 'a comparatively continuous and unchecked development of organic forms' (Wallace, 1878: 123; Fischer, 1960; Wiens *et al*., 2009). A related hypothesis is 'tropical niche conservatism'. It states that most taxa originate at tropical latitudes and rarely colonize higher latitudes because of phylogenetic niche conservatism (Wallace, 1876; Wiens & Donoghue, 2004; Hawkins, Diniz-Filho & Soeller, 2005; Hawkins *et al*., 2006, 2007; Wiens *et al*., 2009). Neither evolutionary age, nor tropical conservatism hypotheses require variation in rates of speciation or extinction to drive species richness gradients (Wiens *et al*., 2009); instead, more species are assumed to accumulate in more tropical regions because of longer favourable periods for diversification. A clear alternative is that diversification rates are higher in more tropical regions, either because of higher speciation rates (Cardillo, Orme & Owens, 2005) or lower extinction rates (Wallace, 1878; Wiens, 2007).

In the present study, we present detailed range maps for a diverse and well-studied Neotropical group, the heliconiine butterflies (Lepidoptera: Nymphalidae: subtribe Heliconiina). We combine these data with molecular phylogenetic information and use them to test evolutionary hypotheses for species richness gradients. The evolutionary age and tropical conservatism hypotheses posit that species-rich tropical clades originated at low latitudes, and that richness gradients are then a product of 'time for speciation' and infrequent dispersal to higher latitudes. Species in less diverse areas should therefore be younger or at the most equal in age to those occurring in more diverse areas (Hawkins & DeVries, 2009). Finding that species in less diverse areas tend to be older would cast doubt on evolutionary age or niche conservatism as the sole explanations for the species richness gradient, and imply that speciation and/or extinction must play a role. We test this hypothesis using the terminal branch length of a relaxed clock molecular phylogeny, which will provide an overestimate of species age as a result of coalescence of gene trees typically predating speciation and extinction pruning tips from the tree (Edwards & Beerli, 2000). Assuming no geographic variation in speciation and/or extinction rates, the evolutionary age and niche conservatism hypotheses both predict that across assemblages mean terminal branch length should either be positively related or unrelated to species richness. If differences in evolutionary age or niche constraints operated relatively recently and affected extant species, then their influence on terminal branch lengths should still be apparent and branch lengths will be positively related to species richness. However, if the hypotheses were relevant only before extant lineages appeared, then recent speciation and extinction events could erase historical phylogenetic signal, resulting in no association between terminal branch length and species richness. In contrast to these patterns, higher speciation rates or lower extinction rates both result in assemblages composed of species with shorter mean terminal branch lengths, resulting in a negative relationship between mean branch length and species richness.

In addition to a global tendency for tropical areas to contain more species than temperate areas, there is substantial variation among different tropical regions, with the Neotropical region thought to contain more species of plants, amphibians, birds, and mammals than either the African or Asian tropics (Gaston & Hudson, 1994; Thomas, 1999). Although many hypotheses consider lowland rainforests as the origin for Neotropical biodiversity (Haffer, 1969, 2008), recent studies have shifted the focus onto the Andes as a source of speciation. The diverse topography of the Andean region may present more opportunities for allopatric speciation than the lowlands and may also provide suitable conditions for ecological speciation (Chapman, 1917), with a recent study suggesting that this could be responsible for the unusually high butterfly species richness of the Neotropics (Elias *et al*., 2009). Fjeldså (1994; Fjeldså, Lambin & Mertens, 1999) suggested, on the basis of bird studies, that Andean valleys and plateaus buffered from climatic fluctuations functioned as a 'species pump' for South America, with new narrowly endemic species arising continuously over time. By contrast, the lowlands were seen as unstable climatically, characterized by frequent extinctions, and appeared to be a 'museum' for widespread, older lineages. To address these questions with our butterfly dataset, we map the distributions of subspecies and intersubspecific hybrids in addition to species richness and phylogenetic branch length. This allows us to identify areas of unusually high polymorphism and subspecies diversity. If subspecies represent incipient species (Mayr, 1942), areas where subspecies richness is high and hybridization between subspecies is common may correspond to areas where speciation is initiated, or to 'suture zones' where evolving taxa meet and form hybrid zones (Remington, 1968).

The heliconiine butterflies (Lepidoptera: Nymphalidae: Heliconiina) are a colourful and diverse neotropical group well known for their participation in Müllerian mimicry rings. Heliconiines have served extensively as subjects for studies in evolution, ecology, and genetics, and are among the best studied insects of no commercial importance (Brown, 1981; Turner, 1981). Heliconiine species are normally recognized not only by differences in wing colour pattern and few intermediates in sympatry, but also by means of genitalic morphology and, in some cases, genetic data (Brower, 1996; Jiggins *et al.*, 1996; Jiggins & Davies, 1998; Giraldo *et al.*, 2008). Subspecies are recognized by geographical differences in wing colour pattern and abundant hybrid genotypes in contact zones (Holzinger & Holzinger, 1994). Many subspecies of heliconiines do not fit the definition of subspecies as geographically separated phenotypes (Brown, 1979; Turner, 1981; Mallet, 2001). Rather, in a number of regions, multiple phenotypes co-occur, likely maintained by mimicry with alternative models (Joron *et al.*, 2001). Previous studies on the biogeography of heliconiines demonstrated broad patterns of species richness and subspecies endemism (Emsley, 1963; Brown, 1979; Holzinger & Holzinger, 1994). Subsequent to these studies, however, many new distribution data have become available and there have been significant revisions to heliconiine taxonomy. In addition, new geographical information system (GIS) technology has enabled us to map distributions at a much finer resolution using data that are easily retrievable and verifiable, resulting in range maps for 434 subspecies in addition to 70 species. These provide the most detailed georeferenced range maps for any Neotropical insect group to date and a useful digital source of data for others interested in heliconiines or insect biogeography in general.

MATERIAL AND METHODS

Point locality data

We compiled a database of point locality records for the Heliconiina, using a modified version of the most recent taxonomic checklist (Lamas, 2004) (see Appendix S1). Our principal sources for data were museum collections [primarily those in two of the world's largest collections, the Natural History Museum in London (NHM) and the Florida Museum of Natural History in Gainesville (FLMNH)], research databases, and the scientific literature. The data sources used are summarized in Table 1. Point locality records in the database refer to individual specimens when sourced from collections and research databases but to localities where the species occurs when sourced from literature. Older museum specimens are often labelled with very generalized localities (e.g. 'Nouvelle Grenade'). To remove imprecise locality

Table 1. Summary of data sources

Source	Website	Number of records
The Florida Museum of Natural History	http://www.flmnh.ufl.edu	20 881
The Natural History Museum (London)	http://www.nhm.ac.uk	8 277
Brown (1979)		6 079
Tropical Andean Butterfly Diversity Project	http://www.mariposasandinas.org	4 078
CONABIO, Mexico	http://www.conabio.gob.mx	3 433
Museo de Historia Natural, Lima, Peru	http://museohn.unmsm.edu.pe	3 067
C. Jiggins research database	http://heliconius.zoo.cam.ac.uk	1 780
INBio, Costa Rica	http://www.inbio.ac.cr	1 682
K. Willmott and J. Hall research database	http://www.flmnh.ufl.edu/butterflies/neotropica	1 479
J. Mallet research database	http://abacus.gene.ucl.ac.uk/jim	1 203
'Butterflies of Colombia', LeCrom (in prep.)		1 179
Butterflies and Moths of North America	http://www.butterfliesandmoths.org	1 118
A. Brower research database	http://frank.mtsu.edu/~abrower	960
speciesLink	http://splink.cria.org.br	618
Other sources (published studies, websites, etc.).		2 228
Total		58 062

data from the analysis, we obtained a crude measure of precision when georeferencing a locality (e.g. a national park) based on the extent of the area to which the name refers. We measured the maximum radius from the inferred central coordinates of the locality to the edge of the area and included these as 'point' localities only where precision was < 40 km. Museum specimens are also often mislabelled, especially if collected commercially, where little importance may be placed on precise localities, or where the locality labels are deliberately misleading (Emsley, 1963). We therefore excluded any localities that appeared to be erroneous, where a data label of low reliability indicated a locality point significantly outside the otherwise known distribution of the species. When unsure of the reliability of data, we consulted with experts with specific knowledge about the particular sampling region.

If a specimen was identified as an interspecific or intraspecific hybrid (i.e. displaying a mix of colour pattern elements from other taxa), we treated it as a locality record for both putative progenitors. All ten putative species of *Philaethria* were excluded (except for maps of sampling effort) as a result of taxonomic uncertainty regarding species limits: recently, a number of cryptic species have been described (Constantino & Salazar, 2010). *Laparus doris* (Linnaeus) also created problems for mapping because it is highly polymorphic and shows wide clinal variation (Mallet, 1999), which has resulted in inconsistent application of subspecific nomenclature to specimens in major collections. We therefore excluded *L. doris* from analysis of subspecies distributions.

DISTRIBUTION MAPS

All GIS-based work was carried out in ARCMAP, version 9.3 (ESRI), unless otherwise stated. We chose not to apply a species distribution modelling approach in the present study because many heliconiine subspecies (and some species) are known from very few (< 10) localities. Instead, we explored two simpler approaches for converting point locality data into predictions of species' distributions. As a first step, we used a minimum convex polygon (or convex hull) method, which estimates a species range as the smallest polygon encompassing all of the data subject to the constraint that no internal angle can be greater than 180° (Sheth *et al.*, 2008). However, the method gives an unrealistic extent of occurrence when a species range includes a real concave boundary. This can result in the extent of occurrence map including large areas of inhospitable habitat where the focal taxon is known to be absent. To correct for this, we used a related method, α-convex hulls, which allows for convex margins and gaps within a species ranges (Edelsbrunner, Kirkpatrick & Seidel, 1983; Burgman & Fox, 2003). A difficulty with the α-convex hull approach lies in deciding what area should constitute a real gap in a species range. This is particularly problematic in poorly sampled areas such as the Amazon. We found that the maps that most consistently met our subjective a priori expectations of species and subspecies ranges were made by applying α-convex hulls with α set to 1400 km, which was the smallest value that resulted in no discontinuous ranges for any species. We obtained this value by increasing the value of α incrementally until all species ranges formed single continuous polygons. Thus, using the α-convex hull approach not only returns similar results to the minimum convex polygon approach, but also allows for ranges to have convex margins at broader scales. We created α-convex hulls in the R package alphahull (Pateiro-López & Rodríguez-Casal, 2010) using point locality data projected to a Lambert Cylindrical Equal Area projection.

Many of the resulting distributions still contained areas where we are confident that a particular species does not occur, so that clipping of the resultant range maps was required. We clipped the polygons to coastlines and the altitudinal ranges of the species using a 30 arc-second altitudinal grid obtained from WORLDCLIM (http://www.worldclim.org). When the polygon for a species indicated presence on islands, we only included the islands with known records for the species. Altitudinal clipping was based on published information (Brown & Mielke, 1972; Brown, 1979; DeVries, 1987) and consultation with experts, as well as on our own field experience and data. We also clipped polygons to exclude well-sampled areas where we could be confident that an absence of records is not an artefact (e.g. in cases where areas west of the Andes were inferred to contain otherwise Amazonian species).

GRID-CELL BASED ANALYSIS

Species richness, subspecies richness, and sampling effort were mapped using a 50 × 50 km grid and Lambert Cylindrical Equal Area projection to ensure equal area sampling. Sampling effort was estimated as the number of specimens in each grid cell. Species and subspecies richness were estimated as the count of species or subspecies ranges that overlap each grid cell. We also present a second map of species richness, which was created by merging the subspecies ranges for each species, and using the resulting distributions to map species richness. The map of subspecies richness therefore differs only from the second map of species richness in that subspecies within species are

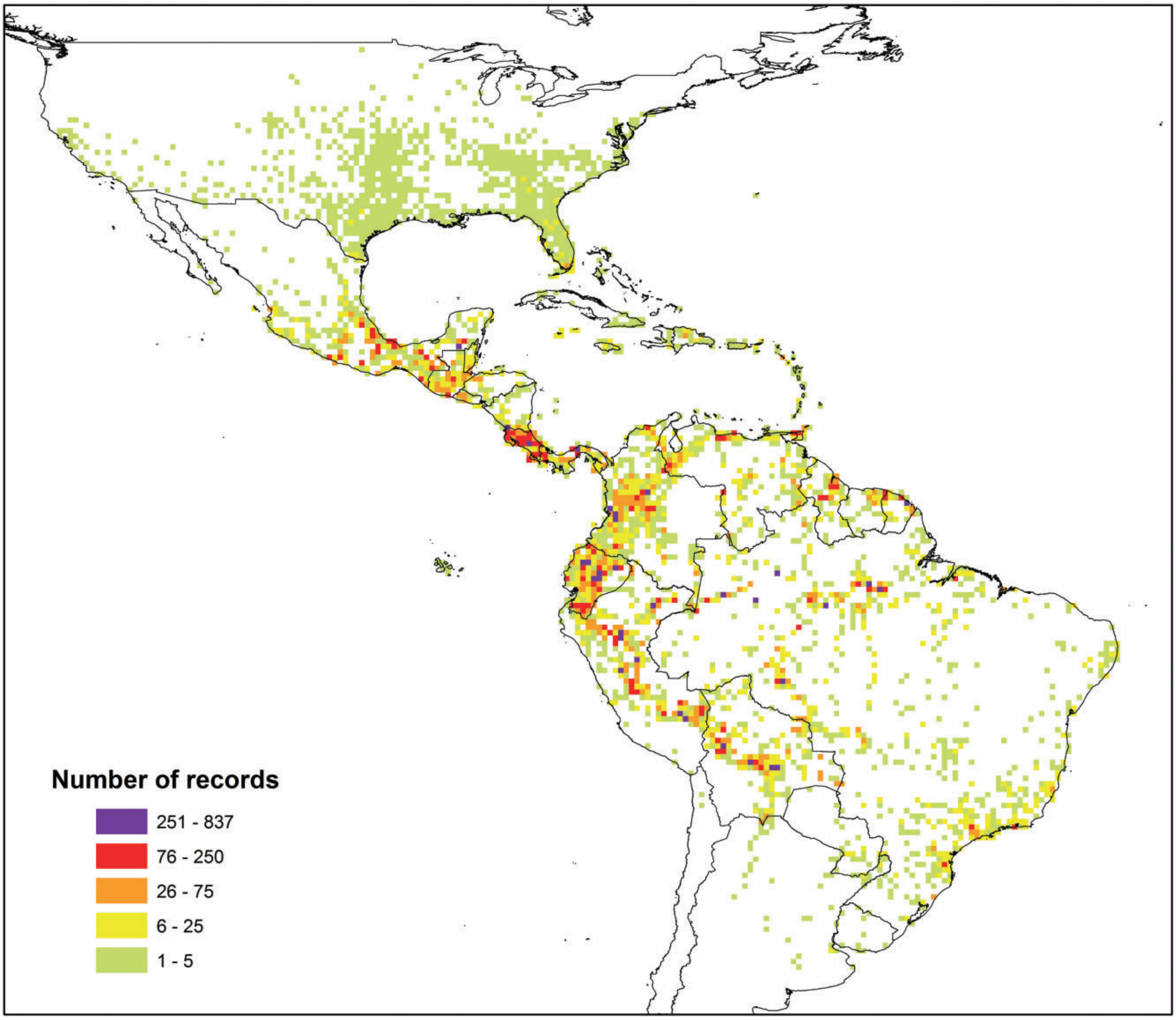

Figure 1. Sampling effort mapped in 50 × 50 km grid cells.

superimposed as a result of polymorphism in traits considered diagnostic for subspecies, allowing direct comparisons between species and subspecies richness patterns. Finally, to identify areas with unusually high numbers of subspecies (i.e. once the number of species present is taken into account), we mapped the mean number of subspecies per species in grid cells.

Molecular phylogenetic information comprised mitochondrial DNA divergence-based branch lengths estimated using a relaxed clock method with a multilocus sequence-based phylogeny of the Heliconiina (Beltrán *et al*., 2007; Mallet *et al*., 2007). We mapped the mean terminal branch lengths of assemblages as described by 100 × 100 km equal area grid cells. To test whether mean terminal branch length is related to species richness we used Pearson's correlation. Given the problem of spatial autocorrelation, we used SAM (Spatial Analysis in Macroecology) software (Rangel, Diniz-Filho & Bini, 2010) to calculate geographically effective degrees of freedom and correct *P*-values (Dutilleul *et al*., 1993). The degree to which two cells/assemblages have similar mean values will be influenced by the number of species that they share. For example, any two cells that share all the same species must have the same mean terminal branch length. Correcting for non-independence among cells as a result of spatial autocorrelation should partially deal with this problem, although some non-independence may remain if the degree to which cells share species does not decline over space at a constant rate.

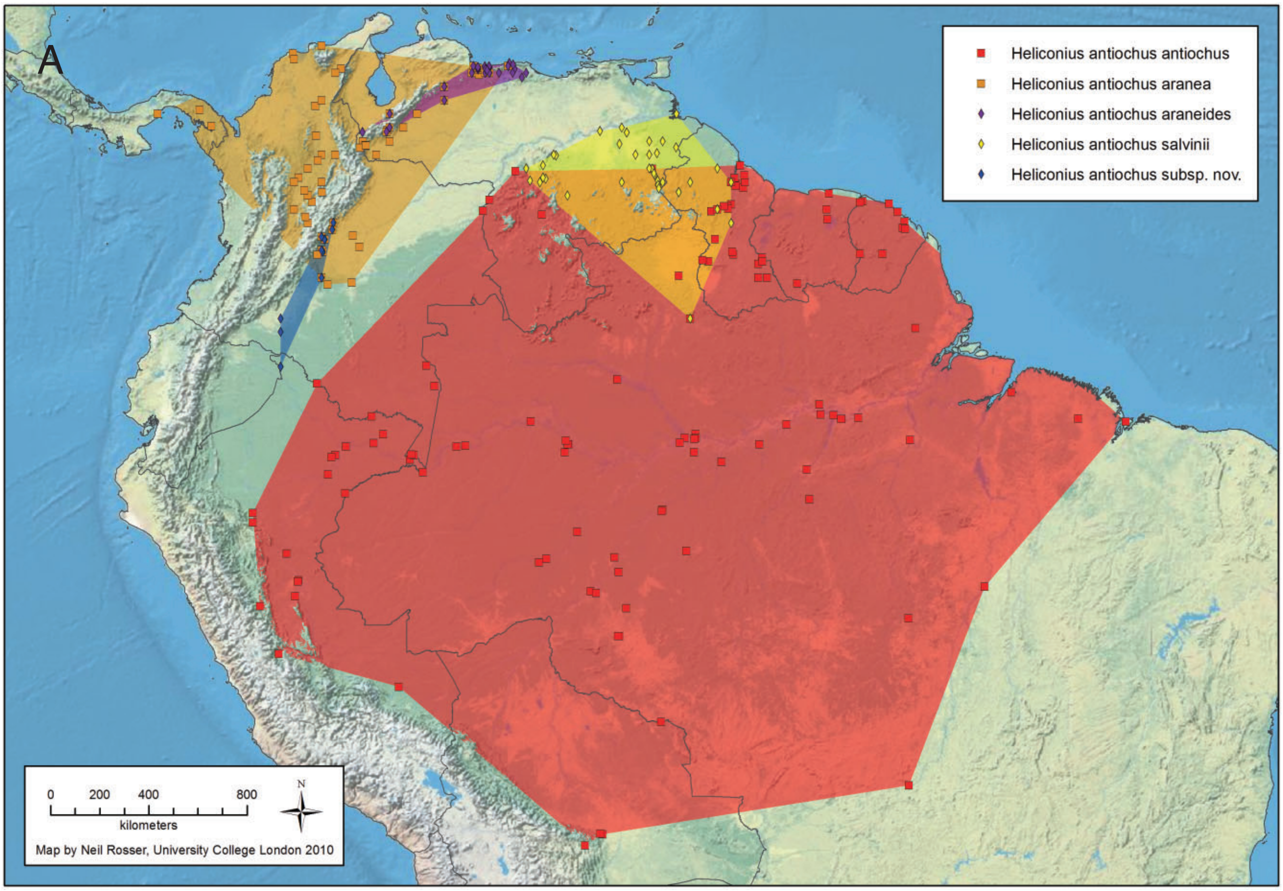

Figure 2. Range maps for the subspecies of *Heliconius antiochus* (A) and *Heliconius telesiphe* (B).

To identify possible intraspecific suture zones (Remington, 1968), we selected all specimens that had been identified either as subspecies hybrids or as taxa listed by Lamas (2004) as hybrids. We then calculated the proportion of species with hybridizing subspecies in equal area grid cells of 100 × 100 km: potential subspecific suture zones can therefore be recognized as grid cells where a high proportion of species have hybridizing subspecies. Although we use the term 'suture zone' here, we note that a high proportion of subspecific hybrids may arise from ongoing divergence, as well as from the meeting of already diverged populations (Dasmahapatra *et al.*, 2010). To account for biases induced by small sample sizes, we only included grid cells with ≥ 20 specimen records. To investigate whether sampling effort affects the proportion of hybrid specimens, we used a general linear model (GLM) treating proportion of hybridizing species as a response and with binomial family error structure and log sample size as a predictor. We back-transformed the residual variation from the logit scale to proportions and mapped it. We then compared the map of residual variation with the map of proportions of hybridizing species.

RESULTS

DATA

We collected a total of 58 059 geographical records for 70 species and 434 subspecies of heliconiines, with information from 10 046 point localities. We excluded 3901 records of dubious authenticity or imprecise locality information. The database will also be made available online via various websites (Table 1), enabling users to download data, add additional records, and plot species and subspecies distributions.

For those localities where a measure of precision could be made, 85% of locality names referred to an area that extended < 40 km from the central coordinates, and 74% to an area extending < 10 km from the central coordinates. Specimen collection date and precision varied among collections. For example, comparing the two collections that provided the largest number of records, specimens in the FLMNH had a

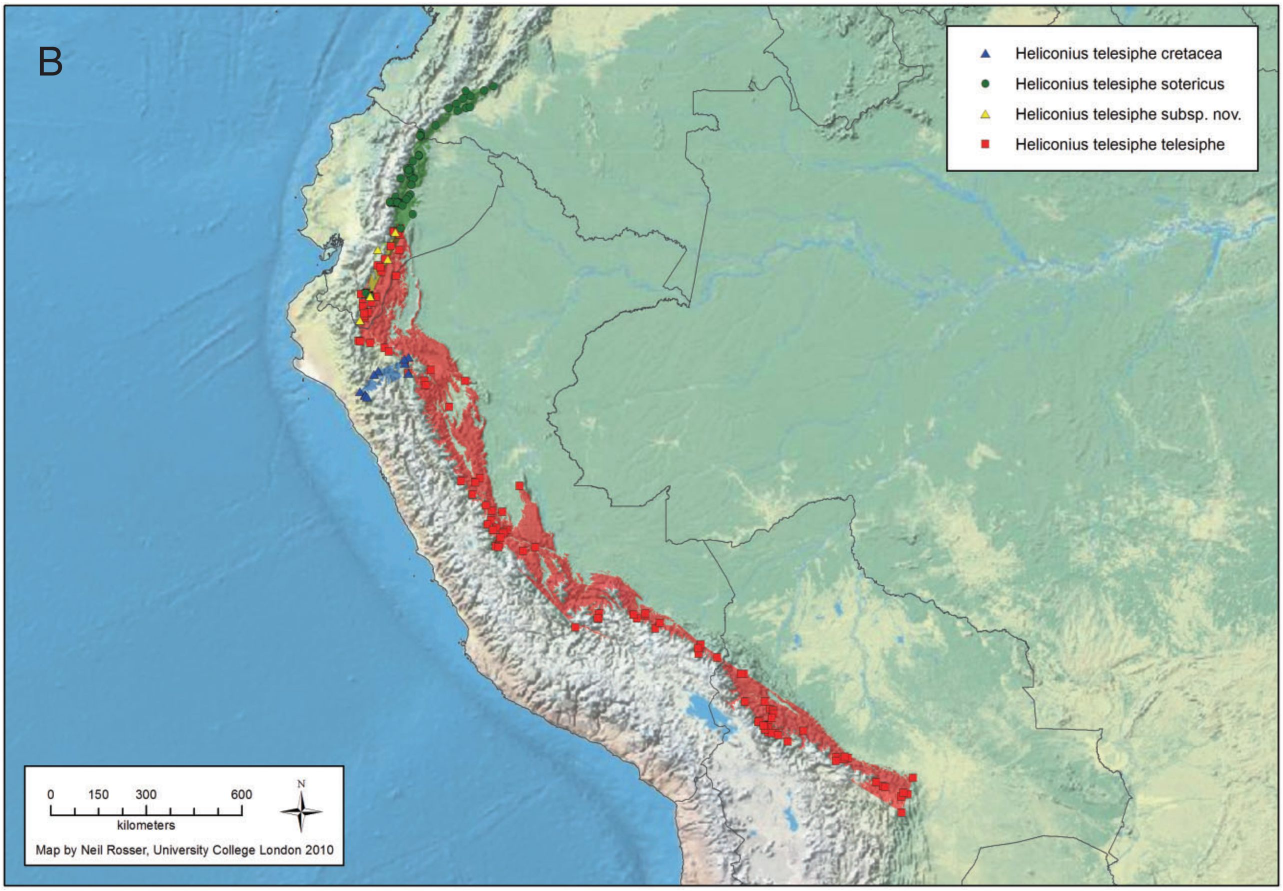

Figure 2. *Continued*

mean collection date of 1984 in contrast to 1921 in the NHM, whereas 87% (FLMNH) versus 79% (NHM) of specimens could be assigned to the nearest 40 km, and 77% (FLMNH) versus 68% (NHM) could be assigned to the nearest 10 km.

SAMPLING EFFORT

A map of sampling effort is shown in Figure 1. Tropical Mexico and Central America are generally well sampled, although there are few data from Nicaragua and Honduras. The dearth of records from the Caribbean islands is probably not a good indication of overall museum sampling effort because few heliconiines occur there. In South America, Andean regions are for the most part well sampled. In comparison, very few collections are available from across vast areas of Amazonia and the Guiana shield. Notable exceptions include various sites along the main course of the Amazon River, the Ariquemes area in Rondônia, Brazil (intensively collected by K. Brown, G. Austin, and others in the 1970s and 1980s), coastal French Guiana and northern Guyana (the latter two well represented in the NHM collections). Eastern Colombia has been poorly sampled, although it is probable that the lack of records from the Llanos of Colombia and Venezuela is partly a result of the scarcity of heliconiines in such ecosystems (where they are confined to gallery forest). Similarly, it is likely that the lack of samples from Western Peru, Chile, and the interior of north-eastern Brazil is as much a result of the fact that few heliconiines occur there as it is to low collecting effort.

SPECIES AND SUBSPECIES DISTRIBUTIONS

Distribution maps for species and subspecies are presented in the Appendix S1. Figure 2 shows example maps for a lowland species [*Heliconius antiochus* (Linnaeus)] and a mid-elevation species [*Heliconius telesiphe* (Doubleday)].

PATTERNS OF SPECIES RICHNESS

Species richness of heliconiines is highest in the Amazon basin and adjacent slopes of the Andes, the Guiana shield, central and western Colombia, and north-west Ecuador (Fig. 3A). Across this area, the

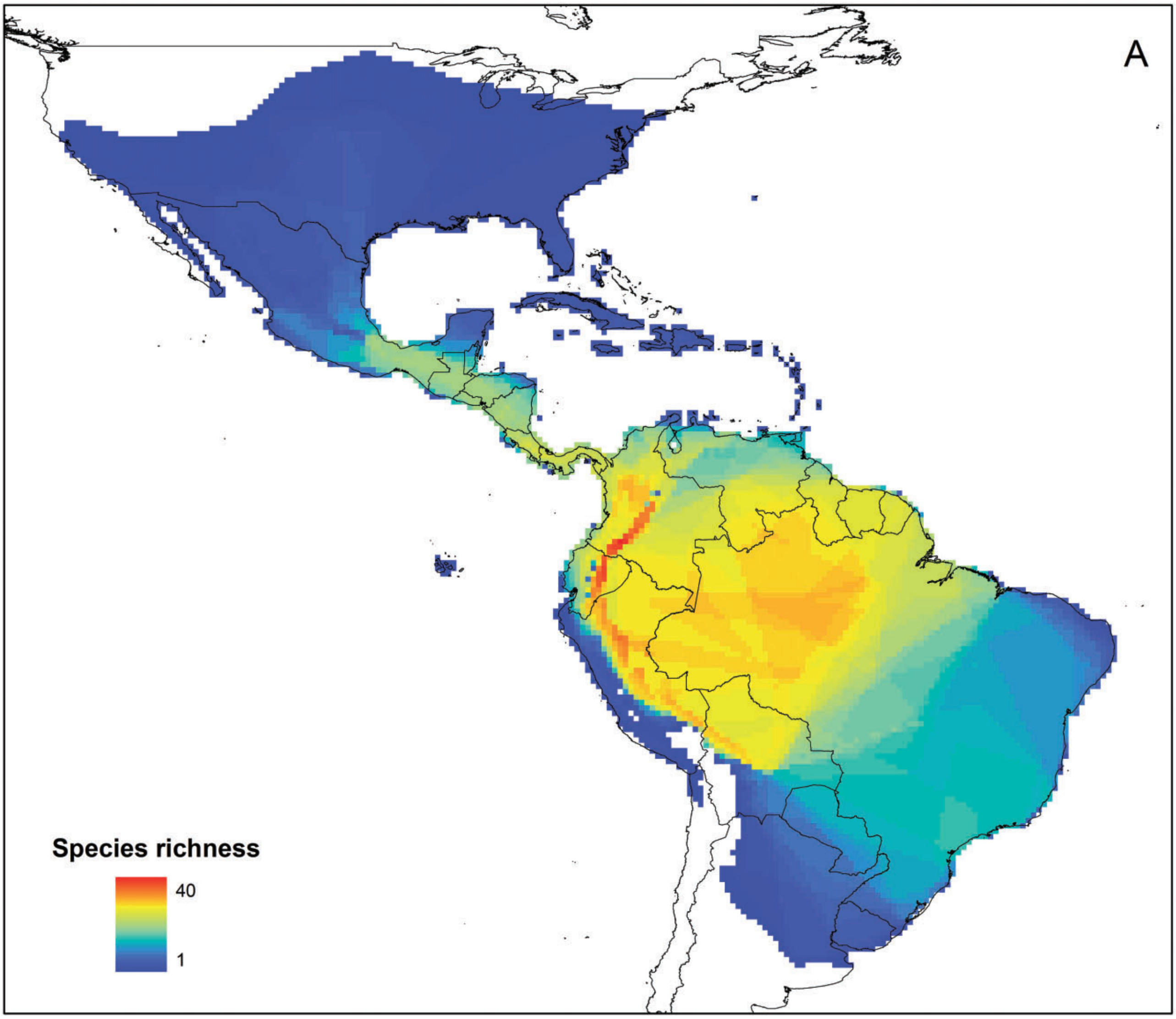

Figure 3. A, species richness mapped using species polygons. B, species richness based on the union of subspecies polygons. 50 × 50 km grid cells.

number of species in 50-km grid cells is rarely lower than 25, with the Andean–Amazonian ecotone of Colombia, Ecuador, and Peru comprising the most speciose region of all (up to 40 species/50 × 50 km grid square in the vicinity of Mocoa, Putumayo). The Manaus area in the central Amazon forms another hotspot, although here richness does not exceed 31 species/50 × 50 km grid square. The Llanos of Colombia and Venezuela have low species richness compared to surrounding areas. Central America and southern Mexico are relatively species rich (up to 24 species/50 × 50 km grid square in Panama), although only four species have colonized the Caribbean. Central and eastern Brazil appear depauperate and the Atlantic rainforests of south-east Brazil do not show up as a richness hotspot for heliconiines, despite having a number of endemics. Very few heliconiine species occur on the Pacific coast of South America south of Ecuador, where rainfall is extremely low. To allow direct comparisons between maps of species and subspecies richness, we present a second map of species richness based on the merged subspecies ranges for each species, rather than the overall species' ranges (Fig. 3B). Note that, in this map, species richness counts tend to be lower because the constituent species range estimates are typically smaller.

Figure 4 maps the mean terminal branch lengths (a proxy for species age) of species in equal area grid cells. Interestingly, there is a weak tendency for terminal branch lengths tend to be short in the regions of highest species diversity, especially on the eastern

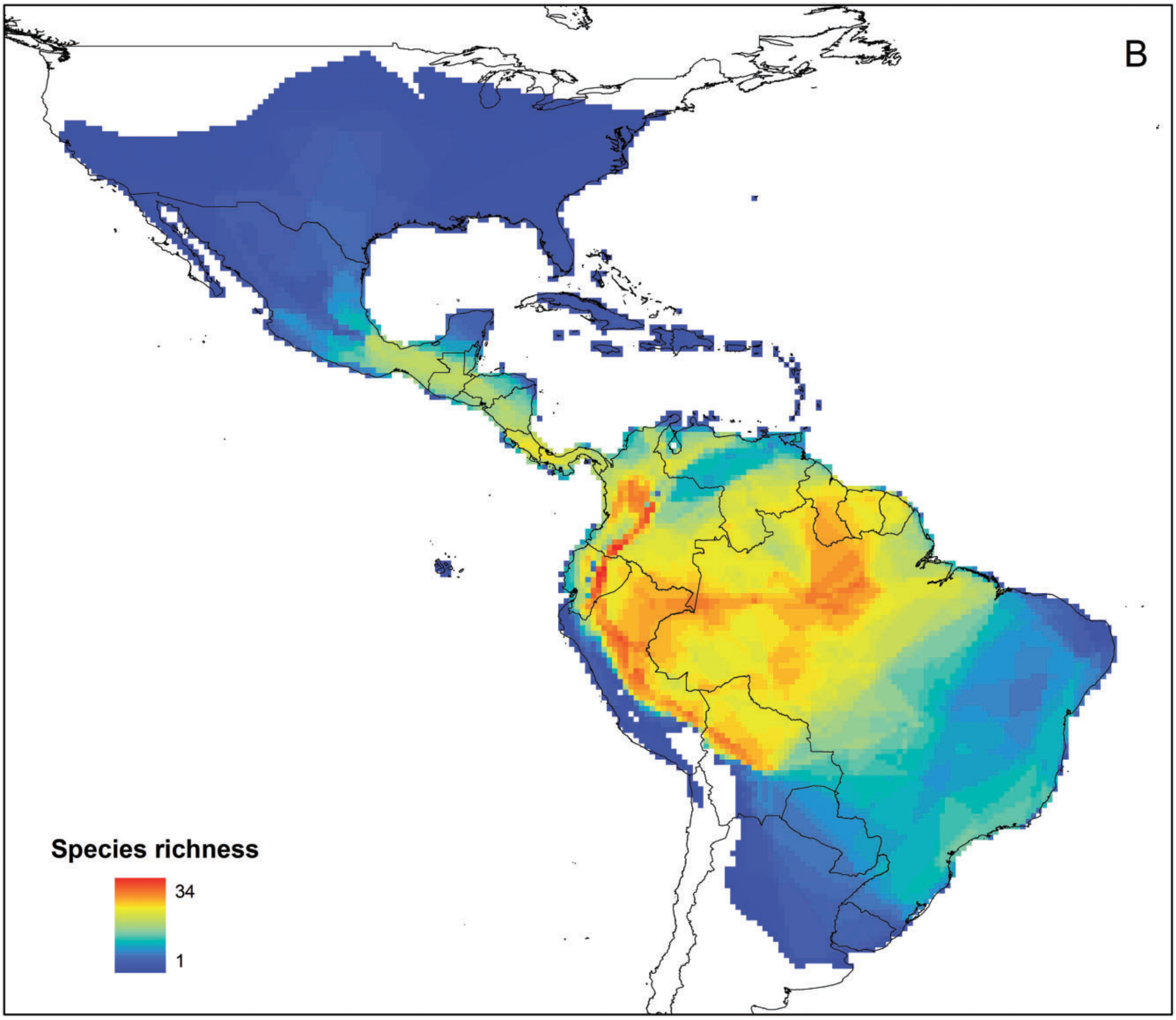

Figure 3. *Continued*

slopes of the Andes, and longer in the less diverse regions. However, there are a few areas on the edge of the distribution where a young mean age is concentrated on very low diversity regions which may be attributed to an increase in the variance of the mean as species richness declines (as in a funnel plot; Fig. 5). Species richness is negatively related to species age (Fig. 5), although the relationship is marginally insignificant when spatial autocorrelation is taken into account (Pearson's $r = -0.704$, corrected degrees of freedom = 5.751, corrected probability = 0.057).

SUBSPECIES RICHNESS AND SUTURE ZONES

Patterns of subspecies richness (Fig. 6A) show several differences from those of species richness. It should be emphasized that this map of richness can differ from that in Figure 3B because multiple subspecies of a single species may occur within a single cell when the cell is located at a subspecies range margin, or because subspecies are sympatric as a result of polymorphism in traits considered diagnostic for subspecies. Notably, regions near the Amazon River appear by far the richest area in terms of subspecies, with as many as 63 subspecies present in a 50-km grid square at Manaus, Brazil. By contrast, richness in the Amazon-draining foothills of the eastern Andes of Ecuador and southern Colombia does not exceed 52 subspecies in a 50-km grid cell, even though species richness is higher there. Figure 6B maps the mean number of subspecies per species. The contours of this map are similar to those of subspecies richness (Fig. 6A) showing not only that areas surrounding the

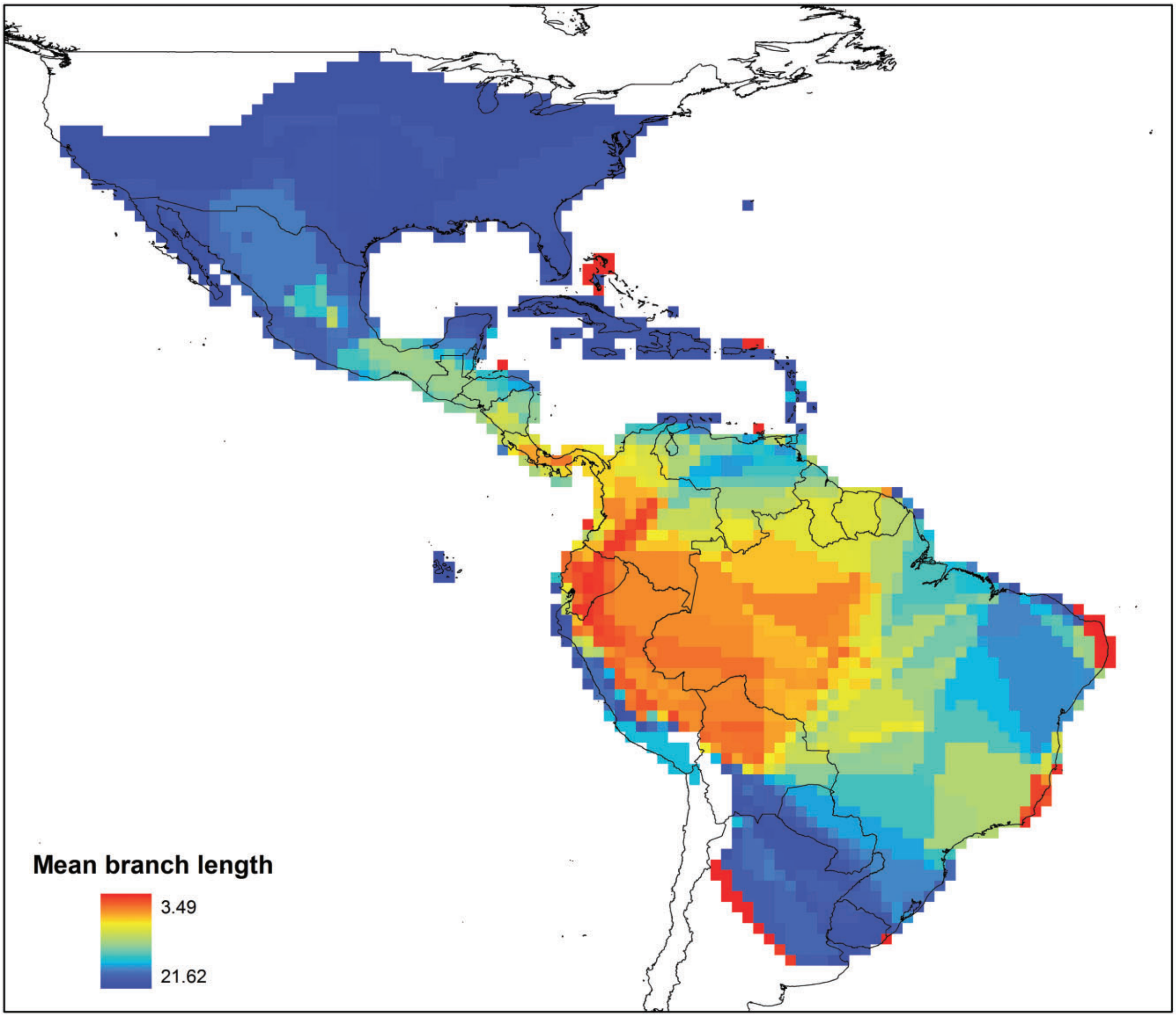

Figure 4. Mean mitochondrial DNA terminal branch length of species mapped in 100 × 100 km grid cells.

Amazon river and its tributaries have the most subspecies per grid cell, but also that these areas are still especially subspecies-rich once species richness is taken into account. In addition, certain patterns emerge that are not apparent in the subspecies richness map. Most strikingly, the eastern Andes of Colombia, Ecuador and Peru, the Magdalena Valley in Colombia, Western Ecuador, and Central America all have relatively few subspecies per grid cell given the number of species that occur there.

We cannot completely discount the possibility that the conflicting patterns of species and subspecies richness are to some extent the result of a sampling artefact. The Amazon basin is much less well sampled relative to the Andes and, consequently, Andean foothill taxa may be more likely to be recognized as species. Indeed, recent ecological and molecular phylogenetic studies of Andean taxa have resulted in taxa formerly ranked as subspecies being elevated to species status on the grounds of bimodality in hybrid zones, as well as the discovery of cryptic species (Brower, 1996; Jiggins *et al.*, 1996; Jiggins & Davies, 1998; Arias *et al.*, 2008; Giraldo *et al.*, 2008; Mallet, 2009). However, we do not consider that these recent taxonomic changes in themselves substantially affect the conclusions drawn here because reclassifying the species concerned as subspecies would at most reduce species richness in the Andes by two per grid cell, and similarly elevate subspecies richness by two per grid cell. Nonetheless, the possibility remains that future studies in the Amazon could show species richness to be

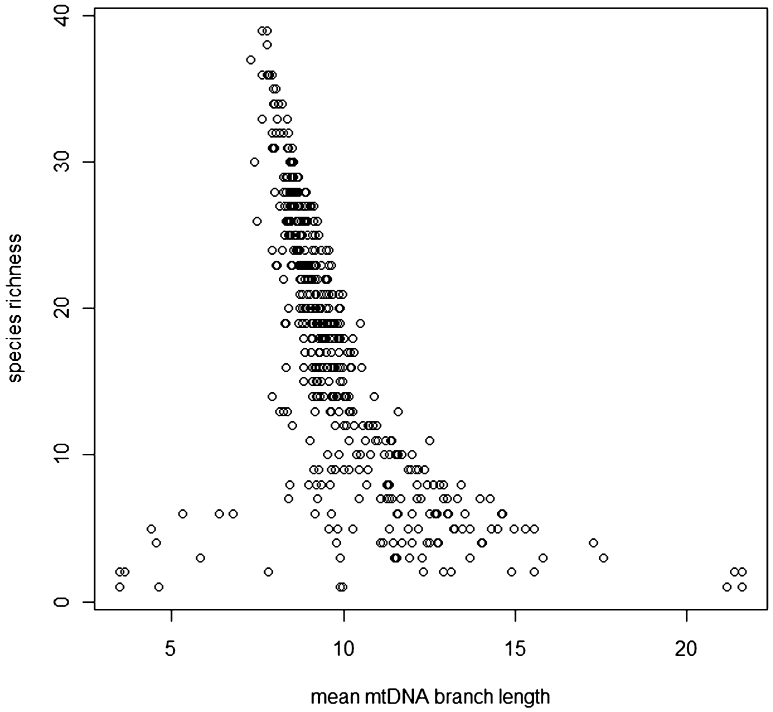

Figure 5. Plot of mean mitochondrial (mt)DNA terminal branch length against species richness in 100 × 100 km grid cells. Note that these data points are not statistically independent as a result of nearby cells often sharing the same species.

underestimated, as well as subspecies richness to be overestimated.

The proportions of species with hybridizing subspecies were mapped in 100 × 100 km equal area grid cells to further identify possible suture zones (Fig. 7A). Most of the cells with high proportions of hybridizing species are found along the course of the Amazon River to the foothills on the Andes in Peru. We used a GLM with binomial family errors to investigate the extent to which sampling effort affects this analysis. We mapped the residual variation from this model (Fig. 7B); cells with high values correspond well to those shown in Figure 7A, suggesting that the geographical patterns cannot be explained solely by sampling artefacts.

DISCUSSION

GEOGRAPHICAL ORIGIN OF HELICONIINE SPECIES RICHNESS

At broad scales, the results of the present study confirm that the widespread latitudinal gradient in species richness also occurs in Heliconiina not only between temperate and tropical regions, but also, importantly, within the tropical region itself (Fig. 3A, B). The mean terminal phylogenetic branch length of species (Fig. 4) tends to be shorter in the eastern Andes and Amazon basin, with terminal branch lengths becoming progressively longer on average through Central America and south-eastern Brazil and then into more temperate regions (a few peripheral regions with young mean age are the result of stochastic variation when the numbers of species tested are very low). Overall, the mean age of species appears to be negatively related with species richness (Figs 4, 5), with the richest areas inhabited by predominantly young species. This pattern is consistent, at a local scale, with variation in speciation and/or extinction rates driving the negative relationship between species richness and latitude (Allen & Gillooly, 2006; Allen *et al.*, 2006; Jablonski, Roy & Valentine, 2006; Wright, Keeling & Gillman, 2006) and argues against evolutionary age or niche conservatism as the sole explanations.

The results reported in the present study are consistent with a recent study of New World limenitidine nymphalid butterflies (Mullen *et al.*, 2011), which also found evidence for higher diversification rates, rather than evolutionary age, driving the marked asymmetry in species richness between tropical *Adelpha* and temperate *Limenitis*. However, in another recent study of North American butterflies, Hawkins & DeVries (2009) found a pattern of increasing ‘mean root distance’ (MRD, an ordinal measure of the mean

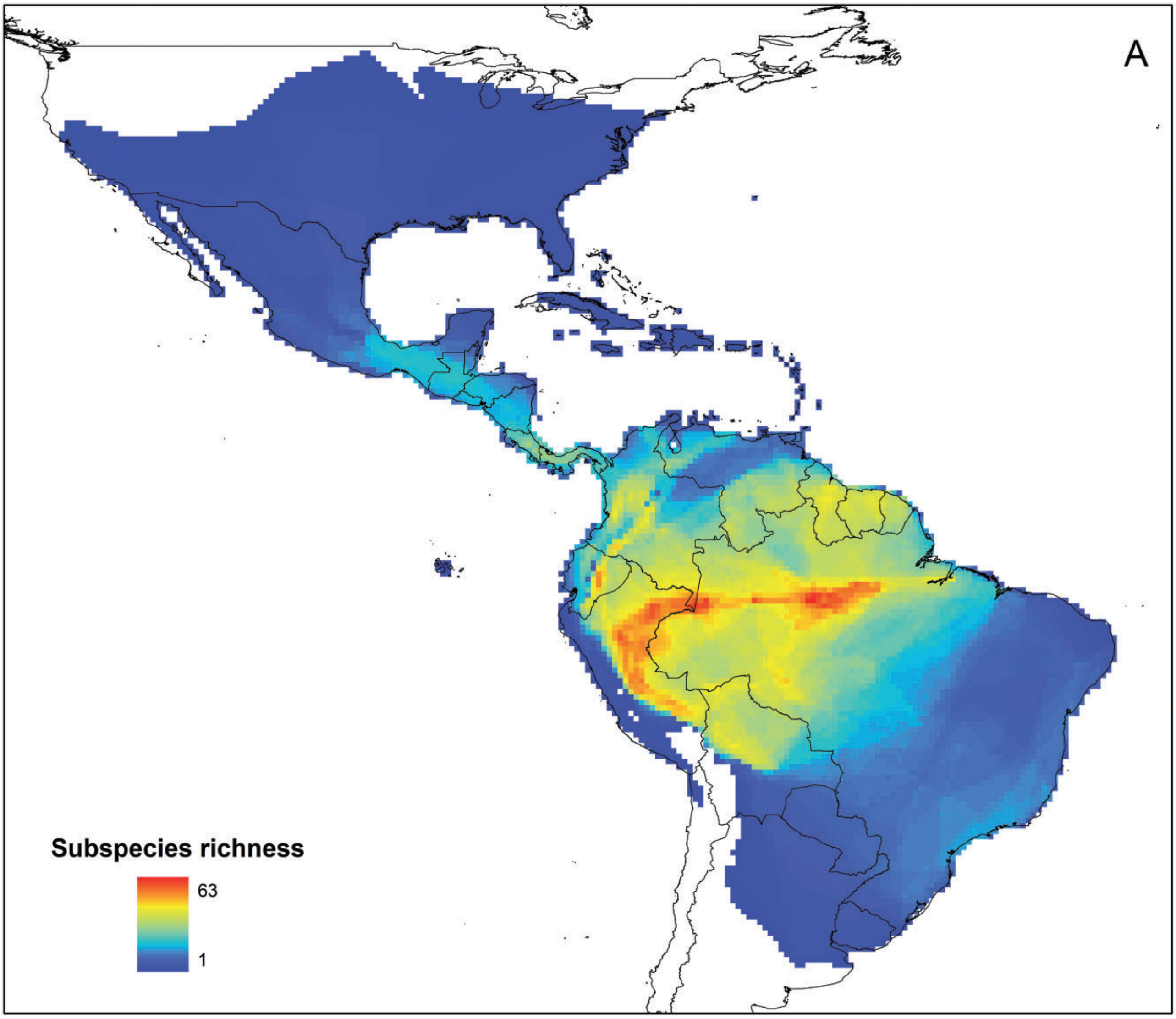

Figure 6. Areas of high subspecies turnover. Subspecies richness (A) and mean number of subspecies per species (B) in 50 × 50 km grid cells.

number of nodes between the root of the tree and the tips) with increasing latitude, which they interpreted as evidence for mainly recent evolution of butterflies into more temperate habitats. This, they argued, was consistent with tropical conservatism as an explanation for low temperate species richness. The latter results might appear to contradict our findings of younger, not older, species in the more diverse parts of the central tropics. However, their clade youth measure was of subfamily 'mean root distance', and there is no necessary reason to expect a relationship with age at the level of species. Furthermore, their data tended to exclude the most diverse, central tropical regions. Nevertheless, the spatial patterns of MRD found by Hawkins & DeVries (2009) are certainly striking. We suggest that these patterns may instead result from evolution of cold tolerance and colonization of relatively recent temperate biomes, in particular grasslands, in only a few clades that contain the greatest potential for rapid adaptation. Such clades are also likely to be those that are diversifying most rapidly (e.g. Nymphalidae, Lycaenidae) and therefore have a higher MRD. An additional requirement of the tropical conservatism hypothesis, namely that most clades have a tropical origin (Hawkins & DeVries, 2009), requires evaluation in most neotropical taxa. In our experience, there is more evidence in favour of the opposite scenario, namely that most neotropical clades evolved from temperate or montane ancestors. For example, the

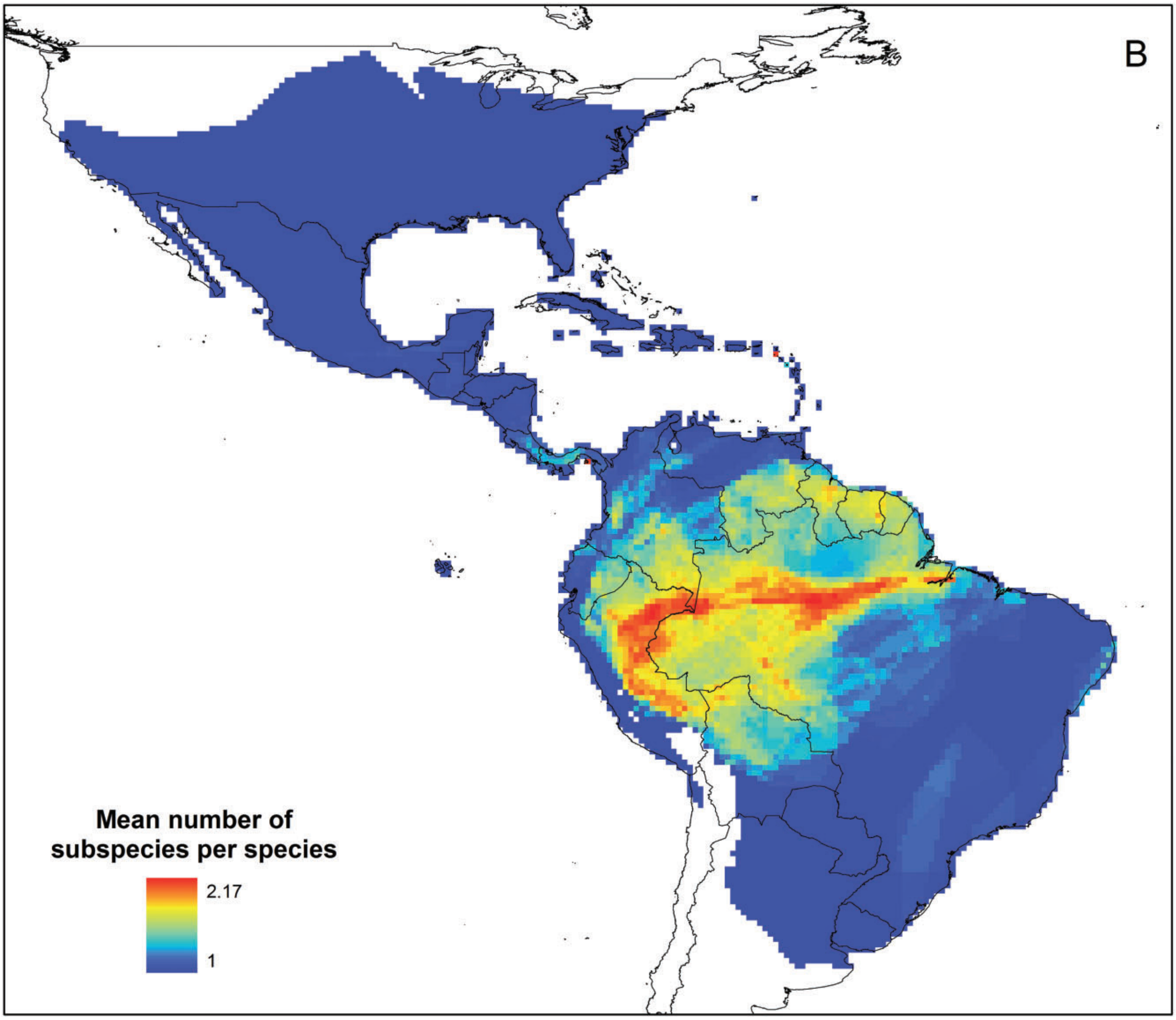

Figure 6. *Continued*

ancestor of *Adelpha* was almost certainly a temperate or montane species because Limenitidini phylogeny supports an Old World origin of the group with subsequent colonization of the New World via the Bering Strait in the middle Miocene (12.5–15 Mya) (Mullen *et al*., 2011). Similarly, northern routes are inferred for the diverse neotropical clades Phyciodina (Wahlberg, 2006; Wahlberg & Freitas, 2007), Euptychiina (Peña & Wahlberg, 2008), and Aporiina (Braby, Pierce & Vila, 2007), and a southern route for *Euryades* + *Parides* (Braby, Trueman & Eastwood, 2005). Clearly, phylogenetic hypotheses for multiple groups are needed to examine the generality of the various evolutionary hypotheses proposed to explain the latitudinal gradient in New World butterfly species richness.

Within the neotropical region, heliconiine species richness clearly peaks along the eastern slopes of the Andes, where richness is highest for species of all ages. This pattern is common in other neotropical groups, including cicindelid beetles (Pearson & Carroll, 2001), birds (Orme *et al*., 2005), and mammals (Willig *et al*., 2003), and corroborates the results obtained in other groups of butterflies, including several aposematic groups (Brown, 1982) and limenitidines (Willmott, 2003; Mullen *et al*., 2011). Partly, this pattern can be explained by high community species richness (α diversity), and partly by high rates of species turnover across the Andean elevational gradient (β diversity), with a mix of montane and lowland species occupying grid cells in the eastern Andean foothills. For example, the large but

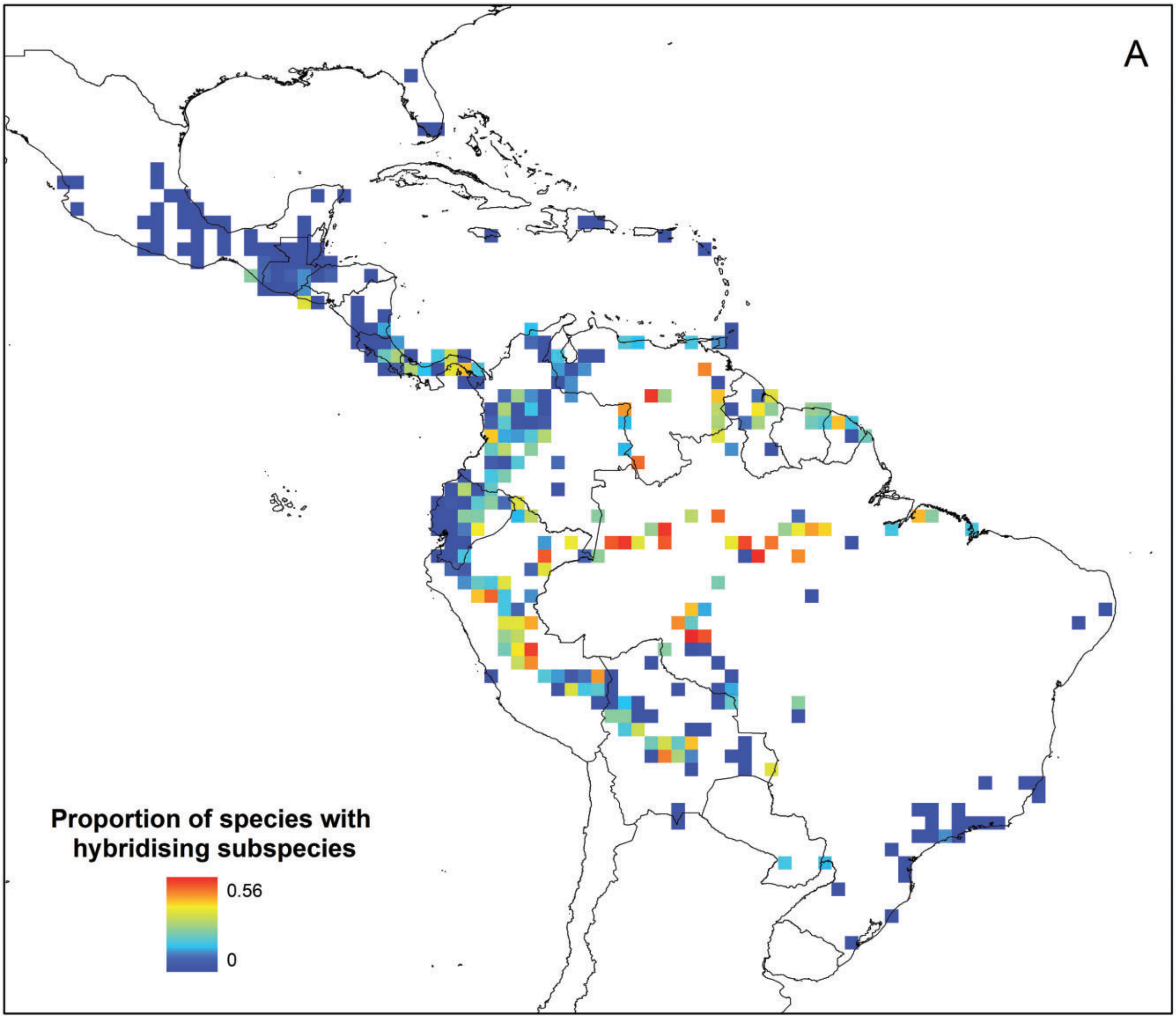

Figure 7. A, proportion of species with hybridizing subspecies mapped in 100 × 100 km grid cells. To account for biases induced by small sample sizes, we only included grid cells with ≥ 20 specimen records. B, residual variation from general linear model treating proportion of hybridizing species as a response and with binomial family error structure and log sample size as a predictor, backtransformed to proportions.

almost entirely lowland butterfly genus *Theope* shows little difference in species richness across the Amazon basin, probably because of a lack of montane species to inflate grid cell totals in the Andean foothills (Hall, 1999).

Whatever the cause of high species richness in the east Andean foothills, our data support an important role of the Andes in the evolution of heliconiines, which is a view bolstered by case studies of Andean heliconiine taxa close to the species boundary (Brower, 1996; Jiggins *et al*., 1996; Jiggins & Davies, 1998; Mavárez *et al*., 2006; Arias *et al*., 2008; Giraldo *et al*., 2008). The radiation of the most diverse heliconiine genera (which comprise approximately 85% of the species) has taken place during the last 18 million years (Wahlberg *et al*., 2009), coinciding with the major period of Andean uplift (Gregory-Wodzicki, 2000) and, notably, species terminal branch lengths are on average particularly short in the eastern Andes. Nevertheless, species richness in the Amazon basin is also high and the mean terminal branch lengths are also short, suggesting that the lowlands have produced much of the heliconiine diversity.

By contrast, 'subspecies richness', reflecting intraspecific polymorphism of colour pattern traits, is highest along the course of the Amazon River, with the Eastern Andes relatively depauperate (Fig. 6A,

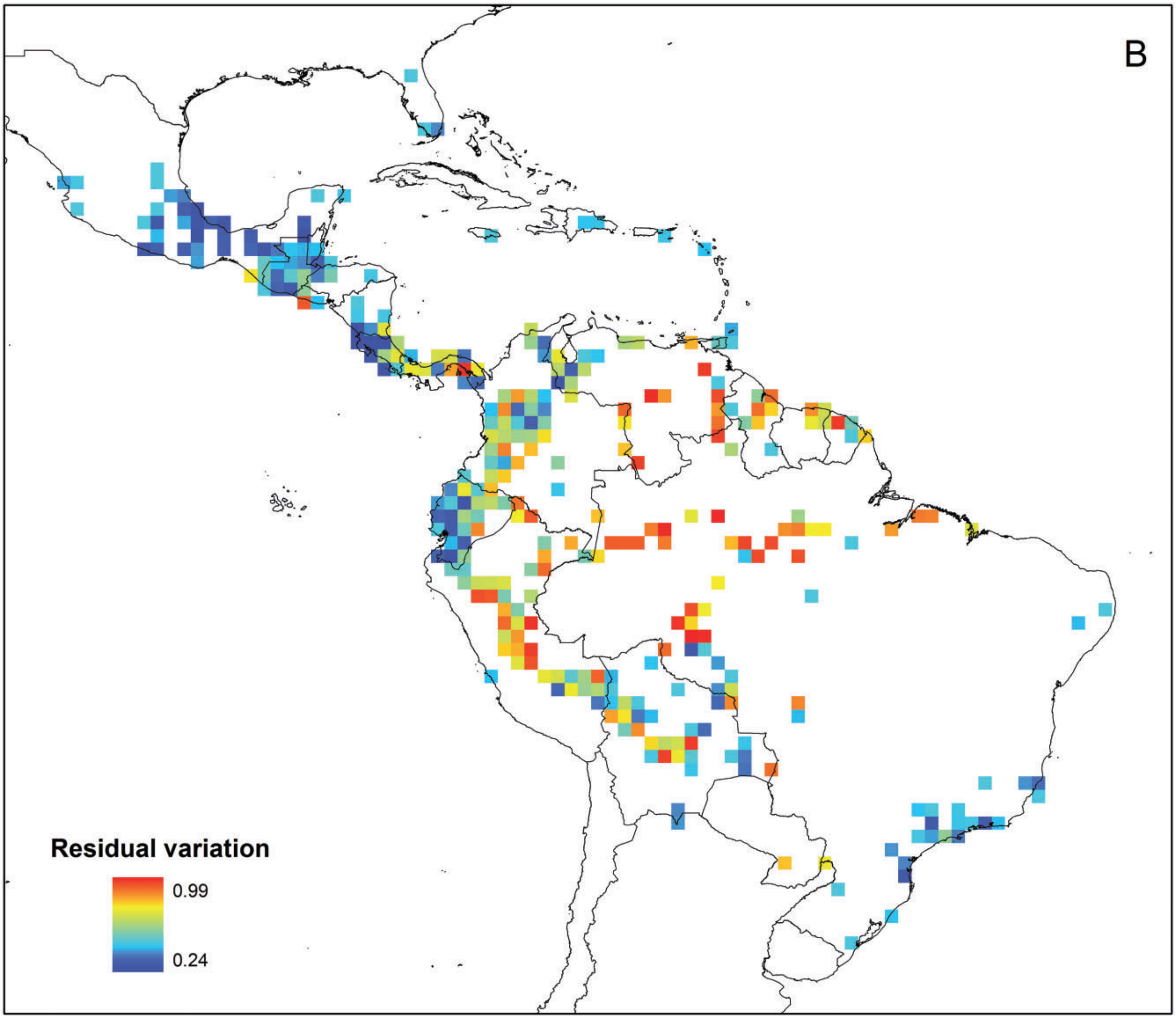

Figure 7. *Continued*

B). Our map of the proportions of species with hybridizing subspecies (Fig. 7A, B) suggests the existence of a suture zone (Remington, 1968), which corroborates this pattern of phenotypic diversity; many grid cells with high proportions are closely associated with the area of maximum subspecies overlap and richness along the Amazon River (Fig. 6A). Many subspecies have their range limits near the Amazon (see Supporting information, Appendix S1), and the region may form a discontinuity between faunas. A partial barrier effect of major rivers is well known from birds and primates (Capparella, 1990; Ayres & Clutton-Brock, 1992; Burney & Brumfield, 2009), although our maps show that species and subspecies ranges often span even the widest rivers. We suggest that, although the Amazon does not form an impregnable barrier for all heliconiines, it may serve to reduce gene flow sufficiently to slow the spread of novel phenotypes on either side leading to the formation of a suture zone.

What might explain the apparent discrepancy between areas with high species richness and those with high subspecies richness and levels of intersubspecific hybridization? A likely explanation is that the majority of lowland subspecies do not represent incipient species. Although many species probably go through a stage of being subspecies, the formation of locally adapted populations does not necessarily result in speciation (Butlin, Galindo & Grahame, 2008). Although speciation in *Heliconius* is frequently associated with a switch in mimetic colour pattern (Jiggins *et al*., 2001; Mallet, 2009), most Amazonian subspecies represent relatively minor variations within broad mimicry rings, and high rates of gene flow may prohibit further divergence among local polymorphs (Joron & Mallet, 1998). By contrast, subspecies occurring close to the Andean cordillera often have more divergent colour patterns, and speciation may more readily be completed here. This could be a result of the Andes providing more barriers to gene flow, or because the more spatially heterogeneous environmental conditions (particularly across elevations) present greater opportunities for ecological speciation (Elias *et al*., 2009) or a combination of the two.

Another possibility that might explain the differences in subspecies and species diversity patterns is that subspecies in the Andes are older, allowing more time for speciation, and thus a greater preponderance of young species and fewer remaining subspecies. It is hard to estimate the ages of subspecies; indeed, the concept may often be meaningless if different parts of the genome have very different histories. However, in both *Heliconius erato* (Linnaeus) and its unrelated mimic *Heliconius melpomene* (Linnaeus), there is now molecular evidence from the red colour pattern and Müllerian mimicry locus, *optix*, that the ‘rayed’ Amazonian colour patterns are very closely related to each other compared to the peripheral, Andean ‘postman’ subspecies with unrayed colour patterns. This suggests that rayed mimetic colour patterns are a recent Amazonian innovation that has spread out, confining older, extra-Amazonian mimicry colour patterns to relictual, and, in some cases, disjunct populations in the periphery of the Amazon (Hines *et al*., in press). This centrifugal spread hypothesis was predicted for *Heliconius* warning colour originally on the basis of disjunct colour patterns in the periphery of the Amazon, and also because it was consistent with other evidence for a ‘shifting balance’ mode of warning pattern diversification (Mallet, 1993, 2010; Turner & Mallet, 1996). Rapid evolution and turnover of new colour patterns in the Amazon, as well as rapid spread to the periphery, followed by slow progress towards speciation of relictual populations in Andean valleys, can explain why subspecies are so diverse in the Amazon, whereas young species are commoner in the more species-rich Andes.

Most likely, some combination of explanations underlies the discrepancies in diversity pattern between species and subspecies. On the one hand, ecotones are probably steeper in the Andes, and the terrain is more conducive to geographical isolation. This contributes to a greater tendency to transition across the species boundary. On the other hand, if the older ages of subspecies characteristics found in Andean *H. erato* (Hines *et al*., in press) are general among the heliconiines, many of the ‘young species’ that we find in the Andes may be relictual ‘older subspecies’ that were left behind after rapid competitive spread of new subspecies variants from the Amazon. If so, the Amazon may be the ‘species pump’, whereas the Andes has high diversity because it is a ‘museum’ of subspecies that have transitioned the species boundary more often as a result of their greater age. This in effect could reverse the directionality of Fjeldså’s (1994) argument, by focusing on sources of speciation in processes below the species level.

CONCLUSIONS

We have found that the heliconiine butterflies in species-rich regions tend to have short phylogenetic branch lengths, and species in depauperate regions tend to have long branch lengths. This result supports a role for variation in speciation and/or extinction rates in driving the latitudinal species richness gradient, rather than just evolutionary age or phylogenetic niche conservatism. An apparent mismatch between areas of high species richness and those inferred to be favourable for speciation as a result of elevated subspecies richness, polymorphisms, and the presence of suture zones, suggests that speciation is more likely to be completed in Andean foothills than in the Amazon lowlands. In summary, most heliconiine species originated in the upper and middle Amazon basin and the eastern slopes of the Andes in Colombia, Ecuador, and Peru, and these comprise areas that have the highest current species richness for this group.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Range maps for Heliconiine species and subspecies.