Mechanisms of Speciation in Reptiles and Amphibians: A Synopsis

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Abstract

Speciation processes have long been inferred from phylogenetic, phylogeographic, and biogeographic pattern-driven perspectives. Now much current speciation research is attempting to more directly describe the underlying processes and mechanisms of divergence leading to speciation. Ideally, researchers should integrate both process- and pattern-based approaches for a more comprehensive understanding of speciation. To this end, a symposium was organized during the 7th World Congress of Herpetology in Canada with the goal of bringing leading experts together to share successful examples of these perspectives and to promote a more cohesive understanding of reptile and amphibian speciation. Here we present a joint paper of short and updated summaries of each of these contributions with the aim of providing a reference source and launching pad for students and researchers interested in speciation in amphibians and reptiles.

Keywords: Speciation, ecological speciation, niche, phylogeography, species delineation.

The manuscript of this paper was prepared by the participants of the symposium “Speciation in amphibians and reptiles: from patterns to processes and mechanisms” organized by Sebastian Steinfartz and Jonathon C. Marshall during the 7th World Congress of Herpetology in Vancouver, British Columbia August 12th, 2012.

Introduction

The emergence of new evolutionary lineages and the loss of such lineages through extinction generate patterns of biological connection and common ancestry. These patterns were often identified by close examination of morphological, genetic, and ecological data sets across a temporal or geographical scale. Historically, processes were inferred from these patterns rather than directly tested and, as a result, much of what we inferred about the process of speciation or lineage splitting came from scrutinizing patterns. 

*Drosophila* served as the original major model system in investigating biogeographic patterns resulting from diversification. For instance, the pattern of distribution of endemic Hawaiian *Drosophila* inspired the idea of the founder-flush mechanism of speciation (Carson, 1974; Templeton, 1980) and greatly influenced subsequent perspectives on speciation in general. Coyne and Orr (1989) endeavored to investigate the ‘time course of speciation’ by comparing 119 pairs of closely related *Drosophila* species and examining patterns of known genetic distances, mating discrimination, hybrid sterility and inviability as well as geographic ranges. This was soon followed up by examples of inferring processes of speciation from pattern scrutiny in amphibians and reptiles. For instance, Sites and Davis (1989) generated phylogenies from allozyme and restriction site data in the *Sceloporus grammicus* lizard species complex to investigate the role of karyotypic variation in the speciation process. Tilley, Verrell and Arnold (1990) compared geographic patterns to levels of ethological isolation in a plethodontid salamander species (reviewed by Garland and Adolph, 1991) whereas other studies correlated phylogeographic patterns with the occurrence and evolution of other traits (e.g.; Rodriguez-Robles and De Jesús-Escobar, 1999; Shaffer and McKnight, 1996; Wiens et al., 1999; García-París et al., 2000) or
tested for the degree of speciation and reproductive isolation in certain groups (e.g. Malone and Fontenot, 2008).

Amphibians and reptiles contain an immense variation in ecological features, genome structure, mating systems, vagility, and other life history traits - and as such serve as a repository for the generation of a vast array of biological and geographical patterns. We believe great potential to fully understand the link between patterns and processes of speciation lies within these taxonomic groups (Camargo et al., 2012). We begin by reviewing several remarkable examples of patterns of speciation from amphibians and reptiles from around the world. We start by looking at the giant tortoises of the Galápagos islands, then the South American lizard family Liolaemidae, and finally the lizard species complexes from South American dry biomes. We then transition to discussions of processes and mechanisms of speciation such as adaptive radiation, the role of the niche in speciation, speciation in the presence of gene flow, traits influencing species diversification rates, speciation driven by intrapopulation color polymorphisms and associated behavioral differences, and finally the interplay of ecology, genes, and behavior in salamanders (Table 1).

Table 1. Summary of topics address in this review divided between patterns and processes of speciation. Contributing authors of each section are given.

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I Patterns Generated by Speciation

Ia Giant tortoises of Galápagos: A model system for demonstrating the classic pattern-based approach for inferring mechanisms of speciation

Oceanic island systems have provided valuable insights into the patterns and processes underlying speciation, acting as “laboratories of evolution” with simplified and rapidly maturing biotas that aid in clarifying evolutionary processes that may be opaque in mature ecosystems (Cowie and
Holland, 2008; Emerson, 2008; Gillespie, Claridge and Goodacre, 2008; Parent, Caccone and Petren, 2008). Moreover, many oceanic island systems provide replicate natural experiments and an explicit temporal component associated with the formation or separation of landmasses. Consequently, the classical pattern-based approach to investigate mechanisms of speciation has proven quite fruitful in such systems. While dispersal has generally been inferred as a primary process leading to island colonization and subsequent speciation (“island progression hypothesis”; Wagner and Funk, 1995), vicariance may also play a role (Geist et al., 2014).

The Galápagos Archipelago occupies a unique position in evolutionary biology, having played a large role in influencing the theoretical underpinnings and empirical testing of evolutionary hypotheses from the time of Darwin through present day (Grant and Grant, 2007; reviewed in Larson, 2001). Located 960 km west of the coast of Ecuador, the Galápagos Archipelago constitutes a chain of volcanic, oceanic islands that have never been connected to mainland South America. At present, the Galápagos Archipelago is composed of 13 major islands larger than 10 km$^2$, six smaller islands, over 40 islets with official names, and many smaller unnamed islets and rocks, covering approximately 8,000 km$^2$ of land spread over 45,000 km$^2$ of water (Snell, Stone and Snell, 1996). They were formed as the Nazca Plate passes west to east over a hotspot such that the islands in the east (Espanõla and San Cristóbal) are the oldest (~3 million years old; Geist, 1996) with progressively younger islands to the west (Fernandina emerged < 70,000 years ago; Kurz et al., 2005).

As typical of isolated oceanic islands, the Galápagos fauna is generally impoverished, with no native amphibians and only seven reptile lineages naturally occurring, four of which diversified after their arrival (Parent et al., 2008). Giant Galápagos tortoises (*Chelonoidis* spp.) are one such group, the ancestor of which arrived in the islands from mainland South America approximately 6-12 million years ago (mya) (Caccone et al., 1999). Presently, there are 16 species of Galápagos tortoises, one of which was only recently described (Poulakakis et al., 2015) and five others that have previously gone extinct largely due to human activities (Fig. 1).

Giant tortoises are the only native, large-bodied herbivores in Galápagos and play an important role in the islands’ ecosystems (Blake et al., 2015; Gibbs, Marquez and Sterling, 2008; Hansen et al., 2010). Giant Galápagos tortoise species diversification has long been considered to follow the progression rule (Wagner and Funk, 1995; Beheregaray et al., 2004; Parent, Caccone and Petren, 2008), however, a recent study that included all extant and extinct species pairing phylogenetic analyses of mtDNA character data and Bayesian inference of species divergence times set forth more complex hypotheses related to patterns of colonization as well as timing and mechanisms of divergence (Poulakakis et al., 2012). Based on this work, the ancestral node to Giant Galápagos tortoise lineages dates back to the late Pliocene (3.2 mya) with lineage diversification beginning ~1.74 mya (Poulakakis et al., 2012). San Cristóbal Island was likely the first to be colonized, giving rise to three major clades of Giant Galápagos tortoise species including: 1) a central-south-western clade from the islands of Rabida, western Santa Cruz, Pinzón, Floreana, Fernandina, and southern Isabela (intraclade diversification beginning ~1.28 mya); 2) an eastern/northern clade from the islands of Espanõla, San Cristóbal, eastern Santa Cruz, Pinta, and Santa Fé (intraclade diversification beginning ~0.82 mya); and 3) a western/northern clade with tortoises from the island of Santiago and northern Isabela (intraclade diversification beginning ~0.40 mya) (Poulakakis et al., 2012). Combining these patterns of relationships and timings of divergence with paleographic reconstructions implicates both vicariance and dispersal as mechanisms of diversification (Poulakakis et al., 2012).

Giant Galápagos tortoises have also provided important insights into the evolutionary dynamics at the early stages of speciation due to their relatively unique life history traits, especially their long generation times (Pritchard, 1996). Several studies have shown the importance of introgressive
hybridization in speciation (Abbott et al., 2013; Schwenk, Brede and Streit, 2008), which may lead to so-called “despeciation”, as in the case of the Darwin’s finches (Grant and Grant, 2014). In Giant Galápagos tortoises, several examples of introgressive events have been found, which are likely to lead to very different evolutionary outcomes. A recent study on the population history of C. becki endemic to northern Isabela Island has shown that the two genetically distinct tortoise lineages that independently colonized the slopes of Volcano Wolf from the island of Santiago are likely to fuse back together after ~50,000 years of evolution in micro-allopatry (Garrick et al., 2014). This finding represents an unprecedented opportunity to look at the fusion/fission dynamics of early speciation, which are rarely captured in study systems with shorter generation times.

Human translocations of giant tortoises are also likely responsible for rare introgression events between allopatric Cheloniaid species and have facilitated the “dispersal” across the archipelago. Early phylogenetic studies of extant Galápagos tortoise species noted the detection of “aliens” on Isabela and Santiago Islands, referring to rare occurrences of individuals with highly divergent haplotypes that were more related to those detected in geographically distinct populations from other islands rather than the local population (Caccone et al., 2002). These detections were most abundant along the slopes of Volcano Wolf on northern Isabela Island where it was suggested that the non-native tortoises were deposited by whalers, a hypothesis consistent with old log books from the industry (Pritchard et al. 1996; Caccone et al., 2002; Beheregaray et al. 2004). Subsequent studies that included population-level samplings of now extinct species (C. elephantopus from Floreana; C. abingdoni from Pinta) by way of historical DNA analysis of museum specimens confirmed the non-native origin of the Volcano Wolf “aliens” (Russello et al., 2007; Poulakakis et al., 2008; Russello et al., 2010; Garrick et al., 2012; Edwards et al., 2013). Given their rarity, these hybridization events will likely not impact the evolutionary trajectories of the species involved as suspected in the case of the C. becki lineages on

Figure 1. A) Distribution of Giant tortoises in the Galápagos Archipelago. Shaded and nonshaded islands indicate the presence of extant and extinct (†) tortoise populations, respectively. Italicized names indicate current taxonomic designations and black triangles denote locations of volcanoes on Isabela Island. B) Schematic of the proposed phylogeographic history of Galápagos tortoises modified from Poulakakis et al. (2012). Arrows represent dispersal and colonization events within Galápagos, with the numbers indicating approximate temporal order in millions of years. Short solid line segments indicate vicariance events. The solid black arrows are hypothesized natural colonization events, while the dashed green arrows represent recent and likely human-induced translocations.
Volcano Wolf (Garrick et al., 2014). Nevertheless, they are of considerable conservation importance, as some of these hybrids contain genomic material from now extinct species from Floreana (C. elephantopus; Poulakakis et al., 2008; Russello et al., 2010; Garrick et al., 2012) and Pinta Islands (C. abingdonii; Russello et al., 2007; Edwards et al., 2013). Ongoing work is devising strategies for using these hybrid individuals for the purposes of genetic rescue as part a broader plan for reintroducing giant tortoises to islands where they have been presumed extinct.

The use of modern and museum samples coupled with the availability of a large reference database that included all extant and extinct populations and species of the iconic adaptive radiation of Giant Galápagos tortoises has provided important insights on the evolutionary drivers of diversification in this system, as well as critical information to guide conservation efforts (Jensen et al., 2016). Ongoing genomic studies Giant Galápagos tortoises will enable new and exciting opportunities to enhance our understanding of speciation within and among islands, the relative importance of introgression and fusion events in species formation and persistence, and the study of the genomic architecture of traits associated with their ecological and morphological diversification.

Ib Liolaemid lizards as a study case: from poorly known taxonomic groups to evolutionary radiations

The South American lizard genus Liolaemus is a large (~ 250 species) temperate zone genus distributed from Tierra del Fuego to north-central Peru, and ecologically ranges from sea level on both South American coastlines to ~ 5000 m in elevation. It offers a wide array of research options in many areas of ecology and evolutionary biology, but some of these options are limited by major gaps in taxonomic knowledge. In 2003, there were ~160 described species, but after the first detailed molecular study of one species complex (L. darwinii complex) Morando, Avila and Sites (2003) inferred that Liolaemus could contain at least twice the number of known species, based on the discovery of multiple well-supported mtDNA haploclades within a single taxonomic species. Since 2003, 95 Liolaemus species have been described (based on morphology and also on molecular data for many), and another ~55 have been identified as "candidate species" (based mainly on mtDNA and allopatry) that require further study. These numbers, added to the 160 known species from 13 years ago, brings the total number to 310 possible species. However, key geographic regions still remain poorly studied, and may contain many additional species. Morando et al. (2013) documented similar patterns in the sister genus Phymaturus, and these studies highlighted the importance of dense geographic sampling and mtDNA as a "first pass" approach for poorly known groups. These species hypotheses ("candidate species") can be used to direct further sampling and the use of integrative approaches to test species limits (i.e. including other molecular markers, morphology, ecology, etc.). For example, based on the integration of molecular, morphological, and ecological “niche envelope” modeling, Aguilar et al. (2013) found and described several new Liolaemus species from Peru, Minoli, Morando and Avila (2014) tested species limits in the Liolaemus fitzingerii group with morphometric and niche envelope analyses, and a similar integrative taxonomic approach validated and discovered new "candidate" species in the L. lineomaculatus section (Breitman et al., 2015a,b).

The "candidate" species in these examples were based on previous taxonomy, but if there is no a priori hypothesis of species boundaries, new methods for single-locus data can add statistical rigor to the identification of candidate species. One of these single-locus methods is the General Mixed Yule-Coalescent method (GMYC, Pons et al., 2006), as extended to incorporate genealogical uncertainty and more conceptually-justified interpretation of results (Reid and Carstens, 2012; Esselstyn et al., 2012).
Other species discovery approaches can be applied to multi-locus datasets including the heuristic, parsimony- and likelihood-based methods in Brownie (O’Meara, 2010), and threshold criteria based on large numbers of loci and dense taxonomic sampling (Birky, 2013). Subsequently, these candidate species can be tested statistically with species validation approaches (Carstens et al., 2013) in a fully-coalescent framework that simultaneously explores species limits and species trees (BPP3, Yang and Rannala, 2014; STANCEY, Jones, 2015), and/or with an integrative Bayesian approach that combines molecular and phenotypic data that allows modeling divergence with gene flow (iBPP, Solís Lemus, Knowles and Ané, 2015). For instance, Camargo et al. (2012) applied several species validation approaches to test for species limits in the L. darwinii complex based on previous taxonomic knowledge of the clade.

For many researchers interested in species delimitation, the collection of large molecular data sets is not feasible, and in these cases an “Integrative Taxonomic” (IT) approach – the use of different kinds of data and methods for species discovery (Padial and De la Riva, 2010; Padial et al., 2010; McKay et al., 2014; Pante, Schoelinck and Puillandre, 2015) – may be employed with productive results. IT approaches based on multiple classes of data and analyses may also be useful when divergence initially occurs along non-molecular axes of differentiation, and/or when divergence occurs with gene flow, as hypothesized between Liolaemus bibroni and L. gracilis (Olave et al. 2011). IT approaches include step-by-step methods based on sequential analyses of independent data types, and followed by a qualitative assessment of species boundaries (Schlick-Steiner et al., 2010; Yeates et al., 2011), or model-based methods that simultaneously evaluate multiple data types, and followed by delimitation of species based on statistical or information criteria (Edwards and Knowles, 2014; Solís Lemus, Knowles and Ané, 2015; see Aguilar et al. 2016, described below for a case study in the L. montanus group). Both approaches can be used for four focal areas of IT: (1) validation of candidate species as evolutionary distinct lineages; (2) inferring species relationships; (3) detecting “cryptic diversity”; and (4) assignment of individual specimens to a species group (Edwards and Knowles, 2014; Leavitt, Moreau and Lumbsch, 2015).

Aguilar et al. (2016) recently applied the IT approach to resolve some taxonomic uncertainties in the northernmost species of Liolaemus, the montanus group in north-central Peru. These authors analyzed molecular (mitochondrial and nuclear gene regions), morphological (meristic, morphometric, geometric morphometric, and categorical attributes), and bioclimatic (“niche envelope”) data, to delimit species boundaries. Aguilar et al. outlined four alternative hypotheses of species boundaries in this group, and then carried out multiple analyses on four classes of morphological data, including: (1) meristic (count), (2) morphometric (continuous measurement), (3) geometric morphometric (quantification of head shapes), and (4) categorical (presence/absence). For these same samples nine of the 19 standard climatic variables (Hijmans et al., 2005) that were not correlated with each other, were chosen for pairwise estimates of “niche similarity” using the Schoener’s D metric (Warren et al., 2010). All of these data sets – multilocus molecular, morphological, and bioclimatic, were combined for Gaussian clustering (GC) to estimate species boundaries. These data were then analyzed by step-by-step methods to test four hypotheses about taxonomic assignments and species boundaries in this group of lizards.

Results of this study revealed that as a rule, older “candidate species” as identified by longer branches on the gene and species trees, were generally more clearly corroborated by other classes of data, and also more strongly corroborated across model-based GC and step-by-step methods, while there was a lack of correspondence between GC and other approaches across younger lineages (Aguilar et al., 2016). This in and of itself is not necessarily a surprising result, but some of this “fuzzy” resolution may be due to small sample sizes (only 1-3 individuals for some localities) for some methods for which a
minimum of five is recommended (Hennig and Hausdorf, 2010). Further, some populations/species are known only from their type localities, which compromises the collection of sufficient bioclimatic, morphological, and/or molecular data for the GC approach. Too few empirical studies have been completed to distinguish between the possibility that these results reflect either: (1) a limitation of the GC analyses for this particular group of organisms, (2) or a more general limitation of the GC approach. Future studies with appropriate sampling designs and simulations can distinguish between these alternatives, but as Aguilar et al. show, multiple low-cost options exist for collection and analyses of many informative classes of data for IT studies, although given the reality of “gray zones” in speciation processes, we will likely always rely upon qualitative judgments of species boundaries in some cases.

Species delimitation in most species complexes of *Liolaemus* has been complicated due to the occurrence of extensive paraphyly in multiple loci *Phymaturus* (incongruence between mtDNA and traditional morphological species limits). Some of these are due to limited taxonomic knowledge (e.g., Avila, Morando and Sites, 2006; Breitman et al., 2012), incomplete lineage sorting (e.g., Avila, Morando and Sites, 2006), while for others, hybridization was suggested as the most likely cause (Morando et al., 2004; Morando et al., 2007; Breitman et al., 2015a,b). In some cases, further study based on nuclear markers has confirmed mtDNA introgression (Olave et al., 2011; Camargo et al., 2012). In cases where parapatry and introgression are limited or absent, some hypotheses of the “drivers” of speciation can be formulated based on comparing patterns of variation in multiple data sets collected from recently diverged sister clades (Camargo, Sinervo and Sites, 2010). For example, molecular, morphological, and niche envelope data for *L. petrophilus* suggest that environmental niche divergence may have promoted diversification in allopatry, for sister clades north and south of the Somuncurá Plateau in Argentina (Fontanella et al., 2012). Nevertheless, some knowledge of approximate species limits is a prerequisite for the study of speciation, yet in both genera these remain poorly-known in many groups, and gene flow between different species in contact zones poses an extra challenge to species delimitation. Future studies will also need to accommodate the effect of sampling design (Rittmeyer and Austin 2012; Camargo and Sites, 2013) and population structure (Niemiller, Near and Fitzpatrick, 2012) on species delimitation studies. Sampling designs may vary between clades depending on variables such as within-species population structure and speciation histories. Consequently, while general recommendations are difficult to make, simulation analyses are one potential tool to assess adequate sample sizes in a specific study (Camargo and Sites 2013).

On a macroevolutionary scale, despite taxonomic limitations, we know enough to be certain that *Liolaemus* is likely to be several times more species-rich than its sister genus *Phymaturus*, so there have been major differences between the two either in rates of speciation or extinction, or both. Several molecular phylogenetic studies of distinct clades of *Liolaemus* have resolved polytomies at nodes that are strongly supported by multiple analytical methods of multi-locus data sets (Morando, 2004; Breitman et al., 2011; Morando et al., 2013), albeit these data sets are small relative to the number of loci needed to distinguish between “hard” vs. “soft” polytomies under some speciation scenarios. Thus, these clades are excellent candidates to implement the new analytical approaches that are being developed to test for rapid radiations (e.g., Rabosky et al., 2007, Rabosky, Slater and Alfaro, 2012) that may have been part of the macroevolutionary history of these clades.

To our knowledge, Olave et al. (2015) first used an explicit model in a statistical coalescent framework to test for rapid radiations in *Liolaemus*, in a sample of 142 species of the subgenus *Eulaemus*. These authors used three species-tree methods (*BEAST, BEST and MDC). Based on simulated datasets under eight explicit evolutionary models (including rapid radiations), and testing them against empirical data, we found support for two rapid radiations as the most plausible hypothesis for diversification of this clade of lizards.
Some parapatric hybrid zones have been discovered in *Liolaemus* (Morando et al., 2004; Avila, Morando and Sites, 2006; Morando et al., 2007) and detailed work on these no doubt will continue to elucidate the evolutionary forces that could have contributed to diversification in this species-rich genus. For instance, a recent study suggested that adaptive processes, linked to episodic ecological opportunity generated by the gradual uplift of the Andes, could account for the extremely rich species diversity of *Liolaemus* (Pincheira-Donoso, Harvey and Ruta, 2015). With these first discoveries and more synthetic studies, a dynamic evolutionary view about the lizard genus *Liolaemus* is emerging that promises to offer many opportunities to study various aspects of different speciation modes.

**Ic Lizard speciation and historical biogeography patterns at the South American dry biomes**

Speciation research has undergone a lot of its progress thanks to integrative taxonomy studies of amphibians and reptiles (Camargo et al., 2010; Florio et al., 2012; Vasconcelos et al., 2012), which greatly benefited from the increased statistical rigor of species delimitation methods over the past decade (Fujita et al., 2012). A smaller portion of these speciation studies focused on evolutionary patterns and processes within the theoretical and spatial framework of historical biogeography (Chan, Brown and Yoder, 2011). Whether indirectly on small scales (Bell et al., 2012) or explicitly over wider geographical scales (Fujita et al., 2010; Pepper, Fujita and Moritz, 2011; Werneck et al., 2012), speciation research has a great potential to enlighten the contrasting roles of geological landscape and changing climate on the diversification of amphibian and reptiles (and other groups).

The degree to which speciation/phylogeographic approaches can make relevant contributions to the understanding of broader historical biogeography patterns at multiple spatial and temporal scales is directly dependent on the coverage of geographical sampling, the biology of the study taxa, and the nature of markers used. Taxa of lower vagility, characterized by deeper genetic structure, dense geographical (individuals and populations) sampling, and multiple independent markers are overall preferable for stronger biogeographic inferences. Some studies integrated densely sampled empirical examples with model-based parameter estimation and hypothesis testing for species delimitation (Camargo et al., 2012). However, integration of model-based approaches with explicit historical biogeographic hypothesis for the Neotropical herpetofauna is still less explored. Such an approach was used recently to study the speciation of lizards across the South American diagonal of dry biomes.

The highly threatened open vegetation biomes of central-eastern South America extend diagonally across a large latitudinal range and include the Seasonally Dry Tropical Forests (SDTFs, with the largest area, Caatinga, in northeastern Brazil), the Cerrado savanna (central Brazil), and the Chaco (southwestern South America; Fig. 2). While early studies have suggested an impoverished fauna (compared to the tropical rainforests), currently these biomes are recognized as having high diversity and endemism levels for amphibians and reptiles (Nogueira, et al., 2011; Werneck, 2011; Valdujo et al., 2012), as well as other taxonomic groups. Recent diversification studies have advanced the understanding of biogeographical processes responsible for the observed speciation patterns of amphibians and reptiles, showing some congruent patterns along the diagonal of open formations, such as: (1) the occurrence of genetic breaks geographically congruent with the limits of the three biomes part of the dry diagonal, indicating complex speciation scenarios that may have been influenced by altitudinal variation (Werneck et al., 2012; Recoder et al., 2014); (2) some geomorphological components that have important roles in the speciation at the dry diagonal, as the Serra Geral de Goiás, the Serra do Espinhaço and the São Francisco river (Werneck et al., 2012; Recoder et al., 2014; Werneck et al., 2015; Guarnizo et al., 2016); (3) deeper divergence events within closely related groups dating to the Miocene-Pliocene transition and assigned to events such as the uplift of the Brazilian...
Shield and marine introgressions (Prado, Haddad and Zamudio, 2012; Werneck et al., 2012; Machado, Silva and Silva, 2014; Santos et al., 2014; Guarnizo et al., 2016); (4) Cerrado lineages appear to have a deeper structure when compared to Caatinga lineages, which have more shallow structures indicating instances of ecological speciation and scenarios of speciation with gene flow (Werneck et al., 2012; Oliveira et al., 2015) or recent demographic expansions (Thomé et al., 2016; Werneck et al., 2015); (5) the prevalence of deep phylogeographic structures with high levels of cryptic diversity and occurrence of species of complexes (Prado, Haddad and Zamudio, 2012; Werneck et al., 2012; Recoder et al., 2014; Santos et al., 2014; Guarnizo et al., 2016); and (6) the existence of a west-east diversification pattern, especially in the Cerrado (Prado, Haddad and Zamudio, 2012; Werneck et al., 2012; Recoder et al., 2014; Santos et al., 2014; Guarnizo et al., 2016). For *Vanzosaura* lizards, the pattern of east-west divergence is congruent with morphological variation, and a new taxonomic arrangement was proposed for the genus with the description of a new species endemic to the Cerrado (Recoder et al., 2014).

Alternatively, other patterns do not show overall agreement between studies, such as the role of Pleistocene climatic and vegetational cycles on population structure or correlation between areas of climate stability and high genetic diversity. For example, Pleistocene climatic cycles were shown to be important for the diversification of Cerrado treefrogs (Prado, Haddad and Zamudio, 2012). On the contrary, other studies that explicitly tested the prediction that areas of long-term stability during Quaternary climatic fluctuations would have greater genetic diversity and corresponding phylogeographic structure, did not find such an effect for the lizard species investigated (Werneck et al., 2012; Santos et al., 2014). Thus, responses to Pleistocene climate fluctuations seem highly variable among taxa, and different evolutionary responses to changing climates other than population extinctions and range shifts may be more common than previously thought. Persistence *in situ* can occur, if changing climate remains within the species' physiological tolerance limits (Davis, Shaw and Etterson, 2005) and the preferred habit persists, as in the case of the rock-outcrop specialist gecko *Phyllopezus pollicaris* (Werneck et al., 2012). In these cases, it is expected for phylogeographic signatures to reflect events that have not been over-written by Pleistocene climate dynamics. Thus, stability is not an exclusive force in generating diversity (species and genetic) patterns and climate change should not be unconditionally associated with loss of diversity (i.e. extinction) without a critical evaluation of each biological system’s idiosyncrasies. The stability-instability dynamics is crucial to generate opportunity that promotes diversification and ultimately speciation along the diagonal of open biomes. Patterns of persistence and/or susceptibility to climatic change provide important insights about responses to future environmental changes and long-term population viability, a critical variable for establishing efficient conservation strategies. However, other taxa associated with the dry diagonal may be more susceptible than others to range oscillations and extinction due to climate change. Allocation of conservation resources will then be more effective if comparative studies can provide evolutionary histories of a diverse array of co-distributed ‘dry diagonal’ endemics.

Werneck et al. (2012) used model-based Approximate Bayesian Computation to test alternative population divergence hypotheses for *Phyllopezus pollicaris* gecko complex that correlated with historical biogeography hypotheses at the landscape level, for the poorly studied South American dry biomes. Three biogeographical hypotheses were outlined, each incorporating different population structures, divergence times, and the presence or absence of gene flow between populations of the three biomes (Cerrado, Chaco and SDTFs/Caatinga): (A) a null model of no speciation reflecting early views in the literature that the dry diagonal biomes would share a single evolutionary history; (B) a speciation model that predicted an ancient divergence event in three major phylogeographic clades (southwest/Chaco, central/Cerrado and northeast/Caatinga) and representing an older speciation scenario triggered by geological events and (C) a speciation model with two divergence events, an initial
separation between populations from southwest/Chaco and all the others, followed by a more recent ecological divergence event between central/Cerrado and northeast/Caatinga populations (Fig. 2). Stronger support was found for model C, which predicts two speciation events (one considered allopatric speciation and the other ecological speciation) between lineages associated with the Chaco, Cerrado, and Caatinga, revealing complex scenarios of diversification among the dry diagonal biomes. Oliveira et al (2015) used ABC to test four alternative diversification scenarios for a whiptail lizard (*Cnemidophorus ocellifer*) in the Caatinga that considered varying divergence times, migration estimates, and demographic histories, and found support for speciation with gene flow along an environmental gradient.

**Figure 2.** Distribution of the eastern South America dry diagonal biomes (top left) and the distribution of inferred Bayesian population clusters and ancestral distribution of *P. pollicaris* with respect to the inferred historical stability surface in yellow (stable areas obtained by overlapping predicted logistic outputs under four climatic scenarios: current, 6, 21, and 120 kyr BP) and a digital elevation model for South America (brown represents higher altitudes). Pie charts represent the posterior probability that a given individual is assigned to a particular cluster. Alternative divergence models tested using an ABC framework (bottom left). Adapted from Werneck et al. (2012).

In summary, we now have a more refined knowledge about the diversity, biogeography, and diversification of the lizard fauna of the dry diagonal biomes. However, many fields of research deserve further attention, and explicit comparative studies are needed to assess the degree of spatial and temporal
congruence for the observed patterns. Future studies should preferably focus on specific knowledge gaps, and on testing explicit biogeographical hypotheses based on their genealogical, temporal, and demographic expectations.

II Processes and mechanisms of speciation

IIa Intrinsic and extrinsic contributors to diversification

Speciation in amphibians and reptiles is thought to be influenced by numerous factors, which can be categorized as extrinsic and intrinsic. Extrinsic factors are represented by environmental variables such as temperature, precipitation (Qian et al., 2007), geography (see section I), and presence of microhabitats suitable for reproduction (Buckley and Jetz, 2007; Vences and Wake, 2007). Intrinsic factors include ecological specialization (Orr and Smith, 1998; Losos, 2009; Sobel et al., 2010), body size (see section IIe and citations therein), metabolic rate (Feder, 1992; but see Wiens, 2015), population density (Tamarin and Krebs, 1969; Gaines and Krebs, 1971; Sinervo, Svensson and Comendant, 2000), structural chromosome rearrangements (Olmo, 2005; Leaché and Sites, 2009; Kitano and Peichel, 2012; Adler, Yadamsuren and Procuinier, 2016), parameters related to reproduction (Blair, 1958; Zeh and Zeh, 2000; Hoskin et al. 2005) or past evolutionary constraints. Extrinsic factors represent the overall opportunity for speciation provided by the environment, while intrinsic factors represent the specific organism’s potential to diversify, in relation to its existing evolutionary constraints (Barraclough, Vogler and Harvey, 1998). Evolutionary constraints are defined as properties of traits that limit the evolution of new phenotypic variants (Blomberg and Garland, 2002), and the organismal potential for future diversification might be related to already existing species diversity (Emerson and Kolm, 2005). In addition, stochastic factors might determine which groups diversify more than others (Slowinski and Guyer, 1993). The dependency of reptile and amphibian diversity on extrinsic factors such as global climate becomes evident when comparing climate maps to amphibian and reptile species richness estimates: amphibian species richness is concentrated in regions with high net primary productivity (Buckley and Jetz, 2007), while reptile species diversity on a global scale is correlated with temperature and topography in the Afrotropics (Qian, 2010).

However, these factors do not operate independently in reptile and amphibian evolution. For example, the interplay between extrinsic and intrinsic factors in shaping species distributions, and patterns of endemism and species richness, is evident in the parameter of geographic range size. The range size of a species can be the result of both extrinsic and intrinsic factors, as it is both linked to environmentally suitable niches (see section IIb and citations therein), as also to intrinsic factors limiting dispersal such as body size (see section IIe and citations therein). Once established, geographic range size itself may act as an extrinsic as well as intrinsic factor that can influence morphological evolution (reviewed in Cooper and Purvis, 2009): small range sizes can be an outcome of population dynamics through gene flow, where peripheral areas of the range experience a demographic sink (intrinsic, Kirkpatrick and Barton, 1997). Alternatively, a small range size limits species in ecological opportunity that may be available for more widely distributed species (extrinsic, e.g., von Hagen and Kadereit, 2003). The high species diversity present in clades that constitute adaptive radiations therefore may be the result of the complex interactions between both factors. In order to obtain a quantitative understanding of the process of diversification in species-rich clades of reptiles and amphibians, the relative importance of both extrinsic and intrinsic factors needs to be assessed. With regards to the possible mode of speciation, timing seems to be important: intriguingly, the present species diversity of some island radiations is an outcome of speciation events that post-date the initial large-scale, often
allopatric divergence events in the early stages of the radiation (e.g., Kaffenberger et al., 2012, Wollenberg et al., 2013). Intrinsic factors may help to explain such more recent speciation events in adaptive radiations, that are often not coinciding with phylogeographic splits associated with hard dispersal barriers. For example, in African cichlid fishes (Wagner et al., 2012), the relative importance of extrinsic and intrinsic factors causing adaptive radiations is better understood than for reptiles and amphibians. If optimum values for both extrinsic and intrinsic factors are in concordance (e.g., solar radiation and lake depth as extrinsic and sexual dichromatism as intrinsic), the likelihood of cichlid lineage diversification can be partially predicted (Wagner et al., 2012). To quantify the relative importance of such different agents of evolutionary change for amphibians and reptiles, integrative data sets of environmental and phenotypic characters need to be studied.

Present allopatric patterns of species distribution lend themselves to inferring past allopatric speciation, whereas speciation with gene flow is not expected to result in similarly obvious spatial patterns. This, or alternatively the prevalence of allopatric sister species, may explain the prevalence of examples for pure allopatric speciation in the literature (see section IIc and citations therein). Present strong genetic divergence among populations across soft barriers to gene flow is not only harder to interpret in terms of pattern versus geographical process of speciation, but also poses the question of whether it represents imminent speciation versus standing local adaptation (section IIc and citations therein). Examples may be found in younger clades separated by barriers that do not fall into the “hard” barrier category – i.e., climatic oscillations or habitat gradients (see section IId and citations therein), such as some sympatric/syntopic and microendemic Madagascan sister pairs of frogs. Some of these recently diverged species show evidence for ecological speciation coinciding with soft barriers to gene flow, divergence in bioacoustics characters (Gephyromantis eiselti and G. thelenae, Wollenberg and Harvey, 2010), body size (Gephyromantis enki and G. boulengeri, Wollenberg Valero, 2015), or functional convergence in color patterns (for a case of mimicry, cf. Mantella madagascariensis and M. baroni; Schaefer, Vences and Veith, 2002).

On small spatial and temporal scales, imminent speciation could be discerned from standing local adaptation by a combination of the following lines of evidence: (1) Assuming that similar processes of selection will result in similar outcomes, divergence patterns can be compared across different taxonomic levels of one clade. For example, if speciation processes are deterministic, then we would expect to find similarities between among-population divergence and among-species level divergence, which would indicate that the populations are on a trajectory to diversify (see section IId). Comparing different clades occupying the same habitat (e.g., different endemic radiations of Madagascar showing similar phylogeographic patterns) allows inferring common evolutionary processes among them (Brown et al., 2014). (2) If signatures of convergent genomic adaptation among populations can be found, this may indicate adaptive speciation (Hohenlohe et al., 2010). Patterns of convergent genomic adaptation are likely the outcome of convergent evolution, even in the presence of gene flow, but some caution needs to be employed: One alternative but less parsimonious explanation for convergent alleles would be the evolution and subsequent selection of such beneficial alleles through multiple, independent mutational events. Also, it is likely that more than one genomic route can produce a phenotypic adaptation responsible for divergence with gene flow, as genomic adaptations in different loci may be functionally equivalent (polygenic). For example, several mutations can cause interruption of different levels of the same metabolic pathway (e.g., Daub et al., 2013). The following paragraphs in this paper highlight the importance of, and interplay between intrinsic and extrinsic factors, and give examples for amphibian and reptile studies that identified their importance in the speciation process.
IIb Speciation and the niche

The niche may play many pivotal roles in speciation. The niche describes the set of abiotic and biotic conditions where a species can persist (Hutchinson, 1957; Holt, 2009). The niche simultaneously includes the environmental conditions that determine the broad-scale distribution of species (Grinnellian niche) and its interactions with other species at the local scale (Eltonian niche).

The niche is critical to speciation in at least two ways. First, the Grinnellian niche plays an important role in geographic isolation. This is clear from first principles, given that the niche determines where species occur. Both divergence and conservatism in the niche may play a critical role in speciation.

For parapatric speciation, divergence of species along an ecological gradient (e.g., different climates along a mountain slope) may lead to some populations becoming locally adapted to some parts of the gradient (e.g., Moritz et al., 2000; Hua and Wiens, 2013). This may then lead to reduced gene flow between these populations, possibly leading to parapatric speciation (e.g., if individuals of one population cannot tolerate the local environmental conditions where the other population occurs, and vice versa). Such a scenario typifies the process of speciation via niche divergence.

For allopatric speciation, niche conservatism may lead to the initial geographic isolation of populations (e.g., Wiens, 2004; Hua and Wiens, 2013). Niche conservatism is the tendency of species to retain niche-related ecological traits over time (reviewed in Wiens et al., 2010). From first principles, niche conservatism should be critical for allopatry (Wiens 2004). Populations become allopatric when they are separated by a barrier of unsuitable ecological conditions. This barrier may be relatively obvious (e.g., oceans for terrestrial species) or more subtle (lowland mesic temperate forest vs. upland mesic temperate forest), but the basic principle is the same. Ultimately, the reason why this barrier functions as a barrier is because the populations separated by the barrier are unable to adapt to the ecological conditions within that area and maintain gene flow across it. Thus, the barrier of unsuitable ecological conditions is maintained by the retention of similar niche-related ecological traits in these populations over time (i.e., niche conservatism). It is very important to note however, that just because niche conservatism was involved in the initial geographic isolation of the populations, this does not mean that they do not diverge subsequently in one or more ecological traits.

There are now many examples in the literature of speciation through both niche divergence and niche conservatism in reptiles and amphibians. For example, there is evidence that in tropical salamanders, sister species tend to occur in divergent climatic conditions (Kozak and Wiens, 2007). At a larger scale, tropical plethodontid clades with higher rates of climatic niche evolution have faster rates of diversification (speciation minus extinction), consistent with the idea that climatic divergence drives speciation (Kozak and Wiens, 2010a). At an even broader phylogenetic scale, levels of climatic niche divergence seem to explain much of the variation in diversification rates among salamander and frog families, with greater climatic niche divergence within families associated with higher rates of diversification (Gomez-Rodriguez, Baselga and Wiens, 2015). Indeed, climatic niche divergence is far better at predictor family-level diversification rates than climatic niche variables alone (i.e., tropical vs. temperate). Several other studies have found divergent climatic niches between closely related species, including studies of frogs (Hua and Wiens, 2010), lizards (Knouft et al., 2006), and snakes (Pyron and Burbrink, 2009). Several studies have also found interesting patterns of within-species phenotypic divergence and environmental variation that may lead to parapatric speciation (e.g., Schneider et al., 1999; Ogden and Thorpe, 2002).

There are also several studies that potentially support speciation through climatic niche conservatism, including analyses of plethodontid salamanders in eastern North America (Kozak and
Wiens, 2006, 2010b), Australian frogs (e.g. Hoskin et al. 2011), and studies of various groups of tropical terrestrial vertebrates (e.g. Cadena et al., 2012). It is also important to note that just because climatic niche conservatism was not supported as driving allopatric speciation, allopatry may have been associated with niche conservatism in other ecological traits (e.g., microhabitat types).

The Eltonian niche may also be important in speciation. For example, many models of adaptive radiation suggest that an important part of the process involves divergence along many different axes of the ecological niche, including axes that involve division of resources at the local scale. For example, many vertebrate radiations involve divergence in microhabitat and body size, suggesting that these are linked to diversification (Streelman and Danley, 2003). However, directly linking variation in some of these traits to speciation (or diversification) has proven more difficult (e.g. Adams et al., 2009). Nevertheless, microhabitat (aquatic vs. terrestrial) seems to explain the majority of variation in diversification rates (~67%) among the 12 major clades of vertebrates (Wiens, 2015). Another important question is whether speciation along Eltonian niche axes might reflect sympatric speciation.

Clearly, the role of the niche in speciation depends (in some part) on the geographic mode of speciation involved. What do we know about geographic modes of speciation in reptiles and amphibians? In general, allopatric speciation is widely considered to be the most common geographic mode (Coyne and Orr, 2004). Several herpetological studies now show some support for this hypothesis. For example, studies of range overlap of species pairs in some groups support the prevalence of the allopatric mode (salamanders: Kozak and Wiens, 2006; frogs: Hua and Wiens, 2010; turtles: Stephens and Wiens, 2003). However, other groups remain largely unsurveyed in terms of their geographic modes and more extensive surveys are needed.

IIc Speciation in the presence of gene flow

Whether divergence and speciation can occur in the presence of gene flow is a fundamental yet controversial question of evolutionary biology (Via, 2001; Butlin, Galindo and Grahame, 2008; Fitzpatrick, Fordyce and Gavrilets, 2008a, 2009; Mallet et al., 2009; Feder et al., 2013). It is widely accepted that speciation requires the interruption of gene flow between populations (Coyne and Orr, 2004), as gene exchange and recombination is a significant impediment to population divergence and the formation of new species. Speciation among allopatric populations is generally straightforward, as an ecological barrier to gene flow is present (see IIb). When a geographic barrier is not present, then a mechanism is required to counterbalance the homogenizing effect of gene flow. Theoretical models have demonstrated a variety of scenarios in which speciation can occur without complete geographic isolation (Gavrilets, 2004; Bolnick and Fitzpatrick, 2007; van Doorn et al., 2009; Flaxman et al., 2014), and empirical examples demonstrate that speciation in the face of gene flow may be more common than previously thought (Nosil, 2008, 2012; Feder, Egan and Nosil, 2012, Feder et al., 2013). Several processes can facilitate genetic divergence during speciation in the face of gene flow, such as direct divergent selection on a few loci of large effect that underlie reproductive isolation (Feder et al., 2013), mate choice that is correlated with a trait under divergent selection (Via, 2001; Gavrilets, 2004), and divergence hitchhiking in which gene exchange is reduced over larger genomic regions as an indirect effect of strong divergent selection on loci involved in local adaptation (Via and West, 2008; Via, 2012). More recently, advances in genomic approaches have extended the study of speciation with gene flow by examining patterns and extent of admixture, divergence, and linkage disequilibrium between taxa on a genome-wide scale (Ellegren et al., 2012; Nosil et al., 2012; Feder, Egan and Nosil, 2012, Feder et al., 2013).
Amphibians and reptiles have been important model systems in recent years in determining the prevalence, patterns, processes, and mechanisms of divergence and speciation with gene flow. Examples of studies that have examined aspects of divergence and speciation with gene flow include tropical frogs (Hua and Wiens, 2010), Andean frogs (Guarnizo and Cannatella, 2014), ranid frogs (Richter-Boix et al., 2010, 2013; Lind et al., 2011; Buskirk, 2014), barking frogs (Streicher et al., 2014), chihuahua frogs (Funk et al., 2016), newts (Nadachowska and Babik, 2009; Pereira, Martinez-Solano and Buckley, 2016), plethodontid salamanders (Kozak and Wiens, 2007; Niemiller Fitzpatrick and Miller, 2008, Niemiller, Nosil and Fitzpatrick, 2010), Anolis lizards (Ogden and Thorpe, 2002; Stenson et al., 2002; Calsbeek, Smith and Bardeleben, 2007), Iberian and North African wall lizards (Pinho, Harris and Ferrand, 2008), Sceloporus spiny lizards (Rosenblum, Hickerson and Moritz, 2007; Leaché, 2011; Leaché et al., 2013a,b; Grummer et al., 2015), whiptail lizards (Oliveira et al., 2015), rattlesnakes (Schield et al., 2015), Pantherophis ratsnakes (Gibbs et al., 2006), and Thamnophis gartersnakes (Fitzpatrick et al., 2008b; Playck et al., 2012). A review of divergence with gene flow in amphibians can be found in Nadachowska (2010). Only a few studies have begun examining speciation with gene flow from a genomic perspective in amphibians and reptiles (e.g., Leaché et al., 2013a; Streicher et al., 2014; Schield et al., 2015).

IId Determinism and convergence

Repeated evolution of similar adaptations to similar environments have been identified in several groups of amphibians and reptiles. For example, snake-like forms and lizard-like forms evolved convergently within squamates (Wiens, Brandley and Reeder, 2006). Frogs from different clades converged into a limited number of eco-phenotypes (Moen, Morlon and Wiens, 2015), and so did Cryptoblepharus lizards in Australia (Blom, Horner and Moritz, 2016). Pythons and Boas have convergently evolved similar head shapes related to their ecological niche (Esquerré and Keogh, 2016). Another classic example of convergent evolution are the circa 140 species of Caribbean Anolis lizards, due to their sequential, repeated evolution of similar sets of ecomorphs on different islands (Losos et al. 1998; Mahler et al. 2010). These ecomorph categories include specialist species of similar microhabitat, morphology, and behaviour, and include the “crown-giant”, “trunk-crown”, “trunk”, “trunk-ground”, “grass-bush”, and “twig” types. They evolved convergently on the four largest islands of the Greater Antilles (Cuba, Hispaniola, Puerto Rico, and Jamaica; Losos et al., 1998). A likely explanation for this in situ diversification of similar ecomorphs on different islands is ecological speciation (Losos et al., 1998; Mahler et al., 2010). Ecological selection can result from extrinsic factors (environmental differences, interspecific interactions) or intrinsic factors (sexual selection, within-species competition, cf. Rundle and Nosil, 2005). However, this adaptive radiation also encompasses many younger speciation events that are not as well studied. In fact, most Caribbean anole species evolved in situ within the Greater Antillean islands (as evident from the high number of terminal taxa per island-branch and ecomorph in published phylogenies, e.g., Mahler et al., 2010). Some studies on Anolis in-situ diversification performed to date confirm Ernest Williams’ (1972) original hypothesis that diversification within this younger portion of the radiation might be explained by environmental differences as an extrinsic factor (e.g., Anolis cybotes, Glor et al., 2003), but also indicate a role of sexual selection as an intrinsic factor to cause spatial divergence among populations (e.g., in Anolis distichus, Ng et al., 2013). Different climatic regions on Caribbean islands are found to harbour different species of anoles belonging to the same clade and ecomorph category, and additional morphological variation with macrohabitat differences is found within ecomorph categories (Anolis cybotes, Glor et al., 2003; Anolis roquet, Thorpe et al., 2008, 2010). The evidence for the speciation process continuing
beyond the emergence of ecomorphs makes Caribbean Anolis a good model system to investigate the mechanisms and degree of contingency vs. determinism in current population divergence. Contingency describes how evolutionary trajectories are influenced by chance events, and thus proceed opportunistically (Darwin, 1875; Gould, 1989), while determinism assumes that evolution occurs along more or less predictable trajectories (Osborn, 1929; Schluter, 1996; Schluter and McPhail 1993; Danley and Kocher 2001; Conway Morris, 2003). Comparing the outcomes of speciation across different taxonomic and temporal scales in Anolis might provide insights on the prevailing mechanism of divergence (Losos, 2010). Widespread populations of the Hispaniolan trunk-ground anole A. cybotes show a strong genetic population structure (Glor et al., 2003), but also evidence of gene flow (KJV, unpublished data). Morphological diversification among A. cybotes populations occur partly along the same axes that account for phenotypic diversification at a more basal taxonomic level of the anole radiation, which is evidence for determinism in evolutionary trajectories. Furthermore, morphological characters converge to similar phenotypes at high elevations. Across the montane areas of the Sierra Baoruco, the Cordillera Central, and the Sierra de Neyba, A. cybotes populations have shorter limbs, wider skulls, higher body mass, and occupy lower perches than in the lowlands, which indicates spatial convergence (Wollenberg et al., 2013). Phenotypic divergence of A. cybotes populations according to spatial differences in environmental variables is an axis of divergence similar to that driving in situ diversification of the more basal Hispaniolan trunk-ground anoles (the clade containing A. cybotes and other species). Divergence in the occupation of structural microhabitat in A. cybotes mirrored diversification on the more basal level of Anolis ecomorphs characterized by divergence into different microhabitats. Overall, these results demonstrate a strong signature of deterministic evolution across this investigated portion of the Anolis radiation (Wollenberg et al., 2013). However, another part of the overall morphological variance among A. cybotes populations was determined by a set of characters that were not observed previously to vary with divergence in anoles, which can be interpreted as contingency. Future endeavours to identify the level of determinism vs. contingency in this or other model systems need to be based on spatially exhaustive population datasets. These need to cover the total extent of environmental, phenotypic, and ecological variance present within the species in question.

IIe Body size in mantellid and other frogs: traits and speciation rates

Even the most superficial look at the Tree of Life immediately reveals the enormous differences in diversification rates among clades (e.g. Ricklefs, Losos and Townsend, 2007; Wiens, 2015; Hedges et al., 2015). Some taxa such as the recent coelacanths, the tuatara, the platypus or the pignose frog (Nasikabatrachus) are the lone representatives of ancient lineages and are sometimes referred to as living fossils. On the other hand, other clades of comparable age may contain thousands of species. Diversification rates are composed of speciation and extinction rates, and it is usually not easy to disentangle these two factors. Species-poor extant clades might have been much more diverse in the past, and suffered from high extinction rates. Differences in species richness among clades can also be shaped by differences in clade ages and by purely stochastic factors. However, it seems likely that some lineages are more prone to speciation than others. Will it be possible to disentangle the reasons underlying these differences in speciation rates, and will there be a common pattern at all? Butlin et al. (2012) flag this as one important unsolved question in speciation research. The most frequent approach to the study of speciation or diversification rates and their possible determinants (which might for example be key innovations, which are intrinsic factors, or alternatively extrinsic factors) is to use phylogenies and comparative methods, but these methods require refinement to be able to distinguish between the effects of speciation and extinction (Mooers and Heard, 1997; Butlin et al., 2012; Fitzjohn, 2012).
The endemic Malagasy frog radiations provide a good amphibian example for an adaptive radiation. They have been extensively studied for their phylogenetic relationships (e.g., Wollenberg et al., 2007, 2008; Vieuxtes et al., 2009; Wollenberg et al., 2011) and biogeographic histories, while little is known about their ecologies (except for general ecological modes like habitat and breeding biology; Glaw and Vences, 2007). These frogs share Madagascar with other endemic clades (e.g., lemurs, tenrecs, vanga birds), and the island is subdivided into several regions of biological turnover (another term for beta diversity, Allnutt et al., 2008; Brown et al., 2014, 2016), thus constituting a good model region to infer the processes causing species diversity, species richness and endemism (Vences et al., 2009; Brown et al., 2014).

Most research in Madagascan frogs has been conducted on extrinsic factors, owing to the collection of large data sets on genetics and species distributions. The general finding for the entire radiation of Madagascan mantellid frogs was that many sister species occurred in close spatial proximity to each other (Wollenberg et al., 2011, PWV Fig. 1). Based on these studies, allopatric speciation across large distances can be rejected, as well as peripatric speciation where distribution area sizes are hypothesized to be dissimilar. Wollenberg et al. (2011) also found a trend for clades of smaller species having higher species diversity, as well as smaller mean range sizes, and higher mitochondrial substitution rates. This hypothesis needs further testing in other radiations that contain a broader range of body sizes, because most mantellid frogs are relatively small. But we can conclude that these results are consistent with other recent studies showing a connection between body size and lineage diversification (van Boxlaer et al., 2010; Zimkus et al., 2012).

In contrast, small body size can alternatively be proposed to limit the number of dispersal events leading ultimately to speciation, so that maybe a putative “optimally speciating phenotype” may be of intermediate size (see below). A complication to infer such links between phenotype and speciation events in many mantellid species is their relative old ages. In order to truly link pattern to process, studies using phylogenetic comparative methods need to be integrated with studies at the level of populations.

Speciation is ultimately a consequence of processes occurring at the population level (Turelli, Barton and Coyne, 2001; de Queiroz, 2007). In the following paragraph, we use the term to refer to (i) species formation and (ii) the origin of lineages within species which may or may not complete the speciation process. Because similar processes may drive patterns of biodiversity both within and among species (Velland, 2005; Velland and Geber, 2005), a straightforward approach is to test whether factors affecting clade diversification might also affect genetic variability at the intraspecific level. Pabijan, Wollenberg and Vences (2012) evaluated the contributions of five variables thought to influence speciation in frogs (body size, range size, reproductive mode, adult microhabitat and skin texture) on mitochondrial sequence variation in 40 species of rainforest frogs (Mantellidae) from Madagascar. Contrary to expectations, four out of five variables (range size, adult microhabitat preference, skin texture and reproductive mode) showed no relationship to (i) regional differentiation or (ii) levels of genetic variation within populations of mantellid frogs (Fig. 3). However, body size was inversely correlated with nucleotide divergence between populations and was coupled with high $F_{ST}$ values and an absence of haplotype sharing in small-bodied and medium-sized frogs. This implies substantial population subdivision and low levels of gene flow in small-bodied mantellids and is corroborated by a lack of haplotype sharing in nuclear genes at least in some species (Vences et al., 2010). On the other hand, most of the large species exhibited low genetic differentiation among populations and evidence of haplotype sharing. Pabijan, Wollenberg and Vences (2012) suggested that low dispersal ability most likely caused higher population differentiation in small-bodied mantellids, although it is conceivable that other mechanisms may have also contributed (e.g. shorter generation times in small frogs or size-dependent
Figure 3. The importance of body size on amphibian diversification, from radiation to population. In Madagascar, the influence of body size on patterns and processes of evolution has been studied on several levels of the radiation including 1) the complete radiation of mantellid frogs. Genera are abbreviated as follows: a, Aglyptodactylus; b, Laliostoma; c, Blommersia; d, Guibemantis; e, Mantella; f, Wakea; g, Spinomantis; h, Boehmantis; i, Gephyromantis; j, Mantidactylus; k, Tsingyamantis; l, Boophis. 2) the community level, comparing communities between sites of high diversity, Andasibe and Ranomafana. 3) A pair of mantellid sister species and 4) populations of one of these species. 1) Mantellid frogs of Madagascar constitute a species-rich amphibian radiation with high diversity of ecology and phenotype (tree). Young pairs of sister species are found in closer spatial proximity than older sister species pairs (top scatterplot), and sister species with different range sizes also differ in their body sizes (bottom scatterplot). 2) Mantellid divergence between sister species of two spatially separated communities is higher for smaller species indicating their more limited ability to disperse. 3) In a pair of ecologically similar mantellid sister species, Gephyromantis enki (smaller) and G. boulengeri (larger), the smaller species shows higher residual genetic variance across the same landscape than the larger species (box plot). Landscape resistance is lower for the larger species (inset maps). 4) Population diversification for the small G. enki is influenced by barriers to dispersal such as the Namorona river (localities on opposite sides of the river are separated by a mutation in cytochrome b). Figure references: Wollenberg et al., 2011; Pabijan et al., 2012; Wollenberg Valero, 2015.
metabolism determining mitochondrial mutation rate). Whatever the mechanism is, the lack of genetic cohesion among populations establishes regional genetic isolation within mantellid species, and thus may have the potential to accelerate rates of allopatric speciation in small frogs relative to large species. Other animals also show signatures of higher diversification in smaller-bodied lineages, but with clearly defined constraint values for very small body sizes (Martin, 2016).

A consequence of higher regional genetic differentiation in small-bodied frogs might include increased speciation rates in clades containing small species. This hypothesis received no support from mantellids, small body size correlates with small range sizes and higher rates of nucleotide substitution, but not increased rates of cladogenesis (Wollenberg et al., 2011). This apparent inconsistency between microevolutionary process and macroevolutionary pattern may stem from the cumulative influence that dispersal has on diversification at short and long temporal scales. In the long-term, “small-bodied low dispersal” species may have fewer opportunities to colonize suitable new habitats (Owens, Bennett and Harvey, 1999; Van Bocxlaer et al., 2010) which in amphibians could further be exacerbated by niche conservatism (e.g. Kozak and Wiens 2006, 2010a). Range expansion in small-bodied species, facilitating allopatric speciation, would therefore be less likely to occur. Moreover, amphibians with geographically limited distributions might have higher extinction rates (Sodhi et al., 2008). Thus, although small body size may potentially accelerate speciation via higher rates of nucleotide substitution and regional differentiation, net diversification may be simultaneously offset by fewer chances of range expansion and higher extinction rates in poor dispersers.

Recent developments in dispersal theory have highlighted that speciation can occur at smaller spatial scales in taxa with low dispersal capacity (Kisel and Barraclough, 2010), whereas high gene flow among populations will usually inhibit speciation (Claramunt et al., 2012). Highest species diversity (and presumably highest speciation rates) may occur in lineages with intermediate dispersal abilities that are sufficient to extend their geographic ranges, yet occur in low enough densities to maintain low levels of gene flow, allowing for population differentiation (Etienne and Olff, 2004; Price and Wagner, 2004; Claramunt et al., 2012; Agnarsson and Kuntner, 2012). Because range size and range filling correlates with body size in amphibians (tested in Madagascar; Brown et al., 2016), it can be hypothesized that intermediate dispersal ability corresponds to intermediate body size in frogs.

Evolutionary trends in body size have been repeatedly hypothesized to influence speciation and diversification in anurans. A decrease in body size has been a hallmark of amphibian evolution since their Devonian origin. An evolutionary reduction in body size has often been accompanied by the truncation of development of some morphological features (progenesis), such as skull elements and reductions in numbers and elements of the digits. Miniaturization in *Batrachoseps* (Plethodontidae) was thought to underlie fractal diversification, i.e. the non-adaptive radiation of morphologically and ecologically similar species through extreme range fragmentation (Wake, 2009). A reduction of body size may have also initiated an ecomorphological radiation in the plethodontid genus *Thorius* (Rovito et al., 2013). However, reduction of body size was not associated with diversification rate in phrynobatrachid frogs (Zimkus et al., 2012). On the other hand, large body size is part of a dispersal-prone phenotype and is linked to diversification in toads (Van Bocxlaer et al., 2010). In general, body size is positively correlated with range size as recently shown in a comprehensive study of Malagasy amphibians and reptiles (Brown et al., 2015), reflecting higher dispersal capacity of large-sized animals, but this association has not yet been analyzed in a large-scale macroecological study in amphibians. Likewise, no large-scale test of habitat associations and body size is available, even though many large-bodied temperate species (anurans and salamanders) seem to be associated with aquatic habitat, many large-bodied tropical species seem to be arboreal, whereas small species from both high and low latitudes seem to be more terrestrial (Wells, 2007).
Besides body size, several other traits may influence speciation and diversification by determining the ability of amphibians to disperse across a landscape. These traits include reproductive mode (direct developers vs. aquatic larvae), and stable vs. unstable breeding habitats with associated variation in clutch size (Inger, Voris and Voris, 1974; Vences et al., 2002a; Dubois, 2005; Chan and Zamudio, 2009; Paz et al., 2015; Rodríguez et al., 2015), although Gómez-Mestre, Pyron and Wiens (2012) found no association between diversification rate and life-history mode across frogs. Direct development, terrestriality, philopatry, territoriality and small clutch size (often tightly associated) are thought to contribute to low dispersal ability (e.g. Dubois, 2005; Cabe et al., 2007; Ryan, Lips and Eichholz, 2008; Fouquet et al., 2012). Aquatic breeders with free-swimming larvae (and usually large clutch sizes) may be more mobile because of periodic migrations of adults to breeding habitats. Mobility may be particularly high in species adapted to dry climates in which breeding habitat is ephemeral and unstable, as suggested by elevated levels of gene flow across landscapes (Chan and Zamudio, 2009; Pabijan et al., 2015). Moreover, dispersal may be facilitated in species that deposit eggs or larvae in rivers, streams or periodically flooded pools, due to occasional long distance transport in lotic waters.

Studies examining the effects of ecology on phylogeographic patterns across multiple species, coined ‘ecophylogeography’ by Paz et al. (2015), may be particularly relevant to identification of intrinsic factors that promote differentiation. Rodríguez et al. (2015) detected a strong association between macrohabitat (forested vs. open areas), elevation, and phylogeographic structure (scaled to geographic distances) in 39 frog species from both the New World (Brazil, Cuba, Central America) and Old World (Madagascar) tropics. Species living in non-forested lowland habitat showed low levels of population structure, whereas populations of rainforest species from mountainous areas were highly differentiated. Differences in dispersal ability were proposed to explain this result, with forest-adapted anurans thought to be less mobile than species dwelling in open areas. One pertinent corollary of these findings is that anurans from topographically complex rainforest areas (e.g. tropical mountains) should exhibit higher speciation rates assuming a predominance of allopatric speciation. This finding is in line with previous suggestions that heterogeneous topographies and mountainous areas may facilitate intraspecific divergence (Guarnizo and Cannatella, 2013) and increase speciation or diversification rates (Wollenberg et al., 2008; Hutter, Guyasamin and Wiens, 2013) in frogs. In a similar-spirited study at a smaller spatial scale in Central American anurans, Paz et al. (2015) identified body size, reproductive mode, landscape resistance, geographic range, and biogeographic origin of the selected lineages as the main predictors of phylogeographic patterns. This study highlighted species-specific life histories that may interact with landscape features and either promote or inhibit speciation, as also suggested in single taxon analyses (Fouquet et al., 2012; Wollenberg Valero, 2015).

Intrinsic factors such as organismal traits that enable colonization of new environments or “ecospace” (cf. Bambach, 1983) are referred to as key innovations and are thought to influence diversification rates. Ecospaces recurrently occupied by amphibian clades are arboreal vs. terrestrial vs. aquatic, and terrestrial (endotrophic) reproduction including viviparity. Which morphological and physiological traits favor these switches is largely unstudied. It is also unclear whether any of these switches are related to speciation rate, which constitutes an interesting avenue for future study. Life history mode was found to be unrelated to diversification rate across frogs (Gomez-Mestre, Pyron and Wiens, 2012), terrestriality did not increase diversification rate in *Phrynobatrachus* — in contrast, the more terrestrial clades showed decreased rates (Zimkus et al., 2012). The presence of aerolate ventral skin has been correlated to increased species richness in South American Terrarana (Gonzalez-Voyer et al., 2011). One interpretation of this latter finding is that more vascularized bellies may have been an adaptation to lower atmospheric oxygen levels, facilitating the colonization of high altitude ranges. In bufonids, a suite of morphological and life history traits has been demonstrated to increase colonization ability and trigger
diversification (Van Bocxlaer et al., 2010). This ‘range expansion phenotype’ includes a terrestrial niche, large body size, the presence of parotid glands and inguinal fat bodies, aquatic oviposition sites, large clutch size and exotrophic larvae (Van Bocxlaer et al., 2010).

Several other intrinsic species traits may influence speciation rates in amphibians, but are unknown in sufficient detail for firm conclusions. For instance, physiological and cellular processes affecting DNA substitution rate may modulate speciation rate in some amphibian lineages. Differences in active metabolic rates scale with substitution rates in mitochondrial and nuclear genes in poison frogs (Santos, 2012), and clade level variation in metabolic rates may also contribute to patterns of substitution in mtDNA in salamanders (Chong and Mueller, 2013a). If nucleotide substitution rates are positively correlated with speciation rates in amphibians, as they are in birds and reptiles (Eo and DeWoody, 2010), then we anticipate that differences in metabolism among clades may also translate to different levels of species formation, although no influence of this trait was found on diversification rates across vertebrates (Wiens, 2015). Other potential but yet little-explored traits that may affect speciation rate include variation in genome size (Chong and Mueller, 2013b) and karyotype instability (Bogart, 1991; Olmo et al., 2005; Schmid et al., 2010). Likewise, the importance of sexual selection in driving amphibian speciation, for example through the evolution of male call divergence and female preference in frogs and how it plays out in different environments (Richards, 2006; Boul et al., 2007; Hoskins et al., 2005, 2011), remains to be analyzed.

II) Intrapopulation color polymorphisms and speciation

The presence of discrete color variants, often called “morphs,” within populations is a prominent feature of many amphibian and reptile species, and may be important for speciation. As such, the topic has attracted the attention of researchers for several decades (reviewed in Hoffman and Blouin, 2000; Hofreiter and Schöneberg, 2010; Olsson, Stuart-Fox and Ballen, 2013). In squamate reptiles, especially lizards, populations of many species include two or more discrete morphs of males, females, or both sexes that differ in secondary sexual coloration. In most cases where the proximate basis of such color variation has been studied, morphs are highly heritable (Sinervo and Zamudio, 2001; Olsson et al., 2007; Huyghe et al., 2010b). In addition to their differences in color, morphs differ in one or more aspects of reproductive behavior in numerous species, including members of the families Phrynosomatidae (Rand, 1988; Thompson and Moore, 1991; Sinervo and Lively, 1996; Bastiaans et al., 2013), Lacertidae (Vercken et al., 2006; Huyghe et al., 2007; Vignoli et al., 2012), and Agamidae (Healey, Uller and Olsson, 2007; Yewers, Pryke and Stuart-Fox, 2016). Males of different color morphs may vary in aggression, dispersal, physiological performance, territoriality, and/or mate choice (Rand, 1988; Sinervo and Lively, 1996; Sinervo et al., 2006; Healey, Uller and Olsson, 2007; Huyghe et al., 2007, 2010a; Vercken and Clobert, 2008a, 2008b; Zajitschek et al., 2012; Pérez i de Lanuza, Font and Carazo, 2013). Female morphs may differ in life history, maternal effects, and/or mate choice (Zamudio and Sinervo, 2000; Sinervo, 2001; Vercken et al., 2006; Bleay and Sinervo, 2007; Lancaster et al., 2007, 2008; Lancaster, Hipsley and Sinervo, 2009; Lancaster, McAdam and Sinervo, 2010; Pellitteri Rosa, 2012; Pérez i de Lanuza, Font, and Carazo, 2013; Lattanzio, Metro and Miles, 2014). Within populations, heritable color and behavioral morphs may be maintained by negative frequency-dependent selection, temporally or spatially variable selection, overdominance, or gene flow between populations differing in coloration (Gray and McKinnon, 2007; Wellenreuther, Svensson and Hansson, 2014).

Across lizards, similar colors occur in many species in which color polymorphisms are linked to variation in behavior, but the same colors are not always associated with the same behaviors. For example, female color morphs in *Uta stansburiana* and *Podarcis muralis* differ in life history strategies,
with females of one morph reproducing as r-strategists (many, small eggs) and females of another morph reproducing as K-strategists (fewer, larger eggs) However, in *U. stansburiana*, yellow females are K-strategists while orange females are r-strategists (Sinervo, 2001); whereas in *P. muralis*, yellow females are r-strategists, white females are K-strategists, and red females display a mixed strategy in which they are K-strategists when young and r-strategists when older (Pellitteri Rosa, 2012). Male lizards with orange or reddish color patches are more aggressive than males of other color morphs in *Uta stansburiana* (Sinervo and Lively, 1996), *Sceloporus consobrinus* (Rand, 1988), and *Ctenophorus pictus* (Healey, Uller and Olsson, 2007), but they are less aggressive than males of other morphs in at least one population of *Urosaurus ornatus* (Hover, 1985; Thompson, Moore and Moore, 1993; Carpenter, 1995) and one population of *Sceloporus grammicus* (Bastiaans et al., 2013). *Podarcis muralis* males exhibit the same three morphs as the females described above, but these morphs do not appear to differ in aggression (Sacchi et al., 2009). The association between a specific color and a suite of behaviors can even vary between closely related populations of the same species. In *Urosaurus ornatus*, orange males were less aggressive than males of other morphs in a population with blue, blue-green, green, orange-green, and orange morphs; but orange males were more aggressive than orange-green morphs in a population where only those two morphs occurred (Carpenter, 1995). In *Sceloporus grammicus*, some populations exhibit an orange/yellow/blue polymorphism in males, whereas other populations exhibit an orange/yellow/white polymorphism. In one population with an orange/yellow/blue polymorphism, orange coloration was associated with decreased aggression and blue coloration with increased aggression; in a closely-related population with an orange/yellow/white polymorphism, yellow coloration was associated with increased aggression and white coloration with decreased aggression (Bastiaans et al., 2013). In most cases, it is not clear why a particular color is or is not associated with a particular set of behaviors. Further studies applying techniques from sensory ecology would help reveal the factors influencing these relationships.

The previous two examples illustrate how color and behavioral polymorphism within populations of squamates connects to variation at the interpopulation level. In numerous color-polymorphic taxa, closely related species (Runemark et al., 2010; Feldman, Flores-Villela and Papenfuss, 2011) or populations of the same species (Hews et al., 1997; Sacchi et al., 2007; Corl et al., 2010) vary in the number or frequency of morphs present (McLean and Stuart-Fox, 2014). Although the causes of this variation are not clear for most systems studied, recent work indicates that environmental variables explain more variation in morph frequencies than the geographic or genetic distances among populations (Cox and Chippindale, 2014; Lattanzio and Miles, 2014; McLean, Stuart-Fox and Moussalli, 2015). These findings suggest that morph frequencies in many populations respond rapidly to local selection, regardless of the underlying phylogeographic structure of the species.

Both theoretical (West-Eberhard, 1983, 1986; Ritchie, 2007; Forsman et al., 2008) and empirical (Gray and McKinnon, 2007; Corl et al., 2010; Hugall and Stuart-Fox, 2012) studies support the idea that taxa in which color polymorphisms or alternative reproductive strategies are common may exhibit higher rates of speciation than taxa in which most populations are monomorphic for these traits. Variation among populations in the number or frequency of morphs present may occur due to selection (as described above) or due to stochastic processes such as genetic drift or founder effects (Alonzo and Sinervo, 2001; Runemark et al., 2010; McLean, Stuart-Fox and Moussalli, 2014). The presence of different morphs in closely related populations may contribute to prezygot (Bastiaans et al., 2014) or post-zygotic reproductive isolation (Corl, Lancaster and Sinervo, 2012) between those populations. Further species-wide studies comparing rates of gene flow between populations differing in morph frequencies would be helpful in empirically evaluating the effect of polymorphism on reproductive isolation between populations. One recent example of such work found that in the lizard *Ctenophorus*
decresii, only limited gene flow occurred after secondary contact between polymorphic and monomorphic lineages (McLean, Stuart-Fox and Moussalli, 2014).

Polyomorphic lineages may also be more diverse than monomorphic lineages if they are less vulnerable to extinction and thus tend to be older. There is empirical evidence that polymorphic clades tend to be older relative to monomorphic clades in snakes (Pizzatto and Dubey, 2012). The presence of multiple morphs may allow populations to occupy more than one ecological niche and/or maintain higher levels of genetic diversity than are present in monomorphic populations (West-Eberhard, 1986; Forsman et al., 2008; Hugall and Stuart-Fox, 2012). However, Bolton, Rollins and Griffith (2015) suggest that some features of color polymorphic populations may make them more vulnerable to extinction than monomorphic populations. Alternative behavioral tactics linked to color morphs constitute suites of co-adapted traits, which may be correlated through pleiotropy, physical linkage, or correlational selection (Wellenreuther, Svensson and Hansson, 2014). When behavioral traits that differ among color morphs are influenced by independent, physically unlinked loci, the action of correlational selection can generate a substantial recombination load on the population, in which individuals with maladaptive combinations of color and behavioral loci are born but fail to survive or reproduce (Sinervo and Svensson, 2002). If mechanisms evolve that maintain trait correlations despite recombination, through changes to regulatory regions or the accumulation of related loci on the same chromosome, this reduced behavioral plasticity may constrain the evolutionary response of polymorphic populations to changing environmental conditions (Bolton, Rollins and Griffith, 2015). In addition, populations in which aggressive interactions occur between individuals of different morphs (Thompson and Moore, 1991; Sinervo and Lively, 1996; Healey, Uller and Olsson, 2007; Bastiaans et al., 2013), or in which morphs play an important role in mate choice (Lancaster et al., 2014; Huyghe et al., 2010a; Lattanzio, Metro and Miles, 2014), may be less able to increase rapidly in size than populations in which fewer aggressive interactions occur or in which mate choice is more flexible (Bolton, Rollins and Griffith, 2015). The relationship between color polymorphism and population persistence, therefore, would benefit from further empirical and modeling studies.

IIf Ecology, genes and behavior – an integrative perspective of ecological speciation in fire salamanders

The adaptation of individuals to new or differing environmental conditions can cause the adaptive divergence of populations leading to speciation, if natural selection strongly favours different ecotypes and reproductive isolation evolves as a consequence of such a differential habitat use (Schluter, 2001; Dieckman et al., 2004; Hendry, 2009; Nosil, 2012). Ecological or adaptive speciation has been identified as a major biological process that has shaped species diversity in quite distinct taxa, ranging from Darwin’s finches, Anolis lizards, sticklebacks to pea aphids and Rhagoletis flies (see Hendry, 2009), and is considered the major process underlying adaptive radiations. During the adaptive radiation of Darwin’s finches across the Galápagos archipelago, for example, 14 distinct species and subspecies have formed starting from a single colonization event from the South American mainland roughly 1.6 million years ago (Grant and Grant, 2014). However, the process of ecological speciation can also occur within shorter time frames. In three-spine sticklebacks repeated and parallel divergence (speciation) of limnetic and benthic forms occurred in small lakes in southern British Columbia following the last glaciation only a few thousand years ago (McKinnon et al., 2004; Vines and Schluter, 2006).

Although many studies showing population subdivision and genetic divergence linked to habitat adaptation are not characterized by complete reproductive isolation, they may mark different stages of adaptive divergence along a continuum reaching from pure adaptive-ecological variation without
reproductive isolation, to ecological-adaptive differences associated with irreversible reproductive isolation (as depicted as a fourth stage continuum by Fig. 4 in Hendry, 2009). It is important to stress that many of the natural systems studied so far might never reach the final stage of complete speciation, such as the outstanding case studies of Darwin’s finches, *Anolis* lizards, etc. mentioned above. Nevertheless, they still represent exciting study systems that may show how ecological adaptation can cause genomic divergence via selection (see Nosil, Funk and Ortiz-Barrientos, 2009) and potentially impact population structure.

In a strict sense, processes and mechanisms of ecological speciation can be best studied in situations where the direct impact of ecological adaptation is measurable with genetic markers, i.e. correlated with genetic differentiation. Furthermore, it is also useful to find situations in which spatial impacts, such as geographic isolation, can be ruled out or mainly neglected as factors causing genetic differentiation. Therefore, individuals or populations under investigation should be in sympatric or parapatric contact. In this context, the study of *Anolis* lizards along environmental gradients separating historical population effects from ecological effects nicely demonstrated that with a rather simple and robust study design, the strong influence of habitat adaptation on genetic population structure can be detected (Ogden and Thorpe, 2002; Thorpe et al., 2008; Thorpe et al., 2010).

To study processes and mechanisms of ecological speciation, one must unravel the phylogeographic history of populations under investigation. Research on ecological speciation in the European fire salamander (*Salamandra salamandra*) by S.S.’s group started initially with studies on phylogeographic patterns in *Salamandra* across Europe, the Near East and North Africa (Steinfartz, Veith and Tautz, 2000). Phylogeographic patterns observed between distinct fire salamander species were found to be quite different. Whereas Near Eastern fire salamanders (*S. infraimmaculata*) showed strong and deep patterns of population structure for the mitochondrial D-loop, differentiation between populations of *S. salamandra* across Europe displayed more shallow differentiation for the same mitochondrial marker. Haplotypes of *S. salamandra* could be arranged into distinct clades occupying separate geographic ranges. Whereas members of the so-called B-clade displayed a disjunct geographic distribution occurring only in northeastern Spain (mainly the range of *S. s. bernardezi*) and southern Italy (region of Calabria), members of the C-clade are distributed continuously across major parts of Europe, except southern Spain (range of *S. s. longirostris*). The genetic distinctness of the B- and C-clades has been now also verified based on both mitochondrial and nuclear genes (Vences et al., 2014). These results suggest that major parts of Central Europe (including all of Germany) have been colonized following the last glaciation by the C-clade. Given that this colonization followed the recolonization by native beech trees (*Fagus sp.*), which make up the natural forest habitats of *S. salamandra* (Eiselt, 1958) roughly 8000–9000 years ago, salamander populations must have become re-established quite recently in Middle Europe. This system therefore offers an ideal phylogeographic framework to study also habitat adaptation and its consequences on population structure. In principle, this system might be best comparable to the three-spine stickleback system in Canada (see above).

*Salamandra salamandra* in Central Europe typically deposit larvae in small permanent streams, in which they undergo development until metamorphosis is completed (Thiesmeier and Grossenbacher, 2004). We have identified a region (called the Ville) composed of old broad-leaf deciduous forests spanning from near Cologne to Bonn where salamanders also use ephemeral habitats (e.g. small ponds, tire ruts, ditches) as larval deposition sites. Because the risk of desiccation is high and food supply is relatively low compared to streams, larvae developing in ephemeral habitats display several habitat-specific adaptations that are absent in stream larvae. These include a greater larval weight at birth, the ability to thrive on lower quality food sources and early metamorphosis to escape unfavorable and non-predictable conditions (Weitere et al., 2004; Reinhardt et al., 2013; Reinhardt, 2014; Reinhardt,
Steinfartz and Weitere, 2015). By performing a detailed phylogeographic analysis of mt D-loop haplotypes across Germany (Weitere et al. 2004), we could show that the Ville has been colonized by the western lineage of *S. salamandra* following the last glaciation. As stream-reproduction is the ancestral state, it can be hypothesized that pond-reproduction evolved locally after recolonization, no more than 8000–9000 years ago (Weitere et al., 2004; Steinfartz, Weitere and Tautz, 2007).

In the Kottenforst, which represents an uplifted forest plateau in the Ville, a comprehensive population genetic study based on microsatellite loci (Steinfartz, Küsters and Tautz, 2004), further showed that genetic differentiation underlies habitat-adaptation, suggesting that salamanders in the Kottenforst are diverging in life-history traits and thus may represent a system of adaptively diverging populations possibly at an early stage of ecological speciation (Steinfartz, Weitere and Tautz, 2007). This genetic differentiation might have been established under possible contact situations (i.e. in sympatry or parapatry) between stream and pond-adapted salamander types, as dispersal rates have shown to be unexpectedly high in populations within the same range (Schmidt, Schaub and Steinfartz, 2007; Schulte, Küsters and Steinfartz, 2007). If stream and pond adapted individuals constantly meet but habitat-based genetic differentiation remains, one might assume that mating is non-random and habitat-specific. Preference tests, showing that females can recognize sex-specific signals via odor cues (Caspers and Steinfartz, 2011), indicate that odor cues can be in general perceived by females. Assuming that the type of habitat adaptation (i.e. stream versus pond-adaptation) of an individual might be also signaled through odor cues, females might be potentially able to discriminate between differentially adapted males for mating. Support for non-random mating of female salamanders in the Kottenforst comes from paternity analysis of mother-larvae arrays collected under fully natural conditions. In nature, multiple paternity in *S. salamandra* is frequent (Steinfartz et al., 2006) and for the Kottenforst population it could be shown that females can gain a fitness advantage in terms of number of offspring by mating with multiple males; interestingly, females seemed to prefer genetically similar males with respect to their own genotypes (Caspers et al., 2014).

Other life history traits, such as larval deposition behavior and maternal investment also reflect differential habitat adaptation in salamanders (Caspers, Steinfartz and Krause, 2015). Pond-type females extend larval deposition over an increased time period and tend to deposit eggs more frequently compared with stream-type females. Over successive deposition events, the body condition of larvae deposited by stream-type females decreased faster than that of larvae deposited by pond-type females. These differences in larval deposition behaviour may represent a bet-hedging strategy, given that ponds are more constrained than streams with respect to likelihood of serious evaporative water loss, as well as food availability. The prolonged deposition period might allow pond-type females to deposit larger larvae towards the end of the deposition period (Caspers, Steinfartz and Krause, 2015).

The fire salamander represents a fascinating system for the study of ecological adaptation as well as population genetics and courtship/egg deposition behaviour in an adaptively diverging population, possibly marking the first step to speciation in this system. In this era of genomics we are now in position to identify genes underlying habitat selection, mate choice, and speciation potential. With the recent development of a *Salamandra salamandra*-specific microarray chip representing the transcriptome of *S. salamandra* (Czypionka et al., 2015), differential gene expression between salamander larvae from the stream and pond habitats will elucidate genes and their functions underlying differential habitat adaptation and potentially also speciation.

**Conclusions**
Various processes and mechanisms of speciation are interconnected and are responsible for the patterns of genetic and morphological diversity that can be observed at different population and phylogenetic levels in nature. The many examples above show the amazing diversity of patterns that can be generated by evolutionary processes, and part of the wide range of processes that can give rise to these patterns. It is only through integrative study of both processes and patterns that we can gain deeper insights into the multiple processes of speciation. This review provides the readers with a quick reference to some of the best-studied systems and well-developed research programs focused on amphibian and reptilian speciation. This obviously is not an exhaustive account as many other excellent systems and research programs have produced impressive results. Our hope is that this review will act as a catalyst for the replication and further extension of such studies, and that this will result in a more profound understanding of speciation across a broader range of taxa.

Acknowledgments
We thank the organizers of the 7th World Congress of Herpetology for providing a venue and symposium for us to come together and share ideas and our research. KWV was funded via National Science Foundation grant HBCU-UP 1435186 and Volkswagen Foundation fellowship 287 (AZ86/447). We would also like to thank John J. Wiens for his contributions on the section ‘Speciation and the niche’.

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