

ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

ГЕОЛОГО-ГЕОГРАФСКИ ФАКУЛТЕТ

Книга 2 – ГЕОГРАФИЯ

Том 110

ANNUAL OF SOFIA UNIVERSITY “ST. KLIMENT OHRIDSKI”

FACULTY OF GEOLOGY AND GEOGRAPHY

Book 2 – GEOGRAPHY

Volume 110

PHYLOGEOGRAPHY AS A SUBDISCIPLINE OF BIOGEOGRAPHY

BORISLAV GRIGOROV, ASSEN ASSENOV

Department of Landscape Sciences and Environmental

Borislav Grigorov, Assen Assenov. PHYLOGEOGRAPHY AS A SUBDISCIPLINE OF BIOGEOGRAPHY

The current paper discusses the importance of phylogeography as a subdiscipline of biogeography and in that respect there exist certain connections between it and other biodiversity sciences. It deals with the principles and processes governing the geographical distributions of genealogical lineages, especially those at the intraspecific level. The beginning of phylogeography is in the late 1970s and 1980s, so it is relatively a young discipline. The cornerstone of phylogeography is the monumental book of John Avise, named ‘Phylogeography’ (2000) in which he underlines the main focus points, concerning the specifics of the discipline. Phylogeography is considered to represent a bridge between population genetics and systematics, therefore a central moment in this field is genetic research. This interdisciplinary science is most often applied to mitochondrial (mt) DNA and Y chromosome lineages, especially emphasizing on the importance of mtDNA. It has a special focus on conspecific populations and on genealogical information.

The present study summarizes some of the most important events in the history of phylogeography. Cameral methods are central to the present investigation and the method of comparative analysis is of special importance. The use of gene trees is another central moment in the field. Empirical phylogeographic patterns are discussed in the paper with the given examples of humpback whales (*Megaptera novaeangliae*), red-winged lackbird (*Agelaius phoeniceus*), American eel (*Anguilla rostrata*), and green sea turtles (*Chelonia mydas*). The investigation ends with the consideration of future development, prospects and issues of phylogeography, adding more value to the fact that the coalescent theory will continue to draw interest and a further expansion is expected.

Key words: mitochondrial DNA, gene trees, genetics, interdisciplinary field, coalescent theory.

INTRODUCTION

The synthetic character of biogeography as a scientific field, is a prerequisite for the formation of different kinds of subdisciplines and a change in the subject of the field in a

rapidly changing world. In order to fully understand the processes, influencing biodiversity and evolution, which are an inseparable part of the subject of biogeography we have to apply interdisciplinary knowledge, giving us access to geography, ecology, and history in their understanding of genetic variation in nature. The questions, concerning evolution remain modern in the present scientific world, and the geographic entity of evolution unites the past and the present of evolutionary biology. A couple of centuries ago Darwin and Wallace applied biogeography as a main instrument for proving evolution, while contemporary scientists use phylogeography as a tool for digging deeper into the subject of biogeography.

In the late 70s and 80s there was relatively little communication between biologists studying the microevolutionary and the macroevolutionary aspects of genetic patterns. The former's domain was population genetics, dealing with changes in population allele frequencies, resulting from mutation, genetic drift, gene flow, natural selection and sexual selection. The latter's was phylogenetics and systematics, while the specifics lay within evolutionary relationships of species and higher taxa. Phylogeography could be regarded as a branch of population genetics, considering the fact that it tends to refer mostly to studies of conspecific organisms and closely related species. However, it differs from classical population genetics by its special focus on genealogy (microphylogeny) and keeping this fact in mind it also could be regarded as a branch of phylogenetic biology. Yet, it departs from traditional phylogenetics by its special focus on population history and demography. Furthermore, systematists rarely considered historical population demography as relevant in reconstructing species phylogenies, but this does not change the fact that historical demographies of conspecific organisms have a considerable impact on how hereditary lineages are apportioned among populations and species during the evolutionary process. However, it differs from traditional biogeography by its special focus on conspecific populations and on genealogical information. It also differs from ecogeography by the fact that it concentrates on historical causation in addition to the selective forces and other ecological processes, operating across more contemporary timeframes. As it was already mentioned, there is a specific interrelationship between biogeography-phylogeography and landscape genetics and, following the work of Rissler (2016), there are some fundamental questions, regarding these fields, concerning: the scale of the reduction of gene flow, required to generate new species and the possibility of reduction of gene flow by geographic distance, natural selection; the presence of species' range limits in the absence of geographic barriers; the relative role of history versus contemporary processes on genetic patterns of species and communities; the making of a prediction regarding species' responses to climate change; the constraining of the evolution species' traits by phylogenetic history etc. This is also a good example of the place of phylogeography among other biodiversity sciences and their links.

The presented article is an example of the type of a current development. The aim of this overview paper is to unveil modern challenges in the research methodology of biogeography by using the emergence and development of phylogeography as a biogeographic subdiscipline.

OBJECT OF RESEARCH

The object in geographic papers is usually a part of planetary space, but in this case, the overview article uses a new, scientific subdiscipline, called phylogeography, as a research object.

Hickerson et al. (2010) state that if we literally interpret the term phylogeography, we'll find that it simply translates as the phylogenetic analysis of organismal data in the context of the geographic distribution of the organism. Yet, the meaning of "phylogeography" and the field that it describes is considerably more far-reaching. In its essence, phylogeography is an interdisciplinary science, a subdiscipline of biogeography, where molecular genetics, population genetics, phylogenetics, ethology demography, climatology, ecology, historical geography, archaeology, anthropology, linguistics, ethology and palaeontology integrate. According to Avise (1996, 1998) phylogeography is concerned with the principles and processes governing the geographical distributions of genealogical lineages, especially those at the intraspecific level, and emphasizes on historical aspects of the contemporary spatial distributions of gene lineages. If the field of population genetics is taken as an example, phylogeography refers to the phylogenetic relationships and the distributions of genetic lineages in the geographic sphere. This interdisciplinary science is most often applied to mitochondrial DNA and Y chromosome lineages. According to Sites and Morando (2009) phylogeography is the study of the spatial and temporal distribution of gene sequences in populations of a single species, or among closely related species. They consider phylogeographic studies to be drawing heavily on geology, palynology, GIS layers of environmental records, population biology etc., and share the opinion that phylogeographic studies are being increasingly applied to study multiple unrelated species that share the same geographic distributions, and a main goal is to identify shared signals of historical events (transgressions, regressions and glacial cycles) that contributed to population divergence and speciation in multiple groups. Phylogeography, as a subdiscipline of biogeography, is a part of geography as a science, despite the lack of researchers of geographic origin, dealing with the problems of phylogeography in Bulgaria. Furthermore, only phylogeography contains the term "geography" in its title, among the other subdisciplines of biogeography.

SUBJECT OF RESEARCH

A research of the historical origins and current development of phylogeography is conducted, following its investigative algorithm and connections with biogeography. The foundation of phylogeography extends back to the late 1970s and the 1980s and since then we observe a real 'phylogeographic revolution'. Today phylogeography is a relatively young research field, dating back to some 30 years. John Avise's remarkable work entitled 'Phylogeography' (Avise, 2000) can be considered as a cornerstone, setting the subsequent rapid developments in molecular techniques. 1997 marked the 10th year anniversary of its birth as a formal discipline, while the very beginning came with the introduction of mitochondrial (mt) DNA analyses to population genetics. There was a shift towards genealogical thinking at the intraspecific level, which is now formalized as a coalescent theory that these methods prompted. The term, that launched the field, arrived with a flourish in 1987 (Avise et al., 1987) and the main aim of the work was to unite evolutionary biologists in the disparate fields of phylogenetics and population genetics. Eleven years later Avise (1998) stressed on the fact that the appliance of phylogeography in evolutionary genetics studies has grown rapidly in the past decades. By 1996, more than 130 papers start using the term 'phylogeography' in their title or as an index word. Empirical phylogeographic analyses deal with the

spatial distributions within and among populations of alleles whose phylogenetic relationships are deduced. Because mitochondrial DNA evolves rapidly in populations of higher animals and usually is transmitted maternally without intermolecular recombination, it has been the workhorse of most of the phylogeographic studies conducted to date. The many distinctions yet connections between notions of phylogeny at the levels of genes vs. populations have made phylogeography a fruitful point of contact between population genetics and phylogenetic biology (Avice 1989; Hey 1994). At the same time, by becoming a major component of biogeography in the last decade, phylogeographic methods have been used to find the answers of many urgent questions. The major achievements of Avice and colleagues were illustrating shared biogeographical patterns in freshwater, terrestrial and marine taxa in Southeastern USA. There was also a remarkable success of phylogeographic methods in deciphering the Late Quaternary history of the European biota, which led to the conduction of similar studies, concerning terrestrial and marine environment in many parts of the globe. Some of the most crucial events in the history of phylogeography are summarized in Table 1 by pointing out several important years for the development of the field.

The phylogeographic studies concerning Bulgaria are mainly focused on the biological part of this field, while geography stays apart in some sense and the participation of Bulgarian scientists is too loose. Kucera et al. (2006) discuss the taxonomy and phylogeography of *Cardamine impatiens* and *Cardamine pectinata* (Brassicaceae) and share the opinion that their populations in Bulgaria are less diverse, and the area has most probably been colonized

Table 1
Important moments in the history of phylogeography (modified from Avice, 1998)

Time	Phenomenon
1974	Brown & Vinograd generate restriction site maps for animal mitochondrial DNAs
1975	Watterson describes some of the basic properties of gene genealogies, which is regarded as the beginnings of modern coalescent theory in the scientific field
1980	Brown provides an initial and very crucial report on human mitochondrial DNA variation
1986	Bermingham and Avice successfully manage to initiate comparative phylogeographic appraisals of mitochondrial DNA for multiple codistributed species
1987	Avice and colleagues coin the word 'phylogeography', define the field, and introduce several phylogeographic hypotheses, which are used as a basis for further investigations
1989	Slatkin and Maddison introduce a method for estimating interpopulation gene flow from the phylogenies of alleles
1990	Avice and Ball introduce principles of genealogical concordance as a component of phylogeographic assessment
1992	Avice successfully summarizes the first extensive compilation, involving multiple species and genetic assays, of phylogeographic patterns for a regional fauna
1994	Moritz promotes the conceptual distinction between 'shallow' vs. 'deep' intraspecific phylogenies by introducing the terms 'management units' and 'evolutionarily significant units'
1996	Avice and Hamrick are editors in Volumes, summarizing many roles for molecular phylogeographic analysis in conservation biology

postglacially. Hoffman et al. (2007) put a hint about the connection between the fire-bellied toads *Bombina* and Bulgaria. In 2010 Valcheva et al. conduct a research about Bulgarian specificity and controversial phylogeography of *Mycobacterium tuberculosis* and they conclude that its spoligotype ST125 is phylogeographically specific for Bulgaria. However, the particular geographic core of phylogeography in these papers hasn't been recognized in that thorough aspect, as it is in this paper. The lack of geographers, as well as biogeographers (whose number is under the number of ten in Bulgaria), in phylogeographic research in our country is due to the shortage of geographic interest in the terms "biogeography" and "biodiversity" and also due to the lack of horizontal connections in the scientific research of different disciplines. This is in contrast with the leading scientific research centers in the world and European indicators for sustainable development, and when it comes to Bulgaria, an obstacle is the existing scientific feudalism from the second half of the last century.

MATERIALS AND METHODS

The central method, applied in the present research, is the method of comparative analysis, which proved to be sufficient enough in the quest to understand the place of phylogeography as a subdiscipline of biogeography. An important role for the analysis of the connection between phylogeography and population genetics plays the work of Avise (2009) about the retrospect and prospect of phylogeography, where he worked on a summarization of the development of the discipline, as well as its future horizons and this investigation proves to be very useful in clarifying the place of this particular field among other biodiversity sciences.

This example of a connection between phylogeography and sciences, dealing with the problems of biodiversity, can be completed with the most comprehensive functional category of biodiversity – landscape diversity, which in fact leads phylogeography to the patterns of geography. Avise (2000) illustrates the connection between phylogeography, biogeography, historical geography, ecogeography and some evolutionary disciplines (Fig. 1).

RESULTS

Alongside with the historical review of the development of phylogeography and its place among other biodiversity sciences, some important breakthroughs and the current focus of the field, will also be regarded. The modern accomplishments in phylogeographic studies extend far beyond the increased statistical rigor that comes with greater computational refinement and molecular advances. The field has witnessed significant conceptual achievements, no matter if they concern inferred biogeographic and demographic processes or the testing of evolutionary and ecological questions that require a historical framework.

The use of gene trees and mitochondrial DNA (mtDNA) (Avise 2009) is indisputable, but the field has moved far beyond this singular approach and the original concept of bridging the traditionally separate disciplines of population genetics and phylogenetics. Today's phylogeographic studies employ a rich pallet of sophisticated methods, as well as molecular tools and more and more complex questions, integrating ecology and history, emerge. Linder (2017) resumes some of the latest studies in the phylogeographic field and states that the search for refuges and range expansion patterns becomes a main subject for investigation.

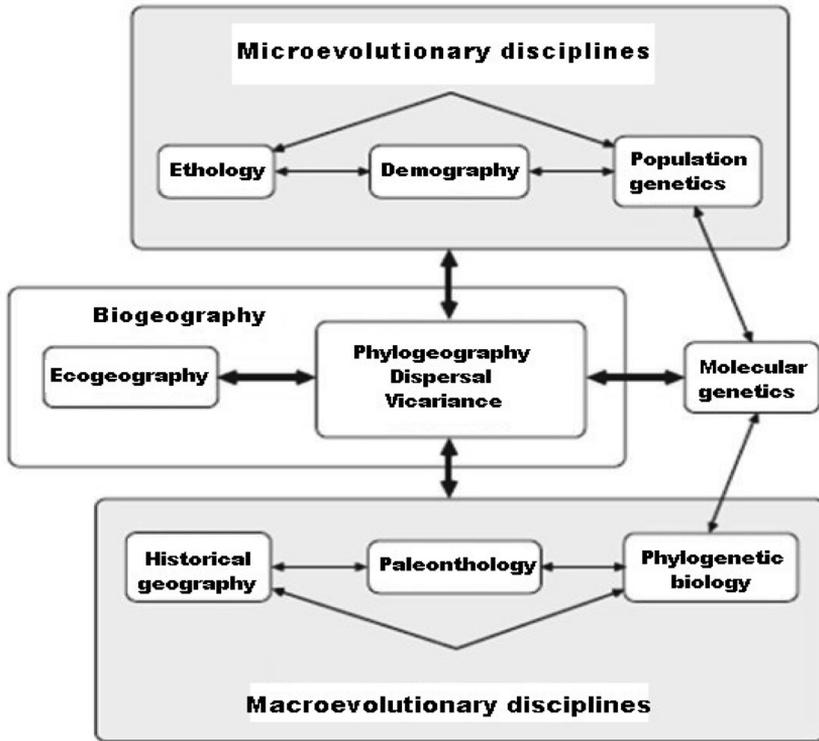


Fig. 1. Phylogeography and connections between biodiversity sciences (after Avise, 2000)

An example of such a study is the work of Gutierrez-Rodriguez et al. (2017), which focuses on southern Iberian refuges with northern expansion for the spadefoot toad, matching species distribution models, with patterns of genetic diversity. Alexandri et al. (2017) continue their exploration of Balkan refuges for wild boar, while Hantemirova et al. (2017) show that far northern Russian and Siberian populations of the common juniperus are largely descendent from populations, which survived in cryptic northern refuges, and that the contribution of southern refuges to these populations is relatively small. The territories where the Aleutian flora survived the Last Glacial Maximum remains unknown, and Hata et al. (2017) show that *Therorhodium camtschaticum* (Ericaceae) probably survived the Last Glacial Maximum on Kamchatka. It has long been suggested that during the Last Glacial Maximum, the African tropical rain forests were restricted to few refugia, and Ley et al. (2017) demonstrate that the genetic patterns in the liana *Haumania* are consistent with these refuges with sub-sequent range expansion and admixture. One of the latest research papers is the one of Habel et al. (2017), who combine morphological data with genetically informed phylogeographic methods to evaluate the divergence in the marbled butterfly species group in the Western Palaearctic.

The current phase in which phylogeography is a part of, is exciting and full of expectations. With regard to data analysis, the field is rapidly moving from descriptive methods into using coalescent models for parameter estimation (Kuhner, 2008), a priori model testing (Fagundes et al., 2007) and even estimation of spatially-explicit demographic histories (Lemmon & Moriarty Lemmon, 2008). Phylogeographic data is being visualized in more and more advanced and sophisticated ways. However, according to Hickerson et al. (2010), deciding which set of models to evaluate and use for parameter estimation remains to be a substantial challenge, when we take under account the perplexing number of possible histories underlying any phylogeographic data set.

Phylogeography has a revolutionary impact on geography and biology. Biogeographers are the ones who can put together evolutionary models of divergency among organisms with models of environmental change in the last millions of years. This is the way to overcome the lack of integration between historical biogeography and ecological biogeography. The place of phylogeography in the structure of biogeographic subdisciplines is presented in Fig. 2.

Phylogeographic scientific results are becoming more and more complex and elaborate and this is commonly incomprehensible for a scientist, representing only one field. The authors of the current study consider phylogeography as a subdiscipline of historical biogeogra-

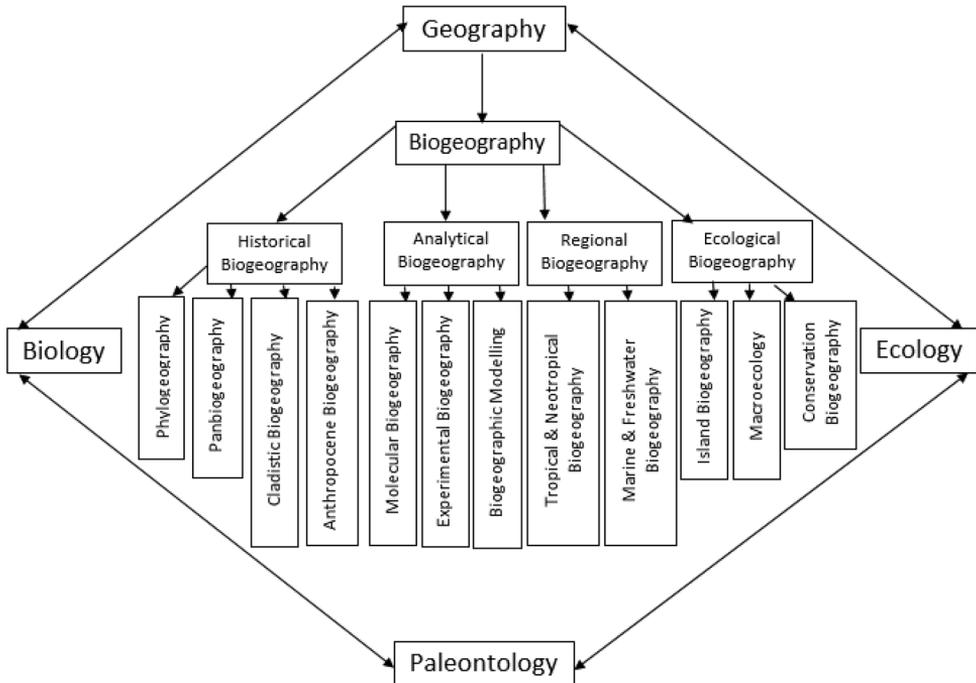


Fig. 2. Distribution of the basic subdisciplines in the structure of biogeography

phy (Fig. 2), and by the application of other subdisciplines, such as analytical biogeography, placed in the center of the scheme in Fig. 2, it will connect with ecological biogeography, remaining isolated since the time of de Candolle.

Coinciding with these methodological advances is the increasing number of opportunities for integrating comparative phylogeographic data with other fields that are also rapidly advancing. The palette includes spatial analysis of genomic signatures in natural selection (Joost et al., 2007), ecological niche models (ENMs) (Waltari et al., 2007), spatial analysis of morphological and functional trait evolution, studies of ecological speciation (Sluter, 2009), and studies of community assembly that make use of approximate Bayesian computation (ABC) (Jabot and Chave, 2009). This interdisciplinary synthesis has enormous potential, therefore comparative phylogeography is already achieving the original prediction of Avise and his colleagues – it is resolving deep-seated puzzles about how climate, geography, and ecological interactions determine and interact with community composition and evolution.

The coalescent theory now has a central role in phylogeographic research, but it took some time before statistical coalescent model-based methods, successfully penetrated into the empirical phylogeographic studies. Knowles and Maddison (2002) are the scholars who started using statistical phylogeographic methods more commonly and it is possible that the greatest impact of phylogeographic approaches has been on the most basic of biological questions, concerned with the meaning of the term species.

When it comes to single species phylogeographic studies, phylogeographic approaches can identify historical hybridization events, hybrid zones, occurrences of introgression (Swenson and Howard, 2005), as well as the geographic determinants of isolation. This can be applied to generate allopatric speciation hypotheses, which can be tested with genetic data in taxa that span the putative isolating barrier. When it comes to multi-species phylogeographic studies, it is obvious that comparative phylogeography has helped elucidate the relative influence of shared Earth history events on current patterns of biodiversity. Although Pleistocene climatic changes were considered as an important contributors to allopatric divergence and range expansion since the turn of the century, Avise (1987) presented this as one of the first results in the nascent stages of comparative phylogeographic research. It managed to predict range shifts arising from future climate change by allowing insights into the dynamics and persistence of communities during Pleistocene climate cycling. According to Hickerson et al. (2010) well-developed regional study systems include several regions of the world like the Australian wet tropics, southeastern North America, the North Atlantic, the Indo-Pacific coral triangle, California, the Pacific coast of North America, the neotropical rainforests, East Africa and Europe, and they stress on the fact that for some reasons southern hemispheric regions have received less attention.

The application of statistical approaches based on coalescent models for parameter estimation and hypothesis testing has been described as statistical phylogeography (Knowles and Maddison, 2002) and in scientific disciplines that investigate historical events, which cannot be observed directly or repeated under experimental conditions, scientists have always proceeded by considering competing hypotheses that serve as separate, but plausible explanations for a given phenomenon.

Although studies of natural selection could be integral to phylogeographic inference, they are usually omitted. Following the availability of genomic data for non-model organisms

(Vera et al., 2008), comparative phylogeographic studies will allow identification of different locus-specific divergent selection patterns between pairs of codistributed taxa.

As for the ecological speciation, researchers are able to uncover the genetic determinants of species boundaries using genetic markers, believed to be associated with reproductive isolation. This represents a great promise for comparative phylogeographic studies to unravel processes behind ecologically driven speciation and the genomic basis of selection, driving these patterns of ecological speciation. Ecologically driven selection is widely found in nature, however cases that entail full reproductive isolation are less common and largely depend on the strength of the divergent selection at single traits or depend on the number of traits at which selection is acting (Nosil et al., 2009).

Empirical systems are also important, as far as phylogeographic approaches and studies are concerned. For example, Moritz et al. (2000) implicated selection driven by environmental gradients as the cause of biogeographic diversity patterns in the tropics, and using emerging genomic tools for comparative phylogeography can help with the examination how selection drives biodiversity patterns in temperate regions that have experienced cyclical range expansions throughout the Pleistocene. Such studies are very useful for identifying and preserving geographic patterns of endemism (Moritz, 2002), as much as the determination whether populations are differentially adapted, is an important issue in conservation biology and restoration ecology (McKay et al., 2005).

The study of Avise (2009) about the retrospect and prospect of phylogeography deals with the essence of mitochondrial DNA and the coalescent concepts. It is important for the subject of the current research to be clarified that mtDNA owes its birth to a protobacterium, which, forced by external and internal factors, managed to merge with a pre-eukaryotic cell by a process, called endosymbiosis in the early history of life, and this event eventually led to the differentiation of eukaryotes. Figure 3 (by Avise, 2009) may be used as an example, illustrating the fact, that any organismal pedigree can be decomposed into numerous gene genealogies, which are analogous to the matrilineal and patrilineal trees. Males are represented by squares, while females are shown by circles and the drawn lines indicate the connection between parents and their offspring. Gene trees play an indispensable role to the emergence of phylogeography as a respected biogeographical subdiscipline.

Multiple populations, distributed across a certain type of a landscape, play a central role in phylogeographic studies, as Avise (2009) states. There are behavioural and/or physical obstacles to the dispersal among some of the populations and these hurdles are observed especially when it comes to species, which capability of movement is restricted and distance, as well as physical barriers (mountains, large rivers etc.) play an augmenting role in the development of the population's structure. Many organisms (winged insects, birds, open-water marine fishes or plant species with wind-dispersed pollen) possess a high dispersal ability, however, even such mobile species face barriers, which cannot be crossed. The Panamanian Isthmus is a perfect example of such a hurdle in historical time, blocking the inter-ocean gene flow between the Atlantic and the Pacific, and no surprisingly deep historical separations in molecular surveys of mtDNA and other genes can be observed in some species of fish, sea turtles etc. (Bermingham et al., 1997).

There are also organisms with high dispersal potential and they show evident population genetic structure, resulting from philopatry (they prefer specific locations) rather than

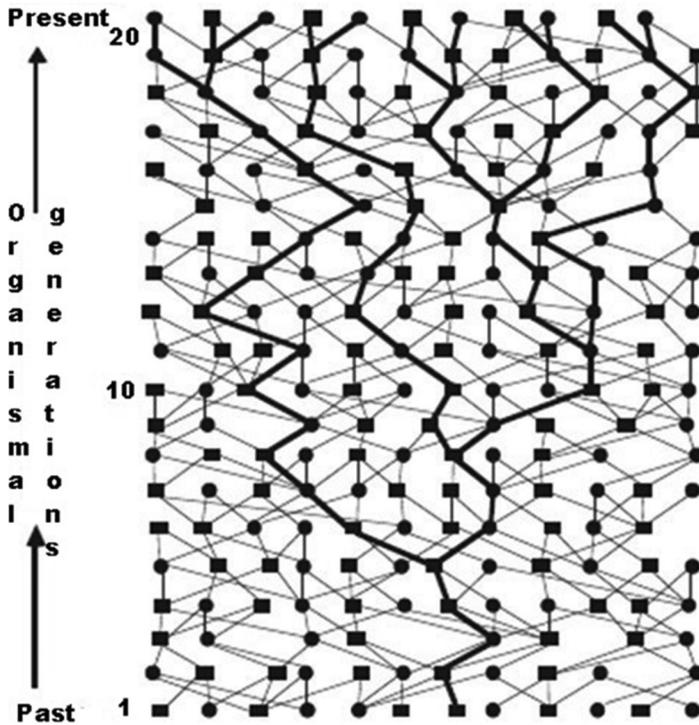


Fig. 3. A gene tree or haplotype tree within a 20-generation organismal pedigree

from physical barriers. Among the cetacean species, there are several, which are matrilineal (socially organized around female kin), one expected genetic signature being a matrilineal population genetic structure. In mtDNA surveys of humpback whales (*Megaptera novaeangliae*) populations within each of three ocean basins did indeed prove to be subdivided into matrilineal stocks and the reason for this is that they probably arose from male fondness of particular migratory destinations and are maintained by it (Baker et al., 1993).

Species with similar ranges or ecologies are also genealogically structured in certain ways, which suggests that biogeographical forces that shaped them, have an impact on the genetic structure of particular regional biotas. The fact that historical processes have also impacted biotic communities are clearly represented if flora and fauna of Europe are taken as an example. The Balkans, the Apennine Peninsula and the Iberian Peninsula are the three southern major European regions that were not covered by Pleistocene ice masses. Many animal and plant species inhabited these refugia and they eventually recolonized the continent in modern time. Phylogeographic trails are present today in the spatial arrangements within many species of distinct genealogical clades that trace back through numerous colonization routes to these Pleistocene refugia.

DISCUSSION

Phylogeographic perspectives have added many different insights into evolutionary genetic processes since the birth of this scientific field several decades ago. Phylogeography is in a phase of rapid growth and many more empirical studies can be anticipated in order to broaden the field's perspective. As a central moment in the phylogeographic paradigm, coalescent theory will continue to draw interest and a further expansion in the utility of the theory, as a formal conceptual thread for tying together more coherently the micro- and macroevolutionary disciplines of population genetics and phylogenetics, is expected. Three areas in the field of phylogeography are set for expansion and each of them is tied to an aspect of genealogical concordance: concordance in significant genealogical partitions across multiple unlinked loci within species; concordance in the geographical positions of significant gene-tree partitions across multiple codistributed species; concordance of molecular gene-tree partitions with geographical boundaries between traditionally recognized biogeographic provinces (Avice, 1996; 1998; 2007).

Most phylogeographic analyses to date have focused primarily on cytoplasmic genomes, which is good, but it is much easier to be achieved, however, advancements in the field may depend on genealogical appraisals of the nuclear genome, which is much more difficult for harvesting.

The second area's relevancy is bound to the strong implication of shared historical biogeographic factors in the formation of the deeper intraspecific phylogenies, especially when it comes to a regional scale and, unfortunately, until now only a few empirical molecular studies have attempted comparative phylogeographic assessments of a variety of codistributed species, because such studies offer the greatest hope for advances in discovering the influences on intraspecific phylogeographic patterns.

The last area's practicability lays in the strong implication of shared historical biogeographic factors as formers of intraspecific organismal phylogenies and patterns of distribution of species. This is the right way to connect phylogeography and especially the molecular aspect of it to other disciplines, such as historical geography, phylogenetic biology and, of course, ecology. Results of conducted studies suggest that historical factors influence species compositions in regional communities, as well as the spatial distributions of genealogically distinct populations within species.

An expansion of the coalescent theory is also advisable, because it has developed primarily with a single-locus orientation, therefore there is a room for addressing interlocus variances in coalescent outcomes as a function of various genetic factors. A meaningful multi-locus coalescent theory also will have to have room for the wide variety of spatial population architectures in nature.

Phylogeographic analyses of additional taxa should also be extended, because only a small part of the biological world has been studied from the perspective of phylogeography. A major research focus should also be placed on comparative phylogeographic appraisals of multi-species regional biotas.

Following Knowles' study about statistical phylogeography (2009), phylogeography will undoubtedly face complications and issues in the not so distant future. For instance, a plausible way for exploring the universe of potential histories, such that their relative probabili-

ties, are considered jointly, as opposed to choosing specific models that might be compared or analyzed sequentially, will have to be investigated. Another example is bound with the needed integration of genomic data (which includes analysis of markers and considers other genomic attributes, such as the chromosomal position) into a phylogeographic framework such that the interplay between demographic processes and selection can be tested. At the same time a framework is needed, in which the potential gains of model complexity can be evaluated relative to the cost of applying complex models and last, but not least, the creation of a synthesis of comparative information across taxa will be an invaluable asset in the struggle for evaluating different historical and ecological theories about diversity patterns and community assembly from different regions of the world and ecosystems.

Phylogeography has had a remarkable start and the greatest benefits and opportunities for the field will continue to rise.

CONCLUSIONS

Phylogeography, which is regarded as a product of historical biogeography, ecology and evolutionary biology, has experienced a rapid evolution in the past decades and unsurprisingly there is more to be done in order to expand the phylogeographic horizon. The field is a subdiscipline of biogeography and it has many blank spots, especially when it comes to its development in Bulgaria. Undoubtedly, phylogeography is of a major importance for Bulgarian biogeography and the evolution of these disciplines is bound together. There are many horizontal connections, which can be implemented, particularly with the fields of genetics, conservation biology and ecology, and more efforts should be put in that direction. The authors of the article stand for the fact, that the increase of the geographic element in phylogeographic research will be more rapid if GIS-instruments are implemented.

REFERENCES

- Alexandri, P., H. Megens, R. Crooijmans, M. Groenen, D. Goedbloed, J. Herrero-Medrano, L. Rund, L. Schook, E. Chatzinikos, C. Triantaphyllidis & A. Triantafyllidis. 2017. Distinguishing migration events of different timing for wild boar in the Balkans. – *Journal of Biogeography*, 44, 259–270.
- Avise, J., J. Arnold, R. Ball, E. Bermingham, T. Lamb, J. Neigel, C. Reeb, N. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. – *Annual Review of Ecology and Systematics*, 18, 489–522.
- Avise, J. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. – *Evolution*, 43, 1192–1208.
- Avise, J., R. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. – *Oxford Surveys in Evolutionary Biology*, 7, 45–67.
- Avise, J. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. – *Oikos*, 63, 62–76.
- Avise, J. 1996. Space and time as axes in intraspecific phylogeography. – In: Past and Future Rapid Environmental Changes: The Spatial and Evolutionary Responses of Terrestrial Biota (eds. Huntley B, Cramer W, Morgan AV, Prentice HC, Allen JRM), 381–388. Springer-Verlag, N. Y.
- Avise, J., J. Hamrick (eds.). 1996. Conservation Genetics: Case Histories from Nature. Chapman & Hall, N. Y.
- Avise, J. 1998. The history and purview of phylogeography: a personal reflection. – *Molecular Ecology*, 7, 371–379.
- Avise, J. 2000. Phylogeography: the history and formation of species. Harvard University Press, London, 453 p.
- Avise, J. 2007. Twenty-five key evolutionary insights from the phylogeographic revolution in population genetics. Phylogeography of southern European refugia (ed. by S. Weiss and N. Ferrand), 7–21. Springer, Dordrecht.

- Avice, J. 2009. Phylogeography: retrospect and prospect. – *J. Biogeogr.*, 36, 3–15.
- Baker, C., A. Perry, J. Bannister, M. Weinrich, R. Abernethy, J. Calambokidis, J. Lien, R. Lambertsen, J. Urban Ramirez, O. Vasquez, P. Clapham, A. Alling, S. O'Brien, S. Palumbi. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences USA*, 90, 8239–8243.
- Bermingham, E., J. Avise. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. – *Genetics*, 113, 939–965.
- Bermingham, E., S. McCafferty, A. Martin. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. *Molecular systematics of fishes* (ed. by T.D. Kocher and C.A. Stepien), 113–128. Academic Press, San Diego, CA.
- Brown, W., J. Vinograd. 1974. Restriction endonuclease cleavage maps of animal mitochondrial DNAs. – *Proc. Acad. Sci. U.S.A.*, 71, 4617–21.
- Brown, W. 1980. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. – *Proc. Natl. Acad. Sci. USA* 77, 3605–3609.
- Fagundes, N., N. Ray, M. Beaumont, S. Neuenschwander, F. Salzano, S. Bonatto, L. Excoffier. 2007. Statistical evaluation of alternative models of human evolution. – *Proc. Natl. Acad. Sci. USA* 104, 17614–17619.
- Gutierrez-Rodriguez, J., A. Barbosa, I. Martinez-Solano. 2017. Present and past climatic effects on the current distribution and genetic diversity of the Iberian spadefoot toad (*Pelobates cultripedis*): an integrative approach. – *Journal of Biogeography*, 44, 245–258.
- Habel, J., R. Vila, R. Voda, M. Husemann, T. Schmitt, I. Dapporto. 2017. Differentiation in the marbled white butterfly species complex driven by multiple evolutionary forces. – *Journal of Biogeography*, 44, 433–445.
- Hantemirova, E., B. Heinze, S. Knyazeva, A. Musaev, M. Lascoux, V. Semerikov. 2017. A new Eurasian phylogeographical paradigm? Limited contribution of southern populations to the recolonization of high latitude populations in *Juniperus communis* L. (Cupressaceae). – *Journal of Biogeography*, 44, 271–282.
- Hata, D., H. Higashi, V. Yakubov, V. Barkalov, H. Ikeda, H. Setoguchi. 2017. Phylogeographical insight into the Aleutian flora inferred from the historical range shifts of the alpine shrub *Therorhodium camtschaticum* (Pall.) Small (Ericaceae). – *Journal of Biogeography*, 44, 283–293.
- Hey, J. 1994. Bridging phylogenetics and population genetics with gene tree models. – In: *Molecular Ecology and Evolution: Approaches and Applications* (eds. Schierwater B, Streit B, Wagner GP, DeSalle R), 435–449. Birkhäuser Verlag, Basel, Switzerland.
- Hickerson, M., B. Carstens, J. Cavender-Bares, K. Crandall, C. Graham, J. Johnson, L. Rissler, P. Victoriano, A. Yoder. 2010. Phylogeography's past, present, and future: 10 years after Avice, 2000. – *Molecular Phylogenetics and Evolution*, 54, 291–301.
- Hoffman, S., C. Spolsky, T. Uzzell, D. Coganiceanu, W. Babik, J. Szymura. 2007. Phylogeography of the fire-bellied toads *Bombina*: independent Pleistocene histories inferred from mitochondrial genomes. – *Molecular Ecology*, 16, 2301–2316.
- Jabot, F., J. Chave. 2009. Inferring the parameters of the neutral theory of biodiversity using phylogenetic information and implications for tropical forests. – *Ecol. Lett.*, 12, 239–248.
- Joost, S., M. Bruford, L. Després, C. Conord, G. Erhardt, P. Taberlet. 2007. A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. – *Mol. Ecol.*, 16, 3955–3969.
- Knowles, L., W. Maddison. 2002. Statistical phylogeography. *Mol. Ecol.*, 11, 2623–2635.
- Knowles, L., 2009. Statistical phylogeography. – *Annu. Rev. Ecol. Evol. Syst.*, 40, 593–612.
- Kucera, J., J. Lihova, K. Marhold. 2006. Taxonomy and phylogeography of *Cardamine impatiens* and *C. pectinate* (Brassicaceae). – *Botanical Journal of the Linnean Society*, 152, 169–195.
- Kuhner, M. 2008. Coalescent genealogy samplers: windows into population history. – *Trends Ecol. Evol.*, 24, 86–93.
- Lemmon, A., Moriarty Lemmon. 2008. A likelihood framework for estimating phylogeographic history using geographically continuous genetic data. – *Syst. Biol.*, 57, 544–561.
- Ley, A., M. Heuertz, O. Hardy. 2017. The evolutionary history of central African rain forest plants: phylogeographical insights from sister species in the climber genus *Haumania* (Marantaceae). – *Journal of Biogeography*, 44, 308–321.
- Linder, H. 2017. Phylogeography. – *Journal of Biogeography (J. Biogeogr.)*, 44, 243–244.
- McKay, J., C. Christian, S. Harrison, K. Rice. 2005. How local is local? – A review of practical and conceptual issues in the genetics of restoration. – *Restor. Ecol.*, 13, 432–440.
- Moritz, C. 1994. Defining “Evolutionary Significant Units” for conservation. – *TREE* vol. 9, Elsevier Science Ltd.
- Moritz, C., J. Patton, C. Schneider, T. Smith. 2000. Diversification of rainforest faunas: an integrated molecular approach. – *Annu. Rev. Ecol. Syst.*, 31, 533–563.
- Moritz, C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. – *Syst. Biol.*, 51, 238–254.

- Nosil, P., L. Harmon, O. Seehausen. 2009. Ecological explanations for (incomplete) speciation. – *Trends Ecol. Evol.*, 24, 145–156.
- Rissler, L. 2016. Union of phylogeography and landscape genetics. vol. 113 no. 29, 8079–8086, doi: 10.1073/pnas.1601073113
- Schluter, D. 2009. Evidence for ecological speciation and its alternative. – *Science*, 323, 737–741.
- Sites, J., M. Morando. 2009. Phylogeography. – In: eLS. John Wiley & Sons Ltd, Chichester. <http://www.els.net> [doi: 10.1002/9780470015902.a0003352]
- Slatkin, M., W. Maddison. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. – *Genetics.*, 123(3), 603–13.
- Swenson, N., D. Howard. 2005. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. – *Am. Nat.*, 166, 581–591.
- Valcheva, V., I. Mokrousov, S. Panaiotov, E. Bachiiska, T. Zozio, C. Sola, N. Markova, N. Rastogi. 2010. Bulgarian specificity and controversial phylogeography of *Mycobacterium tuberculosis* spoligotype ST125_BGR. – *FEMS Immunol Med Microbiol*, 59, 90–99.
- Vera, C., C. Wheat, H. Fescemyer, M. Frilander, D. Crawford, I. Hanski, J. Marden, 2008. Rapid transcriptome characterization for a non-model organism using massively parallel 454 pyrosequencing. – *Mol. Ecol.*, 2371.
- Waltari, E., R. Hijmans, A. Peterson, A. Nyári, S. Perkins, R. Guralnick. 2007. Locating pleistocene refugia: comparing phylogeographic and ecological niche model predictions. PLoS One 2, e563. doi: 10.1371/journal.pone.0000563.
- Watterson, G. 1975. On the number of segregating sites in genetical models without recombination. – *Theor. Popul. Biol.* 7(2), 256–76.

Received April 2017