

TOWARDS AN INTEGRATED HISTORICAL BIOGEOGRAPHY OF THE NEOTROPICAL LOWLAND AVIFAUNA: COMBINING DIVERSIFICATION ANALYSIS AND LANDSCAPE EVOLUTION

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Resumo. – Uma abordagem integrada da história biogeográfica da avifauna Neotropical de terras baixas: combinando padrões de diversificação e evolução da paisagem. – A importância da evolução da paisagem como agente causador da diversificação biológica na região Neotropical é conhecida há tempo, mas mecanismos comuns até hoje não foram revelados. Sugerimos que três fatores principais contribuem para essa ausência de consenso: (i) pouco conhecimento da história da Terra, (ii) problemas com o teste de hipóteses de diversificação, e (iii) representação incompleta da diversidade pela taxonomia atual. Portanto, para estudar padrões de diversificação alguns passos básicos têm que ser observados: (i) as unidades evolutivas têm que ser identificadas através de revisões taxonômicas, (ii) padrões de diversificação precisam ser correlacionados a informações recentes sobre a história da Terra e (iii) hipóteses precisam ser avaliadas em relação a predições diretas e não ambíguas derivadas de cenários históricos alternativos. Neste artigo mostramos como estudos que amostram todas as unidades evolutivas e utilizam abordagens integrativas que combinam análise da diversificação com dados detalhados sobre a história da Terra podem fornecer novas perspectivas sobre o papel dos fatores históricos na diversificação de organismos Neotropicais. Os resultados apresentados mostram que tanto mudanças paleoclimáticas quanto paleogeográficas podem ter sido importantes na diversificação de grupos de aves Neotropicais de terras baixas durante e antes do Pleistoceno.

Abstract – The importance of landscape evolution as driver of biotic diversification in the Neotropical lowlands has long been appreciated, but no clear common mechanisms have been revealed. We suggest that three main issues have collaborated to this: (i) poor understanding of earth history; (ii) problems with testing diversification hypotheses; and (iii) poor representation of diversity by current taxonomy. Thus, in order to study diversification patterns, some basic steps need to be observed: (i) the units of biodiversity need to be properly recognized through taxonomic reviews, with good geographic sampling; (ii) diversification patterns need to be carefully confronted to new information on landscape history and (iii) hypotheses need to be evaluated against direct and unambiguous predictions from alternative historical scenarios. Here we show how studies that sample all evolutionary units and use integrative approaches that combine diversification analysis with detailed data on earth history can provide new insights on the role of historical factors on the

diversification of Neotropical organisms. The results presented show that both paleogeographic and paleoclimatic processes may have had an important role in the diversification of lowland Neotropical avian groups during the Pleistocene and before.

Key words: Neotropical, birds, phylogeography, diversification

INTRODUCTION

Evolutionary biologists have long struggled to understand the causes of high species diversity in the Neotropics, but have not reached a general consensus on the mechanisms underlying observed patterns. In the Neotropical lowlands, the importance of landscape evolution as driver of population differentiation and speciation has long been appreciated (Chapman 1917, Haffer 1969). Although both palaeoclimatic and geological history are thought to have influenced population dynamics, potentially leading to speciation across ecological and physical barriers, many questions remain regarding their roles on the processes of diversification. Here we suggest that three main issues have hindered a better understanding of the mode and tempo of speciation in the Neotropical lowlands: (i) poor understanding of earth history (*i.e.* paleogeographic and climatic processes); (ii) problems with testing diversification hypotheses; and (iii) poor representation of diversity by current taxonomy. These issues have collaborated to create a false and unnecessary dichotomy between processes operating during the Pleistocene (*i.e.* climatic oscillations) and before the Pleistocene (*i.e.* geological evolution), and have eclipsed our views about the influence of the mechanisms underlying diversification.

In the Neotropics, the Pleistocene refugia hypothesis, originally proposed as a speciation mechanism for Amazonian birds (Haffer 1969), rapidly became the dominant paradigm, and remained as such for at least two decades. The main prediction of this hypothesis was that many forest dwelling species originated

due to the cyclical isolation of populations in forest refugia, mainly during the Pleistocene glacial eras. Many aspects of the refuge model have been challenged, including the timing of speciation events and the existence of putative refugia (*e.g.* Lessa *et al.* 2003, Bush & Oliveira 2006). A main criticism was that most diversification events in Amazonia predate the Pleistocene (Hackett & Rosenberg 1990; Mustrangi & Patton 1997; Weir 2006; Hoorn *et al.* 2010), triggering strong reactions against the refuge hypothesis and the Pleistocene paradigm of speciation, and pointing towards a pre-Quaternary origin of lowland Neotropical species (but see Rull 2011a). Some authors, however, challenge this view, suggesting that Pleistocene speciation events may have been overlooked by under-representation of species diversity (Bates & Demos 2001, Tobias *et al.* 2008, Rull 2011a,b). In fact recent studies suggest that diversification rates have been constant during both the Quaternary and the Neogene (Derryberry *et al.* 2011, Patel *et al.* 2011, Rull 2006, 2011a,b), with no signal of decrease in the Neotropical lowlands (Tobias *et al.* 2008, Patel *et al.* 2011, Ribas *et al.* 2012).

Based on these results, we suggest that in order to study diversification patterns, some basic steps need to be observed: (i) the units of biodiversity (independent evolutionary units) need to be properly recognized through taxonomic reviews, with good geographic sampling; (ii) diversification patterns need to be carefully confronted to new information on landscape history and (iii) evaluated against direct and unambiguous predictions from alternative historical scenarios, in a mechanistic framework, bridging the fields of geology, paleoecology, phylogeography and the biology of organisms.

Here, we show how studies that sample all evolutionary units and use integrative approaches that combine diversification analysis with detailed data on earth history can provide new insights on the role of historical factors on the diversification of Neotropical organisms. More specifically, we suggest that new geological and molecular data indicate that both paleogeographic and paleoclimatic processes may have had an important role in the diversification of lowland Neotropical avian groups during the Pleistocene and before. A complete review of current diversification hypotheses is beyond the scope of this study and we only refer to benchmark studies to highlight the increasing importance of phylogeographic approaches in understanding mechanisms of diversification in the Neotropical lowlands.

Underestimation of diversity by current taxonomy – Several authors have highlighted the perils of taxonomic artifacts in biogeographical interpretations (Bates & Demos 2001, Tobias *et al.* 2008, Patten *et al.* 2011). In fact, regardless of the species concept of choice, taxonomic reviews applying modern conceptual frameworks are crucial to document speciation patterns in lowland organisms. Recent studies have shown that many widespread polytypic species actually contain a number of evolutionary independent lineages that deserve species status (Tobias *et al.* 2008, Ribas *et al.* 2011). For example, whereas Weir (2006) suggested that Pleistocene climatic fluctuations were more pervasive on Neotropical mountains than in the lowlands, it remains unclear whether this is an artifact of taxonomic bias (Tobias *et al.* 2008). Similarly, taxonomic uncertainty has led to the idea that species are younger in the Andes than in the lowlands (*i.e.* the museum hypothesis; Fjeldså 1995). Studies of the Amazonian biota that assume that polytypic species do not encompass a

number of good species are likely to neglect part of the diversity they aim to analyze. Indeed, when taxonomic misrepresentation of diversity is fully accounted for by performing taxonomic reviews prior to phylogeographic/phylogenetic analyses on widespread polytypic species, it has been shown that many species originated within the Pleistocene (Tobias *et al.* 2008, Ribas *et al.* 2011).

Evolution of South American climate and diversification of forest birds. Climate, particularly the amount and seasonality of rainfall, is one of the most important factors determining spatial patterns of forest distribution. In the tropical lowlands, including South America, rainforests grow on almost any soil, but only develop where annual rainfall is well distributed throughout the year and is greater than ~1800 mm (Corlett & Primack 2011). Therefore, changes in these variables, associated with climate cycles during the Pleistocene, may have affected the distribution of lowland forests. Based on this assumption, and on the scarce geomorphological and palynological data available at the time, Haffer (1969) proposed the so-called “refuge hypothesis”.

Available data consistently indicates that millennial-scale climate changes in South America were primarily forced by Milankovitch cycles (Vonhof & Kaandorp 2010). Also, significant glacial-interglacial variation is registered in several of the longer Quaternary records (*e.g.* Hooghiemstra *et al.* 1993, Cruz *et al.* 2005). Therefore, it is expected that changes in lowland forests distribution have occurred cyclically and, at least, throughout the Quaternary. Current data also suggest that the intensity of climatic oscillations in South America differed among regions. For example, lowlands and Andean highlands have shown different patterns of rainfall response during LGM (Vizy & Cook 2007). Even among lowland forest regions is also

observed a decoupling of climate patterns. For example, Cruz *et al.* (2009) observed that climate in the eastern Amazon swung between interglacial wet and glacial arid conditions, while western Amazon remained generally humid throughout the last 250 ka (Cheng *et al.* unpublished). Similarly, different regions of Atlantic forest seem to have experienced not only different intensities of changes (Carnaval & Moritz 2008), but also opposite responses - with forest expansion in the south and retraction in the north at the same time (Cruz *et al.* 2005, 2009; Wang *et al.* 2004, 2006), and these patterns are supported by biological data (e.g. Cabanne *et al.* 2008, d'Horta *et al.* 2011). Therefore, it seems that the spatial behavior of climate change during the Quaternary was much more complex than previously thought, generating different expectations regarding the impact of these climate cycles on forest distribution among South American regions.

Although there isn't an integrated regional climate history, a great amount of paleoenvironmental data has become available since the proposition of the Refuge hypothesis (e.g. Auler *et al.* 2006; Wang *et al.* 2006; Cruz *et al.* 2009; Ledru *et al.* 2009). These new data will necessarily improve our knowledge of climate evolution in South America, and allow for a better assessment of the role of Quaternary climate changes in the origin of the biogeographic patterns observed in the continent.

Geological evolution of the Amazon Basin: new data and debates. Based on stratigraphic records from Colombia and eastern Amazonia, several authors (Hoorn 1994, Hoorn *et al.* 1995, Figueiredo *et al.* 2009, Hoorn & Wesselingh, 2010, Hoorn *et al.* 2010) have developed the currently accepted paleoenvironmental history of northern South America, in which the Amazonian drainage system was established during the Middle to Late Miocene (~15 to 7 mya). This implies

that drainage evolution could be regarded as a potential driver of biotic diversification mainly during the Miocene, and has collaborated to the dichotomy between old (rivers/Miocene) and young (refugia/Pleistocene) drivers of diversification in Amazonia.

However, analyses of sediments from central Amazonia and Peru have led other authors (Rossetti *et al.* 2005, Campbell *et al.* 2006, Latrubesse *et al.* 2010, Silveira & Nogueira 2011) to suggest an alternative interpretation, in which drainage evolution was a long process that started during the Miocene, and lasted until the Pleistocene, with the current drainage system being established during the Plio-Pleistocene. This alternative model implies that the Purus arch (Wanderli-Filho *et al.* 2010) was an important barrier for drainage evolution, and that the Amazon basin was divided by it until the Pliocene, with the transcontinental Amazon drainage system being established only after that time (Latrubesse *et al.* 2010, Silveira & Nogueira 2011). Further supporting the dynamic nature of Amazonian drainage, studies employing SRTM data and thermoluminescence dating of sediments at riverine terraces have shown that some of the large Amazonian rivers have recent histories, influenced by sediment accumulation and neotectonics (Almeida-Filho & Miranda 2007, Soares *et al.* 2010).

The evolutionary scenario derived from this alternative interpretation brings the time frame of drainage evolution to overlap with the beginning of the Pleistocene glacial era (last 2.6 Myr). As the dating of diversification events in several Amazonian avian groups indicates that current species originated during the Plio-Pleistocene (Aleixo 2004, Patel *et al.* 2011, Ribas *et al.* 2012, Ribas *et al.* unpublished), this scenario implies a temporal correlation between drainage evolution and biotic diversification. Thus, the possibility that drainage evolution has influenced the diversification of organisms in Amazonia during the Plio-Pleistocene needs

to be further investigated by including all the available geological information into plausible diversification scenarios and adopting sampling schemes designed specifically to test the competing hypotheses.

Hypotheses testing: integrating phylogeographic analyses and landscape evolution. The importance of classical hypotheses in explaining diversification of Neotropical organisms has been historically contentious; the main criticisms stem from the fact that similar biogeographic patterns can be equally well explained by more than one hypothesis (Endler 1982). In fact few contrasting predictions are currently available, or can be derived from alternative hypotheses of speciation (Moritz *et al.* 2000). One way to overcome such hurdles is through the application of integrative approaches that combine information on earth history and landscape evolution with population genetics and coalescent theory analytical methods, which can allow the falsification of hypotheses in a more comprehensive way (*e.g.* Cabanne *et al.* 2011, Maldonado-Coelho 2012, Ribas *et al.* 2012).

The test of competing hypotheses of diversification among and within biogeographic regions is greatly strengthened when available information on paleoenvironmental evolution is incorporated (Carnaval & Bates 2008, Ribas *et al.* 2012). This type of approach can generate unambiguous predictions that can provide clues on how and when populations were affected by earth history such as drainage evolution, glaciations, orogeny, and changes in biome distribution. Paleoenvironmental data may come from palynological (Ledru *et al.* 2006), geological (Campbell *et al.* 2006, Latrubesse *et al.* 2010, Hoorn *et al.* 2011), and paleo-climatic data (Wang *et al.* 2004, Raymo *et al.* 2006). In addition to raw paleoenvironmental data, it is also possible to generate refugia hypotheses based on historical climatic modeling (Carnaval

& Moritz 2008), which can be used to derive explicit spatio-temporal predictions to be contrasted with pattern of genetic variation (Maldonado-Coelho 2012). A conceptual framework combining phylogeography and information on paleoenvironmental data was recently used to disentangle the effects of drainage evolution and glaciations as engines of speciation in a widely distributed Amazonian avian group (Ribas *et al.* 2012). As detailed information on earth history is becoming widely available for many lowland regions, the emergent field of statistical phylogeography also offers the possibility of testing multiple plausible diversification models derived directly from these external sources of data (Knowles 2009, Hickerson *et al.* 2010). In some cases, however, the competing hypotheses predict similar temporal and spatial patterns of population differentiation, and more explicit and detailed spatial-temporal frameworks would be useful. For example, one would need to use sampling schemes designed to test specific hypotheses, as did the classical study of Patton and co-workers along the Juruá River (Patton *et al.* 1994), to derive unambiguous predictions capable of clarifying the roles of relatively young paleodrainage evolution (*i.e.* Pleistocene-Holocene boundary; Rossetti & Valeriano 2007) versus Pleistocene glaciations on population divergence. Different approaches used to test competing hypotheses will vary case by case and will depend on available information on earth history and on the creativity of individual researchers.

RESULTS PRESENTED IN THE SYMPOSIUM

Idiosyncrasies in Atlantic Forest diversification patterns: the case of the Greenish Schiffornis (*Schiffornis virescens*, *Tytiridae*). Studies of Atlantic Forest organisms suggest that the historical distribution of the forest cover generated

demographically stable populations in its central region and unstable populations in the southern and northern regions (Carnaval & Moritz 2008; Carnaval *et al.* 2009). The southern range of the Atlantic Forest was apparently the most affected by forest retraction and formation of refuges during the Pleistocene glaciations (Carnaval *et al.* 2009). This observation led some authors to state that refuge formation might have been important to explain diversification of Atlantic Forest taxa (i.e. Cabanne *et al.* 2007, Carnaval *et al.* 2009, Maldonado-Coelho 2012).

Cabanne *et al.* (unpubl) studied the mitochondrial phylogeographic structure of the Greenish Schiffornis *Schiffornis virescens* (Tytyridae), a passerine endemic to the AF and inhabitant of the forest understory, and evaluated questions and predictions related to the aforementioned hypothesis. The specific questions were: i) does the species present a strong phylogeographic structure, with ii) demographic stability in the northern regions and iii) demographic expansion in the southern populations?

The authors analyzed cytochrome b and control region sequences of the mitochondrial genome by traditional phylogenetic and population genetic methods based on summary statistics. In addition, they used coalescent simulations to evaluate specific models of evolution of the populations of *Schiffornis virescens*.

The results did not support phylogeographic partitions of the genetic variability of *S. virescens*. The time to the most recent common ancestor was 0.4 MY (95%CI 0.2-0.6 Myr), which indicates that the species is relatively young. The overall F_{st} was = 0.32 and gene flow between regions was moderate to high. The analysis suggested that the whole population of *S. virescens* suffered a bottleneck followed by a demographic expansion in the late Pleistocene. The bottleneck might have contributed to the extinction of intraspecific

lineages, hence to the observed lack of a strong phylogeographic pattern and low genetic diversity.

Even though the results of this study are not fully compatible with the demographic predictions derived from Carnaval and Moritz (2008) and Carnaval *et al.* (2009), which were accepted in other AF taxa (Cabanne *et al.* 2007; Cabanne *et al.* 2008; Musturangi & Patton 1997; d'Horta *et al.* 2011; Carnaval *et al.* 2009; Maldonado-Coelho 2012), they still are in accordance with hypotheses of the AF history during the Pleistocene. The fact that *S. virescens* is a relatively young species and is also associated with southern AF might explain why the pattern found differs from other studied groups. Independent of the origin of the phylogeographic structure of *S. virescens*, our results suggest that some AF taxa like *S. virescens* have had all their populations affected by the recent history of the biome, contrary to what has been revealed by other phylogeographic studies that indicated different demographic histories between central and southern AF populations (Cabanne *et al.* 2008; d'Horta *et al.* 2011; Carnaval *et al.* 2009; Batalha-Filho *et al.* 2009, Maldonado-Coelho 2012). Therefore, organisms may have idiosyncratic responses to historical processes, and predictions about the history of the biome should take into account ecological characteristics and distribution of each organism.

Rivers and Refuges in Atlantic Forest and Amazonian taxa: phylogeographic patterns. Surveys conducted in distinct regions containing a suite of environmental conditions constitute ideal scenarios to assess the role of both climatic and geological history as drivers of diversification. Maldonado-Coelho *et al.* carried out a densely sampled study to elucidate the importance of paleoclimatic and geological factors as engines of diversification on the lowland representatives of South American fire-eye antbirds (genus *Pyriglena*). The authors

used mitochondrial and nuclear sequence data to examine the phylogeographic structure of fire-eye populations along the southeastern Amazon Basin and Atlantic Forest, and confronted the predictions of the river and Pleistocene refuge hypotheses against the patterns of genetic variation observed in these populations. A number of phylogeographic and population genetics analytical approaches were employed to address these questions (Maldonado-Coelho 2010).

The phylogenetic/phylogeographic reconstructions and Bayesian dating estimates suggest that the origin of the three major clades trace back to the formation of the modern course of the Amazon, Tapajós, Xingu and São Francisco Rivers, with subsequent diversification fostered by more recent events, such as the origin of the modern Tocantins River course and by Pleistocene climatic oscillations creating opportunities for range expansion and geographic isolation in the Atlantic Forest. More specifically, the role of large rivers as barriers to population differentiation in fire-eyes is apparently stronger in Amazonia than in the Atlantic Forest. A detailed analysis along the Tocantins River valley provides no support for the hypothesis that populations were isolated in glacial forest refuges. Instead, the data provide strong support for a key prediction of the river hypothesis and shows that this river has likely been the historical mechanism underlying population divergence in fire-eyes (Maldonado-Coelho *et al.* submitted). On the other hand, climatic oscillations seemed less important in creating opportunities for geographic differentiation within the Amazon in comparison to the Atlantic Forest.

These results add to mounting evidence that climatic oscillations seem to have played a substantial role in shaping the phylogeographic structure and possibly the diversification of many taxa in the Atlantic Forest (*e.g.* Cabanne *et al.* 2008; Carnaval *et al.* 2009, d'Horta *et al.*

2011). Also, they have important implications for a better understanding of the importance of large Amazonian rivers in vertebrate diversification in the Neotropics and agree with recent geological (Rosseti *et al.* 2005, Campbell *et al.*, 2006; contra Hoorn *et al.*, 2010) and molecular studies (Patel *et al.* 2011, Ribas *et al.* 2011) that have suggested that the modern Amazonian drainage system originated during the Pleistocene (see above). Fire-eyes seemed to have a complex evolutionary history, involving large-scale geological and climatic processes acting over regional and continental scales during the last 2.5 Myr.

A similar pattern for Amazonian birds is revealed by the results presented by Ribas *et al.* Both the genus *Psophia* (Gruiformes; Psophiidae) and the genus *Rhegmatorhina* (Passeriformes; Thamnophilidae) have current species distributions delimited by large Amazonian rivers, and both genera diversified during the last 3 Ma. *Psophia* occurs throughout the Amazon Basin and Ribas *et al.* (2012) showed that the genus comprises eight, rather than the three species previously recognized. Each of these eight species occurs in a different area of endemism, and the sequence of diversification events in the genus suggests a sequence for drainage evolution in the basin, with a first split related to the lower Amazon River, subsequent splits related to the upper Negro and Madeira Rivers, and more recent splits occurring between adjacent interfluvia at the Brazilian shield. The first split within *Rhegmatorhina*, which does not occur in the Guiana shield, corresponds to the Madeira River. In both genera there is higher genetic structure within the Madeira - Tapajós interfluvium (Rondonia area of endemism) when compared to other Amazonian regions. Signal of recent (last 10,000 to 20,000 years) demographic expansion was found for the *Psophia* species that occur at the Guiana and Inambari areas of endemism, while demographic stability was suggested for the Rondonia area of endemism. In *Rhegmatorhina*,

recent population expansion is suggested for all Brazilian shield clades, including the three distinct clades found within the Rondonia area of endemism. Recent population expansion was also detected at the Inambari area of endemism. In both genera there are taxa of similar ages (1.0-1.5 mya) endemic of the lower Negro – lower Solimões – Japurá interfluvium, a region suggested as a new area of endemism (Borges *et al.* 2007, Borges & Silva 2011), and probably influenced by the evolutionary dynamics of the lower Negro river (Ribas *et al.* 2012).

The results obtained for *Psophia* and *Rhegmatorhina*, combined to recent studies of other avian clades (Armenta *et al.* 2005; Eberhard & Bermingham 2005; Ribas *et al.* 2005; Aleixo *et al.* 2009; Patel *et al.* 2011; d'Horta *et al.* in press; Aleixo *et al.* in prep.) show a strong spatial correlation between lineage distributions and large Amazonian rivers. Also, molecular dating in all these groups indicates a relatively recent origin (from about 6.0 to 0.5 Ma) for these Amazonian species. It is becoming clear that these patterns are only revealed when taxonomic reviews are made prior to biogeographic analysis, and when sampling is complete (i.e. includes all basal evolutionary lineages). These results also indicate that species origins predate the strongest glacial cycles (upper Pleistocene), but that these cycles may have influenced species distributions, as there are evidences of population expansions in several forest taxa during this period. It is important to consider the possibility that the Amazonian drainage has been very dynamic during the Plio-Pleistocene and has played an important role on the origin of current species.

The role of rivers in the diversification of birds in the Guiana Shield. Taking a somewhat different approach, Naka *et al.* evaluated distribution patterns and phenotypic or phylogeographic breaks of an entire avian community (Naka 2011, Naka *et al.* 2012). To

evaluate the role of Amazonian rivers in the generation and maintenance of avian species diversity, the authors used pairs of parapatric taxa divided by the Rio Negro. In the Guiana Shield, nearly a third (78 species) of all *terra-firme* forest birds have phenotypically differentiated taxa on opposite sides of the lower Rio Negro (Naka *et al.* 2012). This pattern of parapatric replacement across the river occurs across a taxonomically and ecologically diverse array of taxa, representing 22 different avian families and 63 different genera, and includes groups as diverse as parrots, toucans, woodpeckers, jacamars, and almost every family of passerine birds (Naka 2011, Naka *et al.* 2012). In some cases, these morphologically distinct populations are considered different species (i.e. *Monasa atra* and *Monasa morphoensis*), whereas in others, pairs are regarded as different subspecies of more widespread polytypic taxa (i.e. *Ramphastos tucanus tucanus* and *R. t. cowieri*). Despite the current taxonomic treatment, which in many cases is due to a subjective assessment of the amount of phenotypic differentiation observed, pairs of taxa are geographical replacements that in general do not occur together, thus providing useful pairs to study the role of rivers in dividing their populations. In a recent study, Naka and co-authors (2012) provided evidence showing that the phenotypic variation found in pairs of populations across the Rio Negro is generally accompanied by marked genetic differentiation. These results suggest that by inhibiting gene flow, large Amazonian rivers prevent potential species introgression and, as a result, are important for maintaining current levels of species diversity. It is less clear, however, whether rivers were important in originating current patterns of diversity (Haffer 1992, 1997; Colwell 2000; Gascon *et al.* 2000).

Part of the answer may be found in bird species distributions. Using a comparative phylogeographic approach and extensive

distributional and molecular datasets from the Guiana Shield, Naka *et al.* (2012) showed that only a fourth (21) of the 78 pairs of avian taxa divided by the lower Rio Negro remain isolated by the upper sections of the river, implying that the upper Negro may not represent such an overwhelming barrier for avian populations. Quite interestingly, most of those pairs divided by the lower, but not the upper Negro, are bounded by a second river, the poorly known Rio Branco, which has been shown to divide the distributions of 42 pairs of closely related avian taxa (Naka *et al.* 2006, Naka 2011, Naka *et al.* 2012). Together, these two rivers (the Branco and the Negro) are responsible for dividing the populations of 63 pairs of avian taxa in the Guiana Shield, and offer an ideal setting to better understand the role of rivers in the origin and maintenance of species diversity. Furthermore, because they represent very different rivers, in terms of size, hydrology, color (a proxy for the amount of sediments), geomorphology, extent of associated flooded forests, and history, they provide independent examples to test the riverine barrier hypothesis.

Using a sample of 60 avian populations with phenotypic differentiation across the lower Rio Negro and the Rio Branco, Naka *et al.* (2012) revealed that these pairs present disparate levels of genetic divergences (ranging from 0.5 to 13% of corrected mitochondrial distance), and their origins probably spanned several geological Periods, from the late Miocene to the Pleistocene. Consistent with these observations, preliminary results using Approximate Bayesian Computation analyses do not support a scenario of simultaneous divergence among all pairs of taxa divided by the lower Rio Negro or the Rio Branco (Naka *et al.* in prep). On the other hand, when only pairs divided by the upper Rio Negro are used, a scenario of common diversification cannot be rejected. These results suggest that whereas both the Rio Negro and the

Rio Branco are important barriers for many species/subspecies of birds, their role in the diversification process might have been quite different. This study supports the idea that some rivers may have been important as primary barriers, as suggested by Sick (1967), Capparella (1988 and 1991), Ayres and Clutter-Brock (1992), and Ribas *et al.* (2012), whereas others possibly represent barriers to secondary contact, as suggested by Haffer along the years (1992, 1997, 2002).

Faunal Interchange between Amazonian/Andean and Atlantic forests. The main South American forest disjunction, which isolates the Andean and Amazonian forests from the Atlantic forest, is formed by the Chaco, Cerrado and Caatinga (Oliveira-Filho & Ratter 1995). This region, known as the “open diagonal” (Vanzolini 1963), is covered mainly by savannas, woodlands, and dry forests, which are unsuitable habitats for most lowland forest species.

Dispersal or vicariance can be evoked to explain the origin of the current distribution of sister-species (or even populations of the same species) that occupy Amazonian, Andean and Atlantic forests. In some cases dispersal has been considered a valid mechanism to explain these disjunct distribution patterns (*e.g.* small mammals, Costa 2003), as many forest taxa are able to use forests at the open diagonal (gallery and dry forest in the Cerrado) as corridors. However, the high humid forest dependence exhibited by other taxa makes it unlikely that divergence has occurred by any processes other than vicariance associated with changes in humid forest distribution.

The contact between Atlantic and Amazonian/Andean forests has been analyzed from spatial (Willis 1992, Nores 1992, 1994, Silva 1994, Cabanne *et al.* 2008, Costa 2003) and temporal perspectives (Ribas & Myiaki 2007, Cabanne *et al.* 2008, Costa 2003, d’Horta *et al.* unpublished). Based on distribution

patterns of intra and extra-Atlantic Forest avian species, Willis (1992) evaluated five possible connection routes between these domains. Nores (1992, 1994) and Silva (1994), based on the distribution of avian species, evaluated possible connections throughout the region currently occupied by the Chaco and Cerrado. Finally, Costa (2003), analyzing phylogeographic patterns of small non-volant mammals, pointed to potential historical/current connections between the Atlantic and other forests of northern South America.

Recent palaeoclimate data shed new light over this discussion. Cheng *et al.* (in prep.), analyzing records from western Amazonia and available data from eastern Amazonia and Atlantic forest regions (*e.g.*, Cruz *et al.* 2005, 2009; Wang *et al.* 2004, 2006), described an east-west dipole-like pattern of precipitation (west dry / east wet and vice versa) in cis-Andean South America. This climatic pattern suggests the existence of two preferential corridors between Amazonian/Andean and Atlantic forests through which past biotic interchanges may have occurred: one connecting eastern Amazonia with the northeastern Atlantic Forest (eastern corridor), and the other connecting western Amazonia with the southwestern Atlantic Forest (western corridor). Additionally, the cycles of climate change over the last 250 ky associated to these corridors seem to have been asynchronous (Cruz *et al.* 2009, Cheng *et al.* in prep.), with the pulses of increasing/decreasing of rainfall registered to eastern and western corridors occurring in alternate periods.

d'Horta *et al.* based on available phylogenetic and distributional data of rainforest birds conducted a preliminary evaluation of the predictions derived from the model described above. Results indicated that, first, the spatial patterns of distribution of sister species, or populations from the same species, strongly support the past existence of these two preferential corridors connecting Amazonian

and Atlantic forests. There is a clear pattern of evolutionary relationships linking lineages from southern Atlantic forest with Andean/western Amazonia, and northern Atlantic forest with eastern Amazonia. Second, the scenario of multiple contacts between the two largest forest blocks of South America is also supported by the great differences in divergence times estimated between lineages associated to these forests (*e.g.* Cabanne *et al.* 2008; Ribas *et al.* 2005, 2006, 2007; Derryberry *et al.* 2011; d'Horta *et al.* unpublished). Finally, the asynchrony of the pulses of increased/decreased of precipitation evidenced between western and eastern corridors is also corroborated by the historical demography of populations associated to these corridors (*eg.* Cabanne *et al.* 2008, d'Horta *et al.* 2011).

Despite the poor attention that faunal interchange among South American forests has received so far, the understanding of this process is essential to reconstruct the origin of the current biodiversity patterns of South American rainforest birds.

Seasonally deciduous dry forests and Neotropical arid biomes. Phylogeographic studies on lowland Neotropical birds have been heavily focused on species that inhabit humid forests. While humid forests are very interesting these environments only represent one component of the diverse ecosystems found in the lowlands. The lowlands are highly heterogeneous environments often consisting of multiple habitats types that can turnover across small geographic distances (Stotz *et al.* 1996). For example, a matrix of often highly disjunct arid habitats, known as seasonally deciduous dry forest (Prado 2000), occurs from Mexico through Argentina. Understanding speciation patterns and the tempo of diversification in taxa distributed across these regions and other non-rainforest habitats will provide critical insight into the evolution and assembly of Neotropical avifaunas. Currently,

we do not know whether dry taxa evolved in response to the same mechanisms as humid forest taxa or alternatively, whether dry taxa were impacted by different historical processes. It can be predicted that when climatic conditions became drier during glacial cycles and the ranges of humid taxa contracted, arid biota taxa should have expanded their ranges. Constant phases of expansion and contraction should have left distinct patterns of genetic structure across the landscape.

To assess evolutionary patterns in the more arid regions of the Neotropical lowlands, Smith *et al.* presented findings on spatial and temporal patterns of diversification in *Forpus* parrotlets a group that largely inhabits dry forests and more-open habitats. An objective of the study was to compare diversification rates estimated from multilocus data from estimates using only mitochondrial DNA. Smith *et al.* found that in *Forpus*, multilocus and mitochondrial DNA estimates of diversification rates were similar and that the best-fit model suggested a constant diversification rate across time. This pattern of constant diversification in *Forpus* is similar to the mode of speciation shown in other Neotropical birds, such as ovenbirds (Derryberry *et al.* 2011) and toucans (Patel *et al.* 2011). These findings are contrary to the hypothesis that speciation rates are predicted to decline over time as niche space fills-up (Rabosky 2008, Phillimore & Price 2008). The striking disparities between these findings are not yet clear; however, sampling biases have been shown to impact diversification rate estimates (Cusimano & Renner 2010).

Smith *et al.* assessed the role taxonomic units and sampling may have on the estimation of diversification rates. Diversification rate analyses rely on sampling the extant diversity of a group, but the scale at which diversity should be sampled is not apparent. Should we include biological species, subspecies, or phylogenetic species? Smith *et al.* found that diversification rate analyses may be sensitive

to the user's delimitation of biodiversity. Analyses that used biological species as biodiversity units supported a model of declining rates of diversification over time. Alternatively, analyses that used subspecies or phylogenetic species supported a model of constant diversification over time. Overall, these findings suggest that thorough range-wide sampling of taxa is important for inferring models of speciation and that the current species designations poorly describe the extant diversity. Moreover, this preliminary evidence indicates that humid and dry taxa both exhibit similar patterns of deep genetic structure across the landscape and this structure appears to have been generated at a constant rate over time. Future comparative phylogeographic studies will need to assess whether these analogous patterns in humid and dry taxa have occurred during the same time periods or in contrast, whether these taxa evolved in response to different mechanisms.

NEW METHODS AND PERSPECTIVES

Research on the evolution of the Neotropical avifauna has its roots in natural history, which has since expanded into a highly integrative field utilizing population genetics, computational biology, and paleoclimatology. The molecular age has provided researchers with an unprecedented toolkit for inferring aspects such as historical demography and the timing and patterns of speciation events. Over the last ten years phylogeographic studies have been transitioning from single locus mitochondrial DNA studies to multilocus studies, and now are expanding into genomics using next-generation sequencing. Next-generation sequencing has already shown great promise in avian systematics and has been used to evaluate deep (Faircloth *et al.* 2012) and shallow (McCormack *et al.* 2012) evolutionary questions in the avian Tree of Life.

The number of analytical procedures and software packages available to researchers has greatly increased the questions phylogeographic studies are able to test. The APE (Paradis *et al.* 2004), LASER (Rabosky 2006), and GEIGER (Harmon *et al.* 2008) software packages in the R language allow researchers to estimate diversification rates and test different models of speciation. Reconstructing ancestral areas continues to be an important biogeographic question and range evolution programs have become more complex allowing researchers to incorporate speciation and extinction rates (LAGRANGE; Ree & Smith 2008), phylogenetic uncertainty (S-DIVA; Yu *et al.* 2010), and hypothesis testing of spatial patterns of dispersal (PHYLOMAPPER, Lemmon & Lemmon 2008). The BEAST software package offers an array of different analyses including divergence time estimation (Dummond & Rambaut 2007), species tree estimation (Heled & Drummond 2010), demographic history (Drummond *et al.* 2005), and spatial diffusion models (Lemey *et al.* 2009). For population genetic studies, structured coalescent models have been expanded and can be used to simultaneously estimate multiple population genetic parameters (IMa, Hey & Neilsen 2007; LAMARC, Kuhner 2006; MIGRATE, Beerli & Felsenstein 2001) across multiple populations. The development of programs to simulate genealogies assuming specific population models of evolution, such as SIMCOAL2 (Laval & Excoffier 2004) and SERIAL SIMCOAL (Anderson *et al.* 2005), and of the approximate Bayesian Computation (ABC) approaches offers researchers the potential to test a range of diversification scenarios and complex demographic histories. The ABC packages msBAYES (Hickerson *et al.* 2007) and ABCTOOLBOX (Wegmann *et al.* 2010) have quickly become a popular toolkit for testing for simultaneous divergence in multiple co-distributed taxa (*i.e.* Barber & Klicka 2010). Finally, the recent development

of spatially explicit demographic simulations (DIM SUM, Brown *et al.* 2009) can be used to generate expected diversification patterns across heterogeneous landscapes.

One challenge faced in phylogeographic studies is how to perform comparative analyses on complex datasets. Often phylogeographic studies focus on taxa with widespread distributions that exhibit considerable genetic structure. This complexity often does not fit the assumptions of the available analytical methods. Many of the existing coalescent programs, such as, msBayes are designed to estimate parameters within unstructured populations. Further difficulty occurs when the diversification of lineages encompasses multiple temporal scales. Deeper Miocene-Pliocene divergences are often best analyzed using phylogenetic approaches. In contrast, Pleistocene divergences are better suited for coalescent methods that can account for lineage sorting, gene flow and population size changes. Phylogeographers have been provided a powerful statistical toolkit, but are challenged to decide the most appropriate approach given their data and the assumptions of the existing software packages.

FUTURE DIRECTIONS

A central goal of Neotropical phylogeographers has been to understand how Earth history has shaped biotic diversification. Studies on diversification and Earth history range from climatic changes (Cabanne *et al.* 2008, Horta *et al.* 2011), divergence across forest-savanna ecotones (Cabanne *et al.* 2011), Andean uplift (Brumfield & Capparella 1996, Ribas *et al.* 2007), the closure of the Panamanian land bridge (Smith & Klicka 2010), and formation of the Amazon drainage basin (Ribas *et al.* 2012). However, phylogeographic structure and genetic diversity patterns are not only shaped by these deep historical factors, but also by contemporary (Smith *et al.* 2011) and stochastic processes

(Miller *et al.* 2010). One factor that may play an important role in understanding diversification patterns is species ecology. Foraging strata has been shown to have a significant impact on the degree of genetic differentiation across biogeographic barriers (Burney & Brumfield 2009). Species that inhabit the understory of humid forests have been shown to have higher genetic distances across the Andes, and Amazon and Madeira Rivers than do species that inhabit the canopy (Burney & Brumfield 2009). Moreover, dispersal across biogeographic barriers may be facilitated by changing climatic conditions allowing environmental conditions to become more favorable for certain species (Smith *et al.* submitted).

As more data becomes available, it is becoming apparent that there is wide degree of variance in speciation patterns and timing of diversification across the Neotropical lowlands. For example, results presented in this symposium indicate that some understory birds such as *S. virescens* might present no phylogeographic structure, contrarily to what is expected from other studies on ecologically similar forest birds (Burney & Brumfield 2009, Nyari 2007, Cabanne *et al.* 2008). This variance likely indicates that Earth history and species ecology are not the only important factors. Stochasticity and extinction likely have left a strong signature in speciation patterns and pose serious challenges for reconstructing a shared history among co-distributed species (Haydon *et al.* 1994). Additionally, new data has shown Neotropical speciation is much more complex than the traditional models have proposed. New large-scale analyzes on Amazonian birds are changing our interpretations on the role of rivers as barriers to gene flow (Naka *et al.* 2012). These new findings suggest that Amazonian rivers can be a spatially dynamic barrier and the strength of a river as a barrier changes from the lower reaches to the headwaters (Naka *et al.* 2012). Given that landscapes are ephemeral and ever changing it is unclear how

current landscapes bias our interpretations. Future phylogeographic studies will have to distinguish between the role of landscape evolution in generating biodiversity from the role of landscape features in maintaining genetic differentiation between taxa.

Finally, how robust are inferences of speciation history to changes in our understanding of Earth history? Geology is a dynamic and changing field. As an example, new evidence has suggested that the closure of the Panamanian land bridge and the uplift of the northern Andes may be older than previously understood (Farris *et al.* 2011, Montes *et al.* 2012). Speciation inferences involving these geologic events are often constructed around a Late Pliocene timeframe. Phylogeographers need to be careful to not force the timing of speciation events into an “expected” time period (*i.e.* Rheindt *et al.* 2009) because molecular clocks are often calibrated from the same historical events we study. Ideally, phylogeographers need to assess the robustness of speciation scenarios using independent evidence. Going forward, Neotropical phylogeographers will be faced with the challenge of disentangling the role of Earth history, species ecology, and stochasticity to understand evolutionary patterns in one of the most species rich areas on Earth.

ACKNOWLEDGMENTS

All authors would like to thank the IX NOC Scientific Program Committee for their organizing efforts. CCR thanks FAPESP, FAPEAM, CNPq, CAPES, Frank Chapman M. Fund (AMNH) for funding, and all collectors and collections that have obtained, organized, kept, made available, and loaned samples to the projects that originated the results presented in this symposium. MMC is thankful to J. G. Blake, R. E. Ricklefs, B. Loiselle, J. Bates and C. Miyaki for guidance throughout his PhD and Pos-Doctorate projects, and to FAPESP,

CNPq (overseas doctoral fellowship), National Science Foundation (Doctoral Dissertation Improvement Grant OISE-0555482), Whitney R. Harris World Ecology Center (Parker-Gentry Fellowship) at University of Missouri-St. Louis, and the AMNH (Frank Chapman Memorial Fund) for funding. BTS would like to acknowledge R. T. Brumfield, J. Klicka, A. Cuervo and B. Hernandez-Baños. GSC is thankful to CAPES, Consejo Nacional de Investigaciones Científicas y Técnicas, FAPEMIG, FAPESP and WWF for financial support, and to C. Miyaki for guidance during his PhD. Also, GSC is grateful to E. Sari., D Meyer, F. Santos and C. Miyaki for their participation in the study of *Schiffornis virescens* presented in this symposium. FMH is grateful to F. W. Cruz, who provided insightful ideas, C. Miyaki, D. Meyer and P. E. Vanzolini for their guidance during his PhD project, CAPES and FAPESP for financial support. LNN is grateful to R. T. Brumfield and J. V. Remsen for comments and guidance throughout his PhD project. LNN was supported by a doctoral international scholarship from the Instituto Internacional de Educação do Brasil (IEB; Programa BECA) and an overseas doctoral fellowship from CNPq (202131/2006-8). Funding was provided by NSF (Doctoral Dissertation Improvement Grant 1011512, and grants 0400797 and 0841729 to R.T. Brumfield), AMNH (Frank Chapman Memorial Fund), AOU, IdeaWild, LSU, Sigma Xi, and CNPq (grants 486253/2006-6 to L.M.P. Henriques and 484219/2011-1). LNN would like to thank the financial support provided by the editorial board of *The Condor* and the Cooper Ornithological Society, particularly M. Patten and B. Smith-Patten, who made possible his participation in the IX NOC.

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