GUEST EDITORIAL

Mapping the evolutionary twilight zone: molecular markers, populations and geography

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ABSTRACT

Since evolutionary processes, such as dispersal, adaptation and drift, occur in a geographical context, at multiple hierarchical levels, biogeography provides a central and important unifying framework for understanding the patterns of distribution of life on Earth. However, the advent of molecular markers has allowed a clearer evaluation of the relationships between microevolutionary processes and patterns of genetic divergence among populations in geographical space, triggering the rapid development of many research programmes. Here we provide an overview of the interpretation of patterns of genetic diversity in geographical and ecological space, using both implicit and explicit spatial approaches. We discuss the actual or potential interaction of *phylogeography*, *molecular ecology*, *ecological genetics*, *geographical genetics*, *landscape genetics* and *conservation genetics* with biogeography, identifying their respective roles and their ability to deal with ecological and evolutionary processes at different levels of the biological hierarchy. We also discuss how each of these research programmes can improve strategies for biodiversity conservation. A unification of these research programmes is needed to better achieve their goals, and to do this it is important to develop cross-disciplinary communication and collaborations among geneticists, ecologists, biogeographers and spatial statisticians.

Keywords

Biogeography, conservation biogeography, conservation genetics, geographical genetics, landscape genetics, molecular ecology, molecular markers, phylogeography, population structure.

INTRODUCTION

Ecological and evolutionary processes, such as adaptation, dispersal and genetic drift, necessarily occur in a geographical context. Consequently, biodiversity patterns are usually strongly structured in space, so that biogeography provides a central and important unifying framework for understanding life on Earth (Lomolino & Heaney, 2004). Also, as a consequence of evolutionary dynamics and the continuity of life through time, biodiversity is hierarchically structured so that patterns and processes should be investigated within a framework of spatial and temporal scales (Whittaker *et al.*, 2001).

As a strongly integrative research area, biogeography aims to evaluate both ecological and historical processes that influence and account for the geographical distribution of organisms at different levels of the biological hierarchy. However, due to operational problems, most studies have traditionally focused on species, higher taxonomic levels or even higher aggregates in the ecological hierarchy (e.g. biomes). This picture has changed dramatically in the last few decades owing to the development of multiple types of molecular markers, so that now ecologists, biogeographers and evolutionary biologists routinely use the hereditary information contained in biological macromolecules (proteins and, more frequently now, nucleic acids) to address questions ranging from organismal behaviour up to broad-scale richness patterns at continental levels (Sunnucks, 2000; Avise, 2004).

Nonetheless, the population level of analysis, at which microevolutionary and macroevolutionary processes interact, remains particularly challenging. Understanding patterns and processes at this level has been considered crucial to the finding

of conceptually sound and operational species concepts, allowing more coherent basic biological 'units' to be defined (Isaac *et al.*, 2004; Sites & Marshall, 2004). It has also been called the taxonomic 'line of death' (see Nixon & Wheeler, 1990; Davis & Nixon, 1992), because the reticulation of character states creates problems for inferences based on Hennigian cladistics. Although the use of uniparentally inherited DNA molecular markers, such as mitochondrial DNA (mtDNA) or chloroplast DNA (cpDNA), has minimized this problem, there is still much debate about how molecular markers deal with 'gene trees' or 'species trees' (see Pamilo & Nei, 1988; Page & Holmes, 1998). For example, this discussion is now at the core of the recent debate about the genetic evidence for alternative models of the evolution of modern humans (Fagundes *et al.*, 2007; Templeton, 2007). Although many recent and integrated analytical and theoretical developments have allowed a better understanding of patterns and processes of population differentiation, we believe that this intraspecific level remains a 'twilight zone' defying the imagination of ecologists, geneticists and conservation biologists, and driving them to look for historical and ecological mechanisms triggering variation at multiple levels of biological hierarchy.

The first theoretical framework for the study of intraspecific patterns of genetic differentiation was provided by the field of *population genetics*, which we define here as the development of mathematical models in the first phase of the 'synthetic theory of evolution' led by R. A. Fisher (b. 1890, d. 1962), Sewall Wright (b. 1889, d.1988), J. B. S. Haldane (b. 1892, d. 1964), G. Malécot (b. 1911, d. 1998) and others (see Provine, 2001; Gould, 2002), complemented by the development of the 'neutral theory of molecular evolution' proposed by M. Kimura in the 1960s (Kimura, 1983; see also Ohta & Aoki, 1985). An initial merging of genetics and systematics started a bit later (Avise, 1974; reviewed in Thorpe & Solé-Cava, 1994), firmly grounded on work by Ernst Mayr (b. 1904, d. 2005) and Theodosius Dobzhansky (b. 1900, d. 1975), among others, and on many theoretical and methodological developments of the quantitative systematics (both cladistics and phenetics) that happened in the 1950s and 1960s (Page & Holmes, 1998; Felsenstein, 2004). These developments stimulated the application of new molecular analyses within empirical studies. The greatest development of the field, however, occurred as a result of the coupling of two important technological developments: the financial and technological feasibility of the quick generation of DNA data, and the development of fast personal computers along with powerful new algorithms for phylogenetic and population-level analyses (Hillis *et al.*, 1996; Nei & Kumar, 2000; Felsenstein, 2004).

There are many advantages in using these molecular phylogenies to establish systematic relationships and test biogeographical hypotheses (e.g. Johnson *et al.*, 2006), and this fusion is sometimes called *molecular biogeography* (Schmitt & Hewitt, 2004; Heads, 2005; Brooks, 2006; Moodley & Bruford, 2007). At lower hierarchical levels, the most important advance was that, through the empirical investigation of

phylogenetic patterns of individuals within species, or among closely related species, the new field of *phylogeography* emerged (Avise *et al.*, 1987). This approach was based on the use of molecular phylogenies inferred from uniparentally inherited DNA and allowed the discrimination between patterns produced by contemporary (population structure) and historical (population genealogy) processes (Templeton, 2004). This became possible through the development of coalescence theory and genetic–demographic connections (Tavaré, 1984; Avise, 2000; Edwards & Beerli, 2000; Slatkin & Veuille, 2002). Phylogeography is currently the most popular way to evaluate spatial patterns in genetic data. In illustration, out of 87 papers based on molecular data published in the *Journal of Biogeography* between 2005 and 2007, 43 (49.4%) used a standard phylogeographical approach (*sensu* Avise, 2000).

Nevertheless, there are other research programmes that explicitly incorporate a geographical dimension into modelling or inferential processes. Indeed, the geographical and genealogical dimensions were explicitly incorporated, albeit crudely, into early population genetics theory during the 1930s and 1940s, as exemplified by Sewall Wright's isolation-by-distance (IBD) model (Wright, 1943), or Motoo Kimura's stepping-stone model (Kimura & Weiss, 1964), both of which predict an exponential-like decrease in genetic divergence with increasing geographical distances among individuals or local populations. Since these pioneering works, many theoretical models have been developed that incorporate a geographical dimension into genetic analyses, and that also take into account genealogy and phylogenetic patterns (e.g. Epperson, 2002; Rousset, 2004).

Even more importantly, the availability of molecular data led to an increase in the number of overlapping research programmes that now use molecular tools to empirically evaluate the geographical context of biodiversity patterns by linking genetics, ecology and biogeography. Here we provide an overview of these fields, focusing on their actual or potential interaction with biogeography (i.e. implicit or explicit association with geographical patterns in biological diversity), identifying their respective roles and their ability to deal with ecological and evolutionary processes at different levels of the biological hierarchy.

A PLETHORA OF RESEARCH PROGRAMMES

Starting from a combination of ideas coming from basic fields in biological sciences (i.e. biogeography, systematics and population biology), the molecular revolution allowed the origin of a plethora of approaches to further our understanding of the geographical and historical components of genetic diversity, with slightly different purposes and overlapping research interests (Fig. 1, Table 1). Generally, their goals include the merging of genetics, ecology and evolution into a joint theoretical and methodological framework. The development of these multiple fields was at least in part methodologically driven and was associated with the increasing availability of powerful computational facilities and software (see Excoffier & Heckel, 2006). On the other hand, the
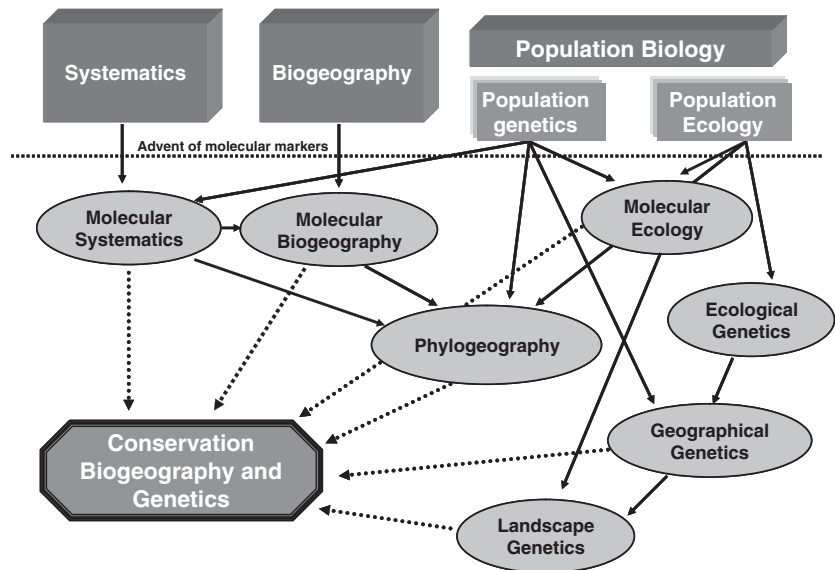
Figure 1 Schematic relationship among the research programmes discussed here (solid arrows) and their relationship with biodiversity conservation (conservation biogeography and genetics) (dotted arrows). See Table 1 and text for details. Rectangles represent broad and traditional research fields, which, after the development and popularization of the use of molecular markers, originated the research programmes discussed in the text (ellipses).

differences in concepts and methods used in each of these fields are due to particular circumstances, including contingencies in the history of science. Thus, there is plenty of opportunity to combine them in the near future. Below we will provide a brief overview of these fields, showing their relationships and common grounds suitable for integration.

Intraspecific differentiation and phylogeography

The simplest approach to evaluating geographical patterns of genetic diversity is by the use of inbreeding (or fixation) indices, such as F_{ST} and related approaches (e.g Φ_{ST} from analysis of molecular variance (AMOVA; see Excoffier *et al.*, 1992). In these methods, the purpose is to evaluate *population structure*, and this is usually done by partitioning the genetic variance into 'within population' and 'among population' components, implying that 'populations' are spatially separated. This approach is based on classic population genetics theory and was very popular in the late 20th century. It is usually applied as a first stage in the description of genetic diversity and commonly used together with more complex approaches. However, it does not really deal with geographical structure, except in the sense that 'populations' (distributed in geographical space and/or evolutionary time) exist, or are assumed to exist. The correlation between pairwise F_{ST} and geographical distances between (pairs of) local populations, assessed with Mantel tests (see below), is still used as important evidence for IBD. Thus, it can be considered as a first step towards an explicit analysis of spatial patterns of population genetic structure (Pérez-Losada *et al.*, 2007; Werth *et al.*, 2007; see below for a discussion on geographical genetics). However, the 'geographical' genetic structure measured by F_{ST} and related statistics may have nothing to do with current patterns of gene flow or microevolutionary processes, since F_{ST} does not discriminate between current and past levels of genetic connectivity (Whitlock & McCauley, 1999; Pearse & Crandall,

2004; but see Neigel, 2002). One of the main drawbacks of the use of these 'frequentist' approaches to the analysis of geographical population structure is the need to define a priori the populations from which to calculate the allele frequencies for each gene studied. This is often done by arbitrarily defining a 'population' as the organisms collected near or at a sampling site.

Multiple hierarchical levels based on geographical classifications can be incorporated into AMOVA, such as grouping populations into broader regions: such analyses can provide a further step towards the explicit spatial analysis of population genetic structure (e.g. Robledo-Arnuncio *et al.*, 2005). An initial way to visualize geographical patterns of genetic diversity is by '*a posteriori*' mapping of groups of populations defined by molecular analyses. Although it was already possible to map results from multivariate approaches applied to genetic data, such as clustering of pairwise genetic distances or principal axes of allele frequencies (the so-called synthetic maps; e.g. Cavalli-Sforza *et al.*, 1994), the idea of understanding genetic patterns in geographical space became more popular with the creation of the new field of *phylogeography* (Avise *et al.*, 1987; Avise, 2000; see also Riddle & Hafner, 2004).

Semantically, phylogeography refers to any evaluation of geographical patterns in the distribution of clades, regardless of how these clades are defined (both in a methodological sense and in terms of level in the biological hierarchy). However, in a narrow sense, phylogeography developed as the study of '…processes governing the geographic distributions of genealogical lineages, especially those within and among closely related lineages' (Avise, 2000; p. 3). At this hierarchical level (i.e. at or lower than closely related species), this has been mainly feasible through the analysis of uniparentally inherited and non-recombining molecular markers, especially the mtDNA in animals and cpDNA in plants, because this solves the reticulation problem of Hennigian cladistics within species.

Table 1 A summary of the main characteristics of the research programmes discussed in this paper, including an overall definition, the taxonomic level usually analysed, the geographical extent (ranging from local to global) encompassed and how geographical dimension is considered (I, implicitly; E, explicitly), the main statistical tools, and some basic references.

Research programme	Definition and aims	Systematic level	Geographical extent and analysis (I/E)	Analytical tools	References
Molecular biogeography	The use of molecular phylogenies to establish systematic relationships that allow a more refined test of biogeographical hypotheses at broad-scales	Higher taxa	Regional to global (I)	Tree-building methods	Moodley & Bruford (2007)
Phylogeography (and comparative phylogeography)	The study of processes governing the geographical distributions of genealogical lineages, especially those within and among closely related lineages and usually based on uniparentally inherited genes (mtDNA or cpDNA)	Variation within species (or among closely related species)	Regional to global (I)	Tree-building methods	Avise et al. (1987), Avise (2000)
Population structure (intraspecific differentiation)	A first analytical derivation from classical theoretical population genetics that aims to partition genetic variability within and among populations	Within and among 'local' populations	Local to global (I)	Variance partitioning (F_{ST}, AMOVA, analysis of variance of allele frequencies)	Hillis et al. (1996), Nei & Kumar (2000)
Ecological genetics	Aims to evaluate the genetics of ecologically important phenotypic traits and their underlying adaptive processes	Various (but typically within species)	Local to global (I/E)	Various	Conner & Hartl (2004)
Molecular ecology	Is the application of molecular genetics methods to solve ecological problems (it may encompass all other research programmes)	Various (but typically within species)	Local to global (I/E)	Various	Beebee & Rowe (2004)
Geographical genetics	The analyses of spatial patterns of genetic diversity using spatial statistics and the inference of evolutionary processes underlying them	Individuals (and eventually local populations) within species	Local to continental* (E)	Multivariate analysis of genetic distances, spatial autocorrelation, Mantel tests	Sokal & Oden (1978a,b), Epperson (2003)
Landscape genetics	Proposes to integrate genetic data and more complex aspects of landscape composition and configuration in a spatially explicit metapopulation context	Individuals (and eventually local populations) at landscape scale	Landscape (E)	Spatial autocorrelation, Mantel tests, assignment tests, discontinuity methods (Monmonier, wombling)	Manel et al. (2003), Storfer et al. (2007)
Conservation biogeography	The application of biogeographical principles, theory and analyses, being those concerned with distributional dynamics of taxa individually and collectively, to problems concerned with conservation of biodiversity	Various	Local to global (I/E)	Various	Whittaker et al. (2005)
Conservation genetics	The application of theoretical and analytical developments in genetics to preserve species as dynamic entities capable of coping with environmental change	Various	Local to global (I/E)	Various	Avise & Hamrick (1996), Frankham et al. (2003)

*But see Handley et al. (2007) for a recent global analysis.

Thus, use of these markers helps in discriminating between current vs. historical gene flow and in the separation between population 'structure' and population 'history' (*sensu* Templeton *et al.*, 1995). In that sense, phylogeography provides a bridge between micro- and macroevolution by seeking to understand how demographic processes of population growth and geographical dispersal, as well as genetic isolation in time and space, trigger speciation events and create broad-scale signatures in the genetic diversity within species or in sets of closely related species.

A more direct link with 'historical' biogeography (see Crisci *et al.*, 2003) can be established if, in a given region, phylogeographical patterns are concordant among several species, in a field usually called *comparative phylogeography* (see Arbogast & Kenagy, 2001; Riddle & Hafner, 2004). This approach allows one to recognize whether multiple groups of organisms distributed in the same region share patterns of geographical genetic structure, presumably due to a common set of vicariance events. If phylogeographical patterns evaluated for several species are coincident, then common processes of population differentiation can be inferred, and this can be directly associated, in a methodological sense, to the construction of area cladograms in cladistic biogeography (see Crisci *et al.*, 2003). Thacker *et al.* (2007) analysed the correspondence of phylogeographical patterns of five *Hypseleotris* fish species in Australia and showed that they are not congruent with previously established relationships among eastern Australian provinces, thus indicating a more complex pattern of divergence among river basins.

At the same time, phylogeography could be linked with other well-studied patterns in 'ecological' biogeography, such as the ecogeographical rules, especially when they are evaluated at intraspecific levels (for recent reviews and discussion see Lomolino *et al.*, 2006; Meiri *et al.*, 2007; Gaston *et al.*, 2008). If two lineages within a species are historically separated across an environmental gradient, phylogeographical analyses could help in establishing and dating those historical events, whereas ecogeographical analysis could evaluate whether these events reinforced adaptive processes creating body size variations following Bergmann's rule, for example (see also Diniz-Filho *et al.*, 2007; Ramirez *et al.*, 2008). Guillaumet *et al.* (2008) recently achieved a valuable move towards this synthesis by using molecular markers and an individual-based approach to investigate the evolutionary and ecological mechanisms driving body size clines in Morocco's larks of the genus *Galerida*. This may provide a link between phylogeography and *ecological genetics*, which aims to evaluate the genetics of ecologically important traits and their underlying adaptive processes (Conner & Hartl, 2004; Quiroga & Premoli, 2007).

More complex analytical and theoretical methods have been incorporated into phylogeography (see Excoffier, 2004). Spatial patterns can be more explicitly incorporated in a phylogeographical context using Templeton's nested clade phylogeographical analysis (NCPA; Templeton *et al.*, 1995; Templeton, 1998, 2004; see also Posada *et al.*, 2000). As pointed out by Templeton *et al.* (1995), it is dangerous to make inferences in phylogeography by a simple visual inspection of how genetic patterns are geographically structured. The idea of NCPA is to use the haplotype (gene) phylogenetic tree to define a nested series of branching events (the clades), which is then cross-analysed with geographical information. This geographical information can be incorporated in NPCA as discrete categories (i.e. localities or regions, as used in F_{ST} and AMOVA) or continuous distances among populations. When a non-random association between clades and space is detected, this can be interpreted as evidence of restricted gene flow, past fragmentation or range expansion. An explicit spatial analysis can be incorporated in NCPA using information about geographical distances among populations (see Posada *et al.*, 2000), allowing one to evaluate the distinct scenarios explaining population structure (restricted gene flow, past fragmentation or range expansion) (see Table 1 from Templeton *et al.*, 1995). However, the NCPA approach has been strongly criticized after tests of its performance over a wide range of biogeographical scenarios and demographic models (Panchal & Beaumont, 2007; Petit, 2008).

Another recent development that is gaining momentum within phylogeography is the application of ecological niche modelling (see Araújo & Guisan, 2006) to generate species palaeodistributional models that are then compared with phylogeographical patterns. This approach was pioneered by Hugall *et al.* (2002), who compared the phylogeography of a land snail with the species' predicted past distribution using palaeoclimatic reconstructions of the Australian Wet Tropics forests. It was also recently used to test refugia predicted by palaeodistributional models with those estimated by phylogeographical patterns (Waltari *et al.*, 2007). Recent developments have gone one step further by adding statistical hypothesis testing to these comparisons using coalescent-based genetic simulations (Carstens & Richards, 2007; Richards *et al.*, 2007).

Geographical and landscape genetics

Despite the popularity and successful applications of the two approaches previously described (i.e. the analysis of population structure based on partition of genetic variance, and phylogeography) in understanding geographical patterns of genetic diversity, they are usually not used in an explicit geographical context (see Templeton, 1998). For instance, Epperson (2003) recently coined the term *geographical genetics* to describe '…the mathematical relationships of spatial statistical measures of patterns to stochastic processes'.

The application of spatial statistics to population genetic data started in the late 1970s, when R. R. Sokal and N. L. Oden established a protocol based on spatial autocorrelation statistics to infer microevolutionary processes based on similarity of gene frequency maps and their corresponding spatial correlograms (Sokal & Oden, 1978a,b). Later, modified indices of spatial autocorrelation were developed specifically to deal with more complex forms of molecular genetic data (Bertorelle & Barbujani, 1995; Smouse & Peakall, 1999). Matrix correlation

and Mantel tests (Manly, 1985, 1997) can be used as a form of spatial autocorrelation if any form of genetic distance matrix (including pairwise 'F_{ST}-like' statistics, as previously discussed) is compared with a geographical (or environmental) distance matrix (see Manly, 1985).

Computer simulations (see Sokal & Wartenberg, 1983; Sokal *et al.*, 1997) showed that this protocol can be successfully used to distinguish among evolutionary processes based on broad-scale gene frequency maps and their spatial signature, revealed by spatial autocorrelation analyses. Diniz-Filho & Malaspina (1995) used this protocol to test alternative models of geographical variation in Africanized honey bees, showing that phenotypic variation could be better explained by hybridization between recently introduced African and pre-established European subspecies, followed by selective north-ward diffusion, than by the relationship between phenotypes and climatic variation. On the other hand, it may be difficult to use the protocol in some situations, such as, for example, to distinguish between alternative scenarios of the Neolithic vs. Palaeolithic origin of current genetic diversity of human populations in Europe (see Sokal & Menozzi, 1982; Richards *et al.*, 1996; Chikhi *et al.*, 2002). Anyway, it seems that the development of phylogeography in some sense shifted the interest of population geneticists from interpreting gene frequency maps and spatial statistics (a more 'current or ecological' approach) to using mtDNA to describe and map genealogical connections between populations or individuals (a 'historical' approach).

More recently, there has been a resurgent interest in the application of spatial statistics to molecular data, frequently by decreasing the geographical extent over which individuals (or populations) are analysed (but see Handley *et al.*, 2007; for a recent review of human geographical genetics at the global scale). For example, Miller (2005) released the software 'Alleles In Space', which is intended for the analysis of inter-individual patterns of genetic and geographical variation. This program avoids the use of arbitrary designation of populations, and performs several spatial analyses such as Mantel tests, spatial autocorrelation, genetic landscape interpolation, allelic aggregation index and inference of genetic barriers through the Monmonier algorithm (see Manel *et al.*, 2003). Epperson (2005) reviewed how spatial autocorrelation analysis based on genetic variation among individuals within a local population can be used to estimate dispersal rates and other popula-tion parameters, and some attempts to link within-population patterns of spatial autocorrelation in genetic data to life-history traits have also been made (e.g. Degen *et al.*, 2001; Arnaud-Haond *et al.*, 2007). This can be important for improving predictions about population viability, which is critical for establishing optimum conservation strategies (e.g. Watts *et al.*, 2007).

These applications directly lead to a connection between geographical genetics and *molecular ecology*, which, in broad ecological terms, involves the application of molecular genetic methods to solve ecological problems (Beebee & Rowe, 2004). Thus, molecular ecology has been defined in a very broad

sense, actually encompassing all other fields discussed here (including conservation genetics, see below). Despite this, it has a special emphasis on dealing with the estimation of population and individual level ecological parameters, such as dispersal rates, mating systems and paternity issues, usually associated with aspects of behavioural ecology.

Another recently created research programme strongly related to geographical genetics has been called *landscape genetics* (Manel *et al.*, 2003; Holderegger & Wagner, 2006; Storfer *et al.*, 2007), which proposes to integrate genetic data and more complex aspects of landscape composition and configuration, including matrix (in a metapopulation context) quality. The development of landscape genetics is based on the methodological integration between landscape ecology approaches [such as high-quality remote sensing techniques and geographical information systems (GIS)] and molecular data, as well as by new sophisticated spatial statistics (such as wombling) designed to detect discontinuities in geographical space (Fortin & Dale, 2005; Guillot *et al.*, 2005; Kidd & Ritchie, 2006). Mantel tests can also be used in a likelihood context, allowing the simultaneous test of hypotheses based on the correlation between different models of landscape structure and genetic data (e.g. Michels *et al.*, 2001; Spear *et al.*, 2005).

The incorporation of complex landscape characteristics into geographical genetics at regional scales appears more clearly in the context of human-modified ecosystems. Because habitat loss and fragmentation caused by human activities usually disrupt diversity patterns in a complex fashion (Fahrig, 2003), geographical distances '*per se*' may not be enough to describe genetic population structure. In fact, the monotonic relation-ship between genetic divergence and geographical distances expected under a pure IBD process, for example, should be viewed as a null model for complex landscape effects. Deviations from this null expectation imply that other forces can be involved in genetic differentiation (Broquet *et al.*, 2006; Telles *et al.*, 2007).

PERSPECTIVES AND INTEGRATION OF RESEARCH PROGRAMMES

Conservation genetics

The convergence of different areas of scientific knowledge towards biodiversity conservation has also become explicit in a more general biogeographical context within the framework of *conservation biogeography* (Whittaker *et al.*, 2005), so that it is expected that a first integration of the research programmes described above occurs in this context. Within and alongside this endeavour, *conservation genetics* may be defined as the 'application of genetics to preserve species as dynamic entities capable of coping with environmental change. It encompasses genetic management of small populations, resolution of taxonomic uncertainties, defining management units (MUs) within species and the use of molecular genetic analyses in forensics and understanding species' biology' (Frankham *et al.*, 2003). All research programmes described above to evaluate

geographical patterns in genetic diversity can be used, in different ways, within conservation genetics (Fig. 1), which coincides with claims to more explicitly incorporate evolutionary processes into conservation practices (Crandall *et al.*, 2000).

The debate over the establishment of MUs and evolutionarily significant units (ESUs) for conservation of genetic diversity at the intraspecific level, for example, happened in an explicit phylogeographical context (e.g. Moritz, 1994; Newton *et al.*, 1999; Fraser & Bernatchez, 2001). Initially, Ryder's (1986) concept of the ESU was developed to determine units of conservation at a lower level than that of species but, subsequently, the concept has been changing according to practical needs and limitations (e.g. Moritz, 1994; Crandall *et al.*, 2000). Moritz (1994) proposed associating the status of ESUs to populations that show reciprocal monophyly in mtDNA strands and significant divergence in nuclear allelic frequencies. On the other hand, MUs are populations that differ in their allelic frequency of neutral loci independently from the phylogenetic relationships among alleles. These two concepts introduced two problems: the first is how to determine a phylogeny that truly represents the phylogeographical history of the species under study, and the second is that the introgressional events that originate in hybrid zones sometimes cause phylogenetic noise.

More recently, however, Fraser & Bernatchez (2001) introduced the concept of adaptive evolutionary conservation, based on the use of different criteria and data (including phenotypic variability) to establish intraspecific units for conservation, case by case. In this spirit, Diniz-Filho & Telles (2002) also proposed that spatial correlograms, widely used in geographical genetics, could be used to define 'operational units' (OUs) for intraspecific conservation when no clear phylogeographical structure can be established. This approach could also be incorporated into optimization methods for reserve selection based on multiple species (Diniz-Filho & Telles, 2006). On a broader scale, comparative phylogeographical analyses can be used for similar purposes, by establishing areas in which genetic diversity of multiple target taxons could be better conserved, and evaluating the evolutionary potential of different conservation unit schemes (Moritz & Faith, 1998). These new approaches to improve conservation efforts exemplify the advantages of integrating methods from different fields within genetics, ecology, biogeography and evolutionary biology.

Theoretical and methodological integration

As pointed out by Slatkin & Veuille (2002) and Epperson (2003), relatively well-developed theories for gene coalescence and spatial and temporal patterns are now available. However, we are forced to recognize that further methodological advances are necessary to merge methods and data currently used to deal with genetic diversity at different geographical scales. The recent developments in the research programmes described above suggest that phylogeographical methods should be explicitly merged with spatial statistics used both to describe continuous patterns of genetic variation and their discontinuities in geographical space.

Different forms of space–time autoregressive (STAR) models can be used to join the effects of geographical and temporal patterns in genetic data, allowing, for example, for the effects of migration through time to be explicitly modelled (Epperson, 2003). These models could be also expanded to incorporate the phylogenetic history of populations and geographical space, although it would be difficult to measure the 'history' to be used in STAR models independently of the genetic data of interest. The palaeoclimatic niche modelling approach coupled with phylogeography can provide an interesting way to combine temporal and geographical structures of genetic divergence and allow a metric for history independent of genetic patterns (see Richards *et al.*, 2007). Geographical and phylogenetic patterns can also be used, for example, to evaluate simultaneously the balance between adaptive and neutral processes in phenotypic evolution in an explicit spatial context (Diniz-Filho *et al.*, 1999). A similar approach could be used to discriminate between competing hypotheses of current isolation vs. deep historical events to explain the observed genetic patterns.

These potential integrations could also benefit from discussion about spatial scale and taxonomic resolution. It is usually believed that a phylogeographical approach may be more appropriate for broad scales (of space and time), whereas landscape and geographical genetics should apply to finer scales (see Manel *et al.*, 2003; Storfer *et al.*, 2007). However, there is no a priori reason for this dichotomy, although the synthesis between these areas will require further work and eventually a better knowledge of the relative resolution of distinct molecular markers. For instance, it may be necessary to improve the integration of historical components of genetic diversity established by phylogeographical analyses with current spatial statistics used by geographical genetics at regional (or even larger) extents. On the other hand, understanding refined genealogical relationships at local levels can improve our understanding of history, and allow its appropriate incorporation into the estimation of population parameters.

Further studies are necessary to show how all the approaches discussed here should converge. However, to meet this goal, as recently pointed out by Storfer *et al.* (2007), it is critical to foster cross-disciplinary communication that will allow the methodological and theoretical aspects of these fields to be advanced, something that in turn can be widely improved by increased discussion and collaboration among geneticists, ecologists, biogeographers and spatial statisticians. Hopefully this will help to lead us through this twilight zone, '…between the pit of Man's fears and the summit of his knowledge'.

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