

# Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models

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## Abstract

Explaining the origins and evolution of Amazonian biodiversity continues to be an outstanding question in evolutionary biology. A plethora of mechanisms for promoting diversification has been proposed, generally invoking ecological and vicariance processes associated with major geological, hydrological and climatic events in the history of the Amazon drainage basin. Here, we review recent advances on this topic in the light of a rich new source of information: molecular phylogenetics and especially phylogeography. We present a comparison of phylogeographical studies covering over 50 clades of amphibians, birds, non-avian reptiles and mammals, focusing on studies where estimates of divergence times were explicitly calculated. We then discuss the congruence of the speciation patterns found in these studies with previous hypotheses of diversification. Based on the estimates of crown group ages, we conclude that a high proportion of present-day diversity is a result of Neogene diversification. The origin of most clades clearly predates the Pleistocene by a considerable margin, refuting the long-held hypothesis that repeated expansion and contraction of lowland forests during Pleistocene climatic changes would be responsible for most of the Amazonian biodiversity. However, some evidence from phylogenetic and distributional patterns suggests that climate cycles did trigger speciation. Speciose lineages of tetrapods tend to be older than groups containing one to a few species, with a few notable exceptions. Considering each tetrapod group alone, amphibians and non-avian reptiles are generally older than birds, while mammals contain both recent and ancient clades of approximately the same number of species. Finally, we make recommendations about future research approaches and animal systems that deserve further attention from phylogeographers.

## Introduction

Tetrapoda is a large and diverse clade of vertebrates with origins tracing back to the Devonian period (360–416 million years ago, Ma). Tetrapods include, in order of increasing number of extant species, mammals (~5400 spp.), amphibians (~6200 spp.), non-avian reptiles (~8000 spp.) and birds (~10,000 spp.). These

eye-catching and diverse animals have captivated naturalists since the earliest European explorations of South America. Not surprisingly, these animals have been the target of much of the biogeographical and ecological research in the Amazon drainage basin. Despite the attention devoted to tetrapods, their diversity has long been underestimated (e.g. Köhler *et al.* 2005) and their origins are more complex than previously thought (Schmitz *et al.* 2001). Phylogenetic approaches have revealed an outstanding diversity linked to a complex biogeographical history. Recent meta-analyses indicate that most vertebrate diversification in the Neotropical region took place throughout the Neogene (Rull 2008), in association with different mechanisms and occurring at different times (Zink *et al.* 2004), but many more comparative studies are needed to

disentangle the processes underlying diversification within Amazonia. Although that region contains a high proportion of South American tetrapod diversity, studies on Amazonian tetrapods constitute a minority of all South American phylogeographical work (Fig. 24.1).

We summarize the findings of recent studies that use molecular data to explore the spatial and temporal aspects of intra- and interspecific diversification of each tetrapod group in the Amazon drainage basin and the influence of Neogene events on these patterns.

### Phylogenetics and dating of speciation events

A phylogeny or phylogenetic tree is a graphic way to depict the history of organisms based on their inferred genealogical relationships. Each node on the tree represents a past divergence event at which point the hypothesized most recent common ancestor gave rise to two or more daughter lineages. For interspecific data, each ancestral node would represent an inferred speciation event. Modern phylogenetics increasingly focuses on testing the validity and robustness of historical hypotheses (Holder & Lewis 2003). Today there are several algorithms to build and test phylogenetic trees. A description of the models and assumptions involved in these algorithms is beyond the scope of this chapter (but see Chapters 23 and 25).

Here we focus on the evolutionary patterns that emerge from phylogenetic studies of Neotropical tetrapods. In evaluating the potential influence of Neogene events on Amazonian diversification, researchers want to know when particular speciation events took place. Controversy surrounds whether and how one may best estimate the ages of nodes in a phylogenetic tree from molecular data. However, such information is vital for testing the correspondence of ancestral speciation events with geological and environmental changes in the past.

In order to date nodes on a phylogenetic tree, several approaches may be used and are most commonly applied to molecular sequence data (Bromham & Penny 2003). The simplest method is to calculate time directly and linearly from genetic distances by

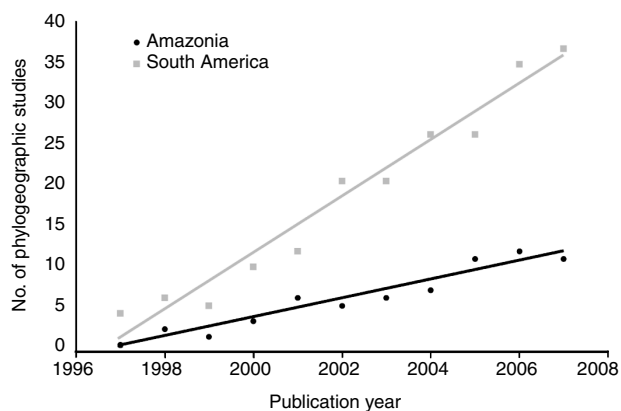
assuming a fixed rate,  $D/T$ , such that  $D$  amount of genetic divergence implies  $T$  amount of time elapsed. However, more advanced and robust methods are available using maximum likelihood or Bayesian inference. Some models assume that the rate of evolution is constant across a phylogeny, while others consider the variation across lineages in rates of evolution to obtain more accurate estimates of divergence times (Thorne *et al.* 1998). The latter methods are referred to as relaxed molecular clocks and may involve a simple minimization of the amount of change across a phylogeny by non-parametric algorithms, or they may involve highly parametric models whose many parameters are estimated by means of Markov chain Monte Carlo (MCMC) simulations (Bromham & Penny 2003). Recent implementations of relaxed clock models can also incorporate multiple genes and temporal constraints based on fossil data or biogeographical events (Thorne & Kishino 2002; Drummond & Rambaut 2007). Population genetic approaches to divergence time estimations account for ancestral polymorphism and assume a probability distribution of gene genealogies rather than a single fixed tree (Hey & Machado 2003). Irrespective of the method used, a crucial, and often highly problematic issue remaining is the calibration behind molecular dating attempts, which relies on the fossil record (unavailable or highly incomplete for many taxa), on biogeographical events (which incurs the risk of circularity if one is attempting to reconstruct said biogeography), or on *a priori* assumptions about evolutionary rates, which may or may not apply to the study taxon.

### Phylogeography

Phylogeography is a discipline focusing on the relationships between geography and gene genealogies, typically at the species level and below, incorporating a phylogenetic and population genetic perspective into biogeography (Avice *et al.* 1987). It also incorporates the effects of historical processes on population-level geographical patterns (Avice 2000). Early studies generally involved overlaying a phylogeny onto geography (sampling sites) and then examining the degree of congruence between clade distributions and geographic barriers (Bermingham & Avice 1986). In animals, most studies have primarily focused on just one kind of genome, the mitochondrial DNA (mtDNA), which has the advantage of relatively rapid rates of evolution, providing resolution for recent speciation events (Moore 1995; Zink & Barrowclough 2008). Recent advances have incorporated nuclear genes (Hare 2001; Crawford 2003), but mtDNA remains the dominant marker, especially as few nuclear markers have sufficiently rapid rates of evolution for intraspecific studies.

### Current methods in phylogeography and phylogenetic biogeography

A number of analytical approaches have been used extensively in phylogeography and phylogenetic biogeography of Amazonian taxa. Analytical methods and tools are undergoing rapid development, and new approaches can be expected to become available in the near future. At present, the most commonly used are the classic interpretation of patterns, tree topology tests, tests



**Fig. 24.1** Number of published papers on phylogenetics of Amazon vertebrate fauna compared to studies in all South America. Data obtained from Web of Science and Zoological Record search engines.

of neutrality and demographic equilibrium, and nested clade phylogeographic analysis (NCPA). We describe briefly each of these in turn.

#### *Tree topology tests*

The simple juxtaposition of phylogenetic information on geography lacks a statistical framework and the ability to test alternative hypotheses. Tree topology tests provide such a statistical framework insofar as different biogeographical hypotheses may predict different phylogenetic trees (e.g. Crawford *et al.* 2007). Optimality scores for trees that are constrained to fit predictions of biogeographic (null) hypotheses can be compared with the optimal gene trees obtained from the data set in question (Quijada-Mascareñas *et al.* 2007; Wang *et al.* 2008). Statistical tests of topology include the Wilcoxon signed-ranks test (Templeton 1983) under a parsimony framework, the Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999) under the maximum likelihood criterion, and the parametric bootstrap (Goldman *et al.* 2000). A statistically significant difference between the observed tree and those expected under the null hypothesis indicates that the data reject the biogeographical scenario. By the same token, the set of post burn-in trees generated by a Bayesian MCMC analysis can be examined for the proportion of trees consistent with the biogeographic predictions, this representing the posterior probability of the hypothesis in question. A comparison of all four methods is found in Crawford *et al.* (2007).

#### *Population structure and historical demographic analysis*

At intraspecific levels, historical events such as dispersal and vicariance leave genetic footprints that may be revealed and interpreted with appropriate statistical tools. For example, analysis of molecular variance (AMOVA) tests for significant differences in pairwise genetic differences among hierarchical levels in the spatial organization of populations (Excoffier *et al.* 1992). Some authors use the level of nucleotide diversity as an indicator of population ancestry (Zink *et al.* 2000; Cheviron *et al.* 2005), assuming that diverse populations must be older or ancestral (although homogeneous populations are not necessarily young). Among the most commonly used summary statistics are the population mutation rate ( $\theta$ ) and nucleotide diversity ( $\pi$ ), two estimators of polymorphism within a population (Tajima 1983). The difference between these two estimators yields Tajima's D, one of the earliest tests of the equilibrium neutral or Wright–Fisher population model for DNA sequence data (Tajima 1989). This and other neutrality tests can corroborate phylogeographical inferences, for example, as statistical tests for historical changes in population size (Ramos-Onsins & Rozas 2002). Evolutionary and biogeographical hypotheses can be tested combining the findings from the application of neutrality tests with other phylogeographic methods (Cheviron *et al.* 2005; Lemmon *et al.* 2007; Quijada-Mascareñas *et al.* 2007). An increasingly active avenue of research in phylogeographical hypothesis testing involves coalescent simulations of samples of haplotypes obtained under alternative biogeographic and demographic scenarios and comparing observed summary statistics against the simulations in order to reject one or more hypotheses (Knowles & Maddison 2002; Hickerson & Cunningham 2005).

#### *Nested clade phylogeographic analysis (NCPA)*

Nested clade phylogeographic analysis uses nested genealogical networks of haplotypes (mtDNA variants) to make statistical inferences about the causes of phylogeographical patterns. Potentially, this approach can discriminate between the different causal hypotheses for observed patterns, such as range expansion, long-distance dispersal and vicariance (Templeton 1998). Despite its popularity, the reliability of NCPA has been questioned, based on simulation studies indicating that NCPA can lead to a high rate of false-positive conclusions (e.g. Knowles & Maddison 2002; Masta *et al.* 2003; Panchal & Beaumont 2007). Some authors (Petit 2007; Beaumont & Panchal 2008) suggest that the method remains unreliable and should not be used until a better understanding of its analytical and test power can be achieved, although this interpretation is contested (Templeton 2004, 2008).

#### *Main mechanisms proposed to explain origins of diversity*

The extraordinary faunal and floral diversity of the Amazon drainage basin has led to the formulation of a number of different biogeographical hypotheses and models of speciation (Haffer 1997; Moritz *et al.* 2000). Here, we briefly review the major models and their predictions as related to phylogenetic and phylogeographic studies of Neotropical tetrapods (summarized in Table 24.1). Repeated habitat shifts, regardless of the underlying causes, are assumed to cause a population centred on a stable core area to speciate by small-scale allopatry. In contrast to the Pleistocene refugia hypothesis, interbreeding barriers could be caused by subtle genetic and environmental differences, rather than vicariant obstacles.

#### *Riverine barriers*

The earliest European biogeographers to visit the Amazon River noticed that this river and some of its tributaries separated the ranges of many forest interior species. Based on these observations, Wallace (1852) postulated what is today called the riverine barrier hypothesis. Under this model, widespread Amazonian organisms are thought to have split into isolated populations due to the formation of the Amazon River network. Salo *et al.* (1986) and Räsänen *et al.* (1987) have shown that lateral river displacement in western Amazonia effectively promotes the isolation of populations. Riverine barrier effects have been invoked to explain distribution limits in a number of vertebrate species in the Amazon drainage basin, including birds (e.g. Bates *et al.* 2004) and primates (Ayres & Clutton-Brock 1992). Other studies (e.g. Gascon *et al.* 2000; Aleixo 2004) have shown that at least some major rivers in Amazonia do not appear to have promoted the diversification of Amazonian animals. Bates *et al.* (2004) noted that meandering rivers arising in the basin might offer more opportunities for gene flow whereas faster flowing rivers arising in the Andes or the Guiana and Brazilian Shields might be stronger barriers.

#### *Pleistocene refugia*

During the past 2.6 million years, the Earth underwent at least 20 major glacial periods, when mean global temperatures were

**Table 24.1** Proposed models of speciation in the Amazon drainage basin.

Model	Description	Biogeographical, phylogenetic or phylogeographic predictions	Temporal predictions
Pleistocene refugia	Speciation due to vicariance in isolated forest fragments during Pleistocene	Species distributions and/or haplotype clades centred on postulated refugia; congruence of patterns among forest taxa; sister clades all Amazonian	Pleistocene dates for most recent common ancestor of sister clades
Taxon pulses	Fine-scale allopatry: speciation due to repeated adaptive shifts to different habitats centred on a stable core area	Closely related species currently occupying the same region	None
Riverine barriers	Speciation in allopatry due to rivers acting as primary barriers to gene flow	Reciprocal monophyly of haplotype clades on opposite sides of river	None, although predicted to be no older than course of river
Disturbance-vicariance	Speciation due to temperature fluctuations and forest heterogeneity	Zones of endemism in periphery of Amazonia	Late Neogene dates for most lineage splits
Marine incursions	Speciation in allopatry due to isolation by encroaching sea	Basal trichotomy for taxa from Guianan, Napo/Inambari and Belém/Pará regions	Consistent with timing of marine incursions and/or sea level changes
Gradient hypothesis	Parapatric speciation due to environmental gradient and divergent selection; no geographical isolation necessary	Sister taxa found in different habitats along same gradient; potential incongruence between mtDNA phylogeny and morphology and/or current gene flow	None

at least 4°C lower than at present (Gates 1993). Even though South America was never covered by ice sheets (as were the high latitudes of North America and Europe), there is strong evidence that these global changes had profound effects on the Amazonian forests (see Chapter 20).

Early authors suggested that the decreased temperatures and rainfall associated with times of maximal glaciation in the north created widespread aridification in the Amazon drainage basin (Ab'Saber 1982; Haffer 1969, 1997). According to some scenarios, the extent of rainforest cover in Amazonia changed repeatedly in response to climatic oscillations, possibly breaking up into isolated blocks (refugia) during cooler (drier) periods and expanding again during warmer (wetter) interglacials, leading to allopatric speciation in forest refugia. First formalized by Haffer (1969) and then developed into a model explaining the diversification of most of the Amazonian biota, this Pleistocene refuge theory gained early support from the subsequent demonstration of similar distribution patterns shared by some taxa.

The Pleistocene refugia hypothesis has become increasingly controversial, as several lines of evidence undermine the general validity of the theory:

- 1 Palynological data provide little evidence of widespread open formations and grasslands, at least during the last glacial maximum (e.g. Colinvaux *et al.* 2000);
- 2 although several taxa do exhibit similar patterns of distribution consistent with refugial scenarios, this is certainly not the case for a wide range of other organisms, meaning that at least in those cases other mechanisms of diversification must be sought (Lomolino *et al.* 2006);
- 3 there is increasing evidence from molecular dating studies that the diversification of most animals and plants occurred well before the onset of climatic oscillations in the late Neogene (see Table 24.2 and Plate 13; Rull 2008).

In a study on small mammals, Lessa *et al.* (2003) did not detect the expected genetic traces of recent range expansion in the southwestern region of the Amazonian rainforest after post-refugial Quaternary range expansions. More recently, the original refuge theory has been extended to include Neogene events on the assumption that climatic oscillations driven by Milankovitch cycles through those periods were of sufficient amplitude and duration to promote forest fragmentation and speciation (Haffer 1993).

#### *Taxon pulses*

Similar to the refuge theory, the taxon-pulse model originally proposed by Erwin (1979) relies on the assumption that repeated expansion and contraction of taxon ranges promoted speciation. Repeated habitat shifts, regardless of the underlying causes, are assumed to cause a population centred on a stable core area to speciate by small-scale allopatry, where the interbreeding barriers are subtle genetic and environmental differences rather than vicariant obstacles.

#### *Disturbance-vicariance hypothesis*

According to this hypothesis, the major factor triggering diversification in the Neotropics was the temperature fluctuations themselves (Colinvaux 1993; Bush 1994), rather than aridification and physical fragmentation of lowland forests as proposed under the refugia theory. The late Neogene climatic oscillations would have caused recurrent displacement of taxa towards lower or higher elevations (during cool and warm periods, respectively). The effects of such displacements would have been most notable in the peripheral parts of Amazonia, which would have served as crossroads for the invasions and counterinvasions of montane

and lowland species. The hypothesis thus predicts a higher level of endemism in the peripheries of Amazonia as compared to its core, implying a diversity gradient, although the precise mechanism of species generation is not clear.

#### *Marine incursions*

During the Miocene, global sea level fluctuations above the present-day level (Haq *et al.*, 1987; Miller *et al.* 2005) periodically influenced large parts of lowland Amazonia (Hoorn 1993; Hoorn & Vonhof 2006; see also Chapters 9 & 26), a hypothesis supported by patterns of fish biogeography and phylogeny (Lovejoy *et al.* 2006). By using topographic maps to identify lowland areas, Nores (1999) argued that during periods of marine incursions two large islands in northeastern South America would have existed around the Guiana Shield. Additionally, a large number of smaller islands and archipelagos would have been formed along the coastal lowlands of northeastern South America and the southern periphery of the Amazon drainage basin. Since the regions where these major islands would have been formed today contain a high level of endemism among birds, Nores postulated that recurrent marine incursions in Amazonia may have increased the opportunities for allopatric speciation and thus could represent a major force driving diversification in the Neotropics. As reviewed later in this chapter, the influence of marine incursions on speciation has been supported by at least some avian and amphibian studies.

#### *Gradient hypothesis*

Contrary to other geographical hypotheses of speciation (e.g. riverine barriers, arid zones, isolating forest refugia) the gradient hypothesis does not require a geographical barrier between stocks of individuals undergoing speciation, and at least some amount of gene flow is expected to occur. Instead, divergent selection is driven by strong ecological forces, and reduced fitness of intergrades and assortative mating may eventually reduce gene flow and ultimately lead to speciation (e.g. Ogden & Thorpe 2002). Thus, this model predicts parapatric speciation across steep environmental gradients (Endler 1977, 1982; Smith *et al.* 1997).

According to Moritz *et al.* (2000), several studies have shown that the gradient hypothesis is satisfactory in explaining particular cases of speciation in tropical Africa and Australia. However, the few papers available for the Neotropics have been contradictory, either refuting (e.g. Cracraft 1988; Prum 1988) or supporting (e.g. Mallet 1993) the pattern. Clearly, more studies are needed to test the role of ecological gradients in promoting speciation in Neotropical lineages.

#### *Andean uplift*

Extending over 5000 km along the western coast of South America, the Andean Cordillera constitutes the largest mountain chain in direct connection with a tropical rainforest. The uplift of the Andes began in the Cretaceous (Milnes 1987), apparently proceeding from south to north and from west to east (Taylor 1991), and took place in episodic bursts (Garzzone *et al.* 2008). In the central and northern Andes, most uplift took place in the

last 20–10 Ma, with some segments of the Eastern Cordillera in the northern Andes having risen as recently as 2–5 Ma (Gregory-Wodzicki 2000; see also Chapter 4).

The Andean uplift may have promoted speciation in several ways, such as by:

- 1 Creating a variety of new Neotropical montane and pre-montane habitats available for colonization by lowland ancestors;
- 2 producing geographical vicariance, and consequently genetic isolation, between populations on either sides of the emerging mountains;
- 3 favouring allopatric speciation among montane taxa, separated by deep valleys or impassable peaks;
- 4 changing the hydrology and climate of the entire South American continent, thus creating new evolutionary pressures on montane and lowland taxa.

Newly formed lineages along the eastern Andes could have then moved into Amazonia and contributed to lowland diversity.

Most molecular dating studies of animals and plants with estimated Neogene divergences have invoked the Andean uplift as a possible candidate for triggering diversification (see below). However, as several authors have stressed (e.g. Antonelli 2008; Dick & Pennington in Chapter 23) it may be extremely difficult to discern confidently between the influence of the Andean uplift and other synchronous palaeogeological and palaeoclimatic events such as the global climatic cooling beginning in the Middle Miocene (Zachos *et al.* 2001).

### **Patterns of phylogeographic diversification of tetrapods in the Amazon**

We review phylogenetic and phylogeographic data for major tetrapod groups in the Neotropics, comparing and contrasting the evolutionary differences between lineages and the differences and similarities in the focus of research questions for each group.

#### **Amphibians**

Half of the world's amphibian species occur in South America (Duellman 1999). As with many other taxonomic groups, the site of greatest alpha-diversity in amphibians is the Amazon drainage basin, in particular the western or upper Amazon drainage basin (Duellman 1978, 1999). In the past decade an increasing number of molecular phylogenetic and phylogeographic studies of frogs have been published with the aim of evaluating the biogeographical models outlined above, and their relative contributions to the diversification of Amazonian amphibians. While some models have been largely rejected or proven to have little relevance to diversification and speciation, we still have no general explanation for the remarkable alpha-diversity found within amphibian communities in Amazonia.

Among the three orders of amphibians, caecilians and salamanders contribute relatively little to Amazonian amphibian diversity compared to frogs, and the former remain poorly known in this

region (Duellman 1999). Thus, this review focuses on Anura. Phylogeographic studies of Amazonian frogs began with explicit tests of the riverine barrier hypothesis (Gascon *et al.* 1996, 1998). Based on allozyme data, the Gascon *et al.* (1998) paper remains a paragon of amphibian phylogeography for its explicit testing of an *a priori* hypothesis and for its comparative approach using sympatric frogs from four different genera. This paper demonstrated that the Juruá River played little to no role in promoting differentiation among frog populations.

More recent studies also have found little support for the role of riverine barriers in driving amphibian diversification, even at the intraspecific level (e.g. Gascon *et al.* 2000). Loughheed *et al.* (1999) suggested not only that rivers played a minor role, but also that differentiation along the Juruá River in *Allobates* (formerly *Epipedobates*) *femoralis* coincided with ancient and now vanished arches that ran perpendicular to the river. A similar pattern was also found in mammals (see below). However, rivers appear to have played a role in population differentiation in some Amazon frogs, as demonstrated in a phylogeographic analysis of *Engystomops* (formerly *Physalaemus*) *petersi* across three large rivers in western Amazon (Funk *et al.* 2007). Among four tests of reciprocal monophyly of haplotypes on opposite river banks, the authors found only one case of obvious rejection of the riverine barrier hypothesis plus one case that fitted very well the prediction.

Even before the advent of molecular zoogeographic analyses, frogs were utilized to evaluate the Pleistocene forest refugia hypothesis put forth to explain the high alpha-diversity in ecological communities in the Amazon drainage basin (Lynch 1982). While considerable disagreement still persists concerning the existence of Pleistocene forest refugia (see above), we can largely reject the hypothesis that refugia played a role in frog speciation. The refugia hypothesis makes predictions on the relationships and dating of speciation events that are readily testable with molecular data (see Table 24.1).

That frog species are older than the Pleistocene seems to be the rule rather than the exception (Crawford 2003; Weigt *et al.* 2005). An early example is the immunological study of Hass *et al.* (1995), which estimated a crown age of about 30 million years for the *Bufo margaritifera* complex, using microcomplement fixation. Some more recent DNA-based estimated ages of Amazonian species include the following: *Rhinella marina* (formerly *Bufo marinus*) crown age 2.7 million years (Slade & Moritz 1998); *Allobates* (formerly *Epipedobates*) *femoralis* crown age of 5–15 million years (Loughheed *et al.* 1999); *Dendropsophus* (formerly *Hyla*) *leucophyllata* crown age 3.8 million years (Chek *et al.* 2001); *Atelopus spumarius* stem age roughly 3 million years (Noonan & Gaucher 2005). While Pleistocene climate changes may not have been drivers of speciation, they have influenced the historical demography and distribution of frogs in South American sites adjacent to the Amazon (e.g. Carnaval & Bates 2007).

Few explicit evaluations of the disturbance-vicariance (DV) hypothesis exist using a frog model. One such study, however, was presented by Noonan & Gaucher (2006), who argued that the DV model explains much more of their data than the refugia model. They note that Amazonian and Guianan lineages of *Atelopus spumarius* had split prior to the Pleistocene and that, while differentiation within Guiana corresponded to the Late Pleistocene,

these recent events corresponded to mountain formations and did not form species.

The role of marine incursions has been little tested in Amazonian frogs, yet clearly such events would have had a significant impact on the history of frogs, especially in the Middle Miocene (Hoorn 1993, 2006). Marine incursions entering the proto-Amazon drainage basin from the north could have divided populations into eastern vs western lineages during the Middle Miocene. Noonan & Wray (2006) pointed out that such a spatial and temporal pattern can indeed be observed today in the phylogeny of Amazonian frogs, but that this pattern has been mistakenly attributed to ancient geological arches (Loughheed *et al.* 1999; Symula *et al.* 2003). Garda & Cannatella (2007) inferred the influence of Middle Miocene marine incursions on the diversification of paradoxical frogs. However, the inferred splitting event ran east to west, separating the Guiana Shield in the north from the Brazilian Shield in the south.

The ecological gradient model for species formation in Amazonian frogs finds little support among recent analyses. Most tests of this hypothesis involved gradients in elevation and, as such, were carried out in the upper Amazon drainage basin along the Andean foothills, not in the Amazonian lowlands *per se*. The model predicts that the spatial axis of morphological and/or genetic differentiation should run parallel rather than perpendicular to an environmental gradient. The null hypothesis would suggest that frog niches are evolutionarily conserved within lineages and that speciation has proceeded without concomitant change in bioclimatic regime (Smith *et al.* 2005; Wiens *et al.* 2006). In support of the null or vicariant model, Roberts *et al.* (2006, 2007) found that closely related species of dart-poison frogs were found at similar rather than contrasting elevations. At the intraspecific level, Funk *et al.* (2007) found no correlation between elevational differences and genetic divergence, again failing to find support for the gradient hypothesis. The best evidence for a correlation between environmental gradients and divergence in South American frogs came from a study of dendrobatids in the Pacific lowlands of Ecuador (Graham *et al.* 2004). In this geographical context, however, any spatial differentiation would have correlated with either the sharp north–south precipitation gradient or the steep east–west elevational gradient. Thus, there was little chance the null hypothesis could have survived this test.

The rise of the Andes certainly promoted speciation in amphibians, as evidenced by the spectacular beta-diversity found there (Duellman 1999). Due to the meagre support for speciation along environmental gradients, classic vicariance would seem to be the most parsimonious explanation for speciation in Andean frogs (Lynch & Duellman 1997; Wiens 2004). Andean speciation can contribute to Amazonian diversity, as evidenced by one widespread Amazonian frog, *Epipedobates trivittatus*, which traces its ancestry back to the mid-elevation Andean slopes (Roberts *et al.* 2006), a pattern repeated throughout the history of dendrobatid frogs (Santos *et al.* 2009).

For Amazonian frogs, as with most groups, no clear geographical pattern or palaeoenvironmental force has emerged as a general explanation for their diversification. Amazonian frog biogeographers may have to content themselves with case studies rather than generalities, or perhaps integrate phylogeographic analyses

with studies of behaviour and sexual selection as potential drivers of species formation (Boul *et al.* 2007; Roberts *et al.* 2007; Vences & Wake 2007).

### Non-avian reptiles

Despite the fact that reptiles are one of the most outstanding features of the Amazon forest, they remain poorly sampled throughout most of Amazonia. Over 100 species of coexisting reptiles can be found in many areas of the Amazon, and new species are still being described regularly. Despite this diversity, few biogeographical and phylogenetic studies have appeared in the literature until very recently, and, compared to birds and mammals, few dated phylogenies are available. From these few published examples, however, a complex pattern of tempo and processes of divergence has emerged. Higher taxa represented in South America may represent either old Gondwanan clades (e.g. South American boid snakes and teiid lizards), more recent immigrants from Central America (e.g. pitvipers, colubrine snakes) or trans-Atlantic dispersalists (e.g. scincid lizards). Unlike in mammals (see below), many dispersal events from Central to South America appear to predate the completion of the Isthmus of Panama (Coates & Obando 1996; see also Chapter 23). Speciation events largely predate the Pleistocene, but several species appear to show evidence of Plio-Pleistocene genetic differentiation.

#### *Turtles and crocodilians*

Only a few phylogeographic studies have been carried out on Amazonian turtles or crocodilians. Mitochondrial DNA sequence analysis of the giant river turtle, *Podocnemis expansa*, revealed very low divergence across the Amazon drainage basin (Pearse *et al.* 2006).

Crocodilians have an extensive Neogene fossil history in South America; however, most of this considerable diversity of clades and morphotypes has since gone extinct (see Chapter 16), leaving only the Alligatoridae as an early Paleogene endemic clade in the Amazon drainage basin itself (Brochu 1999). The genus *Crocodylus*, represented in the New World by an apparently recently diverged and monophyletic group of four species (Brochu 2003), is restricted to the northern coasts and the Orinoco drainage, but does not enter the Amazon drainage basin. Similarly, along the Amazon and its tributaries, the spectacled caiman, *Caiman crocodilus*, and the black caiman, *Melanosuchus niger*, both lack population genetic structure among mtDNA haplotypes (Vasconcelos *et al.* 2006, 2008), whereas populations from the Atlantic versant of French Guiana and Amapá are more distinct in both. However, analyses across the entire range of *Caiman crocodilus* revealed that, throughout the rest of its range, this species shows substantial genetic structure, largely corresponding to subspecies, and also revealed an additional cryptic lineage in Amazonian Peru (Venegas-Anaya *et al.* 2008). The timing of colonization of Central America from South America by *C. crocodilus* corresponds to the completion of the Isthmus of Panama (Venegas-Anaya *et al.* 2008).

#### *Lizards*

The first explicit phylogenetic test of the refugia hypothesis in reptiles involved the genus *Anolis* in western Amazonia (Glor *et al.* 2001). Divergence of mtDNA among species of Amazonian anoles exceeded 15%, indicating that their divergence probably occurred in the Miocene or earlier. Genetic differences among conspecific geographical populations ranged from 3 to 22%, indicating that even the most recent divergences sampled pre-date the Pleistocene, and in many cases the Pliocene. However, the influence of climatic cycles as a causative factor in speciation cannot be rejected solely through the demonstration of pre-Pleistocene speciation.

In the gecko genus, *Gonatodes*, the phylogeographic pattern indicates a mixed influence of climate change and orogeny in the speciation of these lizards (Gamble *et al.* 2008). Most divergences occurred in the Oligocene and Early Miocene. The dramatic climate change and Andean uplift during the Oligocene and Early Miocene were likely factors that drove the burst of diversification in *Gonatodes*.

A pattern consisting of a biogeographic split between the eastern and western Amazon drainage basin is apparent around the species level in several clades of *Anolis* spp. (Glor *et al.* 2001), *Gonatodes* spp. (Gamble *et al.* 2008) and *Thecadactylus* (Kronauer *et al.* 2005). However, large differences in the timing of divergence between the east-west sister groups indicate that these divergences were not the result of a common vicariant event.

#### *Snakes*

Few groups of South American snakes have dated phylogenies, and pitvipers have received the most comprehensive treatment (Wüster *et al.* 2002, 2008). Pitvipers have their origins in Asia (Castoe & Parkinson 2006). A single lineage invaded the New World via the Bering land bridge in the Oligocene or Early Miocene, but multiple lineages subsequently colonized South America from the north, starting in the Miocene. A clade consisting of the genera *Bothrops* (in which we include *Bothriopsis*) and *Bothrocophias* radiated into South America during the Miocene. More recent colonizers of South America include the genera *Lachesis*, *Bothriechis*, *Porthidium* and *Crotalus* (Zamudio & Greene 1997; Wüster *et al.* 2002, 2005a, 2008), all with low diversity in South America. Both *Porthidium* and *Crotalus* are likely post-Isthmian invaders (Wüster *et al.* 2002). *Crotalus* arrived during the mid-Pleistocene and colonized open vegetation formations, both north and south of the Amazonian forests. Because these rattlesnakes do not occur in tropical rainforest, this disjunct but recent distribution has been used to infer Pleistocene fragmentation of the Amazonian forest (Wüster *et al.* 2005a, 2005b; Quijada-Mascareñas *et al.* 2007).

Among other snake families, there are no studies of the large colubrid assemblage that have used sequence-based dating methods. Cadle (1984, 1985) estimated a Late Cretaceous/Early Tertiary origin for the Xenodontinae (Dipsadidae *sensu* Vidal *et al.* 2007), based on immunological distance methods, most probably from the Asian origin postulated for the advanced snakes by Vidal *et al.* (2007).

Boid snakes are an old Gondwanan lineage, and the radiation of the current crown clade of South American boids appears to have

begun in the late Cretaceous or early Tertiary (Noonan & Chipindale 2006). The only species subjected to a detailed analysis, *Corallus caninus*, displays very high divergences between a sample from northern Peru and samples from the Guianas, Rondônia and eastern Amazonia, suggesting a divergence during the Miocene or earlier (Vidal *et al.* 2005), a similar pattern to that displayed by the gecko genus, *Thecadactylus* (Kronauer *et al.* 2005) as well as other lizards (see above).

## Birds

With some 3653 species of birds, the Neotropics hosts more than a third of the world's species (Larsson *et al.* 2008). Birds have attracted much attention from scientists, resulting in a considerable amount of recent phylogenetic research, although ornithological papers from the Neotropics still only represent a tiny fraction of the literature compared with those from the northern hemisphere. Haffer (1969) formulated the Pleistocene refugia hypothesis based on birds, but molecular data have demonstrated that much of this speciation is older than the Pleistocene. Molecular dating studies in birds (e.g. Weir 2006; Aleixo & de Fatima Rossetti 2007) have most often been based on the assumption that mitochondrial DNA of animals in general, and birds in particular, evolves at a rate of 1.6–2% of sequence divergence per million years (Brown *et al.* 1979; Fleischer *et al.* 1998). However, the universality of this rate is still a matter of debate, and indeed large variance has been reported (Garcia-Moreno 2004; Lovette 2004a), so that more recent developments that do not rely on *a priori* assumptions of evolutionary rates (see above) may adjust calibrations. Bates (2001) used available molecular data for birds to support a pattern consistent with marine incursions, but noted that many of the divergence events showing this pattern post-date the Miocene (based on 2% per million years divergence rates).

### Passeriformes

Over half of all bird species are passerines, also called perching birds. Molecular dating analyses place their origins in the Late Cretaceous (~67 Ma), with their first appearance in the fossil record some 30 million years later (Barker *et al.* 2004). The suboscine passerines (Tyranni), a group of some 1000 species mainly confined to the New World, began their diversification in South America near the Cretaceous/Tertiary boundary (Barker *et al.* 2004). Several independent dispersals took place between the Old and New World during the Oligocene and Miocene (34–14 Ma), probably through Beringia (Barker *et al.* 2004).

In the largest phylogenetic and dating analysis of a Neotropical passerine group, Brumfield *et al.* (2007) and Brumfield & Edwards (2007) studied the antbirds (family Thamnophilidae). The first split in the genus *Thamnophilus* occurred between 5.5 and 3 Ma, separating a mainly lowland from a mainly Andean lineage. The Andes were then colonized twice independently, and the *tepuis* once, from lowland-restricted ancestors. Whereas the deepest split in the genus was probably caused by mountain uplift, diversification of lowland taxa was inferred to have taken place in response to the climatic fluctuations and/or marine incursions that periodically interrupted and reconnected the dispersal

corridor around the northern end of the Andes. An important issue for Amazonian lineages is that many currently recognized species comprise genetically divergent lineages (occasionally designated as subspecies), which authors argue deserve species status (e.g. Bates *et al.* 1999; Marks *et al.* 2002). This issue has implications for attempts to study patterns of speciation (Weir & Schluter 2007, but see Tobias *et al.* 2008).

While most species of *Thamnophilus* were probably formed in lowland Amazonia, in the showy genus, *Tangara* (tanagers), many early speciation events (beginning at 6 Ma) took place in the Andes (Burns & Naoki 2004). Andean lineages appear to have dispersed to other geographical areas of the Neotropics, where they eventually underwent speciation.

A third source of passerine diversity in South America is exemplified by wrens in the genus *Campylorhynchus* (Barker 2007). This genus has its origin in North America, where it began to diversify prior to the closure of the Isthmus of Panama. At least one lineage entered South America across the water gap between these two landmasses, approximately 4.7 Ma, with additional lineages following more recently. As a result of these multiple dispersals, South American wrens do not constitute a monophyletic group, a situation analogous to that of the South American pitvipers (see above).

### Piciformes

This group of birds comprises some 400 species, including several commonly seen families, such as toucans and woodpeckers. Diversification of the woodpecker genus, *Veniliornis*, began soon after its colonization of South America at around 3.3 Ma (Moore *et al.* 2006). This estimate coincides with the final closure of the Isthmus of Panama at around 3.5 Ma, and, as in *Campylorhynchus*, suggests post-Isthmian dispersal by a northern ancestor. Speciation may have been correlated to the uplift of the Northern Andes, but most probably the invasion of South America allowed adaptive radiation into multiple habitats, from Amazonian lowland rainforest to high elevations in the Andes and arid lowlands (Moore *et al.* 2006).

### Galliformes

In the Neotropics, this order comprises the guans, chachalacas and curassows (family Cracidae), and New World quails (Odonophoridae). The Cracidae (Pereira *et al.* 2002) appear to have diverged from their sister lineage around 76 Ma. Further diversification began at around 33 Ma and continued until 3.8 Ma, leading to the formation of the 11 genera currently recognized. Speciation among curassows (comprising the genera *Crax*, *Mitu* and *Nothocrax*) started 9.6 Ma, with the youngest species diverging at about 1.5 Ma (Pereira & Baker 2004). Within the genus *Pipile* (including *Aburria*), speciation was more recent, starting at 3.7 Ma until the youngest split estimated at 0.9 Ma (Grau *et al.* 2005). In all these studies of Cracidae, sea level rises were suggested to have played a more important role in speciation than Pleistocene refugia, by causing the formation of marine incursions and wetlands, which in turn caused isolation of populations and fostered allopatric speciation.



### Psittaciformes

All Neotropical parrots (Psittacidae: Arini) seem to form a natural group comprising some 330 species (Sibley & Ahlquist 1990). Molecular analyses based on mitochondrial and nuclear DNA sequences (Tavares *et al.* 2006) indicate that Neotropical parrots shared a common ancestor with Australian parrots about 59 Ma, suggesting Gondwanan vicariance rather than long-distance dispersal. The three major clades of Neotropical parrots originated about 54 Ma. This estimate coincides with high sea levels caused by global warming, creating marine incursions that may have caused the fragmentation of the Neotropical lineages (Tavares *et al.* 2006; see above). Generic diversification continued until about 5 Ma, corresponding to the age of the youngest of the 25 analysed genera. Speciation was presumably associated with the Andean uplift and the subsequent formation of dry, open grassland habitats that would have facilitated ecological speciation via niche expansion from forested habitats. Several other studies focused on a lower taxonomic level, studying the diversification of genera or species complexes (Ribas & Miyaki 2004; Ribas *et al.* 2005, 2006; Eberhard & Bermingham 2005). Estimated ages ranged from 8.7 to 1.3 Ma, and in almost all cases geotectonic events, marine incursions, and river dynamics were suggested to have contributed more to speciation than climatic fluctuations in the Pleistocene.

### Mammals

After the final break-up of Gondwana, the only placental mammals thought to inhabit South America in the early Neogene were xenarthrans (represented today by anteaters, sloths and armadillos). The present-day South American mammal fauna contains a large number of relatively recent invaders from Central America (Simpson 1940). Most of these are thought to have colonized South America subsequent to the completion of the Isthmus of Panama, about 3 Ma, as part of the Great American Biotic Interchange (GABI) (Stehli & Webb 1985; see also Chapter 15), although recent molecular studies suggest that its importance may have been overstated in at least some groups (e.g. Koepfli *et al.* 2007).

#### General patterns

Unlike many other groups of animals, mammals have been the subject of wide-ranging systematic studies of entire faunas in the Amazon drainage basin and surrounding areas, which has allowed the elucidation of general patterns of diversity and population history. The large-scale studies by Patton and colleagues (e.g. da Silva & Patton 1998; Patton *et al.* 2000) have provided a deep insight into the diversification of multiple Amazonian rodent and marsupial species. General patterns to emerge from their studies were:

- 1 The lack of a riverine barrier effect on the Juruá River in almost all taxa examined (see also Jacobs *et al.* 1995);
- 2 divergence levels between clades that strongly suggest pre-Pleistocene cladogenesis, thus negating the role of Pleistocene refugia;

- 3 deep phylogeographic splits between the upper and lower Juruá, which Patton *et al.* (2000) attribute to the ancient Iquitos Arch, which separated Miocene basins that are now buried under later sediments;
- 4 deep phylogenetic splits between the banks of the lower Negro River and the Solimões River.

More recent studies, detailed below, invoke these and other mechanisms of Neogene diversification of the South American mammal fauna.

### Marsupials

#### Opossums

The American opossums (order Didelphimorphia) are the most diverse of the three American marsupial orders, with 17 genera followed by Paucituberculata (three genera) and Microbiotheria (one genus). Their ancestors originated in North America during the Middle to Late Cretaceous and subsequently dispersed into South America (Steiner *et al.* 2005). Costa (2003) and Patton & Costa (2003) found that several genera (*Caluromys*, *Gracilinanus*, *Marmosa*, *Marmosops*, *Metachirus*, *Micoureus*, *Monodelphis* and *Philander*) exhibited great molecular divergence, suggesting cladogenesis before the Pleistocene. Only diversification on *Didelphis* corresponded to the Pleistocene. A recent molecular study (Steiner *et al.* 2005) found that all extant species appeared to share a most recent common ancestor during the Eocene, about 40 Ma. Steiner *et al.* (2005) argue that both geological and climatic changes may have contributed to the diversification of this group. For instance, climatic and vegetation shifts in the Oligocene to Miocene were invoked to explain the lineage splits of the first diverging genera, *Monodelphis* and *Metachirus*, since they are more adapted to terrestrial than to arboreal locomotion. Interestingly, no speciation events were inferred to have taken place after  $4.7 \pm 1.3$  Ma, suggesting that Pleistocene refugia played no role in causing speciation.

#### Placental mammals

#### Primates

All Neotropical monkeys constitute a well-defined monophyletic group, the Platyrrhini. A phylogenetic and molecular dating analysis for 13 species of primates, using eight fossil calibration constraints, indicated that the common ancestor to all Neotropical monkeys dispersed from Africa to South America between 37 and 17 Ma, consistent with the first fossil record of a primate in Bolivia, dated at 27 Ma (Poux *et al.* 2006). The colonization of South America by primates was most likely the result of a founding population rafting from Africa aboard a floating island (Houle 1999). The diversification of all extant lineages of primates from that surviving ancestor began at around 17 Ma.

Within the Amazon drainage basin, primate distribution patterns have been suggested to support the riverine barrier hypothesis (Ayres & Clutton-Brock 1992; Peres *et al.* 1996). Using molecular data, similar patterns were found by Jacobs *et al.* (1995) while testing metachromism in tamarins. Cropp *et al.*

(1999) suggested that the most likely area of origin for the tamarins is somewhere south of the Amazon and west of the Madeira River, in agreement with Hershkovitz (1977). In contrast, Collins & Dubach (2000a) related differentiation in spider monkeys (*Ateles*) primarily to the Andean orogeny, and found little evidence for the effects of Pleistocene forest fragmentation or riverine barriers, except along the lower Amazon. Similarly, Cortés-Ortiz *et al.* (2003) invoked the uplift of the northern Andes as the trigger for the basal cladogenesis in howler monkeys (*Alouatta*), approximately 6.8 Ma, followed by dispersal into Central America as part of the GABI, and suggested that the formation of the modern lower Amazon may have initiated diversification in the cis-Andean clade.

#### Rodents

Rodents are represented in the Americas today mainly by the New World rats and mice (family Cricetidae, ~400 spp.) and by the cavy-like rodents (Caviomorpha, 224 spp.). Like the Platyrrhini, caviomorph rodents are thought to descend from a common ancestor that reached South America from Africa by rafting approximately 45–37 Ma (Poux *et al.* 2006). Diversification of the extant caviomorph lineages started soon after their arrival, and by the end of the Early Miocene all genera analysed by Poux *et al.* (2006) had already diverged. The molecular dating estimates, in combination with a rich fossil record from the Oligocene (16 genera) and Early Miocene (25 genera), give a strong indication that Caviomorpha radiated concomitantly with the Oligocene climate changes. An evolutionarily key innovation proposed to have enhanced their ecological success was the development of hypsodont teeth – high-crowned teeth especially suitable for grazing – which presumably facilitated the colonization of new grasslands opened by these climate changes. Although da Silva & Patton (1993) suggested that Pleistocene refugia were important in the diversification of the family Echimyidae, Patterson & Velazco (2008) suggested a proto-Andean region (9–15 Ma) was consistent with basal cladogenesis in the echimyid genera *Dactylomys* and *Isothrix*. In their data set, only the divergence between *I. negrensis*–*I. orinoci* and *I. pagurus*–*I. sinnamariensis* were considered consistent with Pleistocene fragmentation.

For 10 rodent (and several marsupial) species, da Silva & Patton (1998) found strong phylogeographic patterns between the upper and lower Juruá (not on opposite sides of the river) supporting a vicariance history perpendicular to the river. The geographical placement of the clade boundaries corresponds to the Iquitos Arch that separates the Acre and Central Amazon sedimentary basins, which were formed during the last episode of uplift of the central Andes to the west (see Chapter 4). The uplift of the Iquitos Arch caused important environmental changes in the Amazon drainage basin and acted as a biotic barrier that allowed the development of an isolated fauna and flora (Roddaz *et al.* 2005). da Silva & Patton (1998) also found high molecular divergence values, which suggest pre-Pleistocene cladogenesis, rejecting the effects of Pleistocene refugia. In the same area, Matocq *et al.* (2000) found little to no evidence of gene flow among populations of *Proechimys simonsi* and *Mesomys hispidus* respectively, either on the same bank or across the Juruá River.

#### Bats

The first appearance of bats, estimated from molecular dating analyses, occurred in the Late Cretaceous at around 85 Ma (Springer *et al.* 2003). Unlike New World monkeys, the bat lineages found today in South America do not constitute a monophyletic group. Recent analyses indicate that at least three dispersal events must have taken place into the continent, within the superfamilies Emballonuroidea, Noctilionoidea and Vespertilionoidea (Eick *et al.* 2005; Teeling *et al.* 2005).

Recent reconstructions of the phylogeny and biogeographic history of New World emballonurid bats (Teeling *et al.* 2005; Lim 2007, 2008), based on molecular data and in combination with the fossil record, indicate that the tribe colonized South America in the Early Oligocene (~30 Ma). Diversification began in the Late Oligocene and was followed by a period of rapid radiation in the Early Miocene, with seven of the eight genera radiating within 1.4 million years, and most speciation events occurring before the Pliocene (Lim 2007). The ancestral area of Diclidurini was probably northern Amazonia, and within-area events, and not regional vicariance, appear to have been the major factor driving speciation within the clade. This led Lim (2008) to propose a correlation between speciation and the taxon-pulse model of diversification (see above). Stadelmann *et al.* (2007) reconstructed the evolutionary history of *Myotis*, suggesting that this genus reached South America during the Late Miocene (about 7–10 Ma) and indicated that the presence of other insectivorous bats in the forest might have impeded the diversification of *Myotis*. Webster (1993) indicated Pleistocene refugia were responsible for cladogenesis in *Glossophaga*, but there are currently no estimates of the actual divergence of the genus.

#### Carnivores

Johnson *et al.* (2006) reconstructed the phylogeny of the cats (Felidae, order Carnivora) from an array of nuclear and mitochondrial gene sequences. Their results identified three clades of cats with South American representatives. In the puma clade, the two widely distributed species, *Puma concolor* and *P. yagouaroundi*, diverged approximately 4.2 Ma (3.2–6.0 Ma), presumably in North or Central America before the final completion of the Isthmus of Panama. The ocelot clade (genus *Leopardus*), mostly confined to South America, diverged around 2.9 Ma (2.0–4.5 Ma), broadly consistent with adaptive radiation following isthmian dispersal southwards after the completion of the Panama land bridge. Finally, in the genus *Panthera*, the jaguar (*P. onca*) diverged from its nearest living relative (*P. leo*) around 2.0 Ma (1.2–3.5 Ma), again consistent with post-Isthmian occupation of South America.

Intraspecific phylogeographic studies of South American cats have revealed evidence of a riverine barrier effect along the Amazon and Negro Rivers in ocelots (*L. pardalis*) and margays (*L. wiedi*), as well as a northern South American origin of the Central American populations. *Panthera onca* showed less phylogeographic structure and less influence of large rivers on genetic variation (Eizirik *et al.* 1998, 2001).

## Conclusions

The Miocene-Pliocene period left a profound footprint in the biodiversity of the Amazon drainage basin. Based on the estimates assembled from the literature of crown ages (Table 24.2; see also Plate 13), we can conclude that most of the diversification events leading to present-day tetrapod diversity in Amazonia occurred during the Neogene. The geological evidence coupled with

phylogeographic patterns suggests that most of these speciation events were associated with the uplift of the Andes, marine incursions and global climate change. In contrast, as suggested by Rull (2008) and Antonelli (2008), the Pleistocene accounts for only a small proportion of the cladogenic events sampled, contrary to the expectations of the Pleistocene refugia model. Clearly, the Neogene was an important period of diversification in the history of the Neotropics.

**Table 24.2** Phylogenetic studies of Neotropical tetrapods including divergence time estimates.

Organism	Analysed spp. (total*)	Reference	Crown age (Ma) [boundaries] <sup>†</sup>
<b>Amphibians</b>			
Bufonidae: <i>Rhinella</i> ( <i>Bufo</i> ) <i>marina</i>	2 (2)	Slade & Moritz 1998	2.7
Dendrobatidae: <i>Allobates</i> ( <i>Epipedobates</i> ) <i>femoralis</i>	1 (1)	Lougheed <i>et al.</i> 1999	10 [5–15]
Dendrobatidae: <i>Ranitomeya</i>	4 (24)	Clough & Summers 2000	18 [17.3–18.8]
Dendrobatidae: <i>Epipedobates</i>	8 (29)	Clough & Summers 2000	15 [14.4–15.6]
Dendrobatidae: <i>Oophaga</i>	5 (9)	Clough & Summers 2000	9.3 [8.9–9.7]
Dendrobatidae: <i>Phyllobates</i>	3 (5)	Clough & Summers 2000	13 [12.4–13.5]
Dendrobatidae: Dendrobatinae: clade of four genera	15 (43)	Symula <i>et al.</i> 2003	20 [10–31]
Dendrobatidae	137 (264)	Santos <i>et al.</i> 2009	41 [31–53]
Hylidae: <i>Dendropsophus</i>	2 (2)	Chek <i>et al.</i> 2001	3.8
Strabomantidae: <i>Pristimantis</i>	87 (393)	Heinicke <i>et al.</i> 2007	24.5 [17.3–34.8]
Microhylidae: Gastrophryninae	3 (40)	Bocxlaer <i>et al.</i> 2006	36.9 [27.8–41.0]
<b>Birds</b>			
Cracidae	11 (50)	Pereira <i>et al.</i> 2002	33.3 [26.9–40.6]
Cracidae: <i>Pipile</i> s. lat.	4 (5)	Grau <i>et al.</i> 2005	3.7 [2.6–5.2]
Cracidae: <i>Crax</i> , <i>Mitu</i> , <i>Nothocrax</i> , <i>Pauxi</i>	14 (14)	Pereira & Baker 2004	9.6 [7.5–11.2]
Dendrocolaptidae: <i>Glyphorhynchus spirurus</i>	1 (1)	Marks <i>et al.</i> 2002	3
Dendrocolaptidae: <i>Xiphorhynchus</i>	2 (2)	Aleixo 2006	4
Parulidae: <i>Phaeothlypis</i>	2 (2)	Lovette 2004b	3.5 [3.4–3.6]
Picidae: <i>Veniliornis</i>	10 (12)	Moore <i>et al.</i> 2006	3.3
Pipridae: <i>Lepidothrix coronata</i>	1 (1)	Cheviron <i>et al.</i> 2005	2 [1.1–2.9]
Psittacidae: <i>Gypopsitta</i>	8 (8)	Ribas <i>et al.</i> 2005	8.69 [8.56–8.82]
Psittacidae: Arini	29 (149)	Tavares <i>et al.</i> 2006	53.6 [49.3–59.0]
Psittacidae: <i>Pteroglossus</i>	12 (12)	Eberhard & Bermingham 2005	3.1
Psittacidae: <i>Pionopsitta</i>	5 (7)	Eberhard & Bermingham 2005	6.6
Psittacidae: <i>Aratinga solstitialis</i>	1 (1)	Ribas & Miyaki 2004	4.9
Psittacidae: <i>Pyrrhura</i>	10 (10)	Ribas <i>et al.</i> 2006	1.3
Ramphastidae: <i>Capito</i>	3 (3)	Armenta <i>et al.</i> 2005	3.0
Thamnophilidae	70 (208)	Brumfield <i>et al.</i> 2007; Brumfield & Edwards 2007	6.4
Thamnophilidae: <i>Drymophila devillei</i>	1 (1)	Bates <i>et al.</i> 1999	1.5
Thamnophilidae: <i>Hypocnemis cantator</i> complex	1 (6)	Bates <i>et al.</i> 1999, Tobias <i>et al.</i> 2008	3.4
Tyrannidae: <i>Mionectes</i>	1 (5)	Miller <i>et al.</i> 2008	7
Thraupidae: <i>Tangara</i>	43 (49)	Burns & Naoki 2004	6 [5.47–6.53]
Troglodytidae: <i>Campylorhynchus</i>	13 (13)	Barker 2007	4.7 [3.8–5.6]
Turdidae: <i>Turdus</i> s. lat.	35 (37)	Nylander <i>et al.</i> 2008	2.4 [2.01–2.90]

(Continued)

Table 24.2 Continued.

Organism	Analysed spp. (total*)	Reference	Crown age (Ma) [boundaries]†
<b>Mammals</b>			
Didelphidae	19 (70)	Steiner <i>et al.</i> 2005	39.8 [33.7–45.9]
Caviomorpha	7 (224)	Poux <i>et al.</i> 2006	36.7 [33.0–40.4]
Chiroptera: <i>Carollia</i>	5 (5)	Hoffman & Baker 2003	2.75 [1.0–4.5]
Chiroptera: <i>Diclidurini</i>	20 (21)	Lim 2007	27.1 [25.3–28.9]
Chiroptera: <i>Myotis</i>	30 (38)	Stadelmann <i>et al.</i> 2007	12.2 [10.2–14.2]
Felidae: <i>Leopardus</i>	7 (9)	Johnson <i>et al.</i> 2006	2.9 [2.0–4.5]
Felidae: <i>Panthera onca</i>	1 (1)	Johnson <i>et al.</i> 2006	2.0 [1.2–3.5]
Felidae: <i>Puma</i>	2 (2)	Johnson <i>et al.</i> 2006	4.2 [3.2–6.0]
Platyrrhini	15 (91)	Opazo <i>et al.</i> 2006	26
Platyrrhini	4 (91)	Poux <i>et al.</i> 2006	16.8 [14.5–19.1]
Platyrrhini	15 (91)	Schrägo 2007	20.1 [15.6–28.3]
Platyrrhini: <i>Alouatta</i>	9 (10)	Cortés-Ortiz <i>et al.</i> 2003	6.7 [6.6–6.8]
Platyrrhini: <i>Ateles</i>	4 (4)	Collins & Dubach 2000a, 2000b	3.59
Procyonidae	9 (14)	Koepfli <i>et al.</i> 2007	23.3 [12.1–36.0]
Xenarthra	13 (30)	Delsuc <i>et al.</i> 2004	65 [60–70]
<b>Non-avian reptiles</b>			
<i>Bothrops</i> (including <i>Bothrocophias</i> and <i>Bothriopsis</i> )	28 (53)	Wüster <i>et al.</i> 2002	10–23
<i>Bothrops</i> (including <i>Bothrocophias</i> and <i>Bothriopsis</i> )	5 (53)	Wüster <i>et al.</i> 2008	13.7 [10.2–17.4]
Polychrotidae: <i>Anolis</i>	20 (400)	Glor <i>et al.</i> 2001	15
South American <i>Porthidium</i>	3 (3)	Wüster <i>et al.</i> 2002	3.5–7.7
South American <i>Crotalus durissus</i>	1 (1)	Wüster <i>et al.</i> 2005a	1.5 [1.2–1.96]
Sphaerodactylidae: <i>Gonatodes</i>	11 (20)	Gamble <i>et al.</i> 2008a	35 [28.2–41.8]
Sphaerodactylidae: <i>Sphaerodactylinae</i>	17 (145)	Gamble <i>et al.</i> 2008b	75.5 [44.1–106.9]
Teiidae	11 (120)	Giugliano <i>et al.</i> 2007	59 [54–64]

\*Refers to the estimated number of Neotropical species in each clade; note that this count can differ among publications.

†Crown ages refer to mean ages (in millions of years) listed in the original publications or calculated by us based on implicit information in the articles; boundaries refer to published lower and upper limits of confidence, which may have been calculated in any of several ways.

Conclusive explanations for cladogenic events are difficult for all groups. Amazonian amphibians are particularly challenging, and no clear single force has emerged as a general mechanism. In reptiles, ancient lineages are mixed with more recent ones producing phylogeographic patterns reflecting different timescales and causes of cladogenesis. In the few studies to date, mechanisms include the influence of plate tectonics, orogenic processes during the Neogene, and to a lesser extent recent climate change (Glor *et al.* 2001; Wüster *et al.* 2005a; Gamble *et al.* 2008). These organisms are excellent systems to test general models of speciation, so further research should focus on appropriate sampling of areas and taxa.

As a general rule, it appears that most amphibian and reptile speciation events are old (Johns & Avise 1998) and often predate the Pleistocene by a considerable margin. Typically, even intraspecific divergences reach into the Pliocene or earlier. At higher levels, temporal patterns of diversification are often a result of historical

contingency, such as opportunities for adaptive radiation after the first colonization of the South American continent versus older autochthonous diversification (e.g. Wüster *et al.* 2002; Noonan & Chippindale 2006). Birds continue to be the best-studied tetrapods, but still there has not been enough research to allow for general explanations of their diversification. Divergences in Amazonian birds are variously explained by most of the models proposed: geotectonic events, marine incursions, riverine barriers, and climatic fluctuations in the Pleistocene.

Mammals present patterns similar to those of birds. The main mechanisms proposed include the major phases of the Andean uplift during that period and/or global climatic changes prior to the Pleistocene. At the same time, some mammalian taxa show clear evidence of more recent bursts of speciation, in some cases after the invasion of South America as part of the GABI (e.g. Johnson *et al.* 2006). However, due to the overlap of timescales, it may be difficult to disentangle the effects of adaptive radiation

following the GABI from those of Plio-Pleistocene climatic and vegetational fluctuations leading to speciation due to forest fragmentation or disturbance-vicariance.

In summary, the data compiled here paint a complex picture of the history of the Amazon drainage basin and its biota. Hopefully, additional phylogenetic and phylogeographic studies, coupled with new data and models on past vegetation and climatic changes (Bush *et al.* 2004), will shed new light on this complexity. The data available to date illustrate that there is no cohesive theory of Amazonian speciation. However, some patterns have begun to emerge in comparison with other biomes. In particular, the relatively low number of clear cases of Pleistocene speciation suggests that much of this divergence occurred earlier than initially believed (Haffer 1969). Instead, earlier Neogene events must be invoked as drivers of diversification, but it seems probable that many of those drivers are still operating today.

Generally, it is now clear that the outstanding biodiversity found today in Amazonia has been attained through the combination of two main factors:

- 1 The long presence of lowland tropical forest in the region (dating back to the Eocene, ~50 Ma; Burnham & Johnson 2004) and its modern, wet tropical climate (from at least the Middle Miocene, ~16 Ma; Kaandorp *et al.* 2005), where *in situ* diversification could take place;
- 2 historical perturbations that fragmented and/or altered the ecological structure of such forest (e.g. marine incursions, geotectonic events, wetland systems and climatic fluctuations; see Chapter 26), thus creating new evolutionary pressures that either lead lineages to undergo extinction or to diversify.

### Future approaches and systems

No single mechanism of speciation can account for the enormous tetrapod biodiversity present in today's Amazonia. The ongoing development of analytical tools and comparative phylogeography will permit further hypothesis testing of old models and the proposal of new ones. Therefore, our main limitation will continue to be sample size. Large sampling gaps exist in almost any study to date, and documenting genetic structure in these gaps, following a geographically appropriate sampling programme, is essential to a true understanding of biogeographic barriers and historical events.

### Using non-forest organisms to infer forest history

Most attempts to infer the biogeographical history of the Amazonian forests have involved forest-restricted organisms. However, analyses of non-forest taxa with disjunct distributions interrupted by forest also have much potential to inform us about the history of the intervening forests. Examples in South America include a number of species distributed in open formations both north and south of the Amazonian forests. Analyses of the Neotropical rattlesnake (*Crotalus durissus* – Wüster *et al.* 2005a; Quijada-Mascareñas *et al.* 2007) indicate that the disjunct distribution is relatively recent (Mid-Pleistocene) and that this species probably

dispersed through central Amazonia rather than along the eastern edge. If so, the largely evergreen rainforest of the Amazon drainage basin was likely fragmented at least partially by intervening dry forest or other seasonal formations at that time. Phylogeographic studies involving molecular dating of additional codistributed organisms have much potential for the investigation of the history of forest cover in the Amazon drainage basin (Quijada-Mascareñas *et al.* 2007).

### Evaluation of barriers

The recent development of combining distribution modelling (e.g. ecological niche modelling) and phylogeography is a promising approach to evaluating ecological and geological barriers and their potential role in promoting diversification (Rissler & Apodaca 2007). For instance, a study of Pleistocene speciation attributed to refugia used the combination of phylogeography and ecological niche modelling (Peterson & Nyári 2007). The model of the potential distribution of the thrush-like mourner, *Schiffornis turdina*, demonstrated a conservative preference for rainforest habitat throughout its phylogeographic history, suggesting that dry open vegetation would be a habitat barrier. The model allowed reconstructions of the distribution of *S. turdina* for the Pleistocene Last Glacial Maximum (LGM). The distribution indicated a range fragmentation during the LGM reflected in a species-level diversification in Amazonia. The patterns of differentiation and speciation of Amazonian *S. turdina* are consistent with Pleistocene climate changes. This approach could be used to test other barriers and mechanisms in other species.

### Phylogeographic information systems

Mapping the distribution of organisms has traditionally been an expensive and time-consuming activity. Researchers interested in a particular group have often had to request the loan of preserved specimens from different collections for examination, and almost inevitably needed to visit personally important museums. New technological advances are now facilitating this sort of research. For instance, the Global Biodiversity Information Facility ([www.gbif.org](http://www.gbif.org)) and other web-based data portals (such as FishNet, HerpNet, Manis and ORNIS) are making biological data from all the world's biological collections considerably more accessible. The increasing awareness that biological information should be gathered and made freely available has also led to the development of the Phylogeographical Information System hosted by the National Evolutionary Synthesis Center ([www.nescent.org](http://www.nescent.org)). Here, phylogeographic reconstructions of particular groups can be stored and easily compared with other organisms, providing aesthetically appealing visualizations of the spatial and temporal evolution of taxa.

Nevertheless, a good measure of caution is needed: we will probably never be able entirely to replace traditional taxonomic methodology. The preservation of vouchers will likely continue to be as important in the future as today, as a means of ensuring the reliability of taxonomic identification in any study. Besides, no database is better than the records it contains. In the case of

Amazonian organisms, the limited biological exploration of the region, combined with the fact that many (if not most) records have not yet been registered in databases, may preclude any serious study based entirely on digitalized sources.

In addition to current and upcoming internet-based initiatives, we continue to need, more than ever, the contributions of biologists going out in the field to explore, document and sample the world's most diverse ecosystem, the Amazonian rainforest.

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