

Toward an organismal, integrative, and iterative phylogeography

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Phylogeography involves the analysis of gene genealogies in a spatial context, to infer the historical processes that have shaped the current population structure and distribution of organisms. The field has expanded rapidly in the last three decades, triggered by important technical and methodological advances. However, these technical improvements have not been paralleled by major changes in theoretical paradigms. I suggest that phylogeographic techniques are underutilized, and that adopting an organismal, integrative, and iterative research program in phylogeography will reinforce the explanatory power of the discipline.

Keywords: causality; complexity; explanation; hierarchy; hypothesis testing; organism

Introduction

Evolutionary biology is a complex and pluralistic science.^(1–3) Researchers use evidence from nature, experimental, and theoretical approaches, and a vast array of analytical tools to understand the origin and evolution of the biota on Earth. The aim is dual: first to describe chronologically and in an objective way the events that have occurred through time, *i.e.*, to recover the chronicle of the past events; then, to establish the history of these events, *i.e.*, to infer the causal connections that link these events and that have shaped current and past biodiversity.⁽⁴⁾ Chronicle and history are not conflicting concepts. The accuracy of any history relies on the accuracy of its underlying chronicle. To take a case in point, in evolutionary studies phylogenetic hypotheses diagramming the evolutionary relationships among lineages are first established. These phylogenies are then used as frameworks with which to test evolutionary hypotheses in a causal context (*e.g.* the mechanistic bases of character changes across lineages, the tempo and mode of speciation, or the biogeographic events and processes). In this sense, there-

fore, phylogenies can be viewed as the indispensable descriptive evolutionary chronicles upon which evolutionary history is reconstructed.

Phylogeography emerged three decades ago as a subdiscipline of historical biogeography.^(5,6) Phylogeography concerns the spatial (geographical) distribution of gene lineages. Analysis of the spatial distribution of genealogical lineages is performed in a causal context, seeking to identify the historical (*e.g.* vicariance, dispersion) and demographic (*e.g.* patterns of gene flow, population bottlenecks) processes responsible for the observed distribution of genetic variability. As Kidd and Ritchie⁽⁷⁾ point out, its ultimate goal might be “to understand microevolution and speciation in its geographic or spatiotemporal context”. Phylogeography, thus, emerged as a powerful explanatory tool to recover the evolutionary history of taxa, not just as a descriptive chronicle of past events.

Avise,⁽⁵⁾ while reviewing the course of phylogeography after two decades of developments in the field, suggested three main research lines for future expansion: “(i) the concordance in significant genealogical partitions across multiple unlinked loci within a species, (ii) the concordance in the geographical position of significant gene-tree partitions across multiple co-distributed species, and (iii) the concordance of molecular gene-tree partitions with geographical boundaries between traditionally recognized biogeography provinces”. Similarly, Bermingham and Moritz⁽⁸⁾ suggested three major future challenges for the discipline: “(i) [to] utilize unlinked molecular markers and develop improved analytical approaches for testing for evolutionary congruence (or lack thereof) between nuclear and organelle genes, (ii) to incorporate new developments in coalescence theory, and (iii) to increase the precision with which the timing of cladogenetic (separation) events can be estimated.”

Another decade has passed since those two review articles were written, and the proposed challenges and research lines have been successfully integrated into the discipline; for instance, (i) multilocus approaches in phylogeography,^(9,10) (ii) comparative phylogeographic approaches,^(11,12) (iii) coalescent theory and methods,^(13,14) (iv) temporal estimates from molecular data.⁽¹⁵⁾

However, and in spite of these important advances, no major changes in paradigms have been proposed. For

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example, the majority of published studies invoke retreat to Quaternary glacial refugia and postglacial range expansions to explain the distribution of current genetic diversity. Although not necessarily wrong, there is apparently a need to agree (or disagree) with *a priori* scenarios rather than to challenge the paradigms and explore the explanatory power of phylogeographic techniques.

Here, I argue that phylogeography is a powerful technique in evolutionary biology, but one that is currently underutilized. I identify three mutually dependent factors that are responsible for this: (i) phylogeographic studies are necessarily gene-centered, but they nonetheless have failed to integrate information on the biology and natural history of organisms, which may be essential to understand the genetic and geographic patterns observed in nature, *i.e.* phylogeography needs to recover its essential organismal approach; (ii) phylogeographic techniques have not always captured the hierarchical organization of biological systems and the complexity of the evolutionary process; (iii) given the complexity and hierarchical nature of the systems studied, phylogeography needs to adopt a pluralistic view in terms of hypothesis testing and statistical approach; the classical frequentist approach for hypothesis falsification is far from ideal when dealing with historical processes of this kind.

Complexity and phylogeographic inference

The biota on Earth is organized in complex and dynamic systems. Complex systems are composed of a large number of elements that interact in non-linear ways,⁽¹⁶⁾ which renders the behavior of the systems highly unpredictable. The number of parts, their order and their interactions characterize complex systems,⁽¹⁷⁾ which very frequently take the form of hierarchies.⁽¹⁸⁾ Cells, individuals, species or ecosystems are examples of complex systems. Moreover, these complex systems are not independent, but structured sometimes in a nested design, giving rise to the observed hierarchical organization of the natural world.^(19–22) The interactions between the parts, within and across systems, occur at very different spatial and temporal scales, from the nano-scale and milliseconds in molecular interactions, to hundreds of kilometers and hundreds of years in ecological (and millions of years in geological) temporal scales. The main challenge of evolutionary biology as a science is to face this structural and functional complexity; evolutionary inferences should be distilled from integrating, in a unified explanatory framework, information from the processes and mechanisms occurring at these different organizational levels and scales.

Phylogeography has gained popularity because of its potential explanatory power. Chronicle and history⁽⁴⁾ are estimated in the same analysis, through which some causal

historical explanations are inferred from the genetic and geographic patterns observed. Two main approaches have traditionally been followed in phylogeography: (i) a phylogenetic approach, in which historical scenarios are directly inferred from the branching pattern of phylogenetic trees; (ii) a population genetics approach, in which historical inferences are based on mathematical models and summary statistics characterizing the population structure.^(23,24) More recently, the development of coalescence theory has helped to bridge the divide between the two approaches.⁽²⁴⁾ The coalescent, a demographic mathematical model, is used to trace back the genealogy of sampled alleles or haplotypes; gene trees are, thus, a tool shared in phylogenetic and population genetics approaches.⁽²⁵⁾ The question remains, however: is this set of phylogeographic techniques suitable to analyze the complexity of the evolutionary process? If not, how can we study complex evolutionary systems?

Hierarchies and pluralism in phylogeography: Toward an organismal, integrative, and iterative approach

Phylogeographic studies inquire about when, where, and what kind of processes have taken place during the evolution of a given lineage. The ultimate aim would be to set a causal historical scenario explaining the evolutionary history of the organism studied. The starting points for this query consist of the patterns of genetic variability of sampled representative individuals, the spatial location of the localities sampled, and a set of inference methods, such as statistical tools and mathematical models. It can be argued that the information available comes from a specific level of biological organization (*i.e.* variability of neutral genetic variants), and that the processes occurring at very different temporal and spatial scales are deduced with this information only. Ideally, the study of evolutionary processes should be undertaken under a model of complexity, integrating information from different levels and scales; sometimes this is not feasible. Accordingly, I offer suggestions to capture and incorporate some of the complexity of the evolutionary process into the phylogeographic research program.

Organismal phylogeography

The first suggestion does not require any new analytical tools, but a shift in the emphasis of the research. Although necessarily gene-centered, phylogeography should strengthen its organismal vocation. This shift may be promoted in two ways: (i) highlighting the importance of the sampling design and conducting extensive fieldwork, and (ii) emphasizing the need for including data on the natural history of individuals in the analyses.

Sampling

In every phylogeographic study, one of the most crucial steps is the design of sampling. Unfortunately, the importance of extensive fieldwork and carefully designed sampling strategy is sometimes overlooked. The sampling design must be explicitly justified in the study. How many individuals have to be sampled? What should be the optimal geographic range covered? Obviously, there is no single answer to these questions, since the optimal sampling will depend on the organism and the scope of the study. Nonetheless, some general features apply to any phylogeographic study.

Generally, sampling follows three broad strategies: (i) sampling tries to approximate the range of a lineage by collecting individuals in populations scattered along the distribution; (ii) sampling focuses on specific areas to recover patterns of genetic and/or phenotypic variability revealed in previous studies; (iii) sampling is just contingent on feasibility (*e.g.* endangered species, very restricted population sizes, unknown distribution ranges, and so forth). However, it is important to keep in mind that sampling must meet two goals:

First, individuals included in the study must represent a random sample of the lineage studied, capturing its genetic structure (*e.g.* Box 1). The adequacy of the samples will depend on many factors, such as the population dynamics (*e.g.* metapopulation vs. isolation-by-distance population pattern), demographic parameters (*e.g.* population size and density), and life history traits (*e.g.* dispersal capacity, reproductive behavior), among others. As an example, coalescence estimates from a species showing a great dispersal rate but a strong philopatric behavior (*i.e.* tendency to return to natal sites to breed) may be biased if sampling is focused on a few restricted spots corresponding to breeding habitats for the species. Kinship among individuals sampled will lead to an underestimation of coalescent times.⁽²⁵⁾ Likewise, linear transects in widely distributed species sometimes result in apparent clinal variation in genetic and phenotypic traits. The cline, however, may not reflect a selective or environmental gradient, but may be caused by the sampling bias. The effect is removed when sampling is expanded.⁽²⁶⁾

Second, sampling design and inference methods must be in keeping with each other. Unfortunately, this later aspect is consistently disregarded. Inference methods rely on different assumptions, evolutionary models and specific statistical tests. Sampling must fit the requirements of these tests; otherwise, phylogeographic inferences may be biased. For instance, one of the most popular phylogenetic methods in phylogeographic inference, the Nested Clade Phylogenetic Analysis,^(27,28) requires a uniform sampling along the distributional range of the studied species to get reliable estimates of the historical processes involved. The final phylogeographic inferences rely strongly on the sampling design. Sampling also influences model-based approaches.

They are based on very specific evolutionary models with strong assumptions on population structure, migration, gene flow and demography (changes in population size through time). Therefore, sampling must be designed to test the specific hypotheses underlying the evolutionary models utilized.

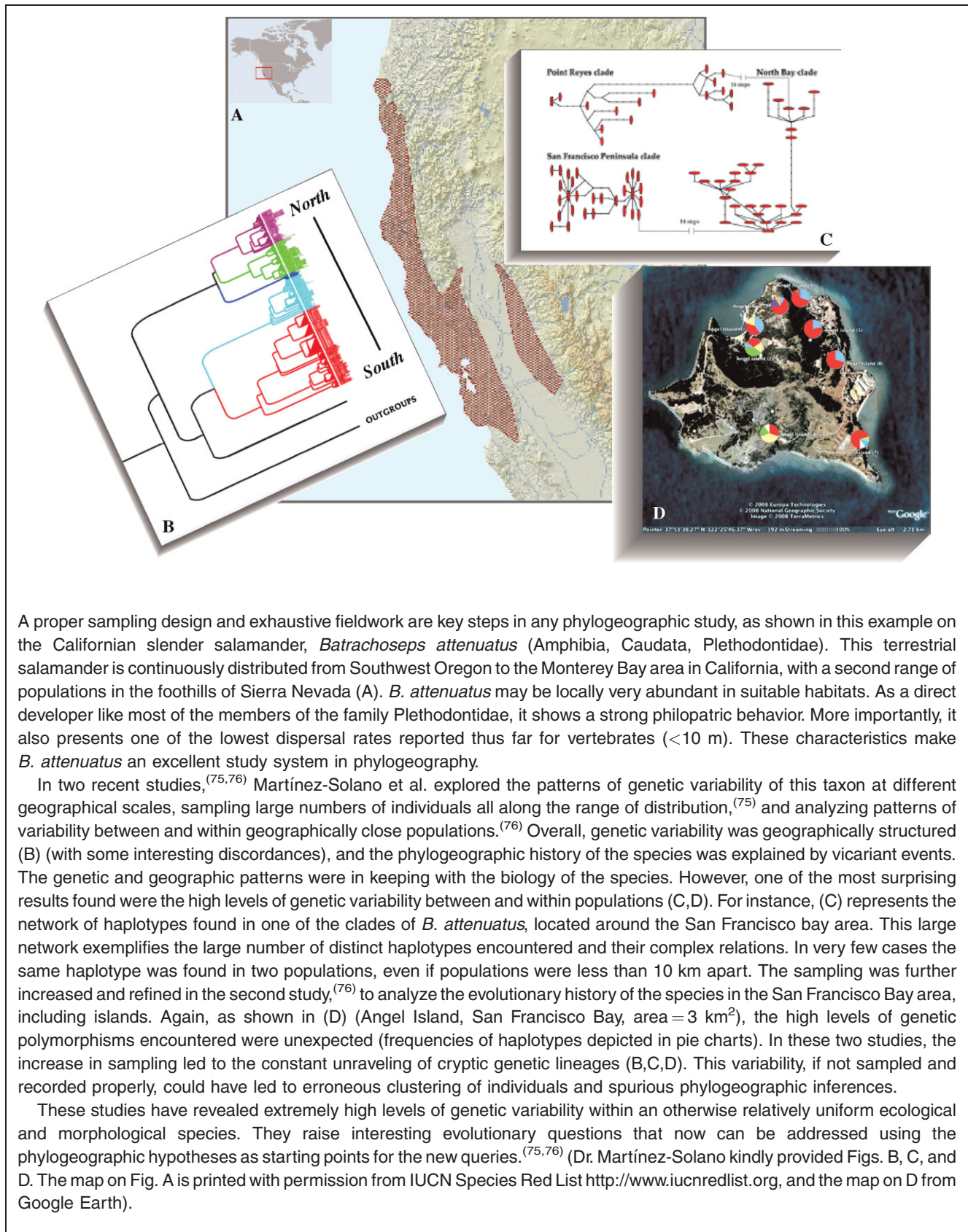
Ideally, sampling should be extensive and the sampling design should be based on previous knowledge of the biology and natural history of the organism. However, natural history information is not always available. Sampling, therefore, must be treated as another parameter in the study, and analyzed as such. Sampling is an explicit hypothesis in the study, a hypothesis about the population structure of the species, the dispersal capacity of the individuals, and their reproductive biology, for instance. The hypothesis must be, thus, explicitly formulated, tested, and discussed. Do the results fit the *a priori* expectation with which sampling was designed?

More data

Fieldwork in phylogeography should not be restricted to obtaining geo-referenced samples for the genetic analysis. It is now a common practice, for instance, to characterize the ecological niche of the species studied by recording a set of representative biotic and abiotic variables (*e.g.* type of habitat, climatic variables). Likewise, the habitat can also be spatially characterized (*e.g.* landscape heterogeneity, fragmentation). Much of this information can be easily integrated in the analysis (*e.g.* landscape genetics approach, see below).

Besides characterizing the habitat and the niche of the species, there is more information that could potentially be integrated, referring to the biology and natural history of the organisms. For instance, dispersal rates are of paramount importance when inferring patterns of gene flow. Despite its utility, information on dispersal is seldom available, which can lead to misleading results in phylogeographic inferences. As an illustration, it has recently been shown that the phenotypic and genetic divergence patterns in two sympatric species of salamanders, occupying the same habitat and presenting similar ecological requirements, were very different. This difference has been linked to the distinct movement behavior in the two species.⁽²⁹⁾ Obviating the ecological information in the study would have led to different interpretations of the historical processes. Differences between species could have been related to different historical demographic processes instead of differences in dispersal behaviors.⁽²⁹⁾

Dispersal is a fundamental parameter. Movement is a complex behavior, driven by processes occurring at very different spatial and temporal scales. Dispersal depends on the physiology of individuals, their biomechanical and navigation capacities, and specific environmental cues.⁽³⁰⁾ Dispersal is thus a dynamic parameter that varies along the lifespan of an individual, and among individuals within and



between populations. Recently, there has been an attempt to formalize a new analytical framework, the so-called “movement ecology framework”, a unified paradigm integrating the relation between all the factors that affect the movement of individuals.⁽³⁰⁾ The “movement ecology framework” has an explicit mathematical formulation and, as such, it could be implemented in the evolutionary models used in phylogeography (see below). For instance, Lemmon and Lemmon⁽³¹⁾ presented a new likelihood method for the reconstruction of historical movements (geographic location of ancestors, centers of origin, or directional migration). In this method, individual movement is modeled as a random walk. The ‘movement ecology framework’ could be potentially incorporated into this new method to obtain more biologically realistic patterns of dispersal.

Given the tight relation between movement behavior, gene flow and population structure, this “movement ecology framework” may serve as a mechanistic and, more important, organismal link between spatial analyses (e.g. landscape genetics), Geographic Information System (GIS)-like analyses (e.g. species niche reconstruction) and phylogeography inference.⁽²⁹⁾ This is just an example of how information on the biology of organisms may be incorporated and may contribute to the phylogeographic explanation. Other pieces of similar ‘organismal’ information could be added, as well. For instance, the integration of physiological data in historical analyses adds invaluable information for reconstructing the historical events that shaped current and past distributional species patterns.⁽³²⁾

In current phylogeographic practice, much of the effort is devoted to increase the genetic information (number of loci) and to the development of new statistical tools. Although necessary, these efforts have not been paralleled by a similar increase in linking and integrating essential biological information from the study of organisms in the field. I stress the importance of the organism as the center of the phylogeographic enquiry. Organisms are fundamental biological entities. They are anatomically and functionally fully integrated units, which can be considered as links between different levels of the biotic hierarchies. Individuals represent the highest level of some hierarchies [e.g. somatic hierarchy⁽²²⁾]. However, individuals are also the basal levels of other hierarchies [e.g. ecogeographic⁽²²⁾]. They must, therefore, play a pivotal role in evolutionary analyses. We need to re-focus the phylogeographic research program on the organisms, incorporating as much information in the analyses as possible.

Integrative phylogeography

In order to analyze a complex system, the most straightforward approach is to deconstruct the system into its constituent parts and to analyze them separately, in

analytically tractable units.⁽³³⁾ Each level of complexity must be analyzed with the appropriate tools and methodology. Individual processes and factors can then be identified. However, the information thus generated must be integrated. By integration, I mean neither reduction to common grounds nor unification of the processes inferred. An integrative approach relies on the use of the appropriate techniques and experimental designs to accurately describe the processes that occur at the different levels of the biological hierarchy to eventually understand the interactions among and between levels that give rise to the patterns observed.^(33–36) Phylogeographic inference will be greatly enhanced by integrating other sources of data and new approaches. This integration could be accomplished in several ways,^(37,38) including (1) the development of new statistical tools, such as new models and priors in Bayesian phylogeographic inference, (2) the expansion of GIS-like technologies, and (3) the development of new analytical tools like network analyses, among others.

(1) Most of the phylogeographic methodologies rely on specific evolutionary models. In model-based phylogenetic approaches, for instance, one of the first steps entails the selection of a substitution model for the genetic samples. Substitution models are a group of stochastic models describing the patterns of base substitutions through time in the samples studied. Similarly, in ‘classical’ population genetics approaches, a population model is used as a null hypothesis with which to test the significance of some summary statistics from the samples. These latter models vary in complexity, from oversimplified (e.g. Fisher–Wright model: one population with infinite size, panmixia, no gene flow or recombination involved) to more complex and realistic ones. Coalescence-based approaches rely on the coalescent as the basic demographic model. The coalescent is a mathematical demographic model that describes retrospectively the genealogy of sampled alleles or haplotypes.⁽²⁴⁾ The coalescent can also be modeled with different complexity, from a simple coalescence process in a population explained only by the effective population size, to scenarios of lineages divergence with migration, for instance.^(39,40)

Models are necessarily simple. They are not intended to reflect the true complexity of natural systems, but to provide a useful mathematical tool capturing reality as close as possible.⁽²³⁾ There is a trade-off between mathematical tractability and how well population structures can be represented. The selection of the model and the inference method are not trivial, though. Several factors, such as the biology of the species studied, the sampling design, and the specific assumptions underlying the models must be carefully considered before the analysis. The model assumptions, for instance, impose restrictions on potential evolutionary inferences. The validity and robustness of the results of the analyses will thus rely on the validity of the models used and their assumptions. Again, it is important to highlight that

the adequacy of the models have to be tested after the analysis⁽³¹⁾ (see below).

There is a growing interest in developing new models in population genetics and phylogeography, especially using coalescence in a Bayesian framework. Bayesian-coalescence inference requires specifying a substitution model describing the evolutionary pattern of the molecular markers, a demographic model (the coalescent), which can vary in complexity, and a set of prior probability distributions summarizing the previous knowledge of the parameters involved. These include both molecular (*e.g.* substitution rates, invariable sites) and population parameters (population sizes, coalescence times), together with the genealogy of the samples. The evolutionary outcome, thus, is inferred from data and models from two different levels of biological organization, genetic variants and populations.

I argue here that information at the organismal level of complexity must be included to complete the evolutionary inference. This can be done under a Bayesian framework by developing new models and priors. Fortunately, there has been a parallel interest in developing Bayesian analysis in ecology, studying processes at individual and population levels. Models and priors have been developed to study species dynamics, individual dispersal, annual survivals, population growth and extinction, and foraging dynamics, for example.^(41–44) The 'ecological' models and priors could be potentially included in a Bayesian-coalescence phylogeographic framework.

A prior is a distribution of possible values for parameters, summarizing information from sources other than the data. Given the formulation of Bayes's theorem, priors have a direct impact on the result, the posterior probability distribution.⁽⁴⁵⁾ The use of this *a priori* subjective information is unique to Bayesian inference and is viewed as either a strength or a flaw by supporters and critics of this statistical inference.⁽⁴⁶⁾ There is a controversy regarding how priors must be specified: either flat or uninformative to minimize the effect on the results (and let the results be driven just by the data), or subjective and informative, to complement the information provided by the data. I argue here that phylogeographic inference would benefit from incorporating information from ecological studies in the form of models and informative priors. Priors are an important part of Bayesian inference and they have to be cautiously specified and tested. The influence of the priors on the final results must be checked. For instance, running the same analysis with and without data can test the impact of the priors on the posterior probability distribution. The development of new Bayesian-coalescence models permitting the incorporation of new kinds of information would enhance the integrative nature of phylogeography.

(2) It has been stated that the 'geographical' part within phylogeographical studies has been somewhat neglected compared to the development of the 'phylogenetic' tools,

reducing the explanatory power of this discipline.^(7,47) The incorporation of the geographic information within a quantitative analytical framework is yet another challenge for phylogeographic and 'landscape genetics' analyses. Landscape genetics focuses on phylogeographic processes but at finer spatial and temporal scales, and targets the individuals as the research units in the analysis. The main aim of this discipline is to decipher the influence of ecological and environmental factors on the demographic history of populations by quantifying the relationship between landscape characteristics and the distribution of genetic variation.^(48,49) Currently, great emphasis is directed toward incorporating information on distribution ranges, climatic and environmental variables and niche models to phylogeographic data, especially through the use of GIS.^(47,50,51)

GIS possesses three important properties: (i) a large capacity for information storage, (ii) high analytical power, and (iii) visualization of spatial information.⁽⁵¹⁾ GIS utility in phylogeography is obvious because its stated purpose is to organize and analyze data contained in different layers of information (*e.g.* biotic and abiotic factors) under an explicit spatial context. The GIS is mostly used for correlation analysis of traits and environmental variables. It is also increasingly being used in hypotheses testing (through the generation of null hypotheses), or as a predictive tool (*e.g.* predictive niche modeling).⁽⁵¹⁾ So far, however, GIS-like technologies in phylogeography and landscape genetics have been used to relate genetic, climatic and spatial information. We lack, however, the mechanistic link that may causally relate all these layers of information. The organism, again, provides this link. Several studies have already included this kind of information in a GIS framework, with promising results.⁽³²⁾ The further development of these kinds of analyses will also require new perspectives and methods for hypothesis testing and interpreting the results from such frameworks (see below).

(3) Complexity in natural systems has been successfully incorporated and analyzed in different contexts [*e.g.* gene and metabolic networks,⁽⁵²⁾ ecological networks,⁽⁵³⁾ social networks,⁽⁵⁴⁾ population dynamics⁽⁵⁵⁾]. In all these studies, complexity is represented and studied through network theory.⁽⁵⁶⁾ Networks are not only very useful as graphical representations of complex interactions, but they are also quantitative and qualitative tools to determine the dynamics and functional structure of the systems.⁽⁵⁷⁾ Network analyses have been developed in a phylogenetic framework to account for non-dichotomous branching processes, such as reticulation, recombination or horizontal gene transfer.⁽⁵⁸⁾ However, no such body of theory has yet been fully developed for the study of networks and natural system dynamics through time, in an explicit evolutionary context.

Networks are defined by 'nodes' (*e.g.* alleles, genes or individuals), edges (defining the interactions among nodes),

and some mathematical models describing the structure (e.g. graph theory).^(53–57) Networks have specific properties (such as stability, connectivity, motifs, position or modularity). The properties define the network behavior and the structure of the system. They also may reveal how the networks do evolve and how, in turn, their structure determines the functionality and the evolvability of the system.^(53–57) Networks could be used to infer and test models of population structure, connectivity and dynamics,⁽⁵⁹⁾ to detect missing links in a structured system⁽⁶⁰⁾ and to understand hierarchical relations of individual within and between populations.

Network theory has not been used in population genetics studies and phylogeography despite its intuitive utility (but see^(61,62)). This possibility should be further explored. Network analyses could also be combined with basic phylogeographic tools. For instance, once the population structure and dynamics are established through a network analysis, the coalescent model could be used as a mathematical tool to project the genetic-spatial networks backwards in time. Network and graph theory may be applied to retrieve phylogeographic parameters from the analysis. Combining network theory and the coalescence mathematical framework may constitute a new and powerful technique in phylogeography.

Network analysis should not be viewed as an alternative to 'classical' phylogeographic inference, but as a complementary approach. New approaches, based on different assumptions and models, may reveal aspects of the system (e.g. interaction among individuals within populations, population structure) hidden in other kinds of analytical tests.

Iterative phylogeography

A major challenge in phylogeography (and, in general, in evolutionary biology) is how to test different evolutionary scenarios. This problem is usually addressed in a classical hypothesis-testing model with estimation of test statistics or, more recently, within a Bayesian framework. While hypothesis testing may be useful when dealing with experimental approaches in which causal factors are manipulated, standard statistical procedures may fail to describe the complexity of historical processes. Questions in evolutionary biology are seldom simple, and highly unlikely to be answered under dualistic paradigms (the null hypothesis H_0 being rejected, we accept the alternative hypothesis H_1).^(33,63,64) One of the main problems under the standard frequentist statistical approach is how to define the hypotheses to be tested amongst all possible alternatives.⁽³⁷⁾ It is too often the case that the null or alternative hypotheses are simple and biologically irrelevant hypotheses chosen *a posteriori*. They will certainly be rejected if we analyze a large enough data set.⁽³³⁾ When attempting to recover an evolutionary history,

there is a vast array of alternative hypotheses that might be tested, which render a simple dualistic frequentist approach unsatisfactory.

Phylogeography is currently moving from a mostly descriptive realm into a fundamentally statistical-testing framework.^(37,38) The developments in coalescent theory in population genetics⁽²⁴⁾ and the implementation of Bayesian frameworks for the study of genetic polymorphism⁽⁴⁵⁾ have greatly contributed to this shift. The statistical merits and demerits of the different approaches in phylogeography, especially the phylogenetic approach implicit in the Nested Clade Phylogenetic Analysis *versus* the model-based approaches labeled as 'statistical phylogeography', have been extensively debated.^(37,38,65–68) Leaving aside this dialectic, it is safe to say that, given the complexity of natural systems and the complexity of the evolutionary process, no single approach will outdo the rest of methods at all the temporal and spatial scales. The explanatory potential will be improved by combining approaches with different resolution power.^(31,68,69)

In addition, there have been several proposals for hypothesis testing under complexity frameworks. Although different in scope, they revolve around two common themes: multi-causality in processes, and iterativity in the hypothesis testing approach. I present here two examples from disparate areas, development and ecology, highlighting the similarities of the approaches and their potential utility in phylogeographic inference.

While discussing the potential role of different processes in driving the evolution of novelties in nature, Wagner⁽⁷⁰⁾ analyzed the relative importance of development as a potential causal factor. He discussed this in terms of the 'explanatory force' of the mechanism (development). The notion of 'explanatory force', as introduced by Amundson,⁽⁷¹⁾ relates to the relative importance of a mechanism as compared to other(s) while explaining an evolutionary phenomenon. It is not an exclusive concept. The occurrence of a mechanism does not preclude the occurrence of others. The main point is to identify the relative importance that different potential mechanisms have.^(70,71) The notion of 'explanatory force' assumes explicitly that we cannot invoke a single mechanism to explain an evolutionary process. Wagner⁽⁷⁰⁾ suggests expanding of the traditional dichotomist hypothesis testing approach to a so-called shifting pluralism, *i.e.* "the idea that there are multiple causes and mechanisms involved in every evolutionary process and that their relative importance for the outcome of evolution shifts from situation to situation". We should look for causal explanatory hypotheses that are not competing but compatible. This pluralistic approach is especially relevant when considering the hierarchical nature of biological systems and the dynamical complexity of evolutionary processes.

Ecosystems encompass dynamics and interactions occurring at very different temporal and spatial scales, and driven

by causal factors that cannot be always distinguished. The utility of classical frequentist approaches for the study of ecosystems has been criticized.⁽⁷²⁾ Instead, the authors propose a process of 'adaptive inference', in which successive rounds of investigation accumulate information on possible mechanisms and hypotheses. The goal is not to contrast and reject hypotheses, but to accumulate a line of evidence that may be consistent with a set of causal mechanisms.⁽⁷²⁾ Again, multi-causality and iterativity are emphasized.

In both cases, the need is to evaluate the probability of a set of alternative hypotheses given the data (observations), not the probability of the data given that a null hypothesis is true. A similar conceptual approach has been recently followed to analyze the influence of landscape in gene flow patterns.^(73,74) In the latter examples, alternative scenarios were tested using least-cost path modeling and partial Mantel tests as chosen statistic. The aim of these studies was to identify the combination of landscape factors that could bring out a causal model for the observed patterns of gene flow.

In all these examples, we usually want to know the probability of a series of alternative hypotheses being correct; comparisons were made using 'classic' statistical tests (e.g. partial Mantel tests in the 'causal modeling' studies). This pluralistic and multi-factorial approach is prone to be implemented in a Bayesian framework, since it entails the computing of conditional probability of a hypothesis given a set of observations. Bayesian inference is a powerful framework with which to improve hypothesis testing in

phylogeographic inference – and Bayesian inference is iterative in nature.

I have been arguing that iterativity must be a key component in phylogeography. This means that phylogeographic inference should not be envisaged as an end to itself. Phylogeographic inference is an evolutionary hypothesis that, ideally, must be empirically tested at all levels in the hierarchy of the system. This means that we need to re-evaluate the parameters and *a priori* information. The phylogeographic inference must be in keeping with the information available. The phylogeographic scenario inferred can challenge *a priori* assumptions on population structure, for instance. This would lead to a reassessment of the utility of the sampling design and the population models utilized. Eventually, this could imply the necessity of more fieldwork. The phylogeographic scenario could also challenge the 'organismal' or ecological information added to the analyses. Are the scenarios inferred consistent with individual survival rates, generation times or population densities, for instance? Again, the phylogeographic study could require a reassessment of the *a priori* information through a specific experimental study or specific fieldwork.

The cycles of testing hypotheses and reconsidering the initial information under the new light of the results do not imply that the phylogeographic inference cannot yield reliable estimate. On the contrary, this iterativity should be viewed as a potential strength of an integrative research program such as phylogeography. The phylogeographic inference, the working hypotheses, the models, and the information included in the

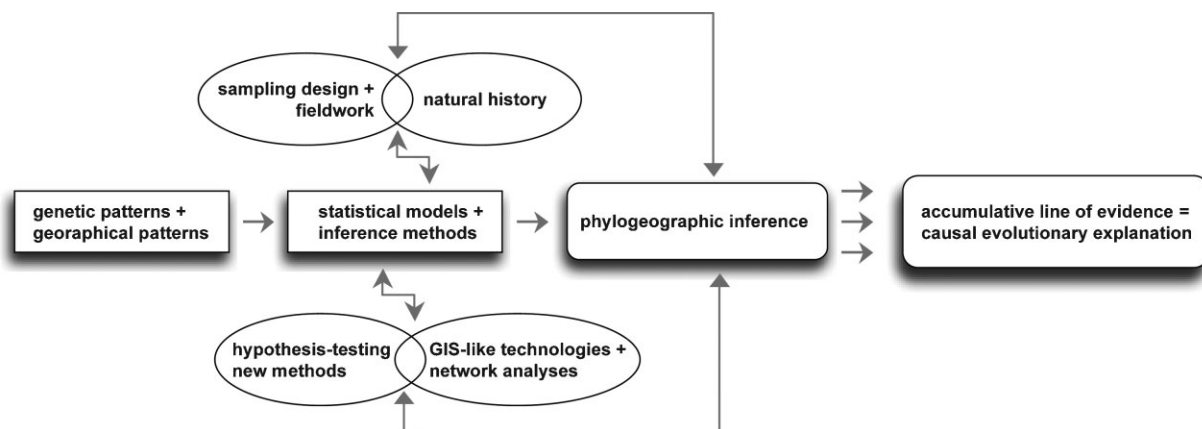


Figure 1. Phylogeographic scenarios are inferred through the analysis of the geographical distribution of genetic variants, using specific statistical models and inference methods. Given the specificity of the inference methods, the models used, and the variables involved, phylogeographic scenarios may be too simplistic and restrictive to capture the complexity of the evolutionary process. The explanatory power of phylogeography could be enhanced by (i) designing carefully the required fieldwork and explicitly discussing the sampling design, including also more information on the natural history of organisms in the study ('organismal phylogeography'); (ii) developing new analytical tools such as GIS-like technologies and network analyses, together with the development of new hypothesis-testing methods ('integrative phylogeography'); (iii) emphasizing the iterative nature of the phylogeographic research; the phylogeographic inference should be regarded as an evolutionary hypotheses amenable to reassessment through re-evaluation of the variables, the parameters and the analyses performed ('iterative phylogeography'). This would consolidate a robust line of evidences that would constitute the causal evolutionary explanation, the main purpose of the phylogeographic research program.

analysis, should be explicitly tested again, integrated then into the study, to generate new phylogeographic hypotheses. This is an iterative and hierarchical approach, so every new datum on any level could potentially modify the results obtained at any other level, encouraging the re-analysis of data under new perspectives. This would eventually lead to the accumulation of a solid body of evidences that would together conform the causal evolutionary explanation for the system studied.

Conclusions

Phylogeography needs to expand its scope, moving toward an organismal, integrative, and iterative approach (Fig. 1). Organisms must be at the center of phylogeographic inference. Organisms are not just a source of molecular data, but the real protagonists. Phylogeographic inferences (the historical explanation) cannot be solely based on statistical analyses of genetic variability. Explanatory power would be greatly increased by the incorporation of information from different levels of the biological hierarchy and development of new analytical tools like network analyses in an evolutionary context. The information is not just added, but integrated, and this is an iterative process.

Phylogeographic inferences are not the end points of the enquiry, but rather are evolutionary hypotheses that must be tested in the field, together with their predictions and the models used. The new tests provide more information that can be integrated in the analysis again, re-evaluating the initial hypotheses. The aim is to capture the complexity of the systems studied. It is necessary to move from the idea of deterministic laws to “non-prescriptive” laws for natural systems.⁽¹⁶⁾ Non-prescriptive laws define a realm of possibilities for a given system, the framework within which the system may evolve, without dictating unique specified ways of evolution. By moving from reductionism and determinism, and by incorporating unpredictability and complexity into the analysis, we will substantially improve our understanding of evolutionary processes.

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