

Comparative Phylogeography: Designing Studies while Surviving the Process

TANIA A. GUTIÉRREZ-GARCÍA AND ELLA VÁZQUEZ-DOMÍNGUEZ

Comparative phylogeography (CP) can be defined as the study of the effects of evolutionary history and biogeography on the distribution of genetic variation of codistributed species. CP studies have intensified in recent years, which is a natural progression from an extensive history of intraspecific phylogeography research. On the basis of a thorough review of published studies that specifically deal with CP, our objective in the present review is to provide a comprehensive guide to the discipline that will help those wishing to develop a CP project. We describe the characteristics that shape a CP study and summarize the field's prime theoretical, methodological, and analytical requirements; frequent hypotheses tested; and current achievements and limitations, including a variety of illustrative examples throughout. We finally highlight some new approaches in CP and briefly discuss future directions for the field.

Keywords: biogeography, evolution, geological history, phylogeny

Over 20 years ago, John Avise coined the term *phylogeography* (Avise et al. 1987), a discipline in which the geographical distribution of the genetic variation of natural populations is studied in a historical context. As such, intraspecific phylogeography helps to decipher spatial and temporal patterns of population structure (i.e., genetic differences within and among populations) and to explain the ecological and evolutionary processes responsible for those patterns (Avise 2000). An astounding number of intraspecific phylogeographic studies have been published since the field's birth (see Knowles 2009), information that allowed the identification of species codistributed in space and time that showed similar or contrasting phylogeographical patterns in gene genealogies (see box 1 for a glossary of terms). This awareness motivated the design of studies to test whether taxa with overlapping distributions share a common history and laid the foundation for what later came to be known as *comparative phylogeography* (CP). It was Bermingham and Avise (1984) who established the theoretical grounds for this new framework in the study of historical distributions of species (Avise 2000). In earlier CP studies, whether a similar evolutionary history would produce shared intraspecific phylogeographical patterns among codistributed species was explored. CP was therefore defined as the study of the effects of evolutionary history and biogeography on the distribution of genetic variation in codistributed species.

Bermingham and Moritz (1998) briefly described the concepts and application of CP and explained how CP

analyses could contribute to broader studies of ecology and evolution. The latter was possible, they reasoned, because congruence among the evolutionary, demographic, and distributional histories of taxa could be described with the use of a CP approach and could, in turn, be explained by the ecological and geological landscape. They also indicated some of CP's early pitfalls (e.g., the use of a single gene system, mitochondrial DNA) while emphasizing some of the improvements needed: (a) to use unlinked molecular markers and to develop new methodologies for testing evolutionary congruence, (b) to continue the development of coalescence theory and to increase the statistical rigor of phylogeographic analyses, and (c) to increase the precision of the time estimation of cladogenesis (Bermingham and Moritz 1998).

Briefly described, the goal of most CP studies has been the search for general patterns in the effect of environmental (historical) changes on several species in a particular space and time. Despite consensus about this objective, several gaps and questions remain: Is there a common methodology for CP? What are the limitations when trying to decipher intraspecific and interspecific patterns? What are the requirements for selecting species, genetic markers, and sampling schemes? In the present review, we have prepared a comprehensive overview of CP to serve as a resource for the design and assessment of CP studies. Accordingly, we first describe the characteristics that shape a CP study, then summarize CP's prime theoretical, methodological, and

Box 1. Glossary.

Allopatric. *Allopatric distribution* refers to species that occur in geographically separated areas; *allopatric fragmentation* occurs when a geographical (e.g., river, mountain) or anthropogenic (e.g., road) barrier separates an originally continuous distribution.

Allozymes. Multiple variants of the same enzyme produced by different alleles.

Cladogenesis. Evolutionary change and diversification resulting from the branching off of new taxa from common ancestral lineages.

Cryptic species. Genetically distinct evolutionary lineages (i.e., individuals along significantly divergent branches of a phylogenetic tree) that are morphologically indistinguishable.

Ecological plasticity. Degree to which an organism can tolerate deviation from its optimal environmental conditions. A term related to *phenotypic plasticity*, which is the potential for a single genotype to develop into multiple alternative phenotypes under different environmental conditions.

Evolutionary significant unit (ESU). A population (or populations) of a species that is genetically distinct from other populations; in phylogeography, an evolutionarily distinct phylogroup can be considered an ESU. ESUs have many applications in conservation.

Gene genealogies. The pattern of similarities between DNA sequences contains information about their evolutionary history. Therefore, the ancestry of a sample of homologous (of the same gene) DNA sequences from a population back to their most recent common ancestor is the gene genealogy of the sample.

Gene tree or species tree. The relationship between species is usually represented as a bifurcating tree with the branching points representing speciation events (*species tree*). The ancestry of genes taken from these species can also be represented as a tree, with the branching points representing ancestral genes (*gene tree*).

Genetic divergence. A process in which genetic changes accumulate over time in an ancestor by the action of different evolutionary processes that cause measurable differences between individuals, populations, or species. Measured by the extent to which two sequences differ from one another.

Haplotype. Refers to half of a genotype. A combination of alleles (for different genes) that are located closely together on the same chromosome and that tend to be inherited together; for example, mitochondrial DNA in most animals is maternally inherited. Therefore, unique haplotypes (DNA sequences) are transmitted from mothers to their offspring. Haplotypes can be detected with molecular techniques and analyzed via gene trees.

Host–parasite. Of or relating to a biological interaction or relationship in which an organism obtains its nutrients from one or very few host individuals, normally causing harm but not causing death, at least not immediately.

Lineage. Descent of an organism; one or a series of populations (*demes*) that share a common history of descent not shared by other populations. A genetic lineage is a series of mutations that connect an ancestral genetic type (i.e., allele, haplotype) to its descendants.

Molecular marker. Fragment of DNA used to generate data from one or more genomes (e.g., mitochondrial and chloroplast DNA). Depending on its location on the genome and mechanisms of inheritance, a molecular marker can be unlinked (inherited independently) or linked (inherited together with other genes).

Molecular clock. On the basis of the idea that DNA sequences evolve at roughly constant rates, the dissimilarity of two sequences can be used to calculate the amount of time that has passed since they diverged. Molecular clocks are incorporated into phylogenetic analyses and calibrated with fossil record data or with rates of molecular change.

Mutualism. Biological interaction between pairs of species that brings mutual benefit; the individuals in a population of each mutualist species grow, survive, or reproduce at a higher rate when in the presence of individuals of the other species.

Taxa (singular: *taxon*). A taxonomic unit, whether named or not (i.e., a population or group of populations of organisms) whose members are phylogenetically related. These units have common characteristics that differentiate the unit (e.g., a geographic population, a species, a genus, a family, an order) from other units.

Sister species. The most closely phylogenetically related species (or taxa) to a given species (or taxon).

Stochastic variance. The coalescent or stochastic variance is the variability in gene divergence time that arises in the evolutionary and demographic history of populations as a natural consequence of genetic drift. As a result of this stochastic genealogical component of divergence, information about the historical demography of the species of study and that of its ancestor need be considered when analyzing and modeling phylogeographic patterns.

Symbiont. Close and often long-term interactions between different species that include mutualistic, commensal, or parasitic relationships and that can be obligate (i.e., both symbionts entirely depend on each other for survival) or facultative (i.e., they can but do not have to live with the other organism).

Sympatric. Overlapping, as in *sympatric distribution*.

Vicariance. Biogeographic process of speciation in which the range of distribution of a species is severed by a geographical barrier, causing the posterior isolation of populations (disjunct populations).

analytical requirements; frequent hypotheses tested; and current achievements and limitations. We end by highlighting some new approaches in CP and by briefly discussing future directions for the field. Illustrative examples are included throughout for clarification and to facilitate understanding.

What is a CP study about?

A CP study consists of a phylogeographic analysis of two or more species or taxa and a comparison of their respective phylogeographic patterns (Avice 2008). As such, CP is not an independent field, but an approach integrating both ecology and evolution. In general, a CP study has two phases (Bermingham and Martin 1998, Victoriano et al. 2008): It begins with a descriptive survey that includes the collection of genetic data from the species (e.g., sequences, multilocus genotypes) and phylogenetic analyses of that data. The intraspecific data obtained at this phase provide insight into phylogenetic relationships of populations, genetic diversity and genetic distance between lineages, effective population size, gene flow, and the timing of diversification events. The second phase is the comparative component and includes diverse analyses to test for congruence between the evolutionary and distributional histories of each species; it also includes evaluation of the geographical, ecological, and biological hypotheses that could explain those histories. The aim at this phase is to evaluate whether the evolutionary histories of these species show a shared response to the same historical events. In figure 1, we have summarized the

information commonly used for developing a CP study following this two-phase model.

Main factors to consider when designing a CP study

Below we outline the major factors that must be considered during the design phase of a CP study.

Choosing the species of study. By evaluating two or more species that share part of or all of their geographical range, one can detect not only dispersal and vicariance events (as in traditional biogeographical approaches) but also hybridization, secondary contact, suture zones, and population and demographic dynamics, to name a few patterns. Most phylogeographic studies are designed on the basis of some initial abiotic and biotic information (figure 1), which is essential in choosing the species of study as well as the geographical scale and method of analysis. Investigating the biology and ecology of taxa is an important first step, because many species-specific characteristics, such as environmental requirements, life history characteristics, dispersal abilities, and ecological associations, can have an effect on the species' response to biogeographical processes and ultimately on patterns of genetic diversity (Ditchfield 2000). Accordingly, species-specific features can be criteria for selecting species to include in a CP study (figure 1). The species' relationship (i.e., sister species or distantly related species) and the distribution of gene genealogies (i.e., concordant or discordant), if they are known a priori, can also be considered in the selection of species for a CP study. Some

general phylogeographical outcomes can be expected on the basis of this information, as is shown in figure 2.

For instance, by studying sympatric sister species or codistributed taxa with comparable ecological preferences and dispersal abilities, one can evaluate whether their phylogeographic structures result from recently derived differences or from more ancient historical processes (figure 2). Species in archipelagos are a good example of the latter, particularly if they represent independent dispersal or colonization events—that is, if they arrived on the islands at different times or from different places. Such species are often exposed to similar environments among the islands and may consequently have similar evolutionary patterns. Kirchman and Franklin (2007) evaluated such a scenario: They studied three bird species

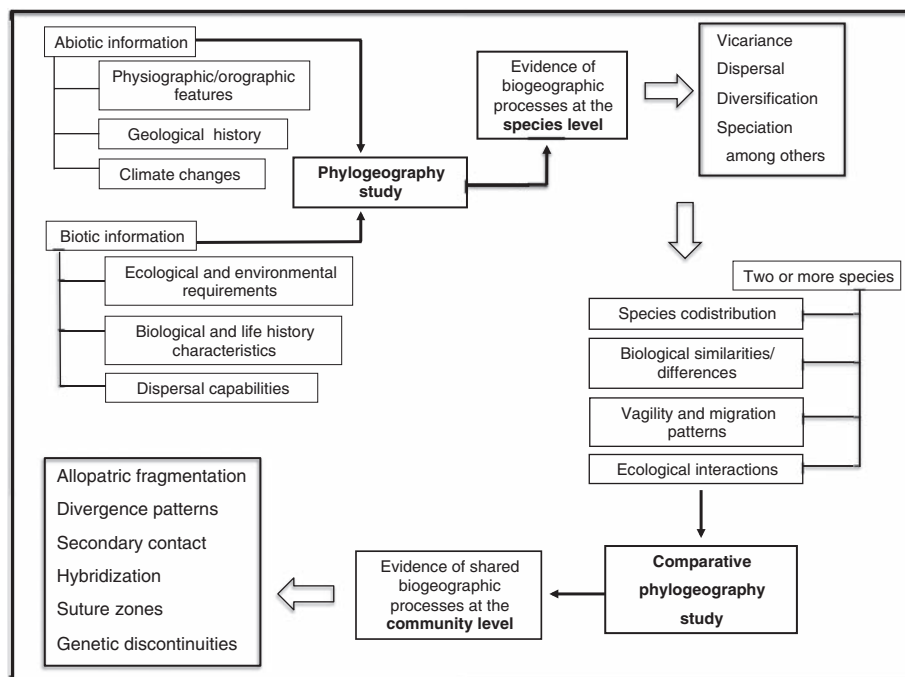


Figure 1. Summary of the information commonly used to perform a comparative phylogeography study, based on the different studies examined for the present review (see also supplemental table S1, available online at <http://dx.doi.org/10.1525/bio.2011.61.11.5>).

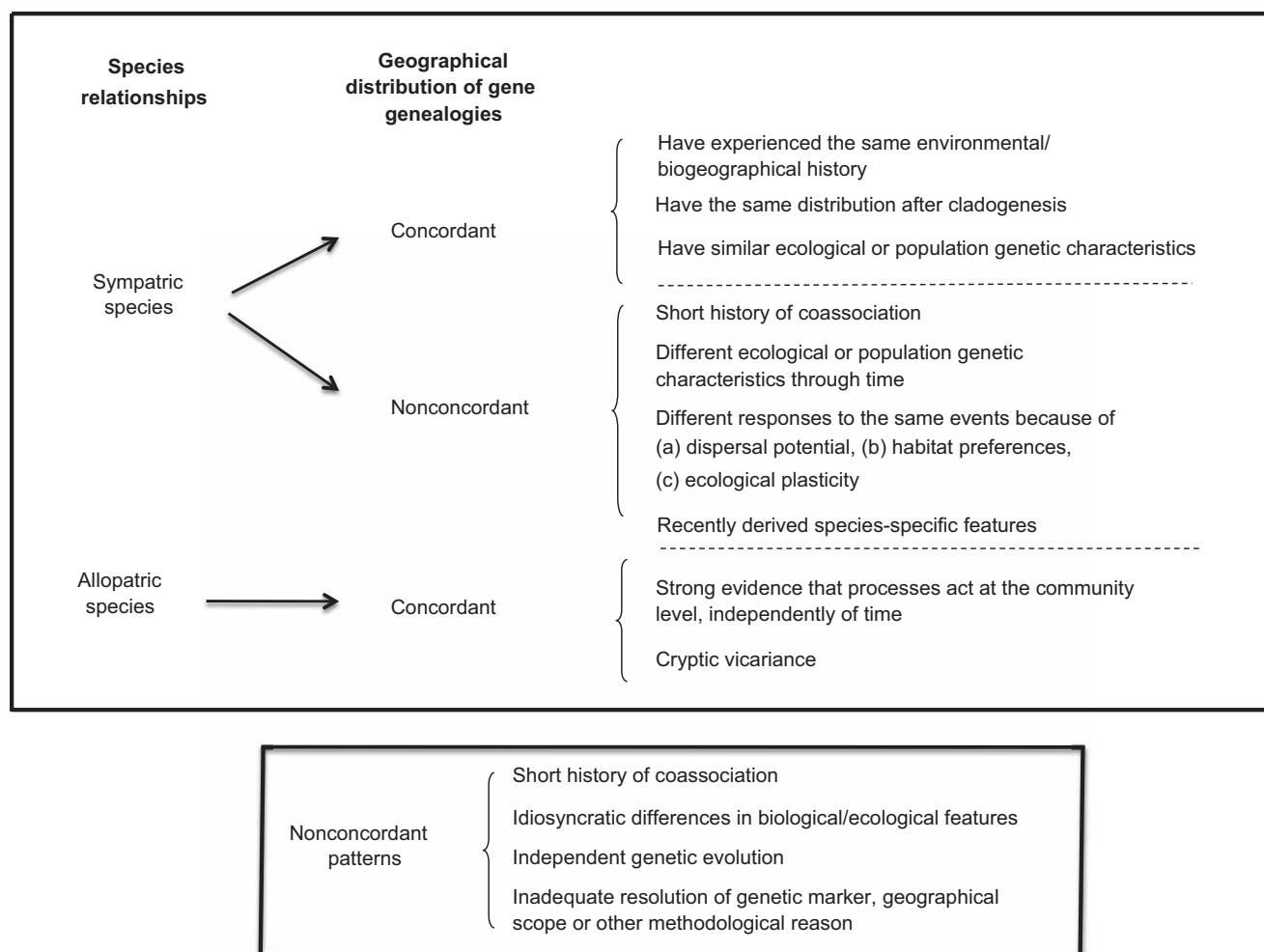


Figure 2. Summary of some simple general outcomes of a comparative phylogeographic study in accordance with the codistributed species relationships and the geographical distribution of their genealogies. More complex patterns occur, but the ones included here are the most frequently found in the literature.

from the Vanuatu islands that are morphologically similar within the islands but have different habitats outside Vanuatu with respect to their distribution, morphology, and degrees of geographic variation. Following a CP approach, Kirchman and Franklin (2007) were able to conclude that these codistributed species had different historical migration and colonization events but have similar levels of phenotypic variation as a result of their parallel evolution on the islands (see supplemental table S1, available online at <http://dx.doi.org/10.1525/bio.2011.61.11.5>).

Even phylogenetically distant species, with an affinity for a particular habitat, are good candidates for CP studies. An example is the study of Fedorov and colleagues (2008), who evaluated the phylogeographic history of the wood lemming (*Myopus schisticolor*), a species closely associated with the boreal forest of the taiga zone in Europe and Asia. Fedorov and colleagues (2008) compared the lemming data with those of other boreal forest species across Eurasia and found that they all had similar phylogeographical patterns associated with late Quaternary glacial and geological events. Their results showed that the primary events that shaped the

historical dynamics of the taiga zone and the evolutionary history of the species were successive range expansions and contractions of the boreal forest (table S1).

Regarding ecological associations and interactions, mutualism and the host–parasite system are ideal systems to explore evolutionary history in CP studies (Razo-Mendivil 2010). Historical processes and geographical barriers that affect both the host and its symbiont can cause parallel differentiation and, consequently, congruent phylogeographical patterns. Nevertheless, because of species-specific characteristics (e.g., dispersal ability), a host and its symbiont can show different histories. For example, Parker and colleagues (2004) evaluated the CP of two species of legumes (*Amphicarpea*) and their root nodule bacteria (*Bradyrhizobium*) and found no evidence of parallel cladogenesis. Their results suggest that different bacterial lineages colonized *Amphicarpea* at different times, after evolving in association with other legume species (table S1).

Some taxa have been less studied under specific comparative phylogeographical frameworks and therefore contain good species to consider; the relatively fewer number of

studies in which fungi, plants, parasites, and marine species were used became evident during our review. Some studies done with fungi—although not explicitly expressed as CP—have shown strong phylogeographic patterns (Printzen 2008), whereas others have described or confirmed new species that originated from cryptic speciation events (Otálora et al. 2010). Nonetheless, still lacking for fungi is an analysis of the distribution of genetic variation and of gene genealogies in a CP context.

CP surveys of freshwater species have primarily involved fish, whereas surveys of other vertebrate and invertebrate species are lacking. Goldstien and colleagues (2006) emphasized the need to evaluate phylogeographic hypotheses among multiple marine taxa; they also highlighted the importance of studying particular regions, such as locations at which currents converge and cause compositional and temperature changes, which may represent biogeographical barriers. Likewise, Schaal and colleagues (1998) stressed the difficulties of working with plants in phylogeography; as a result, considerably fewer comparative studies have been done with plants than with animals.

Finally, choosing taxa for a CP study often depends on the availability of previous phylogeographical studies from the region of interest, as well as on the congruence of the patterns observed therein, independent of ecological history or phylogenetic relatedness (Taberlet et al. 1998). Likewise, although the chosen species must be codistributed, numerous other characteristics are also taken into account, including physiological similarities (Bermingham and Martin 1998), differences in body size and feeding adaptations (Pastorini et al. 2003), habitat preferences and vagility (Hugall et al. 2002), differences in reproductive cycles (Churikov and Gharret 2002), sexual dimorphism (Smith et al. 2000), and ecological preferences and levels of endemism (Huhndorf et al. 2007). Taxon-specific considerations may also be necessary; for example, ecophysiology and seed dispersal forms are important to consider when selecting plant species for CP analyses (Schönswetter et al. 2004).

Geographical scale and sampling strategies. The geographical scale is undoubtedly important when delimiting the region of study in CP. Selection of the study area should depend on the biogeographical question and the distribution of species; however, it often relies on existing geographical, geological, and historical information about the region. Likewise, the availability of samples of the species of interest—be they from museums and public or private collections or from the wild—is an important factor to consider.

The questions evaluated in CP studies are most frequently of a global scale, mainly because the entire geographical range of the species is commonly evaluated. However, some examples actually cover a variety of scales. An example of a global-scale study is that of Albach and colleagues (2006), who investigated eight closely related and sympatric species of the *Veronica alpina* plant complex from alpine habitats across Europe and North America. Following a CP approach,

they described the phylogeographical patterns of species on each continent and located their potential Pleistocene refugial areas. All of the studied species showed concordant phylogeographical patterns, which are directly associated with the similar environmental processes (i.e., Pleistocene climate changes) that affected the Northern Hemisphere (figure 2). In addition, the authors also did an intercontinental comparison and found higher genetic diversity levels and stronger genetic structure for the species from North America, due to less-severe population contractions (table S1).

As for the sampling requirements, both comparative and intraspecific phylogeography undoubtedly require thorough sampling in order to have adequate inference power. Extensive geographical sampling can provide considerable insight into the evolutionary processes of closely related taxa and at different levels of organization, from populations to species (Avice 2000). Different sampling strategies together with a variety of population data have shown that adequate sampling of the genetic variation is crucial for the statistical evaluation of phylogeographical hypotheses (Morando et al. 2003, Bonett et al. 2007). However, as is noted in table S1, sample numbers differ significantly among studies, and the number of samples per locality or population varies as much as the taxonomic level of sampling (Domínguez-Domínguez and Vázquez-Domínguez 2009), which can range from the subspecies level to that of the genus (Taberlet et al. 1998, Zink et al. 2001).

Determining the best sampling strategy is not an easy task. The a priori assessment of sampling adequacy can be particularly challenging, because genetic diversity is not always randomly distributed across a species' geographical range. Increasing the number of samples may not provide a better representation of the distribution of genetic variation, nor does it guarantee better resolution in the analyses performed. However, the higher the number of samples is, the better the framework is for a CP study (Victoriano et al. 2008). Likewise, strong differences in sample size between localities or populations and insufficient coverage of the species' range can both lead to limitations in hypothesis testing or even to erroneous interpretations. If sufficient sampling size and geographic coverage cannot be achieved (as they would be in the ideal scenario), it is acceptable to instead sample a higher number of populations, thereby maximizing the sampling of geographic distribution. Particular attention should be given to locations near putative barriers or filter barriers, which can potentially affect phylogeographical patterns. Previous studies or preliminary sampling might help, for example, to identify specific areas where patterns of genetic variability should be recovered (Buckley 2009). In addition, a careful sampling design (i.e., to try to sample all individuals in the population) must be planned when studying endangered species with limited ranges or reduced populations.

The importance of sample number was highlighted in a comparative study of the salamander *Desmognathus monticola* in the Appalachian Mountains (Bonett et al. 2007). The study design included intensive sampling of a genetically

diverse region and incorporated individuals that were an isolated population. On one hand, the phylogeographical results confirmed that a minimum number of haplotypes, which occur in less than 1.2% of the species' native distribution, was crucial in determining that the isolated population of *D. monticola* was not native to the region (i.e., it was introduced) and that it needed to be extirpated rather than conserved (table S1). On the other hand, examples exist in which few but properly distributed samples have been enough to evaluate demographic histories, the presence of refugia, and processes of colonization and expansion (Ditchfield 2000, Churikov and Gharrett 2002, Kirchmand and Franklin 2007).

Importance of the genetic markers used. The key elements defining a phylogeographic pattern are (a) how much genetic divergence is present among populations, individuals, or haplotypes (i.e., how genetically different are the entities measured?) and (b) how the haplotypes are distributed geographically (Avice 2000, Ditchfield 2000). In addition, the detection of such patterns depends on the genetic structure that can be revealed by the particular molecular marker used. Genetic information in phylogeography has been commonly obtained with the use of mitochondrial markers; for example, cytochrome b has proven useful in the description of intraspecific and comparative phylogeographic patterns of many taxa (see table S1 for other commonly used molecular markers). Nevertheless, the advantages of using more than one gene (mitochondrial, chloroplast, nuclear, or a combination of these) have become more and more evident (Templeton 2002). It is well known that stochastic variance can limit confidence in the interpretation of historical processes, particularly for CP studies, if they are based on only one gene. Accordingly, using a variety of genes is helpful for good phylogenetic resolution among taxa but also for identifying differences among populations or species that were not previously recognized taxonomically (Domínguez-Domínguez and Vázquez-Domínguez 2009), for obtaining a stronger evaluation of geographical barriers and dispersal routes, and for making stronger inferences about different evolutionary significant units. Likewise, if different genes show equivalent divergence among populations, individuals, or haplotypes, it can be considered evidence of a lack of gene flow, the separation of populations, or a history of isolation (Zink et al. 2001). Notably, an important principle in CP is that similar divergence values observed among genes in codistributed species strongly suggests common biogeographical processes (Taberlet et al. 1998, Riddle et al. 2000a, 2000b).

One should nevertheless consider that histories—for example, of local extinction and recolonization—might vary among species, despite a common geographical history. Consequently, genetic differentiation and genetic divergence values can be difficult to compare among genes (Hugall et al. 2002). In addition, it is important to remember that even though some genetic markers are adequate for

identifying genetic differences between populations or taxonomic groups, not all of them provide information about the causes or factors associated with such differences. For instance, allozymes are not always selectively neutral, and allozyme evaluations capture only a small fraction of the variation contained in an organism's genome, because not all variation in the DNA will translate into variable protein products (Freeland 2005). Accordingly, they are not the best choice to determine a geographical pattern of genetic differentiation between populations. Finally, if levels of genetic variation among the species of interest are known at the beginning of a CP study, one can choose the molecular marker that has the highest genetic variability (see Mateos 2005). Another crucial factor to consider when choosing both the species and the genetic markers is undoubtedly the methods of analysis and statistical testing, a topic we review in the following section.

CP analyses: Phylogenetic, genetic, and phylogeographical methodologies

The techniques and methods followed in CP studies vary greatly (see table S1); a detailed survey of methods is out of the scope of the present review, but in this section, we highlight those most frequently used and their primary references.

Phylogenetic approaches. Comparison of the molecular phylogeny, taxonomy, and geographical distribution of several taxa occupying the same area, as is routinely done in CP studies, provides insight into the complexity of the evolution and history of codistributed species (Riddle et al. 2000b, Pastorini et al. 2003). Therefore, obtaining a high-quality phylogeny is a critical element in the search for the repetitive patterns of taxonomic subdivision evaluated in phylogeography. The phylogenetic approaches applied in CP studies are diverse, but in common among them is their use of gene trees instead of species trees. For example, in order to show vicariant events, researchers may use (a) a gene tree for each of the species studied, in which individual haplotypes are grouped by locality (Riddle et al. 2000b, Hugall et al. 2002); (b) a tree in which the branches represent each individual or haplotype, regardless of the species' localities (Mateos 2005); (c) a tree that includes all of the species in each locality (Pastorini et al. 2003); or (d) multiple trees, on which the geographic distributions of the species are overlapped (Riddle et al. 2000b). Phylogenetic methods have been employed for a diverse array of evolutionary and phylogeographical inquiries, such as estimating times of divergence (table S1; see Mateos 2005); discerning taxonomic differentiation at the subspecies, species, or congeneric level (Ditchfield 2000, Demastes et al. 2002); inferring the temporal scale of diversification; testing hypotheses regarding the chronological development of historical events (Leaché et al. 2007); and identifying cryptic species and their phylogeographical histories (Razo-Mendivil et al. 2010). Although diverse types of phylogenetic analyses are necessary in CP, it is important

Box 2. Statistical testing in phylogenetic analyses.

Phylogenetic evaluations must include a statistical component, particularly for support regarding similarities among patterns observed between different genes or among multiple species. Phylogenetic inferences have intrinsic supporting statistics (bootstrap values or posterior probabilities), but other approaches also exist, including comparisons among genetic distance percentages or bootstrap values (Pastorini et al. 2003) or between mean divergence values among localities (Hugall et al. 2002). Specific statistical tests of phylogeographic hypotheses using phylogenetic trees have been done by different means. For example, Sullivan and colleagues (2000) used three statistical methods in order to test competing hypotheses of concerted (i.e., concordant among species) versus independent responses to past climatic fluctuations in the highland rodent *Reithrodontomys sumichrasti* and the *Peromyscus aztecus*–*hylocetes* complex. The results of Kishino–Hasegawa/Templeton test and parametric bootstrap tests that showed that the observed phylogeographic incongruence between the two groups were significant, which supports the independent-response hypothesis. They also used a randomization test of reconciled tree maps, which indicated a significant history of covariance between the two groups, which in turn supports the concerted-response hypothesis. Through combining these complementary methods of analysis, Sullivan and colleagues (2000) concluded that there has been some correlation in the responses of these two taxa to past climatic and geologic events but that the responses have not been entirely concordant (see supplemental table S1, available online at <http://dx.doi.org/10.1525/bio.2011.61.11.5>). Other methods employed for statistically testing phylogenetic results include the Brooks parsimony analysis (Taberlet et al. 1998), a comparison between user-defined trees and empirical ones with statistical tests (Ditchfield 2000).

to be aware of the limitations of these methods and of the need to incorporate new forms of statistical testing (box 2; Vázquez-Domínguez et al. 2009).

Genetic and demographic analyses. Although many CP studies include a qualitative or quantitative comparison of the phylogenies of the species studied, this is not a prerequisite. Indeed, many examples exist in which the species' response to barriers and filter barriers—key elements in phylogeography—have been evaluated using a combination of genetic and demographic analyses (Templeton et al. 1995, Zink et al. 2001, Churikov and Gharrett 2002, Michaux et al. 2005, Templeton 2009). Such analyses have included measures of genetic diversity, divergence, and structure; tests of molecular variance; the use of minimum-length unrooted trees; the application of nested clade phylogeographical analysis (NCPA); the estimation of mismatch distributions and of effective population sizes; and the use of statistical parsimony networks or other algorithms that depict the relationship between haplotypes. One comprehensive example of such an approach is the work of Goldstein and colleagues (2006), who evaluated the CP of three intertidal limpet species distributed along North Island and South in New Zealand. Their analytical approach incorporated estimates of genetic diversity, molecular variance, minimum spanning networks, and NCPA. They confirmed moderate to strong genetic discontinuities among the North and South Island populations due to allopatric fragmentation, a pattern broadly concordant across the three species (table S1, figure 1). Although such methods may be sufficient to detect phylogeographical patterns, there are also theoretical and mathematical methods that have been specifically developed for both intraspecific and comparative phylogeographic analyses and that allow for a more comprehensive description and evaluation of such patterns and their associated processes (Hickerson et al. 2006a, 2006b, Richards et al. 2007, Carstens et al. 2009, Moussalli et al. 2009).

Novel theoretical and mathematical frameworks. The recent increase in CP studies, as well as the use of more genes and a higher number of species, has motivated the development of new theoretical and mathematical frameworks (e.g., coalescence theory; see box 3), together with specific methodological approaches for phylogeographical analyses (box 4). Simultaneous divergence patterns—one of the central interests in CP—are still an analytical challenge because of the confounding effects of the differences among taxon pairs on parameters that affect genetic divergence (e.g., mutation rates, ancestral population sizes, ancestral subdivision, generation time, migration). To cope with this problem, different maximum-likelihood and approximate Bayesian methods have recently been developed that specifically take into account that some phylogeographic information includes multiple data sets that should be simultaneously analyzed (box 4).

As was mentioned previously, inferences in CP require statistical models for hypothesis testing that incorporate the variance present both in demographic models and in coalescent stochasticity. This is important because multiple historical events with temporal and spatial differences can result in similar phylogeographical patterns that appear to be the consequences of a single event (Riddle and Hafner 2006). The approximate Bayesian computation under a hierarchical coalescent model (hABC) developed by Hickerson and colleagues (2006b, 2007) specifically considers the problem of statistically testing biogeographic hypotheses and takes into account coalescent stochasticity, uncertainty in taxon-specific demographic parameters, and the genetic variance associated with coalescent and mutational processes. Leaché and colleagues (2007) applied the hABC method to test for simultaneous vicariance across 12 codistributed taxa that share a common phylogeographic pattern of genetic divergence across the center of the Baja California peninsula. The hABC results did not support a shared vicariant history, and Leaché and colleagues (2007) concluded that genetic

Box 3. Theoretical and mathematical frameworks: Coalescence theory in comparative phylogeography.

Initially, it was considered in phylogeographical analysis that branches on the gene tree could be interpreted as evidence for the occurrence of specific historical demographic events in a geographical context. However, gene trees are random outcomes of stochastic population-level processes, and such randomness has profound implications for the interpretation of the estimated gene tree (Nielsen and Beaumont 2009). The advent of coalescent theory has been fundamental in dealing with this problem, because the theory provides a mathematical framework that describes the distribution of gene trees in populations by relating the patterns of common ancestry within a sample to the size and structure of the overall population (Kuhner 2008, Knowles 2009, Nielsen and Beaumont 2009). The coalescent is a stochastic model that describes the ancestral or genealogical process for a sample of gene copies, which is well suited to data analysis, because it generates testable predictions about variation in a sample and because it yields efficient simulation algorithms. Because the same coalescent process holds for a wide variety of different reproductive schemes, it is considered to be a very robust model. Accordingly, coalescent-based population genetic models, together with diverse analytical and simulation methods, have been developed that account for changes in demography and for stochastic lineage sorting, which have helped to connect demographic models with gene trees.

Box 4. Methodological approaches for phylogeographical analyses.

Maximum-likelihood and Bayesian methods use the full information content of the data and can therefore, in principle, separate simultaneous and variable-divergence histories present in comparative phylogeography (CP) information. However, simulation-based methods, such as approximate likelihood and approximate Bayesian computation, are more suitable to simultaneously analyze multiple phylogeographical data sets, especially those involving complex models and idiosyncratic biogeographical histories (Hickerson et al. 2006b). Moreover, approximate Bayesian computation is a statistical technique that can be used to infer parameters and to choose between models in the complicated scenarios encountered in CP. For example, on the basis of gene-sequence and microsatellite data, approximate Bayesian computation has been used to choose between competing models of human demographic history and also to infer growth rates and times of divergence (Beaumont 2010).

Summary statistics are also applicable to CP analyses (Knowles 2009) and have the advantage that they are relatively unbiased and can summarize relevant information regarding a parameter of interest (e.g., effective population size). As an example, Hickerson and colleagues (2006a) used simulation-based approximation methods to review a set of summary statistics and to estimate their power to test for simultaneous vicariance across comparative phylogeographic data sets: They simulated the behavior of seven summary statistics, considered different divergence times using multiple taxon pairs, and evaluated simple hypotheses to test simultaneous vicariance given variable population sizes. They found that different summary statistics were superior (i.e., achieved the highest statistical power) when testing older divergence-time hypotheses or when testing more recent divergence-time hypotheses.

diversity in this region was structured by two different historical events (table S1, figure 2).

Temporal approaches. The different temporal components of the evolutionary history of species can be inferred by evaluating the phylogeographic signal carried by different levels of genetic diversity: Genotype and allelic frequencies can generally change over a few generations, whereas changes at the DNA-sequence level are relatively slow. Taking this into account, Garrick and colleagues (2008) proposed a framework in which direct and indirect approaches were combined to quantify the temporal elements in a variety of molecular data, which permits explicit assessment of congruence at those variable temporal depths. This approach can be used to evaluate the influence of ancient relative to that of more-recent factors and processes that have an impact on genetic structure. Remarkable examples include CP studies with symbiont species in which these temporal phylogenetic analyses have helped to clarify the changes in specialization in symbiosis between plants and bacteria (Parker et al. 2004).

Conservation approaches. Several years ago, Moritz and Faith (1998) suggested, in a conservation context, the use of a

diversity measure (phylogenetic diversity) based on the branch lengths of phylogenetic trees to evaluate independent evolutionary units. To our knowledge, this approach has only been used in a CP context by Smith and colleagues (2000), who compared the phylogeographic structure of two bird species that have different patterns of endemism across six mountains in Africa. They estimated how much of the total phylogenetic diversity was shared among the regions and how much was unique to each one. On the basis of their results, they identified specific geographical regions that harbor evolutionarily distinct populations, which are highly significant in the conservation of these species (table S1).

Hypotheses in CP

A geographical component—particularly the climatic and geological history of the region of interest—is of course a common feature of all phylogeographic studies. Accordingly, many of the hypotheses that are intended to be tested in CP studies are related to these factors. Frequently tested climatic hypotheses involve global temperature changes; past climatic fluctuations; and glacial cycles, mainly during the Quaternary period. For example, one can assess the classical hypothesis that highland organisms shifted in concert,

elevationally or latitudinally, in response to glacial cycles (Sullivan et al. 2000, Schönswetter et al. 2004). Interestingly, concordant phylogeographic patterns are not necessarily the rule when sympatric species are evaluated. Michaux and colleagues (2005) tested such a hypothesis with their study of two European rodents (genus *Apodemus*), in which they used mitochondrial DNA sequences of samples from throughout the rodents' range. They found that the two species survived the Quaternary glaciations in different ways and from different refugia. This study shows the importance of taking into consideration biological characteristics like ecological plasticity when evaluating survival through climate change. It also confirms that even closely related species can have different phylogeographic histories (table S1, figure 2).

Another source of information to establish hypotheses for CP are geological reconstructions, which are often based on paleontological and stratigraphical data and which have helped to determine the chronology of orogenic processes such as volcanism and river or mountain formation. Such geological reconstructions imply a sequence of evolutionary events from which geographical hypotheses can be established and later tested by evaluating the phylogeography of species. An example of this is the CP study of Huhndorf and colleagues (2007) that revealed the effect of volcanic activity on the fragmentation of montane rainforests in east-central Africa. Using mitochondrial DNA sequence variation, Huhndorf and colleagues (2007) estimated phylogeographic patterns and divergence times for populations of three species of montane endemic rodents. These estimates were supported by molecular dating of the formation of biogeographical barriers shaped by the eruptive events. Their results demonstrated that this type of fragmentation played a major role in the diversification of these montane endemic rodents during the middle to late Pleistocene (table S1).

Historical and phylogeographical hypotheses may be built on the basis of fossil evidence; however, fossils are scarce for most taxa. In fact, we did not find any examples of studies that included fossil evidence for different but codistributed species. There are studies in which species sharing a recent common ancestor were investigated and in which a molecular-clock approach, which facilitates the estimation of divergence times and other events, was frequently incorporated. One example is the study of Hemmer and colleagues (2010), who performed a CP study of the jaguar (*Panthera onca*) on the basis of fossil remains of the lower dentition in combination with modern DNA assessments. Their results showed that the ancestral population was of African origin, from which the jaguar dispersed over Europe 1.95–1.77 million years ago (mya) and that its transcontinental dispersal to North America happened around 0.99–0.78 mya, with a later diversification in South America.

Vicariant and dispersal models are often evaluated in CP studies as biogeographical hypotheses. Although examples are numerous for both models, to illustrate the former, we highlight the work of Riddle and colleagues (2000b), who evaluated the phylogeographic population structures of 12

mammalian, avian, amphibian, and reptilian species. They constructed phylogenetic trees for each taxon, estimated their net divergences, and compared the different phylogeographic groups (called *phylogroups*) that they found. Their results support previously hypothesized vicariant events in the evolution of Baja California–peninsular biota, from the late Miocene to the middle Pleistocene (table S1). Example hypotheses involving dispersal models include instances of continent-to-island migration and recolonization, founder effects, and growth and decrement of population sizes. For instance, Kirchman and Franklin (2007), whose study was discussed previously, combined analyses of haplotype (DNA sequence) diversity and genetic structure with demographic and phylogenetic approaches. They found evidence to support the hypothesis of recent colonization and subsequent expansion in two of the three species of birds that they examined.

One may come across instances in which biogeographical hypotheses do not explain the distribution of genetic variation or the phylogeographic patterns observed. Many factors can lead to this lack of congruence, including the instability of species assemblages or communities through time (Taberlet et al. 1998). Codistributed species can also have different, independent responses because of idiosyncrasies in their biological and ecological characteristics, because they just recently came to share their geographical distribution, or because the gene (or genes) evaluated has (or have) different evolutionary rates in the species studied (figure 2; Sullivan et al. 2000). One should also consider the particular scenario in which the geological events are temporally nested, occurring at the same site but at significantly different times. For example, the repeated elevations of the Rocky Mountains and the most recent glacial cycling associated with those mountains have produced barriers and events of very different ages (Spaeth et al. 2009). This is an essential consideration in CP studies, because it can result in a lack of congruence or, more importantly, in false congruence among species. A potential solution was suggested by Riddle and Hafner (2006) that included temporal nesting within a taxon combined with fossil dating and Brooks parsimony analysis.

New approaches for CP analyses

Several new approaches have recently become available for the performance of CP analysis. We describe them here.

Hypothesis testing. Further advancing the latest methodological frameworks proposed for CP analyses, Hickerson and Meyer (2008) extended the hABC model (Hickerson et al. 2006b, 2007) to develop a new hierarchical approximate Bayesian computation model (HABC). This model uses coalescent population genetics to estimate ancestral demographic patterns (e.g., population size expansion or contraction) across codistributed taxa. Moreover, the HABC model can explicitly test simultaneous vicariant and dispersal events in multiple codistributed taxon pairs. It is also capable of distinguishing simultaneous isolation, even with a few individuals. As was discussed previously, this sampling

limitation is often encountered in phylogeographic surveys. Another advantage of the HABC model is that one can analyze different phylogeographic data sets at once in order to make cross-taxon-pair inferences about biogeographical processes while explicitly allowing for uncertainty in the demographic differences within each taxon pair. Hickerson and Meyer (2008) used the HABC model on two comparative phylogeographical data sets (mitochondrial DNA sequences from multiple codistributed taxa) to test two hypotheses of marine allopatric speciation. Their results showed how the model is able to detect whether either hypothesis is a dominant process across the codistributed taxon pairs.

Multivariate analyses (e.g., principal component analysis) are an efficient tool for the evaluation of genetic variability in different contexts, including phylogeography. They have shown great potential in the detection of spatial genetic patterns and in the exploration of recent phylogeographic events, mainly because of their ability to summarize multivariate genetic information into a few synthetic variables (Jombart et al. 2009). Ciofi and colleagues (2006) explored the challenging scenario of inferring colonization dynamics of populations or lineages that have derived from relatively recent dispersal events within islands and that have limited dispersal. They studied phylogeographical and dispersal events of the giant Galápagos tortoise (*Geochelone nigra*) using mitochondrial DNA and microsatellites and a variety of multivariate, genetic, and phylogeographic analyses. The analyses revealed a strong association between geographical distance and genetic distinctiveness, concordant with the rather recent colonization events on the island (table S1). Previous studies for this species were unable to distinguish colonization processes, probably because of inadequate analytical methods.

Carstens and colleagues (2009) recognized the need to have more means of testing the predictions of null hypotheses in CP. They proposed a method involving information-theoretic metrics in which information theory is used to quantify the probability of multiple hypotheses given the phylogeographical data in question. They used both empirical and simulated data from previous studies of the salamander (*Plethodon idahoensis*) to generate a ranking of 17 models, each of which represented a set of historical evolutionary processes. This approach allowed Carstens and colleagues (2009) to quantify the relative strength of support for each of the 17 models instead of simply rejecting various hypotheses.

Supertrees. Supertrees result from combining many smaller, overlapping phylogenetic trees into a single more comprehensive tree. On the basis of this premise, Victoriano and colleagues (2008) searched for a shared phylogeographical signal in lizards with partially overlapping ranges (three species of *Liolaemus*) using a supertrees method. Their results are consistent with the hypothesis that the species responded in parallel to shared historical processes, which in turn influenced their phylogeographical structure (table S1). This method requires well-resolved phylogenies, with reasonable sampling throughout the species' distribution. The obtained genealogies

are analyzed with the supertrees approach to evaluate whether any combinations of species show significant spatial codivergence. An advantage of these recently developed methods is that they incorporate the assessment of significant statistical signals through randomization tests. Another advantage is that with these methods, one can perform statistical tests of a shared phylogeographical signal between taxa, even if the taxa have only partially overlapping geographical distributions instead of complete codistribution, as is usually required when estimating spatial divergence.

Niche and paleodistribution models. Species-distribution modeling and its historical counterpart, paleodistribution modeling, which both explicitly incorporate spatiogeographic data (abiotic variables such as temperature, precipitation, and topography), are increasingly used analytical approaches in phylogeography (Hickerson et al. 2010). Hugall and colleagues (2002) compared the phylogeography of several endemic vertebrates from the Australian tropical forest with spatial models of predicted species distribution under different paleoclimate scenarios. They identified late Pleistocene–Holocene historical refugia and broad patterns of extinction and recolonization shared among species (table S1). Furthermore, these new modeling techniques, in combination with coalescent simulations, also provide a means for generating realistic phylogeographic hypotheses, even for taxa that have a scarce fossil record (Richards et al. 2007).

Indeed, these approaches have enabled the evaluation of the impacts of past climate fluctuations at a very fine scale, together with speciation mode and migration during Pleistocene cold cycles. In a study aimed at comparing spatial patterns of population persistence and isolation across three species of rainforest skinks (*Saproscincus*) and at investigating their responses to late-Quaternary climate fluctuations, Moussalli and colleagues (2009) found high concordance between molecular data and paleodistribution models, which indicates conservatism of bioclimatic niches (i.e., different codistributed species have similar historical abiotic and environmental characteristics). The study showed that species with broader climatic niches maintained more genetic diversity and that this genetic diversity was more structured as a result of persistence through different historical climates. This result suggests that the observed differences in response to past climate change were associated more with differences in the species' climatic niches than to other ecological traits (table S1).

Conclusions

CP studies have been increasing in number at a much slower pace than those at the intraspecific level. We suggest that data-gathering limitations in conjunction with methodological and analytical difficulties are the primary reasons for this. Time is also a constraint, given that performing a CP study means having to obtain field and molecular data for more than one species and also means using additional and more complex analytical and statistical methodologies.

It is evident that testing and proving congruent phylogeographical patterns among taxa (as a result of a common evolutionary history) is no easy task, considering that even sister species can differ in their ecological characteristics and life-history features. Accordingly, much emphasis has to be placed on developing new analytical methods and on using different methodologies in concert in order to compare species and localities in a quantitative manner. Species-specific demographic and ecological characteristics can result in different outcomes of the same historical and biogeographical events and, consequently, in the loss of concordant phylogeographical patterns between species. Therefore, in the planning of a CP study, the information obtained through intraspecific phylogeographic evaluations is of fundamental value. It is also important to consider, among other things, the stochastic variation of loci; the degree of taxonomic resolution in the species of interest; and their ecological characteristics; together with fossil evidence and biogeographical, geological, and historical information relating to the geographical distributions being studied.

CP is no longer solely descriptive and qualitative, and one can now test more rigorously the comparative evolutionary histories of multiple species. In regions in which sufficient background information is available, *a priori* hypotheses can be statistically tested; moreover, for poorly known regions or taxa, new methods also allow the generation of alternative hypotheses. Many evolutionary and historical questions, such as models of speciation and diversification times, will continue to be answered with CP surveys; the scope of applications will also widen significantly to identify areas of endemism, patterns of hybridization, and evolutionary novelty with conservation value. The identification of selection patterns and that of ecologically driven speciation have been identified by Hickerson and colleagues (2010) as further opportunities for CP studies. Indeed, one of the most powerful properties of CP is that it allows evaluation of evolutionary patterns and processes involving entire communities and assemblages, which may broaden our theoretical and applied knowledge of evolution, ecology, and genetics at the community level. As was mentioned at the beginning of this review, over a decade ago, Bermingham and Moritz (1998) recognized some challenges that would improve the comparative approach in phylogeography. The present review testifies that those challenges have been amply met and, moreover, that many new challenges have been established or are to come. It also provides evidence that we have made considerable gains in our understanding of the influence of past events on current patterns of genetic diversity and on the geographical distribution of species.

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Tania A. Gutiérrez-García (taniagutierrezgarcia@gmail.com) is a PhD student at the Posgrado en Ciencias Biológicas, and Ella Vázquez-Domínguez (evazquez@ecologia.unam.mx) is a researcher with the Instituto de Ecología, both at the Universidad Nacional Autónoma de México. They are interested in population genetics, phylogeography, and conservation biology.