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Genetic Diversity

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GENETIC DIVERSITY

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Contributors

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Meet the editor

Lidija Bitz is a principal research scientist of plant genomics at the Natural Resources Institute Finland (Luke). She has a decade of working experience and research exchange from Bosnia and Herzegovina, Denmark, Germany, the Netherlands, Sweden, and Switzerland. Specifically, Lidija obtained her MSc degree and defended her PhD thesis at the University of Ljubljana, Slovenia. During those times, she was very active in inventory, collection, and genetic diversity evaluations. She is active in the dissemination of achieved results through the authorship and editing of monographs, scientific papers, book chapters, and professional articles. She has also been successful in implementing international and regional scientific and developmental projects, starting from her student days.

Contents

Preface

How do we define genetic diversity and is it going to save the world of hunger and diseases? Let us be reminded what could happen if, for example,- we all humans were identical. One virus strain could easily extinct the entire kind in a very short time. Luckily, we are all different! Genetic diversity is the complete amount of genes and genotypes in a group of organisms. Genetic diversity is the ultimate need to ensure adaptations to dynamic fluctuations of living conditions through evolutionary changes and to answer future unknown needs.

Therefore, we must serve security of wide range of genetic diversities to ensure fitness by preventing losses and erosions. The best way to ensure and secure genetic diversity is by deep understanding of its patterns and continuous persistent questioning of the latter ones. Innovative sustainable use would be another way, and for this, we must not forget our intuitive link to the entirety of the biodiversity.

This book evolved from a joint venture aiming at continuous shedding light on the understanding of variability patterns and their altering. The chapters of the book very much reflect on long-timed engagements of the authors to the very specific topics coming from diverse organisms and their devotion to them.

What are the drivers for genetic diversity generation? What is the dynamic of genetic diversity and how does it influence population structure? How does climate change influence genetic diversity itself? Those are only several questions that authors are triggering in the first section of the book “Toward understanding diversity patterns."

At the beginning of this preface, it was said: “Luckily, we humans are all different!" And indeed the diversity within only one gene seems to contribute to the AIDS resistance! The second section “Gene sequence diversity" is about the functions of the diversity within specific single genes.

How much does the loss of a single variant of any gene cost to the humankind? This is far beyond any calculation. Genetic diversity as a relevant component of the biodiversity is discussed in relation to the sources of ecosystem services, their use, and the legal aspects. Those topics took place in the third section “Biodiversity."

This book is aimed at researchers who are studying or are contemplating starting to act in new research areas, but careful attention was paid to the clarity of writing in order to inspire audience beyond scientific community as well.

Personally, I vastly appreciate the opportunity to work on this book. I learned a lot, thus believing the same for the readers and users, and even more important, I was very often strongly challenged to question my own opinions! For that, I am utmost thankful.

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Section 1

Toward Understanding Diversity Patterns

Chapter 1

Diversity of Plant Virus Populations: A Valuable Tool for Epidemiological Studies

Fernando Escriu

Additional information is available at the end of the chapter

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Abstract

Plant viruses, as any other living organisms, differ genetically from each other as a result of processes (such as mutation, recombination and other forms of genetic exchange) that generate genetic variation in each generation during their reproduction and processes (such as selection, migration and genetic drift) that modulate this variation, determine the distribution of the genetic variants within a population (i.e., the genetic structure of the population) and how it changes with time, in a dynamical phenomenon called evolution. For plant viruses, evolutionary forces that generate and modulate the genetic diversity of their populations are often associated to different phases in their biology and ecology, such as virus-host interactions and host to host transmission. Forces that shape the evolution of plant viruses are at the same time key factors affecting their pathogenic properties, including their ability to cause diseases (an aspect that is studied in the field of epidemiology). The present chapter aims to illustrate how measurement and analysis of genetic diversity and structure of plant virus populations are essential to the current knowledge on the evolutionary biology of plant viruses and how evolutionary factors have a relevant role in the dynamics of virus populations and therefore, in the epidemiology of plant virus diseases.

Keywords: genetic diversity, genetic structure, plant virus evolution, plant virus epidemiology, plant virus resistance, plant virus emergence

1. Introduction

Evolution is defined as the change with time of the frequency distribution of genetic variants in the population of an organism, what is called the genetic structure of the population. In this context, a population of plant viruses may be considered as the group of individuals of the same viral species living and reproducing in a particular and sufficiently restricted environment, so that it represents a single evolving unit (similarly as local interbreeding units of mating organisms, also called local populations or demes, are considered the fundamental evolving units in population genetics; see [1], pp. 45–46). Two different phases may be identified along the evolutionary process: in the first one, genetic variation is generated during reproduction and the second one consists of the modulation of that variability as the result of driving evolutionary forces acting along the organism's life cycle, which are associated to its biological and ecological interactions in a particular environment.

The present chapter does not seek to be a comprehensive review on the evolutionary mechanisms that shape the diversity and genetic structure of plant virus populations, nor on the broad knowledge derived from the significant number of scientific reports published on this subject during the last decades for different groups of plant viruses. There are a number of excellent reviews covering these topics. In the first part of the chapter, these reviews will be cited in relation to the most relevant concepts that are essential to understand evolution of virus populations. This first part is aimed to serve as a general guide for the readers, who if interested, will be easily addressed to the most relevant literature on the topic. The second part of the chapter will emphasize the important implications of these evolutionary mechanisms in the population dynamics of viruses, that is, the epidemiology of virus diseases, which finally determines their development and distribution in the field.

2. Generation and modulation of genetic diversity: driving forces in evolution of plant virus populations

2.1. Generation of genetic diversity in plant virus populations

Genetic diversity of a population can be defined as the probability that two genetic variants randomly chosen from the population are different [2]. Two mechanisms are the main sources of genetic variation in viruses during their evolutionary process: mutation and genetic exchange. Both of them are presented in the following paragraphs.

Mutation is the result of errors during the replication of a genome due to the misincorporation of nucleotides in the daughter strand that do not correspond to those present in the template [3–5]. Besides its relevance in evolution as the initial step for generation of variability in populations, the mutation process has significant practical implications in the design and assessment of virus diseases control strategies (antiviral therapies, vaccinations, etc.). Also, it is a factor for the occurrence of important epidemiological phenomena, such as virus adaptation to new hosts or changes in viral virulence, which may led to the emergence or re-emergence of both animal and plant viral diseases. Therefore, the estimation of mutation rates is an important focus of research efforts [6]. Mutation rate measure the proportion of mutations generated either per round of genomic replication or per infected cell. Its calculation is often more complex than that of mutation frequency, which estimates the proportion of mutations remaining in a population after the action of selection, for instance, a fraction of mutations are deleterious and has been eliminated by purifying selection. Rates of spontaneous mutation of RNA viruses have been estimated to be several orders of magnitude higher that those of DNA viruses [5, 7] and this difference has been attributed to the lack of proofreading activity of virus encoded RNA-dependent RNA polymerases. For plant RNA viruses, direct estimates of spontaneous mutation rates have been obtained for only two viruses, *Tobacco mosaic virus* (TMV) and *Tobacco etch virus* (TEV) [7, 8]. In both cases, measurements were performed in similar experimental conditions of minimum purifying selection against deleterious mutants because the wild-type function was provided by complementation. Also in both cases, values obtained were similar and fell near the lower threshold of estimates reported for animal RNA viruses and bacteriophages, suggesting that plant RNA viruses show, indeed, lower mutation rates than animal RNA viruses [7]. In contrast, these works reported quite different spectra of mutations for the two plant viruses: preponderance (two-thirds) of insertions and deletions and a significant fraction (one-third) of multiple mutants for TMV, whereas most of TEV mutants were single-nucleotide substitutions, with the fraction of transitions being twice that of transversions. These differences could be explained by a different behavior of the respective replicases or by differences in the experimental approach [7]. Lower mutation rates in plant than in animal RNA viruses could partially explain their generally lower rates of molecular evolution and the high genetic stability of plant RNA virus populations [2]. These differences in mutation rates could derive from different selective pressures acting in the mutational strategy of RNA viruses in the two types of hosts, although the role of natural selection on the evolution of mutation still needs to be demonstrated [7]. An alternative hypothesis set out that mutation rates in RNA viruses are not adaptive but are required to replace their chemically unstable genomes [5].

Genetic exchange occurs when genetic information from different genetic variants infecting the same cell is switched between them to form a new variant. It may take place through **recombination**, when the switched information consists on segments of nucleotide strands of different genetic variants, or through **reassortment** of whole genomic segments in viruses with segmented genomes, a process which is also known as pseudorecombination [9].

Once some initial level of genetic variation is created by mutations, the opportunities of genetic exchange between different genetic variants may increase, contributing to the generation of new variability. Recombination and reassortment are frequent in populations of plant-infecting viruses with either RNA or DNA genomes [2, 5, 10]. Analysis of their sequences indicates that both mechanisms contribute significantly to the generation of variability in the evolution and diversification of certain taxonomic groups [11–16]. Recombination and reassortment events may involve members of the same plant virus species [17–19], members of different species [20–24] or even genus [25]. Genetic exchange by recombination or reassortment may have important epidemiological implications of practical relevance, even more than mutation, as it has been associated to host jumps, host range expansion, changes in virulence, breaking of host resistance and finally, the emergence of new viral plant diseases. Outstanding examples of that are the contribution of recombination and reassortment in the development of a severe epidemic of Cassava mosaic disease in Uganda [26] and the appearance of several new recombinant species of begomoviruses in the Mediterranean Basin associated to the Tomato yellow leaf curl disease in tomato [27]. However, in spite of the relevant epidemiological role of genetic exchange, till date, little information is available on the rates with which it occurs in plant viruses in the absence of selection. Recombination rates have been experimentally estimated in coinfections of different genotypes of *Brome mosaic virus* [28, 29], *Cauliflower mosaic virus* (CaMV) [30] and TEV [31]. Although results obtained should be compared with caution, they are similar (particularly those of CaMV and TEV) and as high as mutation rates (at least for TEV), indicating that recombination may be as relevant as mutation in creating variability [31]. It has been shown that selection against heterologous gen combinations increases as host colonization progresses along the infection cycle of *Cucumber mosaic virus* [32], affecting the frequency of genetic exchange. This explains that recombinants and reassortants are often found at low frequencies [33, 34], although the frequency of particular combinations may be dependent on agro-ecological factors [17, 19], and support the hypothesis of co-adaptation of gene complexes within the viral genomes [32, 35].

It has been exposed above that creation of variability is an initial and required step in the evolution of populations. On the other hand, variability may also contribute to effects of evolution that may be detrimental for populations. For instance, a population may become extinct because of an excessive accumulation of mutations, a phenomenon known as lethal mutagenesis [36], which also takes place in viruses and is an interesting mechanism for antiviral therapies [37]. Also, high mutation rates combined to small sizes of asexual populations (as a result of genetic bottlenecks, see below) may led to the progressive accumulation of deleterious mutations and the loss of mutation-free individuals, with a consequent reduction in fitness in populations, which is called the "Muller's ratchet" [9, 38]. In addition to the adaptive relevance of genetic exchange to create beneficial genomic combinations, recombination and reassortment may represent a sexual mechanism contributing to compensate the accumulation of deleterious mutations and the effect of "Muller's ratchet" in populations, and it has been postulated as a theory of evolution of sex in RNA viruses [9, 39]. Alternatively, it could be that recombination, together with mutation, had evolved as consequences of the fast incorporation rate of RNA-dependent RNA polymerases in RNA viruses. It might be the case at least for several RNA viruses, including TEV, for which a highly significant correlation was found between their recombination and mutation rates [31]. Possibilities for evolution of recombination in RNA viruses were reviewed in [9].

2.2. Evolutionary forces that determine the genetic diversity of populations of plant viruses

A key concept to understand evolution, that is, the change with time of the genetic structure of a population, is the fitness of an individual or genetic variant. **Fitness** is a measure of the reproductive ability of each individual or genetic variant with which it contributes to the next generation in a particular environment [40]. Therefore, in an ideal population of infinite size (to ensure that every single variant contributes to the next generation), an estimate of the fitness of a variant is the frequency at equilibrium with which this variant is present in the progeny. Fitness and population size define the meaning of the two main evolutionary forces, selection and genetic drift. **Selection** represents the changes in the frequency of variants in the ideal population: it is positive (adaptive selection) for fittest variants which increase their frequency and negative (purifying selection) for less fit variants which decrease their frequency. **Genetic drift** occurs when the population is not large enough for each variant to have progeny, so that variants might pass to the next generation rather by chance (random effects), not by their respective fitness [2]. Thus, population demography may influence, and even inhibit, the effects of selection through genetic drift. As mentioned above, RNA viruses, including those of plants, are characterized by a high ability to genetic variation, and their populations are subjected to high variations in size along the infection cycle due to fast replication rates (expansions) and transmission between hosts or between tissues within a host (reductions). Consequently, conditions at which mutation, selection and genetic drift operate are determinant for the adaptation or extinction of RNA viruses [36].

2.2.1. Selection

Fitness, the parameter that determines selection, is dependent on the environment. Therefore, changes in environmental conditions (for instance, a change of host) may be determinant for a variant generated by mutation to be eliminated from or fixed in the population, giving it a chance for adaptation. In a population, the proportion of mutations that are beneficial, neutral, deleterious or lethal is known as the **distribution of mutational fitness effects**. For RNA viruses, a large proportion of mutation and recombination events are deleterious or lethal [41, 42]. For neutral and deleterious mutations, the **mutation-selection balance** refers to the relationship between the mutation rate and selective pressures that define the frequency of these mutations in the population (they can be continuously created by mutagenesis). On the other hand, **genetic robustness** refers to a kind of molecular mechanisms that minimize the phenotypic effects of mutations and may become a successful strategy against deleterious mutations or may hinder opportunities of adaptation. Robustness has been observed to occur in experimental populations of a plant viroid [43].

Comparative analysis of genetic diversity of populations in different phases of the infection cycle of plant viruses has allowed the identification of selective pressures associated to each of them, although selection is often difficult to distinguish from genetic drift, as both mechanisms result in the decrease in population diversity. Phases of viral cycle associated to selection were reviewed in [2] and [44]. In summary, selective pressures are related to: i) the maintenance of functional structures, that is, certain amino acids involved in the stability of viral particles or in the secondary and tertiary molecular structures required for replication or other interactions; ii) interactions of viruses with their hosts, resulting in the genetic differentiation of natural populations according to the host, the overcoming of host resistance genes, changes in virulence and co-evolution of plants and viruses [45]; iii) interactions between viruses and their vectors for transmission and between hosts and vectors. The sequence analysis of genes related to some of the functions mentioned above for several plant DNA and RNA viruses indicated that selection on plant virus encoded proteins is mostly negative, as measured by the ratio between nucleotide diversities at non-synonymous and synonymous positions (d_{NS}/d_S) (this estimates the degree of functional constraint for the maintenance of the encoded protein). Two major conclusions were derived from the analysis [2]. First, the degree of negative selection is similar for plant RNA and DNA viruses and does not depend on the function of the encoded protein (in contrast to some proteins of cellular organisms, which are more conserved than others). This suggest the existence of epistatic effects of selection in multifunctional virus-encoded proteins or in genes with overlapping open reading frames, so that proteins are never optimized just for one of the functions. Second, the degree of negative selection in plant viruses falls in the same range of that of proteins of their eukaryotic hosts and vectors, suggesting that selection arise from the necessary triple interaction virus-host-vector.

Observation of changes in the genetic structure of within-host populations that are associated to different degree of compatibility between the virus and the host provides insights into the host-adaptive process (reviewed in [3]). In compatible interactions of highly host-adapted viruses, negative selection tends to maintain virus population in equilibrium, resulting in a high stability of its genetic structure, as found in intra-host populations of *Tobacco mild green mosaic virus* and other plant viruses infecting their susceptible hosts. In contrast, more variability was found in small intra-host populations of *Beet necrotic yellow vein virus* (BNYVV) in partially resistant plants, expected to be under virus-host adaptation, than in large BNYVV populations in susceptible plants. This contributes to explain the sudden stochastic diversification of BNYVV populations in sugar beet after the deployment of resistant plant genotypes in the field, and the higher diversity observed in populations of other plant viruses in their centers of origin, where they are in phase of adaptation to a new host [3]. Similarly, higher between-hosts diversities were found in host-adapting populations in virus' centers of origin (*Wheat streak mosaic virus* in North America; *Rice yellow mottle virus*, RYMV, in eastern Tanzania) or in virus emerging areas (resistance breaking variants of BNYVV in the Imperial Valley of California) than in well-adapted virus populations in other areas (other regions in Africa for RYMV or in USA for BYNVV, see references 3, 51, 114 and 130 in [3]).

Another interesting topic on host-adaptive process is that concerning host-range evolution for those viruses that behave as multi-host parasites. Multi-host parasitism is common among plant viruses, leading to the consideration of generalist and specialist plant viruses [46, 47]. Different hosts represent, indeed, different environments for viruses and, accordingly, fitness differences should be expected for viruses across their host range. Genetic differentiation of virus populations according to the host may indicate host adaptation and detailed analysis show evidence of host adaptation in populations of a particular virus sampled from different hosts, or even from new hosts in which the virus has acquired the capacity to infect [48]. More clear indication is obtained when a virus from an original host is serially transferred to other host and it is observed virus adaptation to the new host but associated to a fitness loss in the original host. This type of host selectivity has been shown even in cases of generalist viruses, supporting the theory of trade-offs across hosts, that is, the virus cannot simultaneously maximize its fitness in all of its alternative hosts [48]. Antagonistic pleiotropy, that is, mutation with positive effects in a given host are deleterious in another one, seems to be the major cause of across-host fitness trade-offs, as reviewed in [48, 49].

In a population, the success in the adaptive process to a new host, that means that any beneficial mutation in the new host become fixed, depends on the distribution of mutational fitness effects (see above), which is highly affected by the environment (the new host species), so that there is a larger proportion of beneficial mutations as the taxonomic relatedness of the new host to the original one decreases. This process is in part explained by antagonistic pleiotropy and may be significantly sensitive to genetic drift [50]. Adaptation is also dependent on **epistasis**, defined as the effect of a mutation in one gene on the expression of other gene in the same genome, being this effect often negative (antagonistic epistasis), that is, the combined effect of both mutations is less pronounced than their individual effects would be, and also dependent on the environment (the host) [51, 52].

Selection pressures are also associated to the transmission process. Many plant viruses depend on vectors for transmission. Thus, virus-vector interaction is a probable source of selective pressures. Evidence of virus-vector selection comes from the loss of vector transmission capacity for viruses that have been mechanically transmitted in experiments of serial inoculations (reviewed in [2]). This phenomenon also suggests that might exist trade-offs for adaptation to transmission, as already commented for host adaptation. Virus-vector negative selection is supported by the lower d_{NS}/d_S ratio observed in the coat protein gene, a determinant for vector transmission of many viruses, for vectored viruses compared to that of nonvectored viruses [53]. More complex interaction among viruses, hosts and vectors may also play a role in selection for transmission. For instance, plant viruses may have mutualistic interactions with their vectors, so that infected plants become more attractive for transmission vectors [54], viruliferous vectors increase their fecundity by partial suppression of plant defense mechanisms against feeding vectors [55] and some circulative-propagative viruses seem to modify their vectors' feeding behavior to increase their transmission rate [56, 57].

2.2.2. Genetic drift

Genetic drift refers to the random effects that result from reductions in the population size. In that context, one must consider what is called the effective population size, defined as the number of individuals that give rise the next generation. Plant viruses exhibit high replication rates; therefore, they may reach large population sizes in infected cells or plants. Though, the effective population size of their populations may be several orders of magnitude smaller, as estimated for TMV, TMGMV and WSMV [2, 44]. This may be considered a probable reason to explain the low genetic diversity commonly found in their natural populations, in spite of their high replication and mutation rates [44].

As shown for selection, genetic drift may be associated to almost every step of the virus life cycle and genetic bottlenecks, which are severe reductions in the effective population size, have been shown to operate during virus colonization of a host and transmission between hosts [58–65]. The multiplicity of infection (MOI), that is, the number of virus particles or genomes that infect a cell, has been estimated for some plant viruses either in local infections or along the systemic colonization of a host [66–68], giving roughly similar results, and showing that MOI may vary during systemic infection. Also, estimates of the size of genetic bottlenecks have been reported to be very low, associated to the systemic invasion of leaves [65], aphid transmission [58, 69] and contact transmission [64] between hosts. In addition, the size of bottlenecks is dependent on the viral load, at least during colonization of the host [70, 71].

Above estimates of MOI and of size of genetic bottlenecks are highly relevant to many important processes in virus evolution. For instance, MOI influence the opportunities for genetic exchange to take place, which at least require two different genotypes coinfecting a cell. Also, the efficiency of complementation of defective genomes may occur at high MOI levels, which may be particularly important when deleterious mutants have pleiotropic effects on other viral functions or on other environments (host jumps) [72]. The direct consequence of a severe population bottleneck (an effective population size very low compared to the total census population, which may be very large [2]) is known as "founder effect", an extreme type of genetic drift implying that the new population (generation) is started (founded) by a few genetic variants randomly sampled from the original population. The overall evolutionary consequence of reductions in the effective population size is a decrease in the genetic diversity within each founded population and a strong spatial structure, derived from the increase in genetic diversity between daughter populations, as observed for TMV even at relatively high MOI [66], with a stochastic spatial distribution of genotypes [73, 74]. Finally, as already indicated in the end of Section 2.1, when bottlenecks lead the population to an effective size below the threshold needed for selection to eliminate deleterious mutations and ensure the transmission of the fittest variants, the fitness of the population may decrease by progressive accumulation of deleterious mutants, leading the population to extinction by mutational meltdown (the "Muller's ratchet" phenomenon) [9, 36, 75]. Interactions between viruses may promote extinction of one of the viruses by mutational meltdown: coinfection of TMV with TMGMV in *Nicotiana glauca* plants from Australia resulted in a reduction in size of the TMV population which led this population to extinction [76].

3. Dynamics of genetic diversity and structure of plant virus populations: implications of evolutionary factors in the epidemiology of diseases

The term evolutionary epidemiology has been coined to denote the link between evolutionary biology and epidemiology [44, 77], which basically describes the integration of ecological and evolutionary concepts, as those already reviewed in this chapter, to better understand specific epidemiological components of host-parasite interactions. Conversely, epidemiological dynamics is also an important factor influencing evolutionary dynamics. This framework is at the base of the most recent advances in the areas of evolutionary biology and epidemiology, some of which are commented below, pointing to the most relevant reviews covering those subjects.

Two of the most important properties of pathogens, with evident implications in epidemiology of diseases, are pathogenicity and virulence. Pathogenicity is defined as the qualitative capacity of a pathogen to infect and cause disease on a host. Virulence is the degree of damage that the pathogen infection causes to the host, but in the context of evolution, it refers to the decrease in fitness of the host caused by the pathogen. Hosts may exert selective pressures on both virulence and pathogenicity of the pathogen, as it happens in agricultural systems in which humans manipulate the host genetic structure by the deployment of host genetic resistance in the field, with the consequent risk of appearance of resistance-breaking variants. On the other hand, the genetic structure of host populations may change in response to the pathogen selective pressures, but mostly in natural ecosystems. The reciprocal evolutionary interaction between hosts and pathogens brings the concept of host-pathogen coevolution. In spite of a broad collection of theoretical models regarding host-parasite coevolution, experimental evidences are scant and some advances have been made in this field to test theoretical hypotheses, which have been recently reviewed [45, 78].

Other epidemiological implications of the evolutionary dynamics of plant viruses deal with the improvement of disease management strategies. Acosta-Leal [3] evaluated possible opportunities for virus-disease control, as resistance genes, natural plant resistance mechanisms, control of coinfection dynamics, modeling virus robustness, etc. and discussed about research advances and needs in relation to a simple theoretical model, which states that an assembly of management measures should be addressed to altogether reduce the effective population size of virus populations, increase their genetic diversity and maximize bottleneck effects, so that a virus population could be gradually excluded from its hosts species.

Finally, a highly relevant epidemiological consequence of plant virus evolutionary dynamics is the risk of emergence of virus diseases, which seriously compromise agriculture production worldwide. Emergence of new diseases may occur either by appearance of new virus species that spill over from wild plant reservoirs or of well known viruses that suddenly show new pathogenic and epidemiological properties (host jumps, resistance-breaking variants). The risk of resistance-breaking was evaluated for a set of representative plant viruses and pathosystems in relation to an index of evolutionary potential, based on the effective population size, the degree of genetic exchange and the amount of gene flow, which was proposed as an important determinant of the durability of resistance against plant viruses [46]. Ecological and epidemiological factors of plant virus emergence often have their origin at the interface between managed and natural ecosystems and are mostly related to a rapid expansion of human activity, including the worldwide distribution of crop species far from their geographic origins, the intensiveness of agricultural practices and the international trade facilitating the spread of damaging viral species, all of them under the effect of global climate change [79]. Factors favoring emergence derive from complex interactions among host plants, viruses and their vectors (for vector-borne viruses) and have been analyzed in the context of evolutionary ecology, genetics and epidemiology [80]. In summary, they result in changes in the ecology and genetic composition of host plant, virus and vector populations during three different temporal phases that describe the process of emergence. In a first phase, viruses spill over from host reservoirs in which they are well-adapted (often reservoirs of wild plants) and jump to the same host species in a new ecological environment or to a new host species. In this phase, ecological conditions for plant hosts, viruses (and vectors) must favor the contact between the original and the new host populations for emergence to occur. This includes the introduction of hosts, viruses (and vectors), often by human activity, in areas where they were not present before. Other factor facilitating new contacts is ecosystem simplification [81], characterized by reduced species diversity in agricultural compared to natural ecosystems, a concomitant reduction in the genetic diversity of crops compared to wild populations and a higher host density. A second phase consists on the evolutionary process of virus adaptation to the new host or environment to the point that new infections and transmission in the new host is ensured, making between-host transmission independent from the original reservoir. As indicated in Section 2.2.1, adaptation to a new host is a process governed by an assembly of evolutionary factors, such the generation of beneficial mutations (and genetic exchange in cases of cellular coinfection), the interaction between beneficial mutations (epistasis) in a favorable environment (a particular new host) that may results in trade-offs across hosts and obviously, stochastic effects. Here, it is important to stress that the symplast, where plant viruses must replicate and evolve, is a high structured environment where virus populations adopt a metapopulation structure, a set of subpopulations, each one occupying different tissues and organs. This metapopulation structure is probably generated by the effect of genetic bottlenecks and might affect the efficiency of natural selection [80]. In the third phase, an efficient epidemiology should optimize between-host transmission in the new host and environment, which implies new adaptation to vectors in the case of vectored viruses. As predicted by theoretical models, the epidemiological potential of a pathogen depends on its basic reproductive ratio (R_0), which represents the number of new infections per infected host in a susceptible population. R_0 value must be larger than unity for an epidemic to occur. Consequently, during this phase of emergence, evolutionary factors determining virus competence for transmission (and adaptation to new vectors in vectored viruses) should maximize transmission rate and reduce virulence.

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Chapter 2

Local Scale Genetic Diversity and its Role in Coping with Changing Climate

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Additional information is available at the end of the chapter

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Abstract

Climate change is thought to alter the patterns of genetic diversity within species and populations. Yet, it is not well-understood how genetic diversity influences organism's adaptation to changing climate. In this chapter, I explore how within-population genetic diversity may be affected by local environmental heterogeneity and to what extent this variation may promote adaption. I focus on mountain ecosystems since they are heterogeneous environments at a fine scale that offer a unique mosaic of highly localized environmental conditions. I start summarizing the drivers of genetic isolation at a local scale and the diversification and adaptation patterns that result from it. I continue discussing these processes in terms of populations' reactions to changing conditions using my own long-term ecological genomic studies. This allows me to demonstrate that local-scale variation, in the long term, may offer safe places for species in a warming world due to their fine-scale topographic variability, which may provide suitable habitats within only a few meters of species' current locations. Yet, such fine-scale habitat variability can also lead to locally genetically adapted populations, so that individuals and populations adapted to a narrow range of conditions may respond poorly to future environments.

Keywords: genome-wide scans, hereditability, evolutionary responses, genetic adaptation

1. Introduction: The mosaic of environmental heterogeneity at a fine scale

Understanding how organisms respond to climate change is a main research area in evolutionary genetics. Organisms, populations or species may respond to environmental change in three possible ways: by migrating, persisting in current locations or going extinct [1]. Persistence in new environments may be mediated by phenotypic plasticity, which is the range of phenotypes that a single genotype can express as a function of its environment [2] or by adaptation from genetic variation by increasing the frequency of existing allelic variants that can cope with the new conditions [3]. Local adaptation to heterogeneous habitats has been documented [4, 5], but the genetics of locally adapted populations are not always well understood [5].

Globally, some of the largest impacts of climate change are expected to occur in alpine environments, particularly near mountain summits, which are dominated by long-lived plant species. In these environments, snow cover and summer temperatures are the major drivers of vegetation composition [6]. The increase in temperature over the last decades has already led to patterns of upward migration in several species [7]. The alpine region is a highly heterogeneous environment that is characterized not only by strong elevational gradients in temperature but also by local topography. Microtopographical features include depressions in which the snow accumulates and disappears very late in the summer (i.e. snowbeds) and more exposed ridges with less snow and where the snow disappears several weeks to months earlier (**Figure 1**). Similar heterogeneities or gradients that may occur across the alpine zone are due to wind exposure, water availability, rockiness and neighbouring shrubs [8], among other factors. Some of these local-scale differences have been shown to cause local adaptation (e.g. in *Dryas* and in *Ranunculus* [9]). For species that occur in heterogeneous habitats, such small-scale variation can have dramatic implications for their response potential to climate change. Small-scale environmental variability may provide new locations with suitable habitats only a few meters away from present locations [10, 11]. Alternatively, such small-scale habitat variability can lead to locally adapted subpopulations [4], and these genotypes that are adapted to a more narrow range of conditions may respond poorly to future conditions.

Figure 1. Mosaic of snowbeds and exposed ridges in the spring (May 2011) on Wannengrat, Switzerland.

Therefore, to understand processes involved in potential responses to changing conditions, it is important to consider not only climate differences between different altitudes but also differences between microhabitats [12–14], because environmental variation at a local-scale can be high [15]. In this chapter, I explore the microhabitat-driven patterns (Section 2) and processes (Section 3) and its impact on genetic diversity. Although there is a focus on alpine ecosystems and sessile organisms throughout this work, the concepts are generalizable. I also summarize the methodologies (Section 4) used to study phenotypic and genetic variation in microhabitats. To understand the interaction among fine-scale environmental variation, genetic diversity and the evolutionary responses of populations and organisms in a changing climate, the following questions can be asked:

- Are patterns of genetic differentiation and gene flow driven by small-scale environmental differences?
- Do morphological and fitness-related traits show heritable variation and is selection currently acting on any of these traits so that they can evolve given changing conditions?
- What is the microhabitat-driven pattern of genomic divergence?
- What is the genomic architecture of ecologically relevant traits at the microhabitat level?

2. Drivers of genetic isolation at a fine scale

The transfer of alleles between populations is known as gene flow. When this allelic transfer is limited or interrupted, there is genetic isolation. Understanding patterns of genetic variation and gene flow across the fine-scale mosaic will help to predict the response of species to climate change. As an example, under climate warming in alpine ecosystems, snowmelt is expected to occur earlier in the season [16]. Restricted gene flow between subpopulations growing in different microhabitats can be linked with local adaptation [17]. In this scenario, genotypes of long-lived dominant species in late snowmelt habitats may have difficulties to persist under warming conditions. On the other hand, genotypes in early snowmelt habitats would need to migrate to new localities, and this might be difficult in long-lived species even if suitable localities are nearby. Alternatively, lack of differentiation between subpopulations in different microhabitats and rampant gene flow between them could lead to genotypes able to grow in both microhabitats and thus persist *in situ* during climate change. Genetic variation contained in subpopulations in early and late snowmelt microhabitats could also differ because of factors such as asymmetric gene flow. This will influence whether genetic variation is lost from one of the microhabitats. In this section, I will cover the main drivers that may limit gene flow across microhabitats. In Section 3, I will consider its major evolutionary and genetic consequences, specifically for adaptation and diversification in populations.

2.1. Mismatch in flowering time and pollen flow

Variation in the timing of flowering between subpopulations in different snow microhabitats can be a major driver of small-scale genetic structuring [9] through the restriction of pollen-mediated gene flow (as compared to seed-mediated gene flow), regardless of whether flowering time is genetically or environmentally regulated [9, 10, 18]. Small-scale genetic differentiation (measured by the F_{ST}, which is the fixation index, a measure of population differentiation due to genetic structure) has been reported in the majority of studies on snowmelt-driven genetic differentiation [11, 19–21].

2.2. Asymmetry in seed dispersal

Although there may be differentiation in populations' phenology (the timing at which periodic life cycle events happen, like bud breaking, flowering, fruiting and bud setting) between microhabitats due to snowmelt timing (Section 2.1, **Figure 2**), sub-populations growing in different microhabitats do not have to be genetically differentiated [23, 24]. This pattern has been observed in *Empetrum hermaphroditum* (Hagerup) [25] and *Ranunculus adoneus* (Gray) [9] but perhaps most extensively examined in *Salix herbacea* L. a clonal, dioecious, dwarf shrub dominant in the arctic, subarctic and in alpine ecosystems in central Europe [26]. In the Swiss Alps, *S. herbacea* is an ideal species for addressing the impacts of climate change, as it grows along a pronounced elevational gradient (2100–2800 m asl) and occupies a wide range of microhabitats such as rocky, early-exposure ridges, and late-season snowbeds.

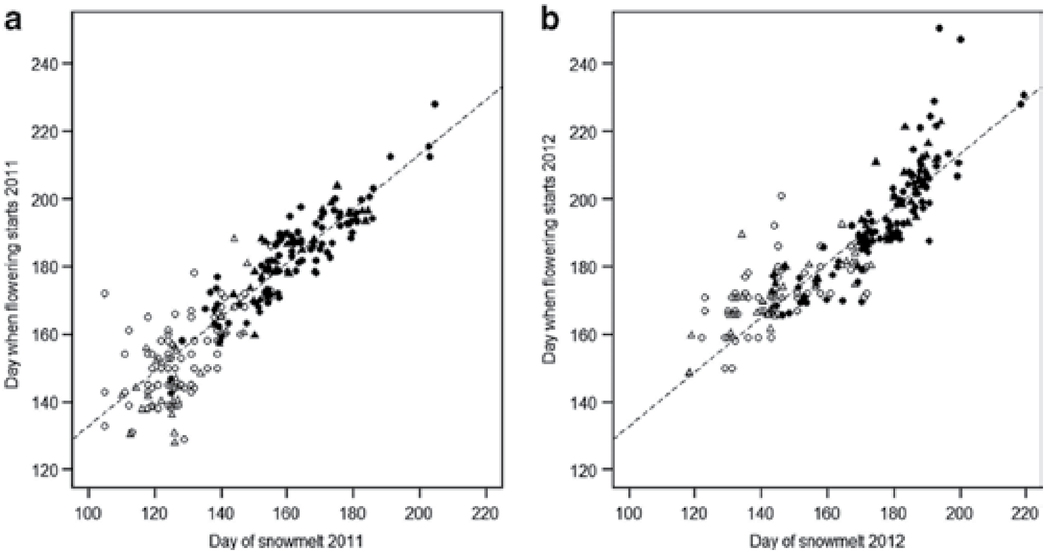

Figure 2. Day of snowmelt predicts when flowering starts for 274 female *Salix herbacea* patches growing on ridges (○) and snowbeds (●) and 85 male *S. herbacea* patches growing on ridges (△) and snowbeds (▲) surveyed in (a) 2011 and (b) 2012. Dashed lines are regression lines ($R^2 = 0.827$, *P* value < 0.001). Modified from Cortés et al. [22].

Although *S. herbacea* populations growing in different microhabitats could be differentiated phenologically, they could not be differentiated genetically [22] using 7 highly polymorphic molecular markers, F_{ST} and N_em estimation and STRUCTURE analysis (see Section 4.4.1). F_{ST} was 0.028 ± 0.003 and 0.035 ± 0.004 for within-microhabitat and between-microhabitat comparisons, P-value = 0.691. Lack of population structure was supported by a STRUCTURE analysis. This absence of population differentiation, even in microhabitats with highly different snowmelt dates, may be mediated by high and asymmetric seed dispersal [22].

Seed dispersal can counteract isolation driven by barriers to pollen flow, like snow, because seed dispersal occurs later in the season when all winter snow has melted [27]. Gene flow via seed dispersal may result in asymmetric source/sink-like patterns driven by wind, topology and the success of seed establishment [28].

In the *S. herbacea* example, late-snowmelt microhabitats (snowbeds) were genetically more diverse than early-snowmelt sites (i.e. allelic richness: 8.93 ± 0.27 and 6.81 ± 0.29 for snowbeds and ridges, respectively), and gene flow, measured as the number of migrants per generation, was asymmetric toward the snowbeds (**Figure 3**). Overall, these results are consistent with snowbeds acting as sinks of genetic diversity and seed dispersal preventing snowmelt-driven genetic isolation [22].

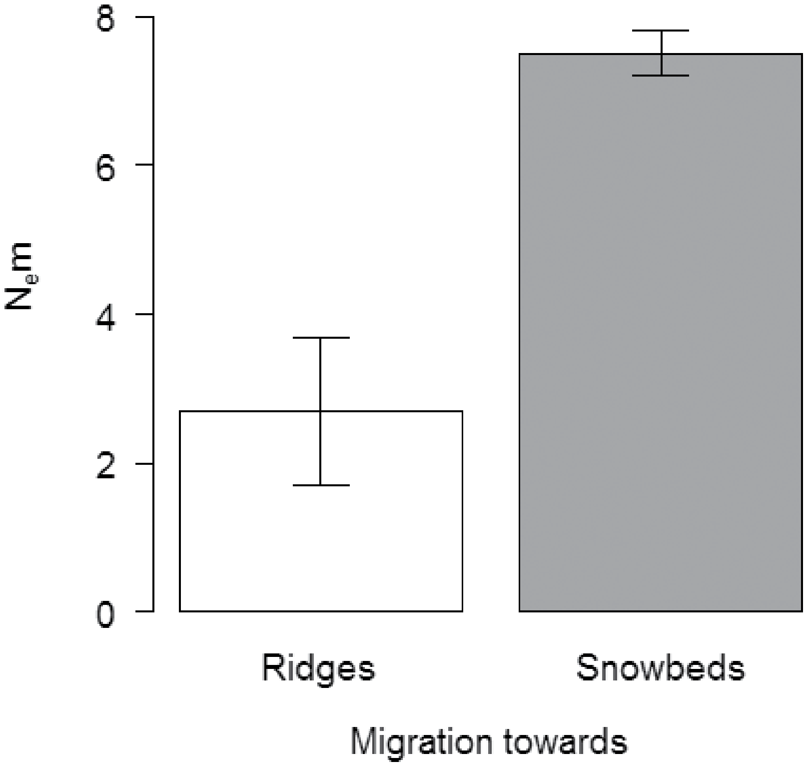

Figure 3. Estimates of the number of migrants per generation ($N_e m$) between microhabitats differing in snowmelt timing (ridges and snowbeds) in *Salix herbacea* from three transects in the Swiss Alps. Modified from Cortés et al. [22].

3. Genetic adaptation and diversification at a fine scale

Most research on the responses of species to changing snowmelt and temperature conditions has focused on species migration toward higher altitudes, where researchers can track the species' climate requirements [29–32]. However, if migration potential is limited, the only way organisms can persist is by adjusting to the new environmental conditions [18, 33]. Adjustment though phenotypic plasticity might be particularly important in long-lived species, as it can occur within the lifetime of an individual [2]. However, plasticity may be constrained or even maladaptive, if populations are exposed to novel conditions outside the range of conditions they encountered in their evolutionary history [33]. Alternatively, adaptation from standing genetic variation may happen by increasing the frequency of existing variants that can cope with the new conditions [34]. While adaptation is dependent on the genotypes, plasticity itself depends on the environment.

Genomic divergence, which is the genetic differentiation throughout the genome, has been studied mostly among species and well-differentiated populations [35, 36] but few of them have been studied at a very local scale from a genome-wide point of view. Genomes are regarded as porous since different regions present multiple signatures and levels of gene flow, drift, selection and ancestral variation [36]. Genome-wide divergence is heterogeneous, with peak-like or plateau-like sections of high divergence surrounded by genomic regions with lower divergence, a landscape described metaphorically like 'islands' and 'continents' of divergence [35]. High divergence in specific sections may be due to disrupting selection from novel or standing genetic variation [37] or random drift [38]. On the other hand, regions with low divergence may be maintained by balancing or uniform selection, continuous gene flow or ancestral-shared polymorphism [39, 40].

An approach that combines between-microhabitat genomic divergence with selection gradients and association mapping of ecologically relevant traits is useful to understand which regions in the genome are likely to differ between populations in different microhabitats, and therefore harbour genetic variation unique to each, and how these genomic regions may relate to phenological, growth and fitness traits (**Figure 4**) [41–44]. Ultimately, this combined approach will allow differentiating plastic and adaptive variation.

3.1. Selection and evolutionary responses

Three essential components are necessary for evolution to occur: there must be trait variation, which must be heritable and selection should be acting on it [45]. A multivariate form of the breeder's equation [46] illustrates this paradigm well and allows for the prediction of the evolutionary response of a trait to selection over one generation ($\boldsymbol{R}$), as expressed in Eq. (1):

$$\boldsymbol{R} = \boldsymbol{G}\boldsymbol{\beta} \tag{1}$$

where $\boldsymbol{G}$ is the variance-covariance matrix of additive genetic parameter estimates ($\boldsymbol{G}$ matrix, or a proxy for heritabilities and traits' trade-offs), and $\boldsymbol{\beta}$ is the vector of standardized selection gradients for the focal traits [47]. The evolutionary response can be calculated using selection-gradient estimates derived from fitness proxies (i.e. fitness regressed as a function of standardized trait values) and marker-based heritabilities [48], using highly polymorphic molecular markers (see Section 4.4.1) as is explained in Section 4.5.3.

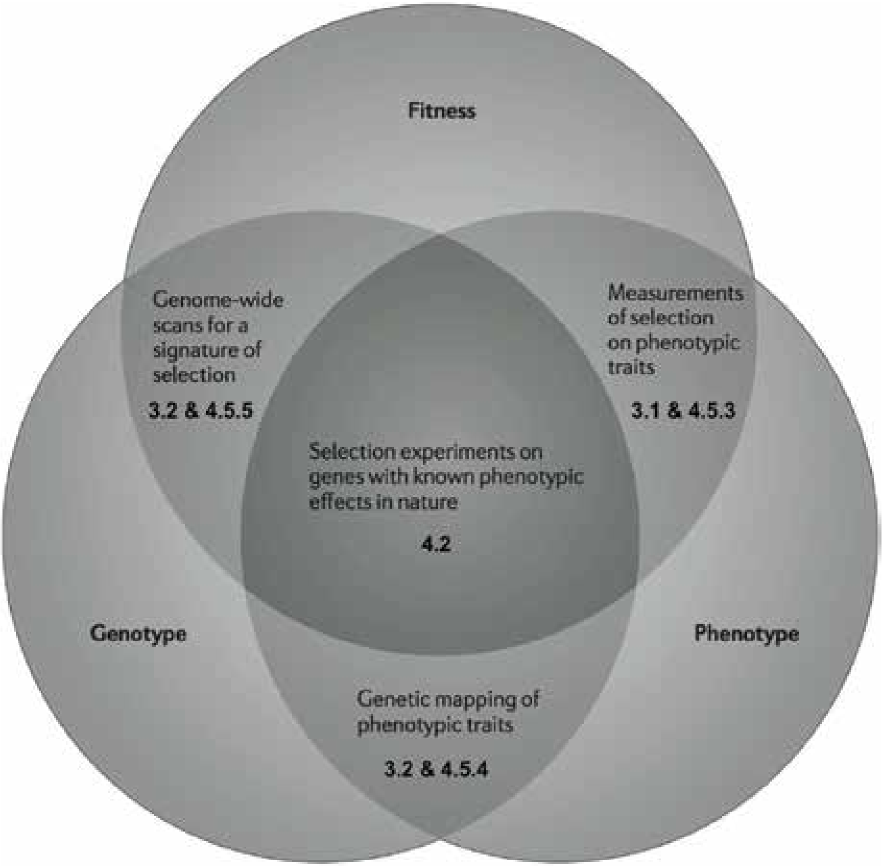

Figure 4. Connections between various approaches for studying the genetics of ecologically relevant variation. Numbers indicate sections in this chapter dealing with the specific concepts. Modified from Barrett & Hoekstra [41].

After the *S. herbacea* case study introduced in Section 2.2, marker-based relatedness estimates in natural populations were used to calculate heritabilities for phenological and morphological traits. For instance, there was selection toward smaller leaves and shorter thermal duration until leaf expansion when using clonal reproduction (change in stem number) as a fitness proxy, in both ridge and snowbed microhabitats (**Table 1**). Conversely, there was selection toward longer thermal durations until flowering in both ridge and snowbed microhabitats when using sexual reproduction (proportion of flowering stems) as a fitness proxy. Selection on thermal durations until flowering diverged in the two microhabitat types when using clonal reproduction as a fitness proxy. This suggests that selection pressures on phenology may vary with ongoing climate change.

Additionally, when using the multivariate form of the breeder's equation (Eq. (1)) to estimate potential evolutionary responses of traits in the *S. herbacea* case study, while accounting for genetic correlations among traits and selection on these traits [12], the strongest predicted response was found for leaf size and the interval between snowmelt and leaf expansion ($R = -5.238$ days per generation) when using clonal reproduction as a fitness proxy. Given these traits, the adaptive potential could allow *S. herbacea* to adapt to climate change. Since earlier snowmelt is the most likely oncoming scenario [49], evolutionary response might shift toward the reaction that is currently observed on ridge microhabitats. Therefore, longer thermal duration until flowering is hypothesized, as a natural way to avoid early season frost damage [12, 14].

Fitness	**Trait (standardized)**	β	**df**	**F**	**p**
Proportion of flowering stems	Leaf size	−0.023	67	0.032	0.86
	Interval snowmelt to leaf expansion	0.029	67	0.342	0.561
	Thermal duration until leaf expansion	−0.041	67	0.426	0.516
	Thermal duration until flowering	0.211	67	4.153	**0.046**
	Leaf size × MH	0.054	67	0.076	0.784
	Interval snowmelt to leaf expansion × MH	0.179	67	1.110	0.296
	Thermal duration until leaf expansion × MH	−0.095	67	0.376	0.542
	Thermal duration until flowering × MH	−0.075	67	0.163	0.688
Change in stem number	Leaf size	−5.232	116	−3.279	**0.011**
	Interval snowmelt to leaf expansion	−3.655	116	−2.217	0.051
	Thermal duration until leaf expansion	−3.646	116	−2.218	**0.020**
	Thermal duration until flowering	2.467	116	1.418	0.85
	Leaf size × MH	3.758	116	1.614	0.073
	Interval snowmelt to leaf expansion × MH	4.058	116	1.308	0.184
	Thermal duration until leaf expansion × MH	1.644	116	0.696	0.805
	Thermal duration until flowering × MH	−4.821	116	−2.047	**0.043**

Linear mixed models were run separately for the two relative fitness proxies: proportion of flowering stems (h^2 = 0.049) and change in stem number (h^2 = **0.071**) and included the traits leaf size (h^2 = **0.386**), interval between snowmelt and leaf expansion (h^2 = **0.178**), thermal duration until leaf expansion (h^2 = **0.469**) and flowering (h^2 = **0.399**), and their interactions with microhabitat type (microhabitat—MH: positive interaction with ridges if $\beta > 0$ and positive interaction with snowbeds if $\beta < 0$), with the plot nested within the transect as a random effect. Estimates of narrow-sense heritability (h^2) are based on the multivariate animal model with a marker based relatedness matrix. Significant values (P-value < 0.05) are in bold based on the F statistic (F), the degrees of freedom (df) and its P-value (p). Significant h^2 values are also in bold. Modified from Sedlacek et al. [12].

Table 1. Standardized selection gradients (β) across microhabitats differing in snowmelt timing (ridges and snowbeds) in *Salix herbacea* in the Swiss Alps.

3.2. Adaptation and plasticity

Persistence of populations and species given climate change may be mediated by phenotypic plasticity [2] or by adaptation from standing variation by increasing the frequency of existing variants that can cope with the new conditions [3]. Epigenetic mechanisms, which are those modulated by environmental factors that switch genes on and off and affect how cells read those genes, may also affect how plants respond to climatic variability [50]. In Section 4, I review ways to access the roles of plasticity and adaptation in explaining trait variation across the fine-scale mosaic. In this section, I discuss the utility of genome-wide analysis to infer microhabitat-driven divergent selection and the genetic basis of trait variation. Although the following results concern *S. herbacea* growing under different snowmelt regimens, this type of analysis also extends to other scenarios such as local scale variation in the occurrence of drought [51–53].

In the *S. herbacea* case study, eight strong, between-microhabitat divergence peaks and two weaker peaks were detected on seven different chromosomes (**Figure 5**). These regions coincided with regions of low SNP (see Section 4.4.2 for a complete definition of this type of molecular marker) density, extensive linkage disequilibrium (a measure of dependency among loci) and negative Tajima's D values (a statistic that describes whether molecular evolution is random). This suggests that novel genetic variation may arise and be fixed in snowbeds and ridges separately in contrast to standing variation that is differentially recruited between microhabitats. The same highly divergent sections persisted when the between-microhabitat F_{ST} was calculated per transect, and they coincided with "valleys" in the F_{ST} when it was computed within microhabitats and across transects. This pattern is an indication that the observed divergent peaks are not due to genetic drift [54], which is the random change in the frequency of alleles. This indicates that genomic divergence can occur in the presence of gene flow and strong environmental differentiation at a very fine geographic scale. The ten between-microhabitat divergence regions spanned a total of 219 genes, which may help to infer functional traits that diverge between microhabitats. This approach is known as forward genetics or bottom-up inference because it makes conclusions regarding unseen traits by first looking at the underlying genetic variation.

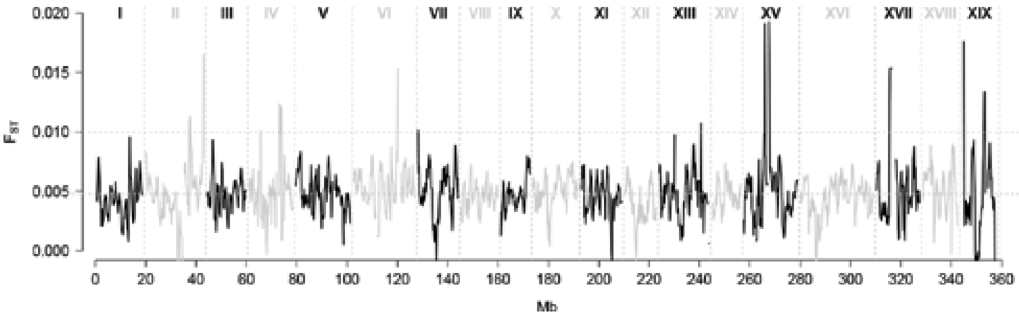

Figure 5. Between-microhabitat genomic divergence in *Salix herbacea*. Sliding window analysis for the average between-microhabitat fixation index (F_{ST}). The window size is 1×10^6 basepairs (bps) and the step size is 200 kilobases (kb). Results of all windowed analyses are plotted against window midpoints in millions of bps. Black and grey colours highlight different chromosomes identified by roman numerals. The lower and upper grey–dashed horizontal lines indicate the genome-wide average and the threshold for the identification of outliers, respectively. Modified from Cortés et al. [54].

In addition to the population genomics approach used to study microhabitat-driven divergence and identify traits that may have diverged between environments, association mapping is usually performed to explore genetically based variation in ecologically relevant traits across microhabitats [54, 55], part of what is known as reverse genetics or top-down inference, as it looks at the genetic basis of specific traits. In the *S. herbacea* case, 57 genomic sections spanning 66 SNP markers were significantly associated with the surveyed traits for which high heritabilities were reported (see Section 3.1). These associated markers explained for each trait, on average, 19% of the observed variation. Ten regions included candidate genes for seven of the nine analysed traits.

4. Heterogeneous microhabitats as a field laboratory to study genetic reactions to climate change

Regions in the world that are experiencing extremely high diversification rates, such as the alpine tundra ecosystems known as páramos [54, 56], and convergent adaptation, like in mountainous microhabitats [57, 58], are good candidates to serve as evolutionary experiments for today's scientists. In this section, I explore the ecological and genetic sampling strategies and the analytical methods commonly used to cover biological variation across altitudes and microhabitats when addressing the main questions suggested at the beginning of this chapter.

4.1. Natural surveys across microhabitats

Plot-based and transect surveys are the two main sampling strategies that allow the study of microhabitat-driven variation. Three-to-five different transects spanning a range of north-east (sun/shade) exposure and covering the main elevational range are the best compromise between sampling effort and feasibility. These surveys at a microhabitat level follow an experimental approach known as space-for-time (SFT) substitution [15], in which current spatial heterogeneity is used as a proxy for predicting ecological time series (i.e. reactions to future conditions).

4.1.1. Plot surveys

In this type of sampling, few representative categorical altitudes (e.g. high and low) are chosen to cover the desired altitudinal distribution. Depressions and ridge-like microhabitats may be chosen at each altitude based on indicators such as topology and vegetation. In each altitude/microhabitat combination, one big plot (~10 × 10 m) is designated. Within each plot, several patches (~100) should be sampled randomly. This sampling is best for assessing isolation between microhabitats (e.g. ecological, trait-based or genetic isolation). Heritabilities and evolutionary responses for different traits are best computed in natural populations with this sampling strategy [12] because it allows including many individuals in close proximity with a high potential of being genetically related in different degrees.

4.1.2. Transect surveys

In this kind of sampling, the main elevational range is covered continuously. Around five to ten elevational bands along transects, with one or more small study plots (~3 × 3 m), must be set up in different microhabitat sites (e.g. early-season exposure from snow and late-season exposure) to grasp a complete overview of the environmental variation. In each plot, patches (at least two) must be selected randomly. When compared to the plot survey, the transect survey has many smaller plots with fewer total patches but covers the desired altitudinal and microhabitat variation at a better resolution by having more total plots. This is ideal to assess ecological responses [15] or genetic isolation-by-distance and to explore trait and microhabitat-driven genomic architecture [15, 22].

4.2. Transplant experiments

To rigorously test how organisms respond to microhabitat-driven changes through phenotypic plasticity, as well as whether populations experience local adaptation (home-site advantage), reciprocal transplant experiments are needed (**Figure 6**) [59]. Transplant experiments are typically carried out across altitudinal gradients. However, reciprocal transplant studies explicitly examining the effects of local-scale variation (e.g. altered snowmelt timing) are scarce. Almost all reciprocal transplants have examined short-lived perennial herbs, and experiments with long-lived woody species are rare due to the difficulty in establishing clones of perennial, slow-growing species. Yet, it is important to understand how long-lived species will respond to changes in snowmelt timing, as they are a dominant functional vegetation type in alpine areas. Transplant experiments can be carried out in long-lived species using long-term monitoring [60] or clonal propagation [59].

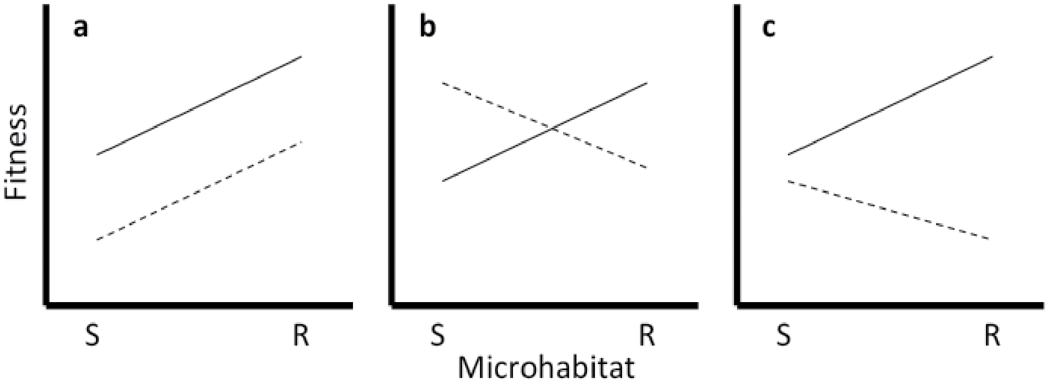

Figure 6. Scenarios where (a) plasticity, (b) local adaptation and (c) plasticity with a genetic basis explain trait variation across different microhabitats: snowbed (S) and ridge (R). Distinct types of lines (S: dashed, R: continuous) are different genotypes reciprocally transplanted to each microhabitat. Illustration based on Sedlacek et al. [59].

4.3. Phenotyping

Phenotyping is the systematic assessment of trait variation. It is an essential component that must be accounted for in ecological and genetic studies. Soil temperature data loggers, nutrient probes and field observations can be used to estimate drought severity [61], frost events [14], snowmelt timing [8], nutrient availability [13] and other soil properties [33]. Monitoring of individuals carried out weekly during the growing season and across microhabitats during several growing seasons is the most exhaustive and informative survey method, although fewer snapshots can also be used.

4.4. Genotyping

Genotyping is the systematic assessment of genetic variation. Adaption and diversification are recognized as important processes that generate diversity [62, 63]. However, their effects on genetic divergence and on the generation of morphological and ecological variation are poorly understood. Low- and medium-throughput techniques (as in Section 4.4.1) together with newly developed high-throughput next-generation sequencing methods (as in Section 4.4.2) offer the promise of major advances in the study of these interactions.

4.4.1. Microsatellite genotyping

Microsatellites loci (also called single sequence repeats or SSRs, which are regions of repetitive DNA that vary in the number of repeated DNA motifs) are commonly used to assess population structure [64], as shown in Section 2.2, and may help to estimate relatedness [65], as shown in Section 3.1, due to their high polymorphism [63, 66]. The PCR (polymerase-chain-reaction, a procedure used in molecular biology to replicate DNA exponentially) reactions are usually multiplexed into several PCR runs. Two or more multiplexed PCR runs can be pooled afterward and separated by capillary electrophoresis. Allele sizes are estimated using software such as GeneMapper v.3.7 (Applied Biosystems).

4.4.2. Genotyping-by-sequencing

Recent developments allow scientists to survey entire genomes to answer questions beyond the population genetics paradigm, in what is starting to be recognized as population genomics (as in Section 3.2). Genotyping-by-sequencing (GBS) is one of the cheapest and most used methods to generate massive amount of SNPs (single-nucleotide polymorphism, a type of molecular marker representing a change in a single base pair). GBS libraries are prepared according to Elshire et al. [67] using different enzymes for digestions. Raw Illumina DNA sequence data from the libraries can be aligned to reference genomes using BWA aligner [68] and processed through the GBS analysis pipeline as in TASSEL-GBS [69].

4.5. Common statistical approaches to compare microhabitats

4.5.1. Linear models

Linear models (e.g. ANOVA, ANCOVA, linear regression) are the first option to assess the effects of microhabitats and altitude on flowering time, and how temperature, humidity and snowmelt vary between microhabitats. In order to assess whether microhabitat and phenological differences trigger genetic isolation, pairwise F_{ST} (fixation index) values can be computed among plots or populations using, for instance, GENEPOP [70]. The number of alleles or heterozygosity, which are standard measures of genetic diversity, may be compared between microhabitats as well (as in Section 2.2), using linear mixed models [71] with the microhabitat as the fixed effect and the transect as the random effect (covariate). These can be done in R (R Core Team), with the packages *lme4* or *lmerTest* [72].

4.5.2. Population structure

Population structure is usually examined using the software STRUCTURE [73]. It is suggested that several independent runs are performed with different K (number of assumed populations) values using an admixture model with a minimum of 100,000 iterations for the burn-in and 100,000 subsequent iterations for the MCMC analysis. The optimal K is posteriorly determined using the rate at which the likelihood changes across different K values [74]. Pairwise migration rates (N_em) and effective population size (N_e) are also meaningful statistics calculated across microhabitats, as in Section 2.2. They can be estimated following coalescent theory and a maximum-likelihood–based approach using software such as, for instance, MIGRATE [75].

4.5.3. Trait heritability and selection

When it comes to trait variation, narrow sense heritability (h^2) is estimated in natural populations using a multivariate animal model [76] with a marker-based relatedness matrix [48]. To test for selection on different traits, proxies of relative (i.e. relative to the mean across all sites or populations) clonal and fixed sexual reproductive fitness are compared against the standardized phenotypic traits using multiple regressions with linear mixed models to yield selection gradients [77]. This analytical approach is illustrated in Section 3.1.

4.5.4. Genetic mapping

To understand trait architecture in natural populations, trait-marker association studies are used [78]. Standard trait-marker association analysis can be easily implemented in FaST-LMM [79] or BiForce [80], the latter detects epistatic interactions (i.e. second-order trait-marker associations) and dominance effects.

4.5.5. Scans for genome-wide selection

As a descriptive approach, genome-wide sliding window analysis can be used to determine F_{ST}, as it is in Section 3.2, and the proportion of variable SNPs that are fixed between microhabitats using, for instance, ARLEQUIN [81, 82]. Linkage-disequilibrium (LD) and Tajima's D [83] are also usually computed in the same windows with the R package *PopGenome* [84].

5. Conclusion

Fast-evolving microhabitat-driven genomic divergence and, at the same time, genetically based trait variation at a larger scale may play a role in the ability of species and populations to persist in diverse and variable conditions in heterogeneous ecosystems. Populations from different microhabitats may be isolated, and they may act as sinks or sources of genetic diversity. From a genomic point of view, multiple genetic regions that diverge between microhabitats may arise at very local geographic scales even in the presence of gene flow, due to strong environmental differentiation. Additionally, regions of high genomic divergence are possibly related to traits under selection that may matter for the prediction of evolutionary responses. In this chapter, I have shown how small-scale environmental variability helps understanding the way organisms may react to changing conditions by looking at processes such as genetic adaptation and diversification at a very fine environmental scale. In the oncoming years, I expect to see an increase in the number of genetic studies aiming to resolve the genomic architecture of environmentally relevant trait variation at a fine scale, improving in that way our understanding on how species and populations may cope with rapidly changing climatic conditions.

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Section 2

Gene Sequence Diversity

Chapter 3

From the Gene Sequence to the Phylogeography through the Population Structure: The Cases of *Yersinia ruckeri* and *Vibrio tapetis*

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Additional information is available at the end of the chapter

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Abstract

Multilocus sequence analysis (MLSA) and multilocus sequence typing (MLST) are nowadays considered as gold standards in the study of microbial systematic, being both techniques based on the interpretation of the sequences of several housekeeping genes. In this context, the sequences can be analyzed from different points of view. On the one hand, the phylogeny of the bacterial species can be estimated using the MLSA approach and on the other hand, the structure of the population can be inferred by means of MLST. Moreover, most species display some degree of population structure that can be interpreted in geographic and chronological contexts, that is, phylogeographic studies. In this review, the phylogeny and population structure of two important fish and shellfish pathogens, *Yersinia ruckeri* and *Vibrio tapetis*, exhibiting very different evolutive patterns will be analyzed. In both cases, the species form robust and monophyletic groups from a phylogenetic point of view. Regarding to the population structure, very different results were found. While *Y. ruckeri* follows an epidemic model of clonal expansion with well-adapted clones that explode to be widely distributed, *V. tapetis* appears to have a mixed structure in where the paradox of clonality and high level of variability coexist. Furthermore, phylogeographical studies provided the evolutionary and geographical context for the species, allowing the determination of historical and spatial influences on the diversification of both species.

Keywords: *Yersinia ruckeri*, *Vibrio tapetis*, MLST, population diversity, population dynamics, mutation, recombination.

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1. Introduction

Phylogenetic analysis has long played a central role in basic microbiology. Sequence data offer direct genealogical information that can be efficiently used to estimate phylogenetic relationships and parameters associated with population dynamics. Furthermore, sequencing methods provide standardized and unambiguous data that are portable through online databases with direct access to the information needed to identify and monitor emerging pathogenic agents [1, 2]. Reconstructing the patterns of descent for a group of organisms can yield important awareness into why and how members of that group have specific characteristics and how those organisms are distributed across the environment. Integrating population patterns with phylogeny knowledge provides insights into epidemiological tracking of an organism at different evolutionary scales, from a single host to across the globe [3, 4]. On the other hand, more recently emerging fields of microbiology, including comparative genomics and phylogenomics, require substantial expertise in phylogenetic analysis and computational skills to handle the large-scale data involved [5]. Understanding the ways in which current and emerging technologies can be used to maximize phylogenetic knowledge is advantageous only with a complete proficiency of the strengths and weaknesses of these methods.

Since the conception of phylogenetic trees [6], morphological comparisons have been utilized to determine patterns of descent. Historically, numerous DNA-based approaches have been used to discriminate, subtype and build phylogenies for groups of organisms. Multilocus sequence analysis (MLSA) represents the standard in microbial molecular systematics. In this context, MLSA is implemented in a relatively straightforward way, consisting essentially in the concatenation of several gene fragments for the same set of organisms, resulting in one matrix which is used to infer a phylogeny by means of purely algorithmic methods [7–9].

For microbial pathogens, phylogenetic analyses are often conducted in order to determinate whether one particular outbreak may be related to another during times of an epidemic. While the clonal nature of an outbreak could be readily measured and predicted, Maynard-Smith et al. [10] pointed out the potential importance of homologous recombination as a determinant in the overall population structure of many bacterial species. These notions are now supported by several typing methods including multilocus sequence typing (MLST). Unambiguous genotyping systems are a key to describing epidemiological and ecological patterns and highlighting the evolutionary processes that shape microbial populations. Levels of genetic diversity are sufficiently high in most of microbial taxa that the sequences of several housekeeping gene fragments can provide a medium-resolution overview of their population genetic structure [11]. For the pathogenic bacteria whose members exhibit varying degrees of virulence, the integration of population genetic, evolutionary and epidemiological studies can provide important insights into the origins and spread of bacterial disease.

MLSA and MLST are based on housekeeping genes, which are subject to purifying selection and slow evolution and the variation within these genes is nearly neutral [12]. Although there are normally fewer polymorphic sites in individual housekeeping genes compared with hypervariable genes, the use of the combined sequences of multiple housekeeping genes has been shown to provide high discriminatory power while retaining signatures of longer-term evolutionary relationships or clonal stability. Furthermore, analysis of multiple loci can buffer against potentially skewed evolutionary pictures obtained by single-locus analysis [13, 14].

Most species display some degree of population structure that can be interpreted in geographical and chronological context [15]. Phylogeography uses genetic information to study the geographical distribution of genealogical lineages, especially those found within species [16, 17]. Because the discipline has deep roots in historical biogeography and population genetics, phylogeography was heralded as a bridge linking the study of micro- and macroevolutionary processes providing the empirical and conceptual link between systematics and population genetics. Based on appropriated sampling of individuals and genes, this approach allows the assessment of the biogeographic hypothesis, the description of the evolution of isolated reproductive population units and the inference of processes underlying the origin, distribution and maintenance of diversity [18]. Detecting concordance of geographical variation in genotypes, or their genealogies and the environment is therefore at the core of phylogeographic studies.

The generation of large volumes of sequence data, combined with the development of novel analytical techniques and conceptual advances, promises a better understanding of the complexity of the evolution of bacterial populations. The application, advantages and constrains of the MLSA, MLST and phylogeographic analysis in taxonomic studies will be illustrated in this chapter with two examples: *Yersinia ruckeri* and *Vibrio tapetis*.

2. MLSA: inferring phylogeny of bacteria species

16S rRNA gene was the most common phylogenetic marker during 40 years. This molecule has a slow rate of evolution so very often it is difficult to establish phylogenetic relationships among taxa with recent divergence. MLSA represents nowadays the novel standard in microbial molecular systematics [19]. This is a rapid and robust classification method to study phylogenetic relationships of very diverse taxa of prokaryotes, including entire genera, by combining the information contained in the sequences of several specific genes [19]. This technique consists essentially in the concatenation of the sequence of several housekeeping genes (more than five), being the relationships among taxa established by phylogenetic inference [7, 8, 20, 21]. The use of such amount of data provides increased resolution power than the use of a unique gene as in the case of 16S rRNA gene, although this marker is considered still useful at taxonomic levels above the species.

MLSA can be used for bacterial identification and classification as well as for inferring evaluative relationships and variability among different groups of bacteria. At identification level, there are several studies demonstrating that MLSA using the concatenation of eight housekeeping genes provides a robust phylogenetic resolution for microorganisms sharing 70–95% of average nucleotide identity (ANI) and therefore, it could distinguish species of the same genus [22]. It has been even proposed to replace DNA-DNA hybridization (DDH), although concerns have arisen about this replacement [23–26]. At systematic level, MLSA is considered an intermediate resolution technique between the 16S rRNA gene and the whole-genome-based approaches [19]. At evolutionary level, MLSA is a useful tool for studying the variability of different evolutionary identities, from families to species, as long as the selected genes for the analysis reflect properly the similarity of the complete genome among the studied group and the evolutionary ratios of the genes represent the evolution of the species.

The critical point for the MLSA is the suitability of the genes chosen for the analysis. In fact, genes that are perfectly informative within a given species, genus, or family may not be useful or even present in other taxa [19, 27]. Ideally, the best strategy to get a reasonable estimation of the species tree is to consider multiple genealogies inferred from unlinked loci and to use multiple individuals per species [28–30]. To date, there is not a general criterion for determining which genes are more useful for taxonomic purposes, but some attributes have been described for the genes to be used in the analysis [9, 26, 31, 32]. Genes should contain enough genetic information and although there is not a specification regarding to length, they should be small enough to be easily sequenced. Very often the fragments used in phylogenetic reconstructions are the same of those employed for MLST, resulting in too short fragments of the studied genes. The genes should also reflect the evolutionary history of the studied taxon [33]. Therefore, conserved genes must be selected for higher taxa and more evolved genes for species or subspecies levels. In that concern, the so-called core genes, the orthologous genes, should be used preferably than the accessory genes [34].

The availability of a universal set of conserved orthologous loci on a given taxon and, therefore, a set of primers that could amplified them across the studied group often precludes the comparative analysis of evolutionary process and patterns among closely related species and genera [26]. The strongest conflicting signals are usually derived from the existence of horizontal gene transfer (HGT) events in the dataset [35–37]. The resulting phylogenetic hypothesis may be distorted since standard treeing methods assume a single underlying evolutionary history [20, 38, 39].

There are no official recommendations about the inclusion of amino acid-base sequence analysis in MLSA studies although it is recommended because the study of the nucleotide sequences by themselves can lead to an "overinterpretation" of phylogenetic differentiation in closely related taxa [32]. Usually, the exchange in a base on the third position of a given codon has no influence in the resulting protein sequence and therefore in the structure and/or function of the protein, but also it can have the opposite effect. Because of that, nucleotide alignments should be done regarding their amino acid sequence. It must be taking into account that a bacterium is not only a sequence of DNA and for taxonomic purposes, the living unit at all its levels should be considered.

3. MLST: establishing the bacterial population structure

Nucleotide sequence data from multiple housekeeping genes in an appropriately sampled population can be used in a variety of analyses to determine population structure. The simplest of these analyses is MLST, which establishes the allele present at each locus and use a clustering algorithm to determine the relationships among strains from the matrix of pairwise differences between their allelic profiles [40]. The major advantage of MLST over others typing methods, such as multilocus enzyme electrophoresis (MLEE), is the unambiguous nature of the data obtained and the simple storage and electronically exchange, meaning that any isolate that is typed using the method can be rapidly compared with all previously typed strains.

The number of alleles obtained for each locus is much higher using MLST than MLEE and the information obtained by MLST is more precise. Publically available databases such as http://pubmlst.org and http://mlst.net provide examples where clinical subtyping has allowed epidemiological, geographical, and/or evolutionary hypotheses were for pathogens like *Neisseria meningitidis*, *N. gonorrhoeae*, *Streptococcus pneumoniae*, *Vibrio parahemolyticus* and *Staphylococcus aureus* [40–43]. National and international surveillance of bacterial clones can be performed using this resource.

Unweighted pair group method with arithmetic mean (UPGMA) dendrograms based on pairwise comparisons among allelic profiles can be structured on the website to detect relationships between query and/or isolates database. However, although clustering algorithms are useful for detecting the genetic relatedness of small number of isolates, they can result infeasible when visualizing larger sample sizes (e.g., >1000) in MLST database. As these methods are not based on an evolutionary model, they are often inaccurate in reconstructing evolutionary events [44]. The recent development of the algorithm eBURST [1] has addressed both issues. The model incorporated into eBURST assumes that, due to selection or genetic drift, some genotypes will occasionally increased the frequency in the population and then gradually diversify by the accumulation of mutation(s) and/or recombinational replacements, resulting in slight variants of the founding genotype. Using allelic profile data, one sequence type (ST) is assigned to each isolate. STs sharing high genetic similarity are grouped into clonal complexes (CCs). The founding genotype for each CC is then identified parsimoniously as the genotype differs from the highest number of the other genotypes in the CC at only one locus. Further diversification will produce variants of the founder allelic profile that differ at two or more locus. Thus, the simple principal underlying eBURST is that bacterial populations will consist of a series of clonal complexes (set of variants of a funding genotype) that can be recognized from the allelic profiles of the strains within a MLST database [1].

While MLST is very effective for establishing which isolates are identical or closely related, the approach will not provide major information about the relationships between more distantly related isolates, unless the population is strictly clonal. However, additional phylogenetic information can be gathered if the nucleotide sequences themselves are studied by analyzing the extent of linkage disequilibrium between alleles and looking for recombination by the congruence of gene trees, or the presence of mosaic structures [45, 46]. Knowledge of the recombination extent in bacterial pathogens is important since low levels of recombination result in a highly clonal population, where lineages persist with little variation over hundreds or thousands of years. At the other extreme, high rates of recombination lead to weakly and/or non-clonal populations in which lineages diversify so rapidly that the isolates recovered in one decade may be completely different from those recovered in the next [47].

For highly clonal species such as *Salmonella enterica* [48], most of substitutions in the genome have appeared by mutations. Alleles that arise independently multiple times in different branches are therefore incongruous with the tree. The phylogenetic relationships between isolates can be inferred from the dendrogram derived from the pairwise differences between STs and independently from a consensus tree constructed from the gene sequences. Characterization of weakly clonal pathogens (e.g., *N. meningitidis*, *S. pneumoniae*) is more problematic since clones diversify rapidly by the accumulation of recombinational exchanges. However MLST is very useful for the identification of the currently circulating hypervirulent lineages because these are recognized as clusters of isolates with identical, or very similar, multilocus sequence types.

4. Phylogeography: putting the geography into phylogeny

Phylogeography attempts to infer history from the geographical variation of genes and genetically controlled characters. In the phylogenetic/population genetic approach, graphical phylogenetics trees, networks, or clades are visualized from the observed variation data [49–51]. Thus, the usefulness of this approach is to integrate both phylogeny and geography within a quantitative analytical framework that encompasses the diverse aspects of phylogeography concordance [16, 52]. In this context, several classes of analytical techniques are used according to their function. The first class of techniques (i.e., AMOVA, Wombling, Monmonier's maximum difference algorithm, cline model by maximum likelihood) extracts spatial pattern from geographically distributed genetic data to identify either geographical partitions or clines (first-order pattern, in the terminology of spatial statistic), or alternatively, patterns of isolation-by-distance (second-order pattern) [53–55]. The second class (i.e., analysis of distance matrices, allelic aggregation index) attempts to infer historical scenarios directly from observed distributions of genes or taxa and one or more phylogenetic model [56]. A third class of techniques, such as Slatkin's distribution, provides statistical testing for the previously inferred scenario [57]. Phylogenetic trees and networks are often visualized over a cartographic background. Spatial interpolation algorithms [58] estimate parameter values at unsampled locations from a spatial distribution of observed points, providing a mean of interpreting and visualization the sampled data at different sets of locations [59, 60].

Many species show pronounced phylogeographic structure, or even regional or continental endemism, which counteracts the previously held paradigm of continuous and global panmixia. However, biogeographic and macro-ecological studies at the community level have shown that relatively few free-living microbial eukaryotes have cosmopolitan distribution [61, 62]. However, prokaryotes are generally smaller and have faster reproduction cycles than eukaryotic microorganisms that were the subject of these biogeographic studies [63]. Several studies have reported clear phylogeographic structuring in bacterial communities including marine, soil and soil-freshwater bacteria [64–66]. Conversely, the absence of spatial structuring in other prokaryotes has been corroborated by molecular data for bacteria from those same environments, including cyanobacteria [67–69]. For microorganisms occurring in extreme environments, phylogeographic structure indicates the effects of strong geographic isolation and dispersal constraints, although not all show clear spatial structure [70, 71]. For the more widely distributed bacteria, biogeographic patterns may result from historical and/or contemporary environmental processes. The importance of these processes in structuring microbial systems is still poorly understood [72] and few studies have focused on the phylogeographic structure and dispersal limitation in bacteria on a truly global scale in discontinuous but globally common habitats.

5. Case studies

5.1. Yersinia ruckeri

Yersinia ruckeri is a Gram-negative bacterium and the causative agent of enteric redmouth (ERM) disease or yersiniosis in salmonid and non-salmonid fish reared in both fresh and marine waters. *Y. ruckeri*, initially isolated from rainbow trout (*Oncorhynchus mykiss*) in the Hagerman valley of Idaho (USA) in the 1950s [73], is now widely found in fish populations throughout North and South America, Australia, Africa and Europe [74]. The pathogen *Y. ruckeri* is a serologically variable, highly clonal species. It includes two biotypes: biotype 1 strains are positive for motility and lipase activity, whereas biotype 2 strains are negative for both tests [75]. The species has been grouped into 6 serovars [76], 5 O-serotypes [77], or 4 O-serotypes with different subgroups [78] by using different serotyping systems. In addition, *Y. ruckeri* strains can be grouped into clonal types on the basis of biotype, serotype and outer membrane protein (OMP) profiles [79]. Strains of serotypes O1a (classic serovar I) and O2b (classic serovar II) cause most epizootic outbreaks and serotype O1a is predominant in cultured salmonids [76, 78].

ERM has been successfully controlled for decades by vaccination with commercial monovalent killed whole-cell vaccines. Although formulations of most commercial vaccines are based only on serotype O1a (Hagerman strain), different degrees of cross-protection among serotypes have been described [76]. In recent years, reports of ERM vaccine breakdown have emerged in Europe and USA mostly attributed to biotype 2 strains [80–82]. Other epizooties have occurred in vaccinated Atlantic salmon (*Salmo salar*) from Chile, caused by serotype O1b/biotype 1 *Y. ruckeri* strains [83].

Molecular techniques have been used to study the intraspecific genetic variability showing a low genetic diversity. By using of MLEE was identified only four electropherotypes for 47 isolates of *Y. ruckeri* indicating that the genetic structure of *Y. ruckeri* is clonal, with one predominant clonal group [84]. The ribotypes, patterns of pulsed-field gel electrophoresis (PFGE) and interspersed repetitive sequences (IRS)-PCR of 30 *Y. ruckeri* O1a strains have been studied, reporting a high level of genetic homogeneity for all the isolates [85]. On the other hand, a total of 44 pulsotypes from 160 isolates identified by PFGE have provided better insights into the relationship between similar *Y. ruckeri* clones responsible for recent ERM outbreak among salmonid [86]. Heterogeneous assembly of phenotypes in serotype O1a *Y. ruckeri* strains with respect to pathogenicity and host has been reported [87]) and the need of expansion of the clonal group theory in this species was suggested, highlighting therefore the existence of new clonal groups [88].

In the context of the genetic approach, none of the studies have focused on the sequencing and analysis of housekeeping genes to understand the *Y. ruckeri* population structure. The existing studies have been limited to MLSA analysis in which few isolates were included for the comparison and description of new species within the *Yersinia* genus [89–91].

Using a sequence-based approach, new studies were developed by our research group to reconstruct the phylogeny and to characterize the molecular epidemiology and population structure with a collection of 103 strains of *Y. ruckeri* (**Table 1**). Studies included the sequencing of six housekeeping genes *gln*A (glutamine synthetase), *gyr*B (DNA gyrase B subunit), *rec*A (recombinase A), Y-HSP60 (heat-shock protein 60 kDa), *dna*J (heat-shock protein 40 kDa) and *thr*A (aspartokinase-homoserine dehydrogenase), as described by [92].

Origin	**Isolates (*n*)**	**Biotype**	**Serotype**	**ST**[*]	**Host/source**
USA	19	1, 2	O1a, O1b, O2b, O3, O4	8	*O. mykiss, S. trutta, O. tshawytcha, Ictalurus punctatus*
UK	6	1, 2	O1a, O1b	4	*O. mykiss*
Portugal	21	1, 2	O1a, O3	5	*O. mykiss,* water, sediment
Chile	11	1	O1a, O1b, O2b	3	*S. salar*
Peru	28	1, 2	O1a	7	*O. mykiss*
Denmark	4	1	O1a, O2b,	4	*S. trutta, Anguilla anguilla, O. mykiss*
Spain	4	1	O1a, O2b	2	*O. mykiss*
Finland	3	1, 2	O1a,	2	*O. mykiss, S. salar*
Canada	4	1	O1a, O1b, O2a	3	*S. salar, Rutilus rutilus, O. tshawytscha, S. malma, Ondatra zibethicus*
Germany	1	1	O2b	1	*O. mykiss*
Scotland	1	1	O2b	1	*O. mykiss*
Norway	1	1	O1b	1	*S. salar*

[*]Established by Bastardo et al. [92].

Table 1. Summary of *Y. ruckeri* strains studied.

Similarity matrix of intraspecific sequence for the individual genes ranged 97.2–100% for *dna*J, 98.5–100% for *gyr*B, 98.8–100% for *gln*A, 99.4–100% for Y-HSP60, 98.1–100% for *rec*A and 99.0–100% for *thr*A. For the concatenated sequence (2786 bp) of encoding-protein genes, the similarity was determined between 98.9 and 100%. The best-fit model of nucleotide evolution was determined using Modeltest 3.7 and following the Akaike information criterion (AIC). The maximum-likelihood (ML) estimation was implemented in PHYML 3.0 without substitution rate heterogeneity correction or invariant estimation as recommended by Modeltest. Clade support was evaluated by analyzing 1000 bootstrap pseudo-replicates. To further probe the robustness of our MLSA-based phylogeny, the DNA sequence data were analyzed by the neighbor joining (NJ), maximum parsimony (MP) and Bayesian inference methods. All three analyses yielded similar tree topologies, which were highly congruent with our previous findings based on maximum likelihood (**Figure 1**).

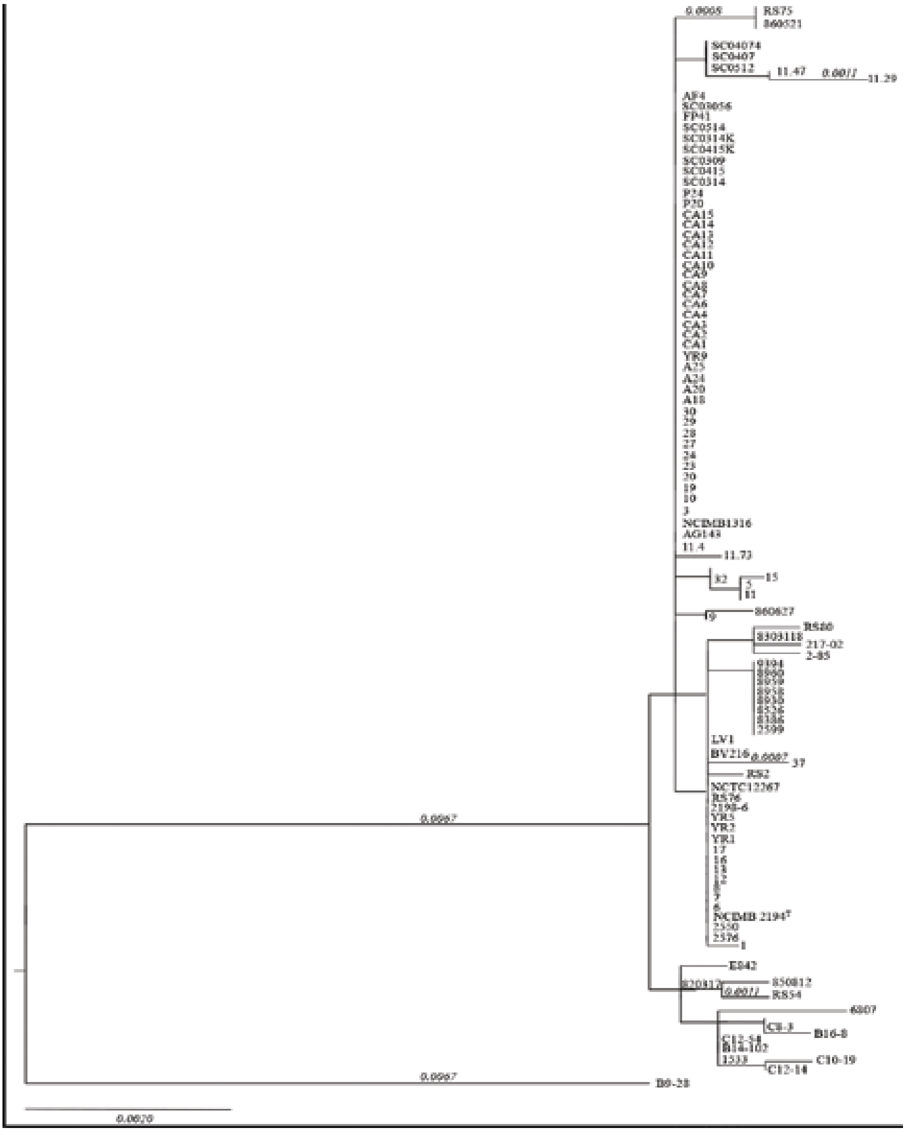

Figure 1. Phylogenetic tree based on concatenated sequences of six housekeeping genes of *Y. ruckeri* obtained by the ML method.

The results of the MLSA analysis confirm that there is significant diversity within *Yersinia ruckeri* isolates showing that they formed distinct clusters. Except one isolate, all *Y. ruckeri* strains joined in a major cluster with a complex topology that does not seem to reflect previous typing schemes of the species [84, 85]. Using MLSA, the ML tree topology suggests major genetic diversity among the isolates of the serotype O1a. Thus, isolates belonging to serotype O1a appear spread among different branches together with other serotype/biotype representatives. Interestingly, only the groups of isolates associated with recent outbreaks in USA, Chile and Peru fall into well-defined branches.

Based on the sequences of the six housekeeping genes available in the public database htpp://publmst.org/yruckeri/, a MLST scheme for *Y. ruckeri* has been developed [92]. **Table 2** shows a descriptive analysis of nucleotide and allele diversity for each locus. Synonymous substitutions (d_S) occurred more frequently than non-synonymous substitutions in every gene (d_N). Furthermore demonstration that all loci are under purifying pressure ($d_N/d_S < 1$) was obtained, which means a strong selection such that most amino acid substitutions are deleterious, as being typically observed for housekeeping genes.

	Size of fragment (pb)	**Alleles**	**Polymorphic sites**	***Pi***	***H***	d_N	d_S	d_N/d_S
*gln*A	416	9	7	0.0064	0.2528	0.0042	0.0123	0.3427
*gyr*B	454	6	8	0.0075	0.3107	0.0043	0.0194	0.2238
Y-HSP60	509	4	4	0.0039	0.0577	0.0000	0.0159	0.0000
*rec*A	472	4	11	0.0125	0.5773	0.0047	0.0354	0.1339
*dna*J	632	7	16	0.0078	0.4717	0.0000	0.0340	0.0000
*thr*A	303	4	3	0.0055	0.4841	0.0074	0.0000	-

Table 2. Genetic characteristics and evolutionary variation among the six loci included in the MLST scheme for *Yersinia ruckeri*. *Pi,* average number of nucleotide differences per site; d_N/d_S, ratio of mean non-synonymous substitutions per non-synonymous site/mean synonymous substitution per synonymous site; *H*, Nei's index of diversity.

Among all isolates of *Y. ruckeri,* 30 different sequence types (ST) were established (**Table 3**), 21 of which were represented by a single isolate, evidencing high genetic diversity. From these MLST scheme, eBURST analysis identified two clonal complexes (CC) showing a common evolutionary origin for 94 isolates forming 21 STs into CC1 and for six isolates of six STs in the CC2. ST 14 and ST 21 were identified as founder sequences into CC1 and CC2, respectively (data not shown). Furthermore, the formation of three STs (singletons), no associated to any clonal complexes, suggests genetic diversification among *Y. ruckeri* strains into the population.

All alleles analyzed showed to be in nonrandom distribution or linkage disequilibrium (I_A value of 0.5563, $p = 0.000$) in the *Y. ruckeri* population, suggesting that mutation drives the diversification of this species and supporting a clonal structure for the population [10]. Furthermore, recombination events were not detected for *gln*A, *rec*A, Y-HSP60, *dna*J and *thr*A when DnaSP5 and RDP4 software was used. However, recombination was found among *gyr*B alleles ($R_{min} = 1$), indicating that the relative low genetic diversity present in all of alleles analyzed could have obliterated the chance of detecting recombination in the other five loci and suggesting that recombination could occur within different subpopulations.

ST	**Profile**						**Frequency %**	
	***gln*A**	***gyr*B**	**Y-HPS60**	***rec*A**	***dna*J**	***thr*A**		
2	1	1	1	1	1	2	43	41.75
1	1	1	1	1	1	1	16	15.53
7	1	1	1	1	2	1	8	7.77
3	1	2	1	1	2	2	4	3.88
16	6	1	1	1	1	2	3	2.91
9	1	3	1	1	1	1	2	1.94
14	1	1	1	1	2	2	2	1.94
23	1	1	1	1	6	2	2	1.94
26	1	5	1	1	2	2	2	1.94
4	2	1	1	1	2	2	1	0.97
5	1	1	2	1	2	2	1	0.97
6	3	1	1	1	1	2	1	0.97
8	1	2	3	1	3	1	1	0.97
10	4	1	1	1	1	1	1	0.97
11	1	4	1	1	1	1	1	0.97
12	1	3	1	1	1	2	1	0.97
13	1	2	1	1	1	1	1	0.97
15	5	1	1	1	2	2	1	0.97
17	7	1	1	1	4	4	1	0.97
18	1	5	1	1	2	1	1	0.97
19	8	6	4	4	5	2	1	0.97
20	9	1	1	1	6	1	1	0.97
21	7	2	1	1	6	1	1	0.97
22	7	2	1	1	4	4	1	0.97
24	1	1	1	1	4	4	1	0.97
25	7	2	1	1	1	2	1	0.97
27	7	2	1	1	7	2	1	0.97
28	1	1	1	1	1	3	1	0.97
29	1	1	1	2	1	2	1	0.97
30	1	1	1	3	2	2	1	0.97

Table 3. Allelic profiles of MLST scheme established for *Y. ruckeri* (htpp://publmst.org/yruckeri/).

Based on the single-locus variables (SLVs) found between the two clonal complexes and the different subgroups identified by eBURST algorithm, the variant alleles can be used to determine the events responsible for the evolution into the population [93]. Thus, the per-allele and per-site recombination/mutation (r/m) parameter was calculated empirically from 25 SLVs identified within the two clonal complexes in *Y. ruckeri*. Twenty out of 25 SLVs arose from a recombination event, whereas only five arose by mutation. This resulted in a per-allele r/m parameter of 4:1. In the case of the per-site analysis, r/m parameter ratio was 7.5:1. These two parameters suggest that the initial steps of *Y. ruckeri* clonal diversification at allele or individual nucleotide sites are 4- and 7-fold more likely to occur by recombination than by point mutation. Recombination appears then to play a greater role than mutation for the generation and maintenance of the genetic diversity of *Y. ruckeri* (**Figure 2**).

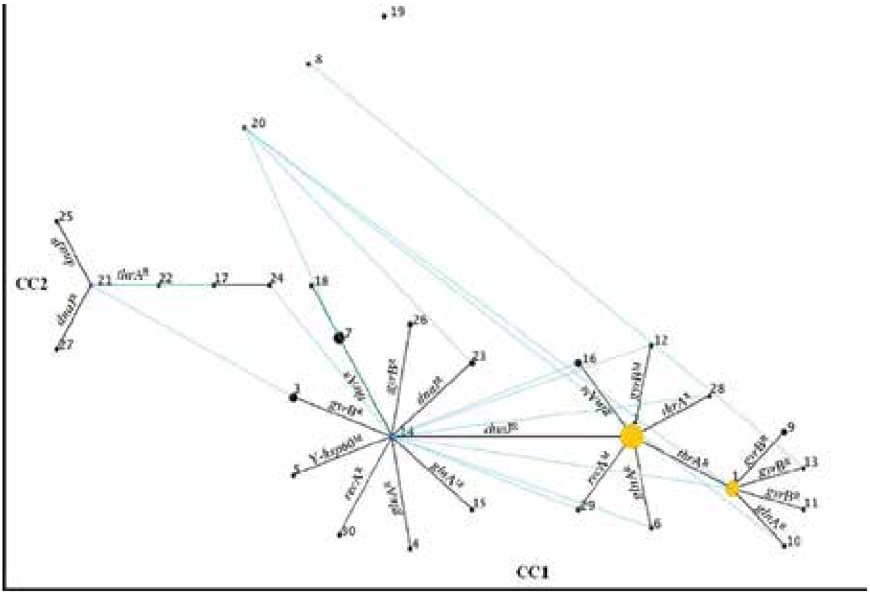

Figure 2. Population snapshot with all the sequence types (STs) included in the MLST database for *Y. ruckeri*. The snapshot was made using the eBURST program. STs that are SLVs of each other are shown connected by black lines. DLV STs are shown connected by aqua lines. Clonal complexes (CC) are marked. The sizes of the circle are related to the number of strains within each ST. The founder and cofounder genotypes are colored blue and yellow, respectively. A superscripted R or M indicates recombination or mutation, respectively (from [92]).

Epidemic model is also consistent with the epidemiology of *Y. ruckeri*, which suggest that ERM started as a geographically isolate disease that relatively quickly became widely disseminated [74, 92]. In an epidemiological approach, the strong association of sub-founders ST 1 and ST 2 to the majority of the ERM outbreaks in salmonid cultures also allows to link these STs to virulence of *Y. ruckeri* strains. On the other hand and although serotypes were not strictly associated with the STs in this MLST study, our results suggest that serotypes O1a and O1b are an example of recently emerged and disseminated variants. In addition, nonmotile *Y. ruckeri* strains (biotype 2), causing recent outbreaks in vaccinated fish, were included into the sub-founders ST 1 and ST 2, indicating that biotype 2 phenotype may have evolved from related motile *Y. ruckeri* strains.

The phylogeographic analysis showed concordance with the eBURST diagram obtained previously for the *Y. ruckeri* population. Thus, it was possible to construct the complete evolutionary networks, showing the missed putative variants linking the established STs separated into two clonal complex and three singletons by using eBURST algorithm (**Figure 3**). The inclusion of the geographical data into this analysis indicates high genetic differentiation into *Y. ruckeri* caused by the fixation of different alleles into one geographical area (data not shown). These findings explain the high diversity of STs found in Europe and USA, including those observed in not salmonid fish species and supports the hypothesis that the majority of *Y. ruckeri* STs has evolved independently in specific areas. Furthermore, the presence in USA, UK and Peru of the different STs grouping nonmotile isolates provides strong evidence of the independent emergence and dissemination of biotype 2 *Y. ruckeri* strains in different geographical areas [92, 94, 95].

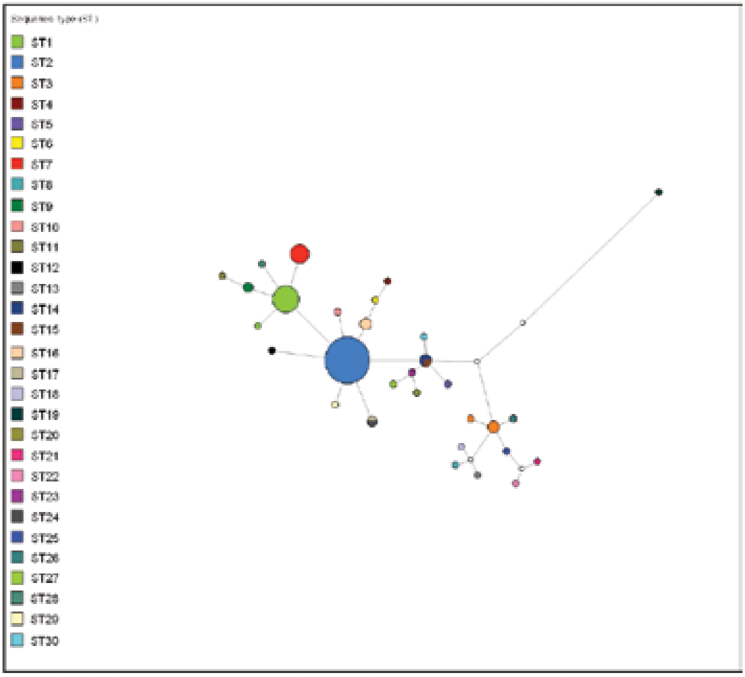

Figure 3. Parsimony network (95%) of *Y. ruckeri* based on the ST alignment. Colors indicate sequences correspondent to STs. A line between STs represents mutational step and open circles represent STs not present in the sample. Radius of the circle represents number of sequences.

The sequence dataset was divided into 29 predefined subpopulations consisting of sequences from STs that present in each geographical origin and the geographical distances between different populations were measured using geographical coordinates. The Mantel test ("isolation-by-distance" analysis) for the matrix of correlation between genetic and geographic distance showed no significant correlation positive for the full dataset ($Z = 18 \times 10^{11}$, $r = 0.0139$ one side $p = 0.5959$), indicating a lack of overall genetic differentiation between the different geographical area. Furthermore, demographical analyses indicate a recent global expansion of *Y. ruckeri* revealed by both Tajima's test ($D = -1.669$, $p < 0.001$) and Fu's test ($Fs = -13.83056$, $p < 0.001$). This fact can explain the emergence of genetic variants that have caused the recent outbreaks in farmed salmonids in several areas, as occurred in Chile, Canada and Portugal where uncommon serotype and phenotype isolates were involved (**Figure 4**) [95].

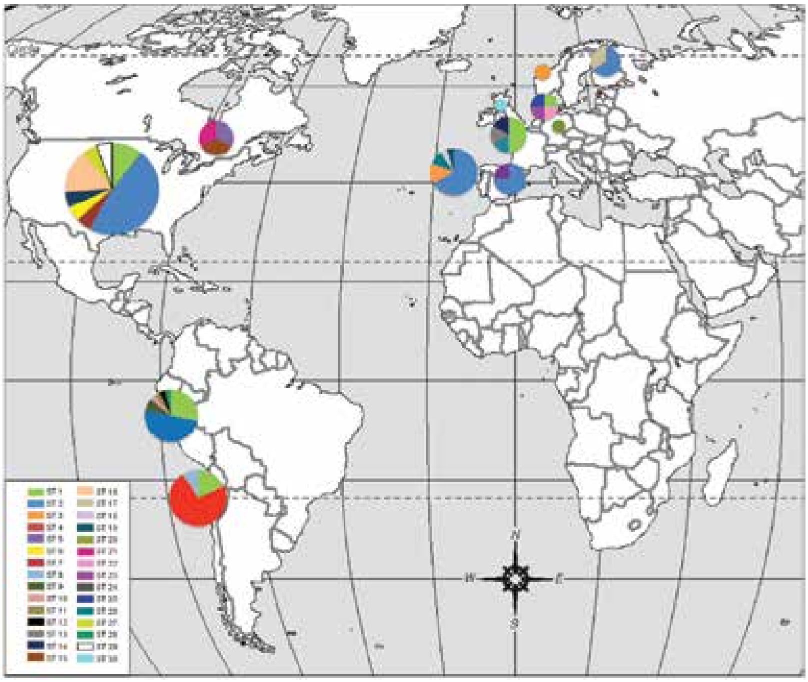

Figure 4. Global distribution of STs of *Y. ruckeri* population based on multilocus allelic profiles defined for the eBURST analysis.

5.2. Vibrio tapetis

Vibrio tapetis is the causative agent of brown ring disease (BRD), a major limiting factor for the culture of Manila clams (*Ruditapes philippinarum*) in Europe which has been associated with large economic losses in the sector [96]. It is considered the only disease with demonstrated bacterial etiology that affects adult clams. This disease was first described in Landeda (France) in 1987 associated with an episode of mass mortality of Manila clam. Since then, it has been detected throughout the European Atlantic coast and occasionally in the Mediterranean and the Adriatic coast as well as in Korea and Japan [97, 98]. The disease received its name because of the most visible symptom in affected animals, the presence of an abnormal deposit of brown organic (composed mostly of conchiolina) on the inner surface of the valves, usually located between the pallial line and the edge of the shell and not subjected to calcification processes [97, 98]. *V. tapetis* was initially considered as a homogeneous taxon but further studies of new isolates with different geographic and host origin, including fish and shellfish species, demonstrated the existence of intraspecific variability, at phenotypic, serological and genetic levels. Serologically, at least three serogroups were detected using slide agglutination and dot blot [99, 100]. At genetic level, differences were first detected in the plasmid content and ribotypes of the different strains [101, 102]. More recently, based on ERIC-PCR (enterobacterial repetitive intergenic consensus), REP-PCR (repetitive extragenic palindromic) and RAPD (randomly amplified polymorphic DNA analysis), three major groups associated with the host and the serogroup were established [103]. The existence of these three groups was confirmed by preliminary studies of MLSA and protein expression by 2D-PAGE studies. This MLSA study was performed on the basis of five protein-coding housekeeping genes but only with three representative strains of the described groups [104].

Population structure and phylogenetic analysis (as well as its relationship with the geography) of *V. tapetis* was performed using thirty strains with different host and geographic origin: seven strains isolated from *R. philippinarum* in France between 1988 and 1994; eight strains isolated also from *R. philippinarum* in Spain in the years 1994, 2005 and 2007; one strain of French cockle isolated in France in 1990 as well as other isolate from the same location and year isolated from Venus clam (*Venerupis pullastra*); two Irish isolates obtained from *R. philippinarum* seed (2005); two isolates from Spanish carpet shell clam (*R. decussatus*) isolated in 1994; one isolate from halibut (*Hippoglossus hippoglossus*) cultured in UK isolated in 2001; three isolates from shi drum (*Umbrina cirrosa*); four isolates from wedge sole (*Dicologoglossa cuneata*) isolated in Spain in 2007 and 2005, respectively; and one isolate from corkwing wrasse (*Symphodus melops*) obtained in Norway in 1999.

The partial sequences of ten housekeeping genes were used: *atp*A (α subunit of ATPase), *fst*Z (cell division protein), *gap*A (glyceraldehyde-3-phosphodehydrogenase), Y-HSP60, (heat-shock protein 60 kD), *pyr*H (uridyl monophosphate kinase), *rct*B (replication origin-binding protein), *rec*A (recombinase A), *rpo*A (α subunit of RNA polymerase), *rpo*D (RNA polymerase sigma factor), *top*A (topoisomerase I). The selected genes were demonstrated to be phylogenetically resolutive in other *Vibrio* species [105] and the results were also compared with those obtained by DDH [106] as well as by other typing methods [101–103, 107]. The concatenation of these ten genes rendered a fragment of 5826 bp in length. The intraspecific sequence similarities ranged between 84.8 and 100% for individual genes and between 93.3 and 100% for the concatenated sequence.

The phylogenetic reconstruction for the concatenated gene sequences was done using three different methods, NJ, MP and ML, using in all cases 1000 bootstraps. Topology of all the trees was the same, showing only some differences at bootstrap values. Visual inspection of the *V. tapetis* concatenated alignment tree reveals the existence of two tight clusters, one formed by most of the isolates and a second one, smaller, composed for three isolates (**Figure 5**). These two clusters shared sequences similarities of 93.3–93.5%, while strains within each cluster showed less than 0.6% of gene sequence divergence variation in cluster 1 and less than 0.4% in cluster 2, being most of the substitutions located at third position of each codon.

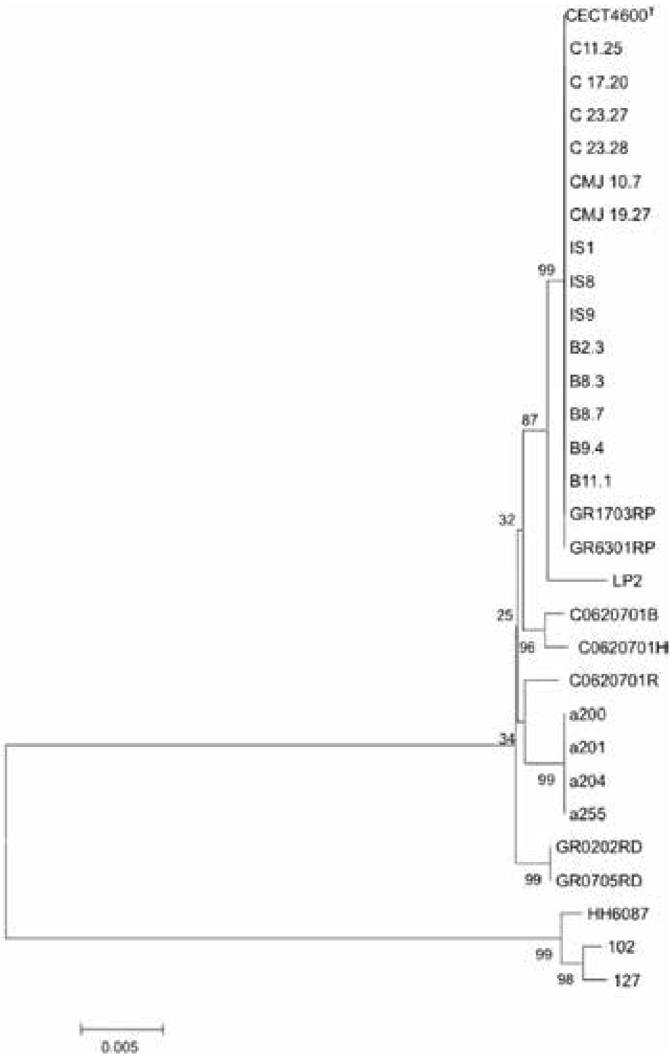

Figure 5. Phylogenetic reconstruction based on concatenated alignment of ten housekeeping genes of *V. tapetis* using the NJ method. Horizontal branch lengths are proportional to evolutionary divergence. Bootstrap from 1000 replicates appears next to the corresponding branch.

In the biggest cluster, high diversity is observed regarding to their host and geographical origin, containing the isolates classified as group one (represented by the type strain) and group two (represented by the isolate GR0202RD) by Rodríguez et al. [103]. As can be observed, different branches are formed, most of them related with host origin: the adult Manila clam isolates together with those from cockle and Venus clam cluster in the major branch and related to them appears the corkwing wrasse isolate. The carpet shell clam isolates fall into an individual branch as well as the wedge sole isolates, which form a cluster close to shi drum isolates. The second cluster, formed by isolates HH6087 (halibut), 102 and 127 (*R. philippinarum* seed), is a very robust branch supported by a 99% bootstrap. These isolates have in common the geographic origin, being all of them isolated in British Isles (UK and Ireland) although from different hosts. The two-cluster topology is supported by the trees generated individually for each gene.

MLST analysis revealed the heterogeneity of the population of this clam pathogen. The high variability of the population is reflected in the number of identified alleles ranging from 3 to 9 depending on the gene analyzed. The allele combination leads to the description of 10 STs (**Table 4**), all of them constituting singletons. Even when the stringent SLV criterion was relaxed (from 9/10 to 1/10 shared alleles), none of the SLV or DLV was found (data not shown). This variability is also reflected in the 450 single-nucleotide polymorphisms (SNPs) detected across the 5826 bp surveyed. The majority of the SNPs were biallelic, being only 7 of them were triallelic. The nucleotidic substitutions found throughout the concatenated sequence showed, as usual for housekeeping genes, more frequency in synonymous substitutions (d_N) than synonymous one (d_S). The ratio d_N/d_S shows that all the genes except by *rpo*D are under positive selection (**Table 5**).

ST	*atp*A	*fst*Z	*gap*A	Y-HSP60	*pyr*H	*rct*B	*rec*A	*rpo*A	*rpo*D	*top*A	n
1	1	1	1	1	1	1	1	1	1	1	17
2	2	1	2	2	2	2	2	1	2	2	2
3	3	2	3	3	3	3	3	2	3	3	1
4	4	3	3	4	4	4	4	2	4	4	1
5	5	4	3	5	5	5	5	2	4	5	1
6	6	1	1	6	6	6	6	1	5	6	1
7	6	5	1	6	6	2	7	1	6	6	1
8	7	1	1	7	1	2	7	1	6	7	1
9	8	1	4	1	7	7	8	3	7	8	4
10	1	6	5	8	8	8	9	1	8	9	1

Table 4. Allelic profile of MLST scheme established for *V. tapetis* (htpp://publmst.org/vtapetis/).

The alleles showed to be in linkage disequilibrium (I_A value for the whole strain collection was 6.3008 ($p = 0.000$)), being therefore the mutation of the main cause of diversification. These data are in agreement with the fact that even when a number of approaches were used to achieve recombination events, only using R_{min} and Phi test some events were found in *atp*A, *pyr*H, *rct*B, *rec*A, *rpo*D and *top*A genes, but not utilizing RDP4 (**Table 5**). However, the SplitsTree generated for all the isolates included in this study shows some structures typical from high recombinatory populations (**Figure 6**). It has been described that the simplest method to detect recombination in aligned sequences is to look for mosaic structures by eye. Significant mosaic structure is indicative of recombinatorial exchange, usually among isolates of the same species [93].

The contradiction between the results inferred for I_A index and those from the SplitsTree can be explained analyzing the deduced genealogies after stripping sequences of recombinational

Gene	Loci			Polymorphisms				Index of diversity			Recombination	
	Length (bp)	G+C content	Alleles	SNPs	SVS	PIS	S_{nonsyn}	*Pi*	d_N/d_S	H	R_{min}	Phi test
***atp*A**	600	46.51	8	25(25)	2	23	1	0.01257	0.0087	0.9556	1	p =0.081
***fst*Z**	609	46.38	6	49(51)	12	37	6	0.01291	0.0204	0.7778	0	p =1.000
***gap*A**	588	45.23	5	8(10)	0	8	0	0.00292	0.0000	0.8000	0	p =1.000
***hsp*60**	525	44.91	8	35(37)	5	30	6	0.01167	0.0270	0.9556	0	p =1.000
***pyr*H**	516	48.42	8	38(38)	2	36	1	0.01332	0.0036	0.9556	1	p =1.000
***rct*B**	600	43.14	8	95(96)	3	92	7	0.02975	0.0095	0.9333	1	p =1.000
***rec*A**	588	45.86	9	64(66)	4	60	6	0.02117	0.0237	0.9778	4	p =0.397
***rpo*A**	600	47.00	3	7(7)	0	7	1	0.00226	0.0528	0.6000	0	p =1.000
***rpo*D**	600	43.52	8	47(47)	2	45	13	0.01598	0.1111	0.9556	5	p =0.586
***top*A**	600	47.75	9	82(86)	5	77	10	0.02633	0.0188	0.9778	1	p =0.650

Table 5. Genetic characteristics, evolutionary variations and recombination among the ten loci included in the MLST scheme of *V. tapetis* population.

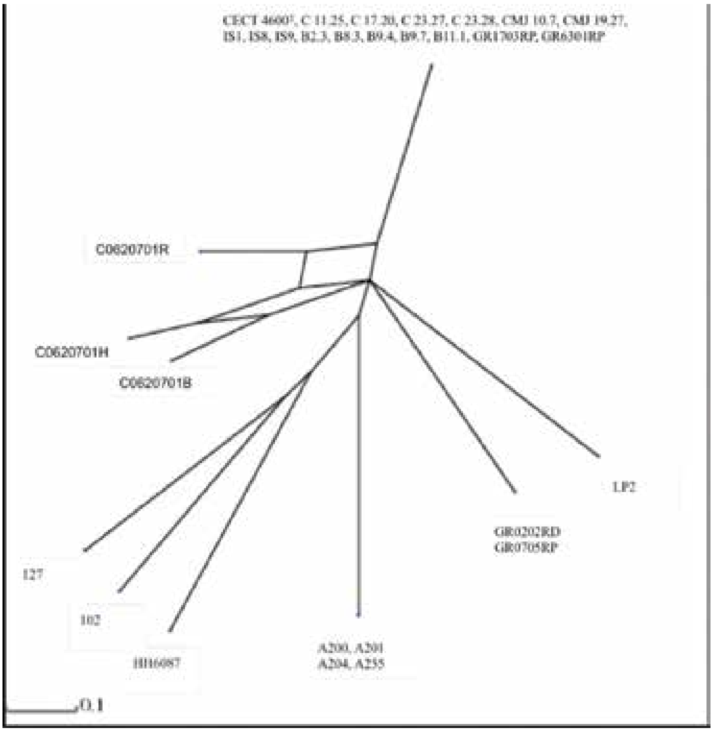

Figure 6. Split decomposition analysis of the concatenated sequences of the 30 *V. tapetis* strains was studied. The splitgraph was generated using SplitsTree v4.

events performed with the ClonalFrame [108]. **Figure 7** shows the clonal genealogy constructed for the 30 isolates (**Figure 7A**) and the evolutionary events for the three nodes tasked in the tree (**Figure 7B**) in where the height of the red line indicates the probability of recombination on a scale from 0 (row bottom) to 1 (row top) and each nucleotide substitution is represented by a black cross. Node A represents the divergence among the two clusters generated by MSLA analysis and it can be seen in the representation of evolutionary events are exclusively generated by mutation events. The opposite occurs at nodes B and C, which represent the diversification between cluster one and two, respectively and their evolution is produced mainly by recombination.

Since the three isolates of the cluster two of *V. tapetis* have in common the geographic origin (British Isles), a phylogeographic approach was used with the aim of correlate geographic

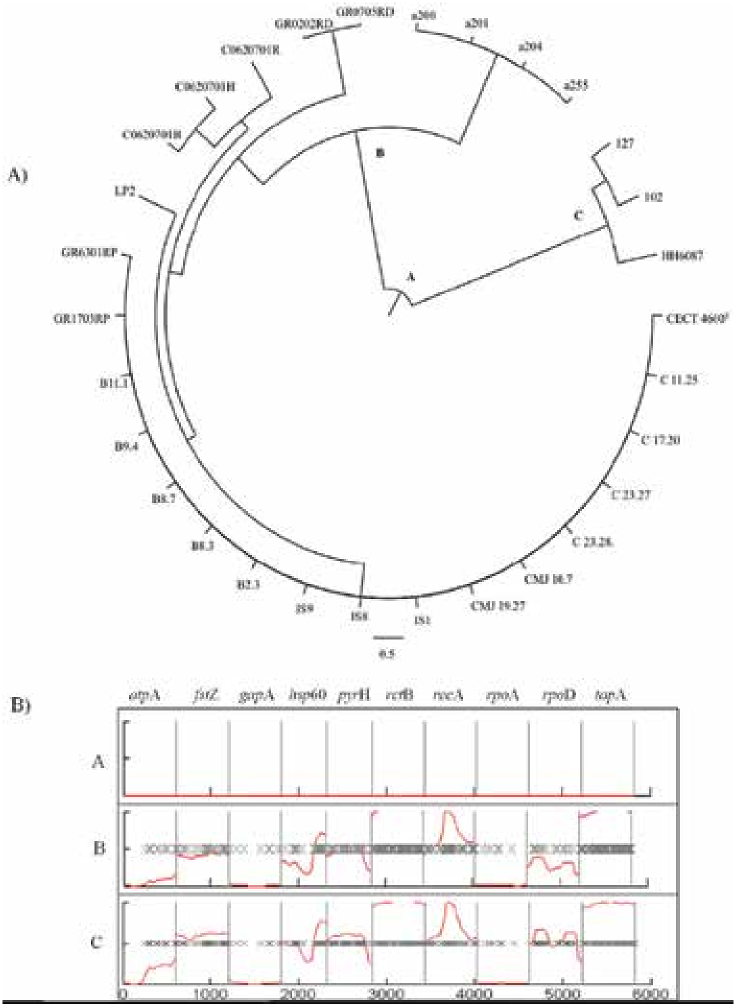

Figure 7. Clonal genealogy (A) and evolutionary events (B) reconstructed by ClonalFrame from concatenated sequences of *V. tapetis* population.

origin and genetic evolution of the isolates. The concatenated sequences were divided into four subpopulations consisting in sequences of STs that belong to each geographic origin. For this study, regions of the same country were not taking in account. Despite the two well-differentiated groups showed in the phylogeograpic network (**Figure 8**), significant correlation was not observed among the identified STs and geographic distance in the "isolation-by-distance" analysis (Mantel test: $Z = 25 \times 10^{11}$, $r = 0.1314$, $p = 0.9750$). Values obtained for Tajima's test ($D = -0.99118$, $p < 0.10$), negative and non-significative and Fu's test ($Fs = 29.227$, $p = 0.000$), positive and significative, are indicatives of population expansion. These indexes indicate that the population has suffered a recent expansion following a bottleneck or a selective sweep.

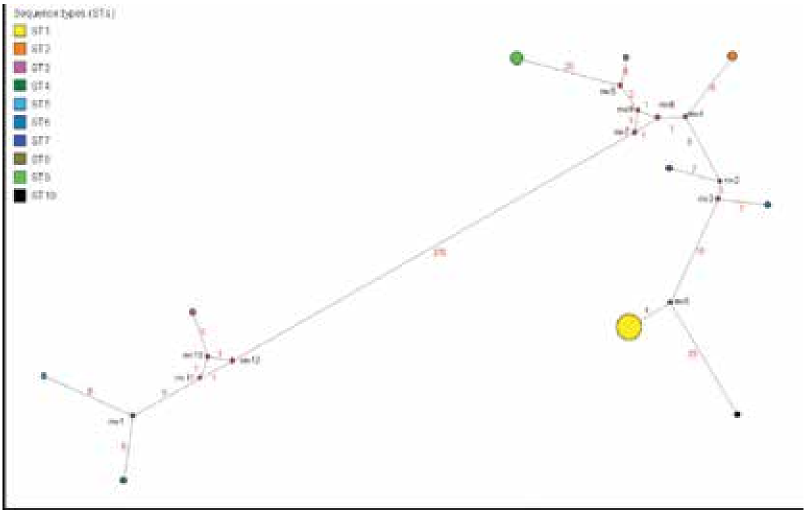

Figure 8. Representation of the evolutionary network using the combination of allelic variants (STs) of *V. tapetis* population. Red dots indicate possible intermediates in the evolution of the species.

The phylogeographic network is very useful to clarify all the previous data. First, the two groups of isolates that can be observed are the same two clusters of the phylogenetic study (MLSA) and in the inferred clonal genealogy. The node A in the clonal genealogy is represented in this phylograph by the long branch (note that this is not an evolutive method, so that length is not representative of distance) generated by 370 nucleotidic substitutions, which according to the reconstructed evolutionary study are likely mutations. At the ends of this branch are located nodes B and C on the clonal genealogy (and the two clusters in MLSA), which are generated essentially by recombination according to ClonalFrame. These recombinatory events can be seen in the topology of the graphic. On the other hand, the inconsistence between the results in I_A index (predominance of mutation) and the splitgraph (predominance of recombination) can be explain on the basis of the amount of nucleotide substitutions produced by mutation between the two clusters (370 substitutions between the two groups) that are probably masking recombination events in the evolution of each group. Moreover, the lack of clonal groups in the population structure obtained by eBURST algorithm can be explain for the amount of intermediated evolutive isolates missed in the population, represented as red dots in the graphic.

To date, the groups defined for *V. tapetis* have been associated to their host origin. In this work, we described species diversification for the first time on the basis of geographic origin. Distribution of identified STs for *V. tapetis* population is shown in **Figure 9**. In the analysis performed, both at phylogenetic and population levels, three isolates appear in an independent group. These isolates show different host origin, halibut and seed of Manila clam, but it have a common geographical origin, British Isles. These groups present huge genetic distance between them, produced mostly by mutation, supporting this finding that the description of two subspecies for *V. tapetis: V. tapetis* subsp *tapetis and V. tapetis* subsp *britanniensis* [106]. The former comprises the majority of the isolates regardless their geographical origin, whereas the latter includes the British isolates. *V. tapetis* shows a non-clonal, panmictic population in where the two subspecies are

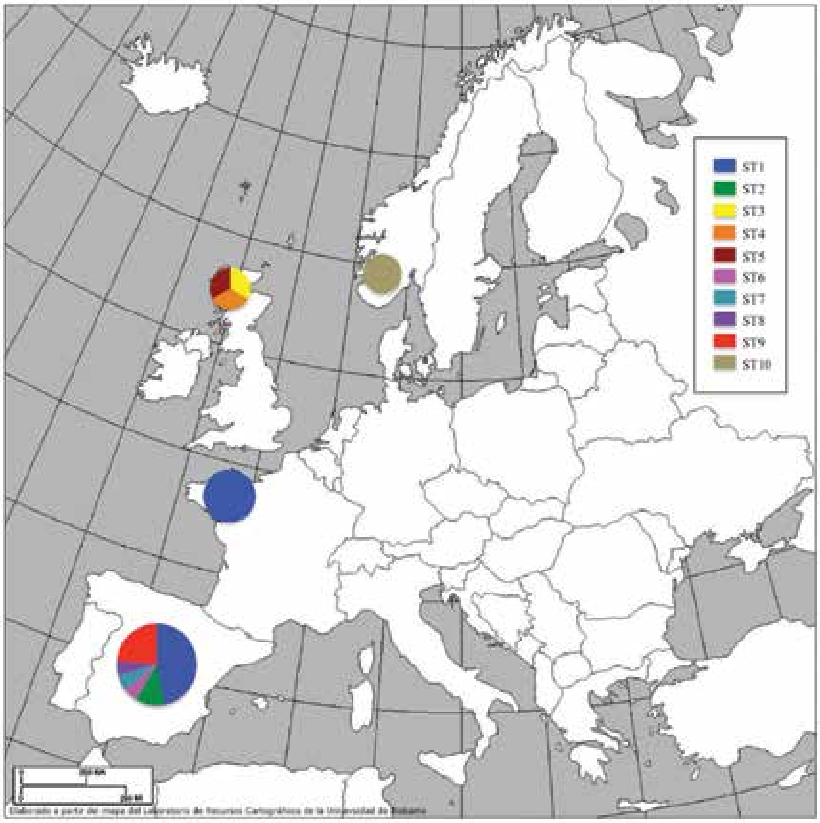

Figure 9. Geographic distribution of the ten STs identified for *V. tapetis*. Regions of the same country are considered as a unique localization.

generated by mutational events but the diversification within each of them is produced mostly by recombination [109].

6. Conclusions

In conclusion, MLSA, MLST and phylogeographic analysis are successful for (i) unambiguously genotyping both *Yersinia ruckeri* and *Vibrio tapetis* species, (ii) establishing evolutionary relationships among the bacterial populations at different levels and (iii) capturing geographical structure of these pathogens. The case studies reviewed here constitute good examples of the usefulness of these powerful tools for understanding the evolution, epidemiology and genetic population/landscape of bacterial pathogens.

The results obtained from our works suggest that the processes involved in the genetic variability and evolution in both species are different. Using the MLST approximation, two different expansion models of population were detected, a mutation-based epidemic model for *Y. ruckeri* and panmictic for *V. tapetis*, where recombination represented the genetic event contributing mostly to diversification.

The phylogeographic approach indicated that well-adapted clones of *Y. ruckeri* exploded to be widely distributed, while *V. tapetis* was divided into two defined groups being one of them associated to a specific geographical area.

It is noteworthy that the observed diversification, no matter the process suffered, could be related with host specificity to some extent, which may be indicating the existence of certain degree of function specialization. Further studies using "omics" techniques will allow to confirm such hypothesis.

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Section 3

Biodiversity

Chapter 4

Biodiversity Studies in Key Species from the African Mopane and Miombo Woodlands

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Additional information is available at the end of the chapter

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Abstract

The Southern African Miombo-Mopane woodlands are globally considered as ecosystems with irreplaceable species endemism, being the most important type of vegetation in the region. Among the approximately 8500 plant species, legume trees play a crucial role in biodiversity dynamics, being also key socioeconomic and environmental players. From the ecological point of view, they contribute significantly to ecosystem's stability as well as to water, carbon, and energy balance. Additionally, legume species represent an immensurable source of timber and nontimber products. Research in Miombo-Mopane biodiversity has been mainly focused on the analysis of ecosystem drivers by means of ecological parameters and models, lacking interdisciplinary with relevant cross-cutting tools, such as the application of molecular markers to assess genetic diversity within the region. In this chapter, the applications and biodiversity dynamics of typical legume species from Miombo (*Brachystegia* spp., *Julbernardia globiflora*, and *Pterocarpus angolensis*) and Mopane (*Colophospermum mopane*) are reviewed. Gaps and challenges are also brought forward in the context of the lack of genetic diversity assessments and the need of an effective and coordinated network of interdisciplinary research.

Keywords: *Brachystegia* spp., *Colophospermum mopane*, *Julbernardia globiflora*, Miombo, Mopane, *Pterocarpus angolensis*, woodlands

1. Introduction

Africa has a vast array of indigenous legumes (Fam. Leguminosae or Fabaceae), ranging from small annual herbs to large trees [1]. The potential of native legumes for multipurposes is high but poorly exploited [2]. This is particularly the case of the woody species from the

Miombo-Mopane woodlands, one of the five ecozones (together with Amazonia, Congo, New Guinea, and the North American deserts) with irreplaceable species endemism [3]. Miombo is the most widespread and important type of vegetation in Southern Africa covering approximately 2.4 million km^2 across seven countries: Angola, Democratic Republic of Congo (DR Congo), Malawi, Mozambique, Tanzania, Zambia, and Zimbabwe [4, 5]. Mopane extends over 0.48 million km^2 in Botswana, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe, constituting the second most important type of vegetation in the region [4]. The woodlands are the main source of woody species and a wealth of resources to the livelihood systems of millions of rural and urban dwellers that depend on these ecosystems to meet their food, health, energy, and housing needs [6, 7]. Environmentally, Miombo and Mopane add to biodiversity and have a global impact in energy, water, and carbon balances.

Miombo woodlands (**Figure 1A**) are dominated by legume trees belonging to three Caesalpinoideae genera: *Brachystegia* (Miombo in Swahili), *Julbernardia*, and *Isoberlinia*. On the contrary, Mopane woodlands (**Figure 1B**) are characterized by monospecific stands of *Colophospermum mopane* (Benth.) Léonard (also belonging to the Caesalpinoideae subfamily of legumes) [6, 8]. From the environmental point of view, these plants are determinant for energy, carbon, and water balance [9, 10]. At the socioeconomic level, Miombo-Mopane legumes are key providers of goods and services [11]. The woodlands are also very important to the national economies as they provide timber for exportation [5].

The ecological dynamics of Miombo-Mopane is strongly influenced by a combination of climate, disturbances (e.g., drought, fire, grazing, and herbivory primarily by elephants), and human activities [12, 13]. The growing population in the region over the last 20–25 years has resulted in increased woodlands degradation and deforestation. Slash-and-burn agriculture and charcoal production are the major causes of forest loss and degradation [14–16]. Additionally, the region is experiencing several major investments in mining, commercial agriculture, and infrastructures, which have further increased the pressure on the woodlands [17].

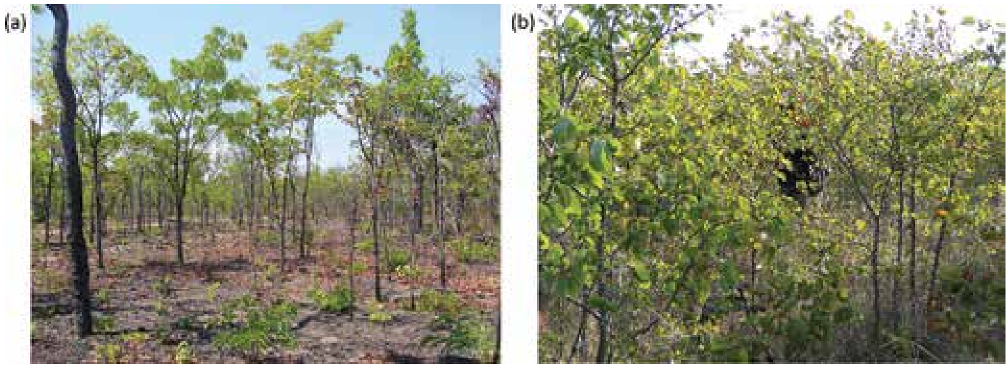

Figure 1. Images of Miombo and Mopane woodlands. (A) Miombo regrowth in burned area; (B) Shrub-like *C. mopane* specimens.

Changes in the global climatic pattern constitute another major threat for these ecosystems. They are mainly associated with more extreme wet and dry seasons as well as with extreme temperatures, which may change disturbance regimes (e.g., fire, shifting cultivation) and thus the prevailing biodiversity status [18–22]. For example, [19] predicts 5–15% reduction in precipitation for Southern Africa, while Green and coauthors [23] hypothesize that the combined effect of climate changes and disturbances may cause the loss of ca. 40% of the woodlands by the middle of the century. In line with these predictions, field studies combined with remote sensing and Geographic information system (GIS) methodologies indicated a decline in vegetation richness of 10–30% across Sahel and a southward shift of Sahel, Sudan, and Guinea zones due to shifts in temperature and precipitation regimes [21]. This may impose changes in biodiversity and biomass with associated modifications on the pattern of goods and services offered by the ecosystems. Under this scenario, several researchers are currently investigating (i) the impacts of the different ecosystem drivers on the woodland biodiversity, (ii) the capacity of biodiversity to supply and underpin goods and services, (iii) the patterns of genetic diversity of important species across environmental gradients, (iv) how different land cover types affect the existing patterns of biodiversity, and (v) how the changes in biodiversity will affect the availability and accessibility of resources to rural and urban dwellers.

In this review, we present the current efforts, gaps, and challenges toward biodiversity conservation of key legume trees from the Miombo and Mopane woodlands, respectively: *Brachystegia* Benth., *Julbernardia globiflora* (Benth.) Troupin, *Pterocarpus angolensis* DC. (Miombo), and *C. mopane* (Mopane).

2. Brief description and major applications of typical Miombo-Mopane species

2.1. *Brachystegia* spp. (subfamily Caesalpinioideae)

Brachystegia (Miombo) is a large genus of trees confined to tropical Africa, representing major components of deciduous (Miombo) woodlands of central and Southern Africa. This is a taxonomically difficult genus, since some species hybridize freely with others, making classification difficult [24]. The most frequent species in dry Miombo woodland are *Brachystegia boehmii* and *Brachystegia spiciformis*. *Brachystegia* species are generally slender and graceful with a clean bole, a crown of handsome delicate leaves (**Figure 2A**) and a fibrous stem bark. The genus as a whole is noted for the great range of red colors of the young foliage, later turning into various shades of green [24]. *Brachystegia* species play an important role in formal and informal economies in the region. For example, barks, which are particularly fibrous, are commonly used for construction, being especially favored for weaving, fishnet, beds, and rope making. Bark is easily peeled from a number of species and is used for fabricating beehives. The ash is used as fertilizer in shifting cultivation, and the wood is used for domestic tools, canoes, firewood, and charcoal; timber is usually heavily susceptible to borers but when treated is suitable for several purposes [6, 25]. *Brachystegia* is also an important nectar-producing genus for apiculture [26]. Additionally, several species of *Brachystegia* are

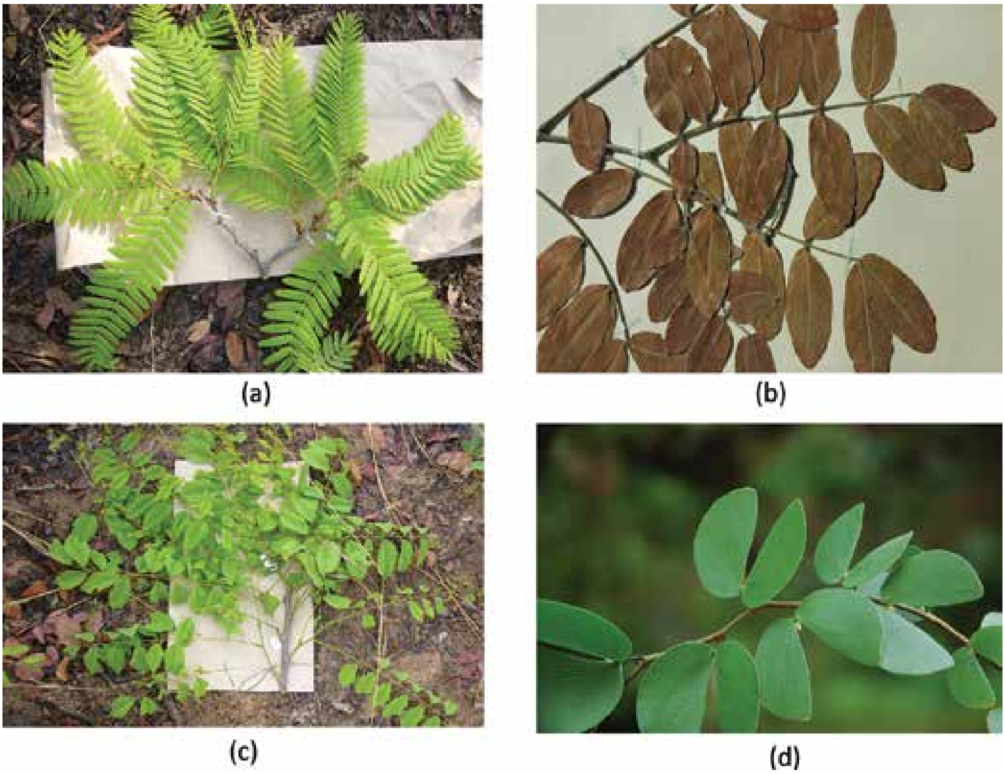

Figure 2. Details of the leaves from (A) *Brachystegia boehmii*, (B) *Julbernardia globiflora* (Barbosa and Carvalho, 2825, 1949 LISC Herbarium), (C) *Pterocarpus angolensis*, and (D) *Colophospermum mopane* (B and D credits to Maria Cristina).

used in traditional medicine [27–29], with antimicrobial, anti-inflammatory, and antidiabetic activities (**Table 1**) [30–32].

2.2. *Julbernardia globiflora* (subfamily Caesalpinioideae)

J. globiflora, known as Mnondo [6], is a common species of sandy woodlands, often codominant with *Brachystegia* species. It is distributed from DR Congo, Burundi, and Tanzania southward to Botswana, Zimbabwe, and Mozambique [33]. *J. globiflora* is usually recorded as a small- to medium-sized deciduous tree with a rounded crown that can grow up to 18 m in height, with alternate and compound leaves (**Figure 2B**) and thick fire-resistant bark [6]. Like *Brachystegia* species, *J. globiflora* is a useful local source of timber and nontimber products. The wood is hard, with a heartwood resistant to borers, and is widely used as a general-purpose timber and also for the construction, tool handles, mortars, and canoes as well as fuelwood and for making charcoal [24, 25, 33]. Ropes, fishnets, and other items are made from the bark fiber, which is fairly good, although of poorer quality than that of the *Brachystegia* spp. [6]. The bark yields tannin used for dyeing [33]. The tree is an important fodder species for early-season browsers [34]; it is also a bee forage, yielding honey of very high quality. Together with *B. boehmii*, *J. globiflora* is among the most important hive trees and nectar sources [26]. The leaves of *J. globiflora* are important food for edible caterpillars [33]. Although roots, barks, and leaves

Species	Phytochemical and pharmacological studies	Genetic diversity studies
Brachystegia boehmii Taub.	Leaves: antibacterial [30]	ISSR markers [70]
	Leaves: anti-inflammatory [32]	
Julbernardia globiflora (Benth.) Troupin	No studies reported	
Pterocarpus angolensis DC	*Antischistosomal* [90]	RAPD [79, 80]
	Stem, stem bark, leaves: anthelmintic [40]	
	Seeds: antibacterial [91]	
	Stem bark: antibacterial and anti-inflammatory; lack of mutagenicity [92]	
	Stem bark, leaves: anthelmintic, antibacterial, and cytotoxic [41]	
	Antibacterial [93]	
	Stem bark: antibacterial; epicatechin and derivatives identified [37]	
	Stem bark: antibacterial; leaves, stem bark: antifungal, HIV-1 reverse transcriptase inhibitory [38]	
	Leaves, stem bark: anti-inflammatory [94]	
	Stem bark, roots: antibacterial; tannins and saponins identified [39]	
Colophospermum mopane (Benth.)	Bark and seeds: significant cytotoxicity against a human breast cancer cell line [48] Heartwood: mono-, di-, and triflavonoids; leaves: beta-sitosterol and stigmasterol; aerial part: essential oils that comprise mainly alpha-pinene and limonene; bark and seeds: diterpenes, including dihydrogrindelaldehyde [48]	Allozyme markers [83]
	Seeds: anti-inflammatory and antioxidant activities [95]	
	Bark and seeds: significant cytotoxic activity against a human breast cancer cell line (aldehyde) [96] Bark and seeds: three new diterpenes isolated [96]	
	Oligomeric flavonoids [97]	
	Leaves: antimicrobial activity [49] Seed husks and leaves: five labdane (1–5), an isolabdane (6), and five clerodane diterpenoids (7–11) [49]	

Table 1. Phytochemical, pharmacological, and genetic diversity studies on the tree species *Brachystegia boehmii* Taub., *Julbernardia globiflora* (Benth) Troupin, *Pterocarpus angolensis* DC., and *Colophospermum mopane* (Benth).

of *J. globiflora* have been recorded to be toxic, various plant parts are used in traditional medicine, mainly externally to treat ailments such as snake bites, leprosy, and conjunctivitis [28, 33].

2.3. *Pterocarpus angolensis* (subfamily Papilionoideae)

P. angolensis (commonly known in English as bloodwood, kiaat, and African teak) [6] is a medium-sized to large-sized tree up to 16 m in height but reaching 20 m under ideal conditions with a spreading crown and a single trunk. *P. angolensis* is widespread in southern tropical Africa, from Angola, DR Congo, and Tanzania south to northeastern South Africa and Swaziland [35]. The bark is dark gray to brown, rough, and longitudinally fissured. The drooping leaves have 5 to 9 pairs of subopposite to alternate leaflets (**Figure 2C**). The flowers are orange-yellow in large, branched sprays. The fruit is a very distinctive, indehiscent, and circular pod holding 1–2 small seeds [24]; the leaves are browsed by elephant and kudu; and fruits are eaten by baboons, monkeys, and squirrels [6]. *P. angolensis* serves a number of purposes, both utilitarian (as multipurpose timber, dye, forage) and in African folk medicine. It is the main Miombo and one of the most valuable timber trees in Africa heavily sought after for export and local use. The heartwood is golden or reddish brown, makes high-quality furniture, shrinks very little in drying, and is very resistant to borers and termites [24]. The wood is also used for construction, carpentry, flooring, boats, and wood carving. It is occasionally used for firewood [35]. The sap from the wood makes a permanent red stain on clothing and can be used as a dye. The powdered red inner bark of the roots, mixed with fat, is used to anoint bodies and faces by some ethnic groups in northern South-West Africa [6]. Traditionally, all parts of the plant are used for human and animal health purposes. For example, the bark is used as a powerful astringent to treat diarrhea, heavy menstruation, nosebleeding, headache, stomachache, schistosomiasis, sores, and skin problems; the root is believed to cure malaria, blackwater fever and gonorrhea [6, 24, 28, 35, 36]. Research studies reveal promising results concerning their antibacterial [37–39], antifungal [38], anthelmintic [40, 41], and HIV-1 reverse transcriptase inhibitory properties [38] (**Table 1**). At the environmental level *P. angolensis* is able to establish nitrogen-fixing symbiosis with rhizobium bacteria and therefore relevant for soil fertilization. It is also planted for soil conservation, for dune fixation, and as live fencing [35]. Flowering trees are good sources of pollen and nectar [35].

2.4. *Colophospermum mopane* (subfamily Caesalpinioideae)

C. mopane is the single species in the genus *Colophospermum*, which only occurs in Africa, being characteristic of hot and dry river valleys, where it is often dominant and may form almost pure stands over large areas—the "Mopane woodlands" [42]. Commonly known as mopane, it is widespread in Southern Africa, where it occurs in Zambia, Malawi, southern Angola, northern Namibia, northeastern Botswana, Zimbabwe, southern Mozambique, and northern South Africa [43]. Mopane is adapted to a wide variety of soils and temperatures, presenting different growth forms, from shrub-like to tall slender trees. It is deciduous or sometimes semideciduous, as water availability determines leaf drop [44], with beautiful autumn and spring colors. The highly sclerophyllous compound leaves (**Figure 2D**) have two leaflets, large and butterfly-shaped, which fold together in the hottest time of day [6]. Leaves and fruits are very glandular and smell strongly as turpentine. As a fodder species, mopane is vital in areas of low rainfall. Like most Miombo legume species, *C. mopane* is a true multipurpose tree, not only important for its wood but also as source of medicine, forage, and edible caterpillars [43]. Cattle and wild animals browse the foliage eagerly and sometimes eat the dry leaves and seeds from the ground [6]. Seeds are consumed by humans as famine food [43]. Mopane heartwood is very hard, and it is known to be very durable and resistant to insect damage. It is traditionally used for posts and poles in the construction of houses and palisade fences. Mopane wood accounts for more than 90% of the wood used for living and storing huts in large parts of Southern Africa [43]. It is suitable for flooring and for carving and is excellent for turned objects, and to a lesser extent, due to its weight and hardness, it is used for joinery and furniture. Mopane gives excellent firewood and makes high valuable charcoal [43]. The rough bark is used to make twine and for tanning [6, 43]. Larvae of mopane moth (*Gonimbrasia belina*) feed on the mopane leaves. Those large caterpillars are rich in protein and considered a local delicacy [6]. Mopane worms are also traded to generate income providing a good economic return [45]. The tree also acts as a food plant for a wild silk moth (*Gonometa rufobrunnea*). Cocoons of the moth are harvested as wild silk and processed to make cloth [43]. *C. mopane* is an important medicinal species, and different parts of the plant are used in the preparation of traditional remedies [43, 46, 47]. Research into Mopane-active compounds revealed biological activities with potential for human and animal health [48, 49] (**Table 1**).

3. Diversity and population structure studies

Miombo and Mopane woodlands face major threats related to climate, human, and animal pressure which in the midterm may reduce tree species abundances and, thus, ecosystem services [17, 43, 50]. Thus, understanding the interaction between the main woodland's drivers is crucial for the development of effective and sustainable management strategies for biodiversity conservation and resource use. In this section we focus on diversity and structure of the key legume trees referred above, i.e., *Brachystegia* spp., *J. globiflora*, *P. angolensis*, and *C. mopane*.

In the Niassa National Reserve (NNR), one of the most pristine and least disturbed areas of Africa's deciduous Miombo woodlands, notable alterations in vegetation structure and composition were reported in areas with high fire (mostly anthropogenic) frequency [51, 52]. These included a decrease in woody parameters and a replacement of typical Miombo species (*J. globiflora* and *Brachystegia* spp.) by subdominant species (*Combretum* spp., *Terminalia sericea* Burch. ex DC, and *Diplorhynchus condylocarpon* (Muell. Arg.) Pichon [51]. In southwestern Tanzania, [50] reported a tree species diversity score (Shannon-Wiener diversity) of 3.44 *versus* 2.86 in the Miombo woodlands subjected to low and high rates of resource use, respectively. In areas of moderate resources utilization, tree diversity was maintained, but the structure of the vegetation showed a reduction of Class 1 (diameter at breast height (DBH) < 10 cm) trees, indicating low recruitment. Key Miombo species from the genera *Brachystegia* and *Julbernardia* were present in all sites, but the frequency of the former declined by 60% from low to high utilization sites. The resilience of *Julbernardia* spp. to disturbances might be due to vigorous resprouting after cutting as observed in *J. globiflora* [33]. On the other hand, *P. angolensis* adult trees were harvested throughout the study site, and only immature specimens were recorded, suggesting that it is commercially extinct for the foreseeable future [50]. *P. angolensis* is widespread in many parts of Southern Africa; however, overexploitation endangers natural populations in all countries. None of the four species considered in this paper is listed in the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora Appendices. Only *P. angolensis* is reported by the International Union for Conservation of Nature (IUCN) as being near threatened. Technically, it does not meet the Red List criteria of a vulnerable or endangered species, but is close to qualifying for vulnerable [53].

Despite the factors referred above, natural factors, i.e., soil fertility, topography, and local hydrology, are also important determinants of biodiversity variation, although these factors are less studied [54, 55]. The vulnerability of the woodlands to climate changes is particularly high [19]. Species distribution models developed for *P. angolensis* under two different climate change scenarios suggest that potential and realized distributions are very similar in Southern Africa, except for Madagascar where the species could grow but does not occur, and that species distribution may be particularly threatened in Namibia and Botswana [56]. Accordingly, pollen records from *Brachystegia* spp. suggest a retraction of Miombo in *ca.* 450 km over the past 6000 years [57] and that such changes in population dynamics may be associated with shifts in temperature and moisture regimes [7]. Based on the historical range shifts in *B. spiciformis*, an ecological niche retraction of ca. 31–47% in the continuous Miombo woodlands from Zimbabwe and southern Mozambique was predicted by 2050 [58]. However, considering the resilience of *B. spiciformis* to extreme environments (e.g., low precipitation, high temperatures), such retraction may be less exaggerated than the model predictions, which no account with genetic adaptation [58].

Contrary to Miombo, Mopane woodlands are constituted by nearly monospecific stands of *C. mopane* [59], resulting in extensive areas with low compositional and structural diversity [60]. Due to the same factors mentioned for the Miombo woodlands, some parts of Southern African Mopane woodlands are experiencing a decline in natural stands [43]. For example, [61] analyzed the effects of heavy land utilization (mainly grazing) on vegetation structure communal woodlands of the Mopane Bioregion of South Africa. The authors observed prominent effects in plant structure, i.e., reduced canopy and height, transforming the woodland into a shrubland. A significant decrease in biomass in post–boom charcoal production in southern Mozambique was also reported [62]. Govender et al. [63] analyzed the effect of two fire management strategies in one of the most important areas of mopane, the Great Limpopo Transfrontier Park (GLTP), which crosses two countries: South African component Kruger National Park (KNP) and Mozambique Limpopo National Park (LNP). KNP has a very structured and intensive fire management (FM), while in LNP, the fire regime is unstructured and highly associated to the large number of people living within its limits. Even so, in LNP natural fire frequency did not affect the ecological weight of Mopane, whereas in the KNP, frequent managed fires reduced the ecological value in 100–200 [63]. These results indicate that natural fire regimes are important to maintain the ecosystem's equilibrium. Although less studied, *C. mopane* biodiversity shifts may also be affected by the environmental conditions. Stevens et al. [60] used MAXENT modeling to investigate which environmental variables may determine the distribution limits of *C. mopane* within Southern Africa. According to the results, both nonclimatic (dryness or hours of light) and climatic (temperature) variables may limit the regional distribution of *C. mopane*, which is restricted to warmer sites.

Genetic diversity is the basis for stability and survival under the ever-changing environments. Populations with high levels of genetic variation offer a diverse gene pool from which breeding and conservation programs can be designed. The over exploitation of the reported species may threaten their genetic diversity in the future and hence might limit their ecological and evolutionary development. Therefore, genetic diversity and structure studies are of utmost importance for designing appropriate conservation strategies.

The use of molecular markers constitutes an effective approach to evaluate genetic variation within and between species and populations, because they are expedited and precise and are not affected by the environmental processes. Polymerase chain reaction (PCR)-based markers, like random amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLPs), inter-simple sequence repeats (ISSRs), simple sequence repeats (SSRs), or single-nucleotide polymorphisms (SNPs), are commonly used in plant science for a wide variety of purposes such as genome mapping, gene tagging, phylogenetic analysis, taxonomy, marked-assisted selection, and genetic diversity studies [64–73]. However, the analysis of the genetic variation and structure is an incipient issue in Miombo and Mopane research. Regarding the species selected for this review, up to our best knowledge, only four reports are available in the scientific literature (**Table 1**).

Maquia et al. [70] have used ISSR markers to assess the genetic diversity in *B. boehmii* and *Burkea africana*, another typical legume tree from Miombo, across a fire gradient in NNR wherein the northeastern side is affected by annual fires and the western by bi- to triannual fires. The authors observed that the levels of genetic diversity were lower in *B. boehmii* (average genetic diversity, He = 0.1965) than in *B. africana* (He = 0.2972). Such difference was attributed to fire tolerance and adaptation, as *B. africana* is a typical fire-tolerant species, while *B. boehmii* is more sensitive to fire, particularly at young stages [74, 75]. Interestingly, in *B. boehmii* high fire frequency resulted in higher variability (i.e., He = 0.2059 in eastern *versus* He = 0.1482 in western NNR), while in the case of *B. africana*, the opposite was observed (He = 0.2977 in the west and 0.2184 in the east). Based on this observation, Maquia et al. [70] proposed that the increase in *B. boehmii* variation driven by frequent fires may be part of its evolutionary response, a phenomena called pyrodiversity-like effect [76], associated to a higher proportion of seed-derived propagation in detriment of vegetative reproduction. Overall, the study concludes that the genetic variability within and among populations as well as the estimated gene flow between populations represents a strong genetic pool of the two species in NNR, agreeing with the fact that the reserve is one of least disturbed areas of Miombo. Similar results were obtained with other species of the Fabaceae family, e.g., *Afzelia quanzensis* Welw. [73], *Astragalus rhizanthus* Royle ex Benth. [77], and *Glycyrrhiza uralensis* Fisch. ex DC [78], from conservation areas.

RAPD markers were used to characterize accessions of *P. angolensis* from Zimbabwe, Zambia, and Tanzania [79, 80]. Chisha-Kasumu and colleagues [79] characterized the genetic structure of different populations from Zimbabwe and Zambia. According to their study, the variability within each population was high (Shannon's information index, I = 81%). In Tanzania, [80] used the same strategy to analyze genetic diversity and population structure in six natural populations. In line with the results of [79], genetic diversity within populations was high (I = 77%), and in both cases, an effective gene flow was suggested. The results are within the range of those reported for other tree species [81, 82].

Villoen and collaborators [83] have used 13 allozyme markers to analyze five populations of *C. mopane* in South Africa: these included aspartate aminotransferase (AST), alcohol dehydrogenase (ADH), esterase (EST), isocitrate dehydrogenase (IDH), guanine deaminase (GDA), glucose-6-phosphate isomerase (GPI-1), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), mannose-6-phosphate isomerase (MPI), peroxidase (PER), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), and superoxide dismutase (SOD). As for *B. boehmii* [70] and *P. angolensis* [79, 80], most of the variation in *C. mopane* was observed within populations. Additionally, gene flow between populations was also effective. The results were also comparable to those obtained in the legume *Virgilia oroboides* (Berg.) [84]. The authors suggest that *C. mopane* developed an effective outcrossing mechanism that avoid inbreeding and maintain a considerable level of diversity necessary to ensure adaptation and survival under a context of ever-changing environments.

It should be highlighted that except for [70], the studies of [79, 80, 83] did not address the impact of environmental and/or anthropogenic drivers, which are determinant to understand ecosystem's dynamics. In fact, the use of molecular markers to assess tropical tree biodiversity dynamics across environmental gradients is an issue that deserves more attention. Using ISSR markers, [85] examined the effect of three different environmental gradients on the genetic diversity of the semishrub legume *Caragana microphylla* Lam. The authors observed that higher levels of genetic diversity were correlated with optimal humidity, soil fertility, and to a less extent temperature. Dai et al. [86] examined the genetic variation of marginal *Bombax ceiba* L. (silk-cotton tree) populations in China and South Asia, based on the sequences of nuclear and chloroplast genes. The results revealed extremely low levels of genetic diversity without significant differences between cultivated and natural populations. This might reflect a small number of individuals (or other founder effects) during the establishment of populations or genetic drift. Addisalem et al. [87] examined the genetic structure of *Boswellia papyrifera* (Del.) Hochst across threaten tropical dry forest (*Terminalia-Combretum*) woodlands in Ethiopia, where populations retain high levels of genetic diversity despite the diverse environmental conditions (including harsh environments, i.e., high temperatures and low precipitation) as well as progressive deforestation and degradation.

Altogether, these studies [70–87] reveal that the response of tropical trees to environmental and anthropogenic pressure is highly variable and that, in general, most of the species are resilient to extreme soil and climate conditions, being able to retain high levels of genetic diversity. This in turn highlights the relevance of integrative molecular analyses at a regional scale, to understand the mechanisms of species adaptation and evolution within the context of climate changes. A good example of the potentialities of such studies is the work developed in *Hevea brasiliensis* (Willd. ex A. Juss.) (Rubber tree) by de Souza and collaborators [88, 89], which have established a foundation of molecular markers (microsatellites and SNPs) highly valuable for breeding and conservation programs.

4. Concluding remarks

African woodlands support the livelihoods of millions of rural and urban people, providing valuable sources of wood, edible products, fibber and related products, insect products (honey and beeswax, edible insects), and medicinal plants, among others.

Based on the available scientific information, it is our understanding that major gaps and challenges need urgently to be addressed. The development of coordinated research throughout the region to assess genetic diversity and structure as well as to define common conservation strategies, adapted to each country needs and facilities, should be prioritized. For that, effective networking between Southern African institutions and their partners from Europe and the USA seems to be the most appropriate approach. However, such interactions are not reflected in a considerable number of scientific publications. It is our conviction that collaborative work is the best way to consolidate and/or promote partnerships, resulting in mutual benefits, e.g., scientific excellence, critical thinking, team playing, access to funding, broadening information sharing to prompt innovation, translation of knowledge from "local to global," and from "global to local" contexts. The involvement of the Miombo Network for Southern Africa would be of utmost importance to incorporate key issues, such as genetic diversity and bioprospection in Miombo and also Mopane, in its research strategy plan. The inclusion of these two issues in Miombo and Mopane research will not only ensure the sustainable use and conservation of key species but also allow the establishment of modern biotechnology platforms toward the incorporation of the most valuable species into bio-based economy schemes.

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National and International Conservation of Biological Diversity in Terms of Administrative Law "Sample of Turkey"

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Additional information is available at the end of the chapter

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Abstract

Living beings in nature spend their lives within a certain environment. The most significant threat on the diversity of living beings on earth results mainly from human beings, who have the potential to manipulate the environment in which they live. The maintenance of the components of biodiversity is possible with a consistent and an effective legal protection. For this reason, various local, regional, and international arrangements have been made. Possessing a high rate of endemism in terms of plants and other creatures, Turkey also has a rich biodiversity. This study, on the basis of basic institutions of Turkish Administrative Law, investigates the legal regulations towards protecting biodiversity in Turkey in relation to international law, and the contributions of these regulations to the conservation of biodiversity. In this respect, the study further explores the public service dimension of biodiversity, law enforcement authorities in the scope of protection precautions and sanctions, the inadequacies of the existing arrangements, and the amendments to be made in the rule of law in Turkey and in international contracts towards providing the maintenance of biodiversity. Moreover, the activities to encourage private law people's and nongovernmental organizations' activities for developing biodiversity will be presented.

Keywords: Biodiversity, administrative sanctions, penal sanctions, legal protection, legislation, environment

1. Introduction

Earth could contain nearly 1 trillion species, with only one-thousandth of 1% now identified, according to the results of a new study [1]. The earth has encountered with an unprecedented loss of biodiversity in recent years. Species' extinction rates are now around 100 times higher than those shown in fossil records and are projected to accelerate. Presumably one-tenth of bird species on earth, approximately one-fourth of mammals, and 70% of plant species are in danger of extinction. The net loss of forests has slowed substantially; however, each year a forest area larger than Zimbabwe has been disappearing since 2000. Coastal and marine ecosystems are greatly affected by human activities. Inland water species have decreased by 50% and marine and land species have decreased by 30%. Special bird species in certain habitats, coastal bird populations in the whole world, the range of forests and sea grasses, and the situation of coral reefs have been getting worse in each year. One-fourth of world plants are in danger of extinction. The existence of vertebrates has decreased at a rate of one-third in the last 30 years [2].

Nearly 12,000 plant species, 2750 of which are endemic, live in the whole Europe. Man-made damage on biodiversity in Europe is far more than in other continents. At the species level, 42% of Europe's native mammals, 43% of birds, 45% of butterflies, 30% of amphibians, 45% of reptiles, and 52% of freshwater fish are threatened with extinction [3].

Surrounded by three seas, shaped with high mountains and possessing approximately 200 natural lakes, Turkey has a surface area of 779.452 square kilometers. The country is at the crossroads of three plant geographies, the origin and diversity and cultivation center of many plant species, and a migratory route. The ecosystems of wetland areas in the country follow tropical forests in terms of biodiversity. There are nearly 9000 plant species in Turkey, almost 3000 of which are endemic [4]. Being at the ninth row in Europe in terms of biodiversity, Turkey has a great wealth in both flora and fauna. The country is home to three of 37 different flora regions of the world. Being located on the meeting point of these three different plant geographies is the most significant reason of this botanic wealth. As the plant taxon has 34% of endemic type, Turkey constitutes one of the most significant plant diversity centers in the world. Endemic plant types are narrow range on a global scale, 70% of which is hung by thread according to red list criteria and some species are unfortunately extinct today.

Wetland areas are of vital importance for migratory birds and many water bird species. As Turkey is located on bird migratory routes, it has an important position for many bird species. It boasts 80,000 animal species. The country is also a very rich in terms of continental, aquatic, and marine invertebrates and 252 of 694 fish species are in the red list of International Union for Conservation of Nature and Natural Resources (IUCN). About 61% of inland water fish are endemic. There are 164 amphibian and reptile species in Turkey, 10 amphibian and 17 reptile types of which are endemic. Among 476 bird species in the country, three of them are critically endangered, five of them are endangered, eight of them are delicate, and 17 of them can get into danger while among 169 mammals, nine of them are endemic, 15 of them are endangered, and 11 of them could be endangered [5]. To illustrate, Anatolian Leopard (*Panthera pardus tulliana*) which used to live only in Turkey and Indian Elephant (*Elephas maximus*), Caucasian ox (*Bison bonasus caucasicus*), Asian lion (*Panthera leo persica*), cheetah (*Acinonyx jubatus raddei*) and tiger *(Panthera tigris*) which used to be found in Anatolia are completely extinct [6].

On earth, apart from natural selection, the most significant threat on biodiversity is man-made. The reasons can be as the following; increasing demand towards biological sources as a result of increasing population, economic development and overconsumption, using improper technologies, the increase in international trade, the failure of markets in understanding the real value of biodiversity, and the failure of governments in finding out and solving problems about overusing of biological resources, migration, and mobility [7].

Climate change and invasive alien species pose a threat in the acceleration of habitat and species losses. Industrialization, global warming, outrageous utilization of natural resources [8], and overhunting speed up this period. Unless these threats are overcome, biodiversity worldwide will decrease to an unprecedented level soon [9].

Social, economic, and environmental problems cause the decrease and even the extinction of biodiversity in many regions of Turkey. Due to factors such as industrial development, agricultural activities, and urbanization, natural habitats of species are destroyed. In this process, some misapplications such as cutting off wild fruit trees in the mountains by forestry administration also take place [10].

In terms of precautions to be taken about preventing the decrease of biodiversity and providing conservation, the state has both advantages and disadvantages, since the property of areas where many living beings in the country live, that is, coasts, seas, forests, mountains, and almost all lakes, belongs to state [11]. As these areas belong to the state and no ownership problems will be faced, the precautions to be taken will be implemented easier and faster results will be obtained. However, the political will governing the country should be determined in order for the public services to be successful [12]. It is because public properties are less preserved than private properties, and sometimes the property of these areas is transferred to the people who have occupied these properties and has built settlement on these areas and actually use them. In the scope of this study, the legislations towards protecting biodiversity in the world and in Turkey will be comparatively examined, entities in charge of the protection of biodiversity in Turkey, the duties of these entities, the decisions of supreme courts about protection, judicial decisions, their contributions to the protection, and the deficiencies in the legislation in Turkey will be examined.

2. The definition of biodiversity

Biodiversity is the variety of life on earth—comprising ecosystems, species, and genes [13]. Biodiversity, which is used to represent genetic diversity and the diversity of habitats (natural living area) along with the species diversity of the living beings on earth, is one of the most significant conditions for protecting natural balance [14]. Biodiversity is the indicator of a healthy environment and the abilities of ecosystems to maintain life support processes necessary for the welfare of humanity. It consists of three components; genetic diversity, which is hereditary and determines the physical and biochemical features of existence; species diversity which means mutual reproducing of a group of organisms genetically similar and naming species from this reproducing; and ecosystem diversity comprising of inanimate beings such as plants, animals, soil, water, air, and minerals [15].

On the second article of Convention on Biological Diversity (CBD), biological diversity is defined as the diversity among living organisms from all resources including land, sea, and other water ecosystems and the ecologic complexes being a part of these ecosystems.

The definition also covers the diversity within the species themselves and among themselves and ecosystem diversity.

Briefly, biological diversity states local and common global diversities of every kind of organisms among themselves and with each other, including genetic diversity, and states genetic differences in relation to the environmental conditions (ecosystems) that they are a part of [16].

3. Regulations towards protecting biological diversity

It is seen that first regulations towards protecting living beings in nature are about protecting living beings of economic importance. It is observed that these protection rules starting from eighteenth century have expanded to a wide range, comprising also species that do not have economic value today.

Today, there are more than 300 multilateral environmental conventions and nearly 30% of them are entirely or partially related to biodiversity [17]. Most of these conventions aim at protecting specific kinds and habitats/ecosystems or lands and in arranging particular activities. Moreover, while some of the conventions about biodiversity are international, most of them are regional. When the formation and development of CBD, which was opened for signature at Rio Conference in 1992, is considered, it is seen that the studies of International Union for Conservation of Nature and Natural Resources (IUCN), United Nations Environment Programme (UNEP) and other nongovernmental organizations have a significant role in the formation of these conventions. For the purpose of preparing a convention, Convention on Biological Diversity was negotiated at 10 separate intergovernmental negotiations. These negotiations, the first of which began in the late 1988, lasted until May 1992 and finally the convention was signed [18].

3.1. Regulations in the Turkish law concerning the conservation of biodiversity

There are many regulations in Turkish law about the direct and indirect protection of biodiversity. It can be said that the origins of these regulations can be traced back to the last 50–60 years. Although the title of the legislation about protection is not directly under the name of conservation of biodiversity in essence, it can be said that the regulations essentially serve to the protection. However, legislation about conservation does not directly contain every single part of biodiversity in today's context; and regulations are seen to be related to certain topics. The Constitution is at the qualification of the top legal norm in almost all countries in the world. It is the same for Turkey as well. According to the Turkish Constitution, the effectiveness of international conventions comes at the second rank after the constitution text. However, as conservation of biodiversity is shaped mostly with international conventions in Turkey and in the world, international conventions are examined under a separate title from the national law regulations in the study.

3.1.1. The view of Turkish Constitution to biodiversity

The Constitution is the most supreme legal norm in Turkey regarding the hierarchy among legal regulations. It is followed by international conventions, enactments, delegated legislations, regulations and by-laws, respectively. There are no direct provisions for protecting biodiversity in 1982 Constitution Act.

The Article 56th of the Constitution states that "everyone has the right of living in a balanced and healthy environment; and developing environment, protecting environmental health and preventing environmental pollution, and that it is the duty of the state and the citizens alike to maintain it."

Environmental right, defined as a separate right since 1970s, is a part in Turkish Constitution, too. This right, the subject of which is human beings, animals and plants, inanimate organisms in relation to human beings and other living beings and ecosystem arranging the relations between living beings and inanimate organisms, is directly related to the conservation of biodiversity.

Environment is composed of elements free to individuals such as air, water, soil, fauna, flora, and cultural environment [19]. Environmental right, being a balanced and coherent right creating a kind of property right on the common wealth of humanity, turns into actualization of freedoms and entity condition by constituting a common area and reconciliation environment for other freedoms [20]. This right and duty cannot be separated from each other. The right holder is under the duty of not destroying the environment and taking action against those who destroy the environment, and has the right of making formal requests from the state, being informed about the changes around, taking part in the decisions around and petitioning. Providing everyone with a life on humanly conditions is in the core of this right [21].

We can say that environmental right is the closest regulation that can be linked to biodiversity in Turkish Constitution. As the Environmental Law is regulated by the Constitution's "Social and Economic Rights and Duties," the state will carry out its duties in the scope of this article to the extent of the sufficiency of financial resources defined by the 65th article. That the environmental right is included in Constitution does not give subjective public rights to people that they can demand through judicial remedy [22, 23].

Evaluating biological diversity in the scope of environmental right is insufficient. The significance of the concept explained above necessitates a separate and special regulation. Therefore, it can be said that biological diversity should be subjected to a separate regulation by getting it out of environmental right scope. Yet, the idea of public welfare existing in conservation of biodiversity should become an independent phenomenon and get out of environment-oriented public welfare approaches. It is because protecting biodiversity and activities carried out in that way involves public welfare itself [24].

Moreover, it must be stated that some provisions of Constitution indirectly serve to the conservation of biological diversity. It is clear that provisions which are indirectly related to the protection of biodiversity such as the 168th article of Constitution that natural wealth and sources are ensured and in the possession of state, 169th and 170th articles about the protection and development of forests and protecting forest villagers, 35th article that the right of property which is accepted as a right for everyone that cannot be used against the benefit of society, 57th article about right to housing, 63rd article about protecting history, culture and nature entities, and even 17th article which states that works having risk to human health can be accepted as violation for right to life, 45th article about protecting meadows and pastures, 43rd article about making use of coasts, and 44th article about fight against erosion are also significant in terms of protecting biodiversity.

As it is seen, Turkish law has set forth liabilities for both state and individuals by including regulations to reach this aim although it does not regulate the protection of biodiversity as an independent provision. These regulations have great significance in guaranteeing biological diversity.

The regulation at the 63rd article as "State provides protection of history, culture and nature entities and takes supportive and encouraging precautions to reach this aim" can be shown as another regulation contributing to the conservation of biodiversity [14].

3.1.2. Other regulations toward the conservation of biodiversity

The most secure way of protecting biodiversity is possible by making regulations in Constitution and international conventions. The Constitutional Law is the top norm in Turkish law in the hierarchy of legal rules itself. According to the current constitution, international conventions are at the equal level with laws, following the Constitution, and have the effectiveness of legal power. International conventions become a norm of national law after they are accepted in due form. In the study, the conservation of biodiversity will be examined first in international conventions and then in regulations in national law.

3.2. Conservation of biodiversity in international conventions

The boundaries of states founded by human communities and habitats and boundaries of nonhuman living beings are not always the same. Moreover, the effects of some activities human beings carry out in their countries do not only affect their own countries but also cause harm to their neighbors and sometimes even the whole world. Therefore, protection of nature and biodiversity is not a simple issue to be left to only the initiative of states, and global regulations must be carried out in order to take immediate and efficient results in stopping destruction and protecting biodiversity. As the loss of biodiversity comes up as a global environment problem and precautions foreseen by states at national law systems are not adequate by themselves, regional and global cooperation have become inevitable [14].

It is seen in protection of nature and biodiversity conventions, as is in many other international conventions, that states can make regulations on natural resources independent from other states, which underlines the principle of domination. Problems related to this principle becomes more apparent with the regulations and usages concerning lakes, rivers, forests, international waters, and even atmosphere that form common borders of states. Signatory countries, which are the natural extension of this principle, always have the power of suspending the provisions of the convention and resiling. The principle that comprises the states' cooperation on conventions about nature without causing harm to other state, avoiding damage, consultation and meeting in good faith is a good neighborhood principle. The principle of protection, which is continuously emphasized on nature and environment conventions, providing the protection of flora and fauna; the sustainable improvement principle that necessitates taking required precautions for protecting environment in plans that are made to carry out economic improvement, and sometimes the principle of common heritage of humanity are emphasized on international conventions [16].

Legal arrangements on protection of forests are known to have existed in France in the tenth century, while it was in the eleventh and twelfth centuries that such arrangements were made in Germany. The first restrictive provisions regarding forests in the UK are about hunting [25].

As the forests started to disappear at the end of eighteenth century, it was realized that the forests should be protected. In most of the European countries, legal arrangements on the protection of forests were made in the nineteenth and twentieth centuries.

Territories of Turkey have hosted many civilizations such as Egyptians, Sumerians, Hittites, Phrygians, Lydians, Persians, Seljuks, Ottomans, but these territories were deforested by the mentioned civilizations. In the Ottoman Empire, the initial arrangements regarding the forests were composed of ordinances and edicts showing the solutions for some problems. The forests had been haphazardly utilized in Ottoman Empire by the nineteenth century, but deforestation was limited, thanks to the people's religious sentiments, manners, and customs. The first arrangement regarding forests was made in Land Code of 1858, and in 1869 Forest Regulation was enacted. Unauthorized tree-cutting, debarking, collecting stones, soil, fresh and dry leaves without permission, unauthorized grazing and setting fires in the forest were prohibited by the regulation [26].

The first international agreements towards protecting biodiversity were made in the eighteenth century in order to end hunting of some species of economically precious living beings in open seas in an order and prevent extinction of migratory fish because of overhunting, In 1900, The Convention for the Preservation of Wild Animals, Birds and Fish in Africa was accepted in order to prevent the extinction of some species like lions and zebras in Africa, which had been under colonial domination of European states. This convention can be said to be the first protection agreement with regional quality [16].

In 1950, European states accepted the International Convention for the Protection of Birds and in 1951, The International Plant Protection Convention was accepted. Many regulations that aim directly and indirectly at protection of biodiversity were made within European community and between other countries. While some of the conventions concerning biodiversity are international global, many of them are at regional scale. Today, there are more than 300 multilateral environmental conventions and the majority of them aim at protecting particular species and ecosystems or areas and arranging specific activities. In some international conventions, monitoring and reporting mechanisms towards adaptation to convention provisions are envisaged. Such mechanisms foreseen in convention provisions provide both better adaptation to the convention and seeing the violations against the convention better.

In Turkey, when we look at the place of international conventions in norms, it is seen that "international conventions that are implemented in order are statutory" according to the 90th article of Constitution. The last subarticle provision of the 90th article of Constitution does not envisage a special transformation procedure for the conventions to bring forth provisions in national law [27]. Therefore, international conventions are in the same class with laws in norms hierarchy [22, 28]. However, in the 90th article of Constitution which regulates international conventions, it is stated that especially when the provisions of international conventions concerning basic rights and freedoms contradict with laws, the provision of international convention will be applied. Moreover, prosecuting to Supreme Court for the cancellation of international conventions is not possible whereas it is possible to prosecute in order to cancel laws. Therefore, it is more important to regulate any subject with international convention for the effect of the content of the subject and rules in Turkey. We observe that regulations about a subject that concern not only a specific region but also the whole world are mostly arranged with international conventions; and regulations made in national laws of states are made after the acceptation of international letters. In spite of that, we see that especially developed countries sometimes resist signing letters accepted by international community. When we look at the subject from the perspective of Turkey, it is seen that extra regulations in national law are made after a regulation concerning international society has been made.

Before international conventions on biodiversity, some regulations have been made in Turkey. However, these regulations are for the protection of either a specific subject or a specific kind. Moreover, many of these regulations are not even at the power of a law. Therefore, it is very important to organize regulations about biodiversity or any kinds of subjects on international letters. Although states have the rights of sovereignty in international relationships, they cannot resist international repressions, and promise that they will obey liabilities envisaged in these letters some time later. The attitude of the USA about Kyoto Protocol can be shown as an example.

Some international regulations actively contributing to the conservation of biodiversity are as follows: Turkey is not a counterparty to some conventions as is the case with The Convention on the Conservation of Migratory Species of Wild Animals, also known as the 1979 Bonn Convention. The Convention on Wetlands of International Importance, also known as 1971 Ramsar Convention, came into effect on May 17, 1994 in Turkey. The Convention on International Trade in Endangered Species of Wild Fauna and Flora, also known as 1973 Washington Convention (CITES), came into operation on December 22, 1996. Convention on the Conservation of European Wildlife and Natural Habitats, that is 1979 Bern Convention, came into operation on February 20, 1984 after confirmed by Cabinet Decree. 1992 Convention on Biological Diversity (CBD), the basic objective of which is to provide the protection and sustainable use of biologic and genetic resources, came into operation on December 27, 1996.

Convention on the Protection of the Black Sea Against Pollution, in which Turkey is a counterparty, and Biodiversity and Landscape Conservation Protocol on this basis, Convention concerning the Protection of the World Cultural and Natural Heritage (Paris Convention), European Landscape Convention, The Convention for the Protection of the Mediterranean Sea Against Pollution (The Barcelona Convention), the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean, Protocol for the Protection of the Mediterranean Sea Against Pollution from Land-Based Sources and Activities, The Convention on the Protection of the Black Sea Against Pollution (Bucharest Convention) and Additional Protocols, International Plant Protection Convention, The International Treaty on Plant Genetic Resources for Food and Agriculture, and International Convention for the Protection of Bird and European Cultural Convention can be shown as conventions including direct or indirect provisions about biological diversity.

Among the prominent legal regulations constituted by European Union about protection of biological diversity are Endangered Species Act, The Directive on the Conservation of Wild Birds, Wild Birds Directive and Council Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora, that is, Fauna-Flora-Habitat Directive (FFH). Along with these regulations, European Union became a part of the following conventions by signing Bern Convention, Bonn Convention, and Convention on Biodiversity (**Table 1**).

Convention on Wetlands of International Importance (Ramsar Convention)
Convention on International Trade in Endangered Species of Wild Fauna and Flora (Washington Convention) (CITES
Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention)
Convention on Biological Diversity (CBD)
Convention on the Protection of the Black Sea Against Pollution
Convention concerning the Protection of the World Cultural and Natural Heritage (Paris Convention)
European Landscape Convention
Convention for the Protection of the Mediterranean Sea Against Pollution (The Barcelona Convention
Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean
The Convention on the Protection of the Black Sea Against Pollution (Bucharest Convention) and Additional Protocols
International Plant Protection Convention
International Convention for the Protection of Bird and European Cultural Convention
Protocol for the Protection of the Mediterranean Sea Against Pollution from Land-Based Sources and Activities
Biodiversity and Landscape Conservation Protocol
Protocol on Special Protection Areas in Mediterranean Sea and Biological Diversity
Cartagena Biosafety Protocol
International Treaty on Plant Genetic Resources for Food and Agriculture
International Agreement on Plant Genetic Sources for Food and Agriculture

Table 1. Legal regulations implemented in Turkey for the protection of biodiversity.

From these conventions, Ramsar aims at particular habitats, Paris aims at particular areas, CITES aims at particular activities and Bonn aims at particular species [17]. It is not possible to say that all of these conventions do not provide the same protection result for biodiversity. For instance, it is possible to meet with ideas claiming that Rio Convention integrates and damages international environment law and policy and international economical law and innovation law [29, 30].

When we look at international regulations such as 1972 Stockholm Declaration, 1982 World Charter for Nature and 1992 Rio Declaration, it is observed that applying these regulations on national law systems are perpetually emphasized. In some international regulations, states are demanded to develop a protection policy and implementation strategy and in some conventions, it is demanded that some units should be founded in order to apply liabilities effectively, penalize violations, and control the application of convention texts [16]. Turkey, which is assessed among developing countries, tries to contribute to the protection with its financial means by being a counterparty to the conventions on protection from the beginning.

3.2.1. Protection of biodiversity according to the Convention on Biological Diversity (CBD)

The foundations of conservation of biodiversity in international law was laid with the formulation of a program in World Conservation Strategy in 1980 and biodiversity was first given place as a legal principle in the report of World Commission on Environment and Development in 1987.

The Convention on Biodiversity, besides being an international convention, privately regulates the issue of biodiversity and provides a forum by bringing the countries and groups having various benefits and expectations close together, and approaches in an ecologic perspective to innovation by contacting between the usage of environment and resources, between protection and forestry, and between fishing and agriculture [31].

The independence right of states on their natural resources and the operating right of these resources through their own environmental policies are identified with the Convention. Although this regulation is appropriate for international law, shaping the convention on common heritage of humanity would be a more suitable approach.

Future targets and means were tried to be revealed with Strategic Plan for Biodiversity 2011–2020 and the Aichi Targets, accepted on the 10th Conference of the Parties in 2010, may be being the most significant progression after the convention was accepted. The scope of 2020 targets was expanded and not only a scientific point of view but also the benefits of biodiversity to ecosystem and its social benefits were taken into consideration. Moreover, commercial fishing was included and some precautions such as removing economical subventions damaging biodiversity were taken. Relatively more concrete and scheduled targets were determined [32].

However, that the target of 2010 Conference of Parties was deprived of clarity, that it advanced very little from a general intention although defined a date and that it could not bring to an end can be problems concerning the target [32]. As the 2020 targets are not binding, they do not strengthen current liabilities of Convention on Biodiversity but are seem far applicable means for states. CBD should focus on applications encouraging especially national and local concrete results more than developing policies [33]. Biodiversity should be brought forward innovation and strategies for reducing poverty. It should be integrated in all sector policies, especially economics and trade policies [7]. The relation between trade and biodiversity should be founded more tangibly, the knowledge, invention, and practices of native and local communities in relation to genetics resources should be protected, accessing to genetics sources and illegal disposition of genetics sources should be analyzed. Problems that biodiversity is face to face in international area seem hard to solve, especially because of the locations of developed and developing countries concerning the relation of biodiversity with trade, intellectual property right, transfer of technology, and environmental problems [18].

3.2.2. Turkey's fulfilling of liabilities generating from the convention

In Turkey, after the acceptation of Convention on Biodiversity in 1992, either with the scope of the convention or with other international or regional conventions that Turkey is a counterparty, topics about biodiversity took part in the sectors of environment and agriculture in development plans, policies through sustainable protection, development and gaining economic value were put forth, necessary precautions were determined, action plans were prepared, and targets, priorities and means were tried to be identified by means of these plans [34].

Plans such as the Strategic Action Plan for the Rehabilitation and Protection of the Black Sea (1996), Mediterranean Action Plan, National Plan for In-Situ Conservation of Plant Genetic Diversity in Turkey (1997), National Environmental Strategy and Action Plan (1998), Turkey's National Biodiversity Strategy and Action Plan (2001) are basic documents in terms of policies and implementations about this topic [9]. On these action plans, the main focus was on the protection of inland waters, coastal and marine ecosystems and agriculture and forest ecosystems, and especially the protection of endangered species and endemic species. However, putting these action plans into practice completely was not possible because of the lack of resources, due to the fact that existing resources could not be transferred to appropriate places in time, lack of coordination and integration between development program and protection program, and because of infrastructure problems. Since Turkey's National Biological Diversity Strategy and Action Plan (NBSAP) has not been accepted by relevant institutions yet, it does not carry a binding quality [35].

That the court decisions have not been applied properly creates anxiety in public opinion and that civil society has not been counseled sufficiently are deficiencies stated in European Commission 2015 Progress Report of Turkey. It is also stated that Turkey has not sent bilateral conventions draft concerning cross-border cooperation about Environmental Impact Assessment to related countries and provisions about access to information, civil participation and access to judgment on environmental issues that have been established with the United Nations Economic Commission for Europe (UNECE) Aarhus Convention have not been harmonized with Strategic Environmental Assessment Directive (SEA) [36].

National Biodiversity Strategy and Action Plan in Turkey was prepared in 2007 in order to fulfill liabilities in the scope of the UN Convention on Biological Diversity, to put into practice applications compatible with the regulations of European Union on nature protection scope and to reach protection and sustainable usage targets by embracing biological diversity in our country with an extensive and holistic approach. However, it is emphasized in progress report that framework legislation about nature protection, national biodiversity strategy, and action plan have not been accepted yet. It is stated that regulations permitting reconstruction in wetland areas, forests, and natural protected areas are not compatible with EU acquis. Draft Nature and Biodiversity Conservation Law is requested to be harmonized with EU acquis. As abolishing current National Parks Law is envisaged with relevant draft law, it is stated that accepting related implementation legislation in time is of great importance in order to avoid any legal gaps. Potential Natura 2000 areas should be determined and hydroelectric investments, especially in areas of high nature value, should be compatible with environmental legislation.

Studies concerning biological diversity are maintained through projects supported with international institutions and credits in Turkey. Turkey is assessed among developing countries within the scope of CBD. With this status, Turkey can receive grant support on the basis of project about the application of the convention from Global Environment Facility (GEF), the financial mechanism of the convention [37].

Gene Bank was established in 1936 for the first time in Turkey under Ankara University. Seed samples of plant genetic resources of the country started to be protected in National Seed Gene Bank, which was established in 1972 under the organization of the institute affiliated to the Ministry of Agriculture and Rural Affairs. National collection is composed of area races, wild and herbaceous relatives, and other wild plant species with economic importance, and endemic plant species. Moreover, seeds of the plants collected within the scope of national and international projects have been taken under protection in the National Gene Bank. There are almost 70,000 materials in gene banks belonging to approximately 2700 species. About 20,000 of the mentioned materials belong to 2221 wild species. Also there are very much research about propagation and using of endemic or endangered plant species [38].

Seed stands which have trees with higher qualities in terms of their character, located in a certain geographical area and subject to a special management and operation for seed production, aim at obtaining high-quality seeds whose source is known. The number of seed stands is 339 for 27 species. Gene protection forests are natural stands which are selected and managed with the aim of protecting genetic diversity of a species in its natural habitat (*in situ*). There are 214 gene protection forests in 28 species. There are also a lot of scientific researches about the genetic diversity in Turkey [39, 40].

Forest Trees and Seeds Improvement Research Directorate affiliated to Ministry of Forestry and Water Affairs carries out *ex situ* protection for forest trees. One hundred and sixty-nine seed gardens from eight species, 35 seed plantation from 19 species and 13 clone parks from five species have been built until now.

The areas where the wild animals live naturally are taken under protection. Some species are produced in such areas. There are 80 Wild Life Development Sites in Turkey. Fifty-two mammals and 415 bird species have been taken under protection by Land Hunting Law. Protection work is carried out within the scope of a project for the species in danger of extinction such as Anatolian mouflon (*Ovis gmelinii anatolica*), black vulture (*Aegypius monachus*), The great bustard (*Otis tarda*), Caucasian grouse (*Tetrao mlokosiewiczi)*, Gazelle (*Gazella subgutturosa*), red deer (*Cervus elaphus*) and hermit ibis (*Geronticus eremita*). In addition, the habitat of Mediterranean monk seals and particularly spawning area of sea turtles have been protected through special environment conservation areas, which contributes to the protection of biological diversity.

3.3. Regulations concerning conservation of biodiversity in Turkish National Law

The first samples of legislative regulations about protection of natural life and environment were encountered in Babylon and Egyptian laws before Christian Era [41]. There is some information about the fact that nature conservation areas were established by Old Egypt laws. First arrangements about forestry date back as early as 400 B.C. in China. Almost 2000 years ago some arrangements were made in India and Sri Lanka with regard to management of forest resources, tree-cutting and taking hunting under control. Some measures were taken by the Romans with the aim of preventing deforestation [42].

In the administration of Prime Ministry, Ministry of Culture and Tourism, Ministry of Environment and Urbanization and Ministry of Forestry and Water Affairs are the responsible ministries for the protection of the biodiversity in Turkey. There are many legislative regulations in Turkey for conservation on biodiversity. Taking the inventory of biodiversity has been started and the data has been put into service on the database named Noah's Ark within Ministry of Forest and Water Management, which undertakes the most significant task on the protection of biodiversity. Noah's Ark Biological Diversity Database established under the supervision of Ministry of Forestry and Water Affairs is the internet-based national biological diversity database which is open to public access and consolidates the observation data on biogeography of Turkey where one can search by "species," "habitats" and "areas." This database allows any individual and institution-citizens, nongovernmental organizations, scientists and researchers willing to contribute to nature conservation of Turkey- to use, to enter data and to make searches. All observation data entered are accepted to the Database. However, the data are recorded in the system if approved in line with the decisions of Expert Groups and Scientific Consultation Board. Noah's Ark Biological Diversity Database groups the individual users in terms of whether they can enter data and see data. The users may be promoted to the higher user groups provided that they increase reliability percent of the biological diversity data approved by the System Administrator [43].

Some regulations in the legislation about conservation on biodiversity and provisions on these regulations are briefly as the following: The 181–184th acts of Turkish Criminal Law embody crimes against environment. According to this, emitting wastes deliberately to soil, water, or air thus damaging the environment is considered a crime and the penalties are increased in the event of committing these events with wastes causing the emergence of illnesses hard to cure for human beings and animals, the atrophy of reproduction ability and the change on natural features of animals and plants. There are some other special legal regulations.

It is possible to encounter provisions about conservation on biodiversity in 1956 Forest Law about forests, constituting one of the habitats of wild life and endemic plants. On the 25th Act of the law, in sum, the following statement is involved: "General Directorate of Forestry assigns forests and forest areas to the use of science, protects the nature, meets various sports and relaxation needs of the society, enables touristic activities, and separates, organizes, directs and in case of need, manages forests and forest areas as national parks, nature parks, natural monuments, nature conservation areas and forest recreation areas." On the 14th Act of Forestry Law, some conservation precautions towards saving forests are included, as well [44].

Concerning the protection of biodiversity, on the 9/a act of Environmental Law, it is stated that protection of biodiversity and ecosystem, constituting the natural environment, is fundamental and the essentials of the protection and usage of biodiversity will be determined by taking the opinions of local administrations, universities, nongovernmental organizations, and other related institutions. On the 9/d act of the law, it is stated that, with the purpose of conservation on biodiversity, "the Cabinet is authorized to determine and declare soil and water areas which are sensitive to environmental pollution and deterioration, possessing an ecological importance on the country and world scale, as Special Environmental Protection Area, and specify the protection and usage essentials to be applied on these areas and determine the ministry which will prepare and implement the plans and projects, in order for making necessary arrangements to secure the transference of biodiversity, natural resources and cultural resources to the next generations." On the 9/f act of the law, the following provision is included "In order to maintain the sustainability of biodiversity, the protection of endangered species and rare plant and animals is essential and it is prohibited to make them an issue of trade against legislation."

Another important arrangement about biodiversity is Land Hunting Law. Wild life protection and development areas defined at the 11th and 12th subarticles of the 2nd article of the law carries special importance in terms of our topic. While wild life protection areas are places where necessary habitats having wild life values are absolutely protected together with plant and animal species and their continuity is provided, wild life development areas are places where game and wild animals and wild life are protected, developed, game animals are settled and habitat healing precautions are taken. In the 4th article about the protection of these areas, the following provisions are included: "In wildlife protection and development areas, wildlife cannot be destroyed, ecosystem cannot be ruined, institutions that can have negative effect on these areas cannot be permitted even out of wildlife protection and development areas and reproduction stations, the wastes of current institutions cannot be released without refining, no construction or institution can be founded except from those defined in approved plans and easement cannot be established. If needed, additional prohibitions can be implemented by the Ministry. No prohibitions can be implemented by other state institutions and organizations without the approval of the Ministry." It is also stated in this article that natural habitats enabling the feeding, sheltering, reproduction, and protection of game and wild animals cannot be poisoned, wetland areas cannot be polluted or drained, and their natural structures cannot be changed [45].

Another regulation about biodiversity is National Parks Law the number of which is 2873. In the 1st article of the law, it is pointed out that the purpose of the law is to choose and determine national parks, nature parks, natural monuments, and nature protection areas possessing the values at the national and international levels in Turkey, to arrange the essentials concerning to protect, develop, and manage them without damaging the features and characters. What is important in providing sustainable utilization of National Parks is doing studies that cause the least harm to the natural structure of the area [46]. In this law, four different protection statutes are envisaged under the names of national park, natural park, nature area, and nature protection area and special protection provisions that will be valid in areas of this statute are included at the 14th article. According to this, in the places in the scope of this law, (a) natural and ecological balance and natural ecosystem value cannot be damaged, (b) wildlife cannot be destroyed, (c) any kinds of intervention that causes or may cause to lose or change the features of these areas, and tasks and operations that would create soil, water and air pollution and similar environmental problems cannot be performed, (d) production of any kinds of forest products that would destroy natural balance, hunting, and grazing cannot be performed, (e) as far as there are no indispensable and exact obligation for public welfare, except from structures and institutions indicated in approved plans and institutions necessary for defense system to be needed by general staff, no structures or institutions can be founded or managed under no circumstances or can be settled apart from existing residential areas in these areas." When we have a look at regulations and applications about national parks in the world, it is seen that areas declared as national parks are protected originally as far as possible and there is hardly any human interference. However, in our country such activities as mining, touristic institutions etc., are allowed and these areas cannot be protected properly [47].

Another regulation comprising provisions about the conservation of biodiversity is Animal Protection Law. According to the 4/e subarticle of the law, "it is essential to protect endangered species and their habitats." Moreover, the following statements are included at the 8th article "Any kinds of interference to exterminate an animal generation is forbidden. Apart from medical purposes, giving an animal hormone or medicine in the form and dosage that would make it contrary to its kind and ecological features, doping it with various substances and changing the behaviors and physical features of animals typical to them with artificial methods are forbidden.

It is stated in the 2/b article of Law on the Organization and Duties of Forestry and Water Affairs that the identifying, directing, protection, development, managing, and ensuring being managed of national parks, nature parks, natural monuments, nature protection areas, recreation areas in forests and biodiversity, game and wildlife areas are among the duties of the Ministry.

Another regulation involving provisions about the conservation of biodiversity is Law on Conservation of Cultural and Natural Property. Regulations about the protection of cultural and natural properties are related to real estate, moveable estate, cultural assets, natural assets, or only the protection of plant or animal species [48]. Cultural and natural assets are the wealth of the whole humanity [49]. In the regulations about the protection of cultural and natural assets, natural and unnatural regulations are acknowledged in the same category.

Some duties of General Directorate of Cultural and Natural Heritage founded for protecting natural properties are as the following: (a) determining the methods and essentials for registration, approval, and announcement of national parks, nature parks, natural monuments, nature protection areas, wetland areas and similar areas that have the statute of protection and register the borders of these areas. (b) Determining the methods and essentials about the identification, registration, approval, modification and announcement of nature properties, natural protected areas, and special environment protection areas and identification and registration of the borders of these areas and management and providing to be managed. (c) Determining the resolutions towards the usage and structuring of national parks, nature parks, natural monuments, nature protection areas, natural protected areas, wetland areas, special environment protection areas and other areas having similar protection statutes, and making, modifying, applying landscape, general plan and implementing development plan in every kind and scale. (Item 13/A)

There are many directives in Turkey concerning the conservation of biodiversity, along with constitutional and legal regulations. Some of these directives are related to the implementation of provisions emerging from Turkey's international conventions while others are made in order to apply these laws and identify ordered tasks. The most significant ones of these directives are the following: Directive through the Implementation of Convention on International Trade in Endangered Species of Wild Fauna and Flora, National Parks Regulation, Wetlands Protection Legislation, Regulation on Protecting Habitats of Game and Wild Animals, Regulations about Game and Wild Animals and Keeping, Producing and Trading their Products, Regulation about the Foundation, Management and Control Essentials and Methods of Hunting Grounds, Regulation about the Protection and Development of Wild Life Areas, Regulation about Duties, Working Essentials and Methods of Central Hunting Commission and Province and District Hunting Commissions, Regulation about Collecting, Saving and Using Plant Genetic Resources, Regulation about Recording Plant Diversity, Regulation about Producing, Picking up and Exporting of Natural Flower Bulb, Regulation about Protection of Animal Genes Resources and Regulation about the Welfare of Farm Animals.

In Turkey, the genes belonging to the plant and animal species have been taken under protection by Seed Growing Law, Biosafety Law, Animal Improvement Law, By-law on Protection and Sustainable Use of Fisheries Genetic Sources, By-law on Protection and Sustainable Use of Pet Genetic Resources, By-law on Genetically Modified Organisms and Their Products, and By-law on Collecting, Keeping and Use of Plant Genetic Sources.

Apart from the above agreements, Turkey is a party to the Protocol on Special Protection Areas in Mediterranean Sea and Biological Diversity, Cartagena Biosafety Protocol, International Agreement on Plant Genetic Sources for Food and Agriculture (**Table 2**).

Law	Directive
Turkish Criminal Law	
Forest Law	
Land Hunting Law	Regulation about Duties, Working Essentials and Methods of Central Hunting Commission and Province and District Hunting Commissions
	Regulation about the Foundation, Management and Control Essentials and Methods of Hunting Grounds
	Regulations about Game and Wild Animals and Keeping, Producing and Trading their Products
	Regulation on Protecting Habitats of Game and Wild Animals
	Regulation about the Protection and Development of Wild Life Areas
Environmental Law	Wetlands Protection Legislation
National Parks Law	National Parks Regulation
Animal Protection Law	
Law on Conservation of Cultural and Natural Property	
Law on the Organization and Duties of Forestry and Water Affairs	
Seed Growing Law	Regulation about Producing, Picking up and Exporting of Natural Flower Bulb
	Regulation about Recording Plant Diversity
Biosafety Law	Regulation about Genetically Modified Organisms and Their Products, By-law on Collecting, Keeping and Use of Plant Genetic Sources
	Regulation about Collecting, Saving and Using Plant Genetic Resources
	Directive through the Implementation of Convention on International Trade in Endangered Species of Wild Fauna and Flora
	Regulation about Protection of Animal Genes Resources and Regulation about the Welfare of Farm Animals
	Regulation about Protection and Sustainable Use of Pet Genetic Resources
	Regulation about Protection and Sustainable Use of Fisheries Genetic Sources

Table 2. Regulations under Turkish national laws and directives concerning conservation of biodiversity.

In Turkey, there are no regulation that has been made just for this aim and directly for the conservation of biodiversity in constitutional and legal regulations. The Environment Protection and Biodiversity Conservation Act draft was accepted in 2011 after having been negotiated by related Parliament Commissions but the draft could not become a law. It is aimed with the draft to protect biodiversity and natural values of our country with national and international importance in land, coast, water, and sea areas and to specify the statutes of protected areas again transparently and apprehensively. Moreover, it was envisaged to found National Biodiversity Board and local biodiversity boards in order to enable the protection of nature and biodiversity and to found Nature Conservation Science Panel in order to provide leading scientific support to the Ministry of Environment and Forests. The draft, possessing great significance as it is directly for biodiversity, was criticized with the changes it brings and problems it will generate. It was stated that the draft which separates protection of cultural assets from protection of nature assets, would create jurisdictional dispute and this would pave the way for using nature and cultural assets more defenseless [50]. National Biodiversity Panel is authorized with revising previously announced decisions about natural protected areas with the provisional article of the draft. Thus, previous decisions about protected areas can become open to changes and natural areas that become strict preservation zones can be gone out of conservation [51]. Moreover, it is pointed out that the draft brings regulations that make "usage" as the aim under the protection-usage balance and it is emphasized that this will destroy the most important values of our country. It is asserted that the draft is against 1982 Constitutional Law and international conventions like the Convention on Biodiversity that we are a party of [52].

However, some changes mentioned in the draft were put into practice through some legislative decrees accepted later. The effects of newly ensured legal and institutional changes in terms of biodiversity are still being argued [35]. It is asserted that environment cannot be managed as is required with the new environment management layout envisaged to be founded with the legislative decree number 636 and any kinds of environmental values can be plundered more easily than previous terms.

4. Efforts by nongovernmental institutions concerning biodiversity

It is seen that nongovernmental organization`s, too, attended activities for the protection of natural life after the First World War. In 1922, International Committee for Bird Preservation was founded by American ornithologists. Again in the USA, The American Committee for International Wild Life Protection was founded and International Office for the Protection of Nature (IOPN) was founded in 1934 with the support of Dutch environmentalists. Especially after the Second World War, activities for the protection of environment and nature accelerated through United Nations and subinstitutions supported by it, and they became topics that world public opinion was interested in. In 1948, The International Union for Conservation of Nature (IUCN) was founded. Man and the Biosphere Programme was initiated in 1971 as a result of studies started by UNESCO about the protection of environment and species. United Nations Environment Programme (UNEP) was founded within United Nations [16].

In the 1970s, nongovernmental institutions actively joined the preparation and application stages of conventions and the institutional identities such as the secretariat and administrative infrastructure originated in order to control the implementation of conventions. In the following years, the role of nongovernmental institutions gradually increased. The Rio Conference held in 1992 can be said to be the work of nongovernmental organizations.

It is observed that international institutions having functions and responsibilities about various dimensions of biodiversity have come to the forefront at CBD negotiations. United Nations Environment Programme (UNEP), which promotes the beginning of negotiations, played an important role as the Secretariat, and the UN Food and Agriculture Organization (FAO), which is directly related to the use of genetic sources and have already had regulations about this topic, actively participated in the process. The International Union for Conservation of Nature and Natural Resources (IUCN), which is an environmental organization, has played an active role in the emerging and shaping of the convention. FAO and IUCN prepared and presented their own draft contracts during the negotiations. World Wildlife Fund (WWF), one of the nongovernmental organizations, took part in almost all negotiations. Other nongovernmental organizations such as World Conservation Monitoring Center, World Resources Institute, Genetic Resources Action International (GRAIN), and Greenpeace joined some of the meetings and contributed to the process by providing data and documents [53, 54].

There are many nongovernmental organizations in Turkey serving to the protection of nature, environment, animals, plants; in other words, the biodiversity. According to the legal order in force, finite number principle is valid for juristic persons; and associations, charitable foundations, and cooperatives can be regarded as private law persons by the legal order. It should be stated that professional associations and unions contribute to the conservation of nature and biodiversity in Turkey as well. It is observed that these associations sometimes take more part than political parties in revealing the activities damaging biodiversity and informing public opinion. They contribute to the political parties in raising their opinions and policies about nature and in developing these policies [21].

Charity foundations and associations which carry out works about nature and biodiversity in Turkey and are generally known by everyone are the following: Turkish Nature Protection Association, The Foresters' Association of Turkey, Society of Environment and Natural Life Protection, Turkish Foundation for Combating Soil Erosion for Reforestation and the Protection of Natural Habitats.

It can be said that the contributions of these nongovernmental organizations are sometimes more than the state institutions. It is because while the units in the administrative hierarchy have to obey the decisions taken by the political will, nongovernmental organizations do not have such an obligation. Therefore, they can do more objective studies.

The public officers cannot generally act objectively in the face of any damages in nature regarding violation of biological diversity, they avoid negative reports and are concerned about these negative situations having a media coverage. Therefore, world public opinion is not informed of harmful acts on the living creatures until after it becomes an environmental catastrophe which the state cannot overcome alone or it is put on the agenda by the other states. However, public opinion is informed of mentioned violations thanks to the nongovernmental organizations which operate on biological diversity-related issues in national and international area. In Turkey, national nongovernmental organizations such as Association for Stray and Street Animals, Federation of Animal Rights, Foundation for Protection and Promotion of Environment and Cultural Values as well as international nongovernmental organizations such as Greenpeace contribute to protection of nature and biological diversity.

In Turkey, charitable foundations and associations benefit from exemption from tax when their activities are identified to be for public welfare. Nongovernmental organizations are deprived of financial possibilities. Therefore, their activities should be supported. The participation of public in the nongovernmental organizations' legal and administrative decisions about biodiversity should be enabled and access to the actual data about the topic to be participated should be provided in a reasonable time. At the 13th and 14th items of CBD, the participation of public to the preparation process of Environmental Impact Assessment (EIA) and to be informed are obligatory. However, the authorization of nongovernmental organizations by convention organizations, enabling them to give information about applications against the convention in the country by giving financial support and providing petition right will contribute to the applicability of the convention.

5. Conservation of biodiversity through judicial means

It is no doubt that every step towards conservation of biodiversity, if well intentioned, is absolutely precious. However, violations against these regulations should be controlled by judicial positions in order to protect both regulations accepted in national law, the international conventions and acquisitions gained as a result of activities performed by nongovernmental organizations for conservation of biodiversity and to provide continuity of these studies. There are many social order rules in everyday life to keep people out of unrecognized activities. However, among these rules, the state just applies sanction in the event of violation of legal rules. Biological diversity is protected in Turkey thanks to the protection mechanisms included in national law rules and international agreements. Law enforcement authorities often impose fines in the event of a violation. However, the objections raised against the fines imposed by the law enforcement authorities are inspected by the courts, and any violation causing a severe damage is punished by the penal courts as a result of the lawsuits filed by the public prosecutor on behalf of the public.

5.1. The inspection of conservation of biodiversity by international judicial bodies

It is observed that international judiciary bodies do not propose directly an independent conservation regime like Turkey about biodiversity but assess this topic with the protection of the environment. Problems emerging about biodiversity and environment are mostly subjected to judgement call. It is because while the states accept conventions about environment, they do not accept the authority of UN Court of Justice and it is viewed that they feel desire to find a solution to problems about this topic with an arbitrator [55]. In 1982, for the first time in the world, the subject was brought to international arbitral tribunal in order to solve the controversy between the USA and England about seal fur hunting in North Pacific and a protection regime was proposed by the court. About international trans boundary rivers, Lake Lanoux Arbitration between Spain and France and Gut Dam Arbitration between the USA and Canada are samples for other arbitral awards. [16]. After 1990s, there were increase in cases about environment in UN Court of Justice. The dam project claimed to harm the environment, pulp mill project built on the river, whale hunting in the Antarctic and road construction project by the river are among the cases that the court handles. An increase was recorded in the cases handled in International Permanent Court of Arbitration in the same years. Apart from these courts with general power, some special authority courts such as World Trade Organization Dispute Settlement Court, The Court of Justice of the EU and The International Tribunal for the Law of the Sea referred partly or completely to basic principles of environmental law in the cases they handled [16].

In Turkey, for the solution of any legal conflict, instead of going to law, it is possible to apply to arbitration boards where the parties reach an agreement provided that the subject matter is not related to public order. However, arbitration cannot be recourse in any matter where the parties cannot dispose freely.

International arbitration is applied to the conflicts which has the element of foreignness in the legal relation and for which the place of arbitration is Turkey. As for international arbitration, arbitration is not referred for any matter which the parties cannot dispose freely and for which they settle or make an agreement; arbitrator(s) do not make a decision in such cases.

The conflicts about any act damaging biological diversity and those prohibited in environment law are related to public order in Turkey, referring to arbitration for such matters is not possible. Consequently, as for any legal problem where Turkey is one of the parties, there is no ruling made by any arbitrator until today.

5.2. The inspection of the conservation of biodiversity by national judicial bodies

In Turkey, cases about the protection of biodiversity are handled in constitutional jurisdiction and administrative justice. Administrative fine is implemented by law enforcement authorities many times in order to protect biodiversity. Administrative sanctions implemented for the protection of biodiversity can be through administrative fine, confiscation, transference of ownership to the public, decision of demolition, closing of the workplace, and disqualifying from the profession and performing art. These are the general sanction types that can be implemented by the administration. However, sanctions about the violation against biodiversity are not limited to them. Penalty of imprisonment related to violations about biodiversity are sentenced by judicial justice. They can be aligned as administrative fine and penalty of imprisonment [56]. For instance, in the event of leaving waste that causes harm to the environment, penalty of imprisonment is sentenced to those doing the activity by judicial courts. When the implementations of law enforcement authorities are observed, 23,000 Euros compensation is demanded from a person in the event of hunting an Anatolian wild sheep without permission and also administrative fine is adjudicated. In the event that some species are harmed, the legal sanctions will be more severely imposed. Poaching is a major threat to many species existing in Turkey [57]. Sanctions are given to many people to prevent many species from poaching especially species which have the status EN (Endangered) or extinct.

Anatolian wild sheep (*Ovis orientalis anatolica*), with a limited population in eastern Turkey, with an isolated population in south-central Turkey, phylogenetically belong to a subspecies of Asian mufflon (*Ovis orientalis*) and is good an example to this [58].

Conservation of biodiversity is mostly examined in the adjudication and appeal controls of Constitutional Jurisdiction or Council of State. There are not any decisions directly about biodiversity in the decisions of Constitutional Court. However, in the cases concerning the cancellation of regulations about control area of the court or in forming cancellation or denial justification, conventions about biodiversity are taken as a reference, and regulations to ruin biodiversity or damaging to protection are cancelled.

It is observed in the decisions of Constitutional Court that the environment and biodiversity are generally considered together. The court decided to the cancellation of the regulation that makes alteration in the Environment Law with its 15.1.2009 date and no E: 2006/99 K: 2009/9 decision by indicating that search operations out of the scope of Environmental Impact Assessment (EIA) may cause differences on biodiversity and nature, these differences may have long-term impacts, it carries risks for the environment and in order to take away and prevent the existing risks in search operations EIA should be envisaged, and it is the requirement of the liability of environment protection given to the state at the 56th article of Constitutional Law.

In another decision dated 3.7.2014 and numbered E: 2013/89 K: 2014/116, the Constitutional Court decided that turning back to previous stages by stopping the production and management of a public investment project, the planning and decision phases of which have passed according to the appropriateness of the investment to the environment and production and management stages have started, does not contribute to the identification of environmental effect beforehand and to take precautions if it has negative effects on the environment. It is also decided that if an investment which has begun production causes harm to the environment, the state's liability to control the investment and to apply sanctions continues, even public investments in the scope of exemption has begun employment, production and management, in other words, even it has been carried out with significant financial resources and has begun to contribute to the economy. With the contributions of these investments to the economic and social life in mind, they can be the object of serious sanctions such as stopping their activities if they cause harm to the environment. However, giving up their productions and management will create more negative effects than the environmental effect that is possible to be removed. In conclusion, it is decided that it is not necessary to take EIA Report for these kinds of investments and it does not constitute an immoderate intervention to the right of living in a healthy, balanced environment [59].

The inspection on the conservation of biodiversity is mostly performed by administrative jurisdiction. In Turkey, there is a separation between civil and criminal jurisdiction and administrative jurisdiction, as is in Germany and France. As being trial courts, administrative jurisdiction becomes general authorized courts and works as council of state appellate courts except from specific cases. It is viewed that court decisions towards the conservation of biodiversity in Turkey do not date back a lot but decisions in this sense have begun to be given especially for the last 5 years. In legal precedents of state council concerning the topic, it is seen that administrative jurisdiction is very sensitive about the protection of biodiversity and has contributed significantly to the conservation. It is deduced that legislation and enforcement take subjective decisions with mostly political vote concerns and political future concerns of politicians while jurisdiction can evaluate the case independently and objectively [21]. When the precedents of state council are examined, it is observed that the most significant task belongs to Ministry of Forestry and Water Affairs. It is viewed that while constituting the administrative structure of the ministry and opening organizations in the provinces, the structuring is formed by taking the genetic resources and biodiversity into consideration, and organization against it is illegal. The decision of 10th Circuit of State Council dated 10.02.2016 and numbered E: 2012/349, K: 2016/667.

In another decision of 10th Circuit of State Council dated 13.10.2015 and numbered E: 2011/7033 K: 2015/4313, the request of cancellation of declaring a dam lake which possesses natural landscape in terms of sheltering, feeding, and living conditions of wild animals as wildlife development area was refused so as to enable the conservation and rehabilitation of biodiversity.

In another decision dated 17.02.2014 and numbered E.2009/1713, K. 2014/895, 10th Circuit of State Council refused the Cabinet Decree about removing the nature area protection status of Yumurtalık Lagoon, which enables better conservation status and determining it as a national park, which enables poorer protection on the grounds that the area is among the most significant wetland areas of our country in terms of biological diversity, the area is turned into a lower-level protection status with the Cabinet Decree at issue and the established process is against the international conventions that our country is a party of.

In another decision dated 22.06.2011 and numbered E. 2011/5290, K.2011/266, 14th Circuit of State Council decided that it was wrong to announce bird sanctuary in Çamaltı Salt Field, which was accepted as the first degree of protected area by the Ministry and had scientific and cultural significance with rich biological diversity, as Coastal Zone Tourism Center on the grounds that the area in question should be included to the List of Wetlands of International Importance because of its biological diversity, the area could lose the constructive functions of natural areas in the ecosystem because of the activities in the scope of tourism center, and sand dunes, coast flora and natural Mediterranean flora just behind could be damaged along with wetland areas as a result of tourism activities, the applications were opposite the nature protection criteria. Moreover, the court states in its decision that the case should be evaluated together with its environment as a part of the whole instead of being alone, in order to protect the environment for the public welfare.

In another decision dated 10.6.1997 and numbered E. 1996/5259 K. 1997/2806, 6th Circuit of State Council decided that an area which shelters 308 bird species, is rich in flora-fauna and biologic diversity and forms a natural residential area in terms of regional recreation should be protected from pollution reasoning from unauthorized structuring and domestic waste, and natural balance and texture should not be destroyed.

6. Discussion

CBD distinguishes itself from both previous international conventions on biodiversity and international conventions of its time as it has the objective of sustainable use and fair sharing of the benefits obtained from genetic sources along with its objective of conservation of biodiversity.

Since the Rio Conference where the convention was opened for signature, processes causing losses in biodiversity have increasingly continued and problems in the implementation of the Convention have not been overcome. As it is seen with the targets put forth at the 10th Conference of the Parties, the efforts of developing the Convention for shaping the future continue at the normative level but when reaching the targets is taken into consideration, the decisions of the Conference of Parties are viewed as less concrete, less organized, and less applicable.

The Convention just imposes obligation on declaration, information exchange, and consultation to the possible activities for the negative impact on the biodiversity on places which are not dependent on national judiciary of states. However, it can be said that this regulation is quite imprecise. Yet, it is seen that the provisions of the convention are not implemented even in this state and the states do not make bilateral, regional, or multilateral agreements appropriate for general collaboration. The provisions of the convention should be revised in this sense and sanctions should be imposed in case of breaking the convention provisions. Compensation should be the first and foremost sanction, not the last, to be imposed.

In the conventions concerning the conservation of nature and biodiversity, the principle of common heritage of humanity, which developed countries insistently oppose, should be used instead of the principle of domination of states on natural resources. This principle means the fair and peaceful usage of natural resources without causing any harm to the environment, by taking into consideration the needs of the overall international community.

Although it is viewed that biological studies on biodiversity are carried out in Turkey through many legislative regulations and institutions, increase in conservation studies in specific regions are provided through national and international projects and the case of international provisions are tried to be met, it is not possible to say that biodiversity can be completely protected and the devastation can be prevented.

In Turkey, industrial premises are more common in certain places, and the people constantly migrate from rural areas which are rich in biological diversity to the cities. This is an advantage for the creatures living in the nature and in need of protection, and today there are still many virgin areas with high variety of creatures. However, in the works such as construction of the roads, airports, industrial premises, etc., which are built for development of the country, any act that may jeopardize biological diversity should not be allowed. Nevertheless, considering the decisions made on this issue and the judicial decisions made as a result of relevant lawsuits, it is likely to see that importance of biological diversity is not clearly understood by the government and the Supreme Court.

When provisions in Turkish law towards the conservation of biodiversity are assessed as a whole, it is observed that a great majority of legislations are based on international conventions. Legislative regulations in national law are seen to be located discursively in different legal texts. There are many legislative regulations about biodiversity in Turkey and the aforementioned legislation is tried to be adjusted according to Turkey's national and international provisions with the amendments. However, the legislation is insufficient in meeting the protection needs of biodiversity because of the disorders or deficiencies of legislative regulations. When the legislation about biodiversity is observed, it is viewed that there are gaps in some topics while there are implementation and sanction deficiencies in some other topics.

There is not a special legislative regulation in Turkey that regulates the subject of conservation on nature and biodiversity. It is seen that legal regulations in legislation about the conservation of biodiversity are attached to different legal letters later and are disconnected to each other, they are insufficient in meeting the biodiversity protection needs and they are deprived of dissuasive sanctions that would guarantee their effectiveness. That there are not dissuasive sanctions is an obstacle for the application, or sanctions that are envisaged for some crimes do not have the implementation capability.

Regulating the conservation of biodiversity with different laws and carrying out precautions concerning conservation by different institutions cause conflict of duties and problems in the implementation of laws. In terms of organizational structure, coordination problem takes an important place as many different institutions are assigned. As different units are assigned in implementing sanctions envisaged in the conventions and laws, a law enforcement officer authorized to prevent a violation do not take any action when he encounters violations that he is not authorized. Moreover, the number of law enforcement officers in charge of conservation is rather limited in Turkey. As Günes and his colleagues mentioned [60], in a nature conservation law to be prepared, regulating thoroughly the features concerning protection of biodiversity and precautions to be taken about conservation contribute significantly to the protection of biodiversity.

In Turkey, environmental sources having different functions are used intensively because of the crowded population. As Belkayalı and her colleagues mentioned in their studies [61], even the activities performed in protected natural areas, although they seem to be nature friendly activities, the area where the activities are performed is inevitable to be under effect. The effects are felt in all source values of the protected natural area. The level of the impact differs depending on the sensibility of source value, the type of the activity, the intensity and time of usage. At this point, the continuity of resources and productions and services presented to the benefit of community gain significance. Hence, in recent years, the concept of sustainable development has drawn the attention as a concept which emphasizes the need of protection of environmental needs and to keep the environmental quality at a reasonable level. Therefore, as Belkayalı and her colleagues stated in their studies [8], identifying the affected resource value, permitting the usage of the resource value according to the sensibility level and following it are quite significant for enabling the sustainability.

As Belkayalı and Kesimoğlu stated in their studies [62], presenting participatory approach in studies carried out in protected areas where biodiversity is large and including the local people in the process are significant for the protection of resource values in the area. It is stated in previous studies about this subject that including local people to the process is significant in enabling the sustainability and protecting resource values, accordingly.

In developing countries, providing participation, and freedom for expression and proclaiming scientific studies correspondingly, presenting impartial reports of governmental organizations are quite difficult. Therefore, nongovernmental organizations comprised people who act in accordance with convention aims and who are specialists in their fields should be authorized by convention bodies and information report should be required from these institutions at least twice a year. Thus, independent reports that do not liable to state hierarchy can be obtained out of formal state bodies.

While providing developing countries with financial support, the awareness of the public should be raised, strict rules should be implemented in the usage of guidance services and the control of the application should be provided.

"It is obvious that protecting public properties such as coasts, mountains which compose the area of biodiversity is very important in terms of convention on biodiversity, as well." It is because the majority of living beings that have to be protected are on public properties. It is both an advantage and disadvantage. It is because conservation of biodiversity can be neglected while state investments such as airport, road on public properties are planned and, occasionally unauthorized structuring becomes legitimate with amnesty laws enacted from time to time. The precautions to be taken by state about the protection of biodiversity will have the opportunity of being implemented on public properties easily when compared with the private property areas. It is because the implementation of some precautions to be taken in the private property would necessitate to limit some rights that the property right holds in itself.

7. Conclusion

In order to provide the conservation of biodiversity effectively, along with legal sanctions, political and commercial sanction mechanisms should be formed with provisions to be attached to the convention so as to prevent activities of party countries against the convention. In order to enable the implementation of CBD, nongovernmental organizations authorized in party countries to the convention and the staff working there should be financially supported by convention bodies and semester reports should be required from these organizations. Including the nongovernmental organizations that may be founded for performing any legal activity will contribute to protection of biological diversity. In Turkey, the arrangements on environment are not subject to unilateral arrangement by the government, and participation of the citizens in the decisions is encouraged to some extent. Legal arrangements made regarding the participation of the area stakeholders in the decisions made provide improvement to a certain extent.

A special regulation handling the protection of nature and biodiversity in Turkey should be performed urgently. With the regulation, the decisions to be taken about the protection of nature and biodiversity and the implementation authority of these decisions should be given to a single organization and each law enforcement officers should be assigned to identify the activities against the protection of biodiversity and to take legal actions about criminals.

Protection net of Natura 2000 which is regarded as the heart of EU Nature Conservation Legislation, taking a key role on the prevention of the loss of biodiversity, should be identified in Turkey as immediate as possible and the objectives and targets defined in action plans towards the convention of biodiversity should be taken into consideration by not only a single organization but also by all public and private organizations. As protecting the biodiversity by just the State is not possible, participant approach should be performed in decisions to be taken about the protection of biodiversity and public should be included in the process. The public should also be trained and their awareness should be raised about conservation.

The legal regulations adopted to ensure the implementation of the convention in domestic law should refrain from including exceptions which are in compatible with the provisions of the contract; besides, political and administrative units should be made to comply with the court decisions.

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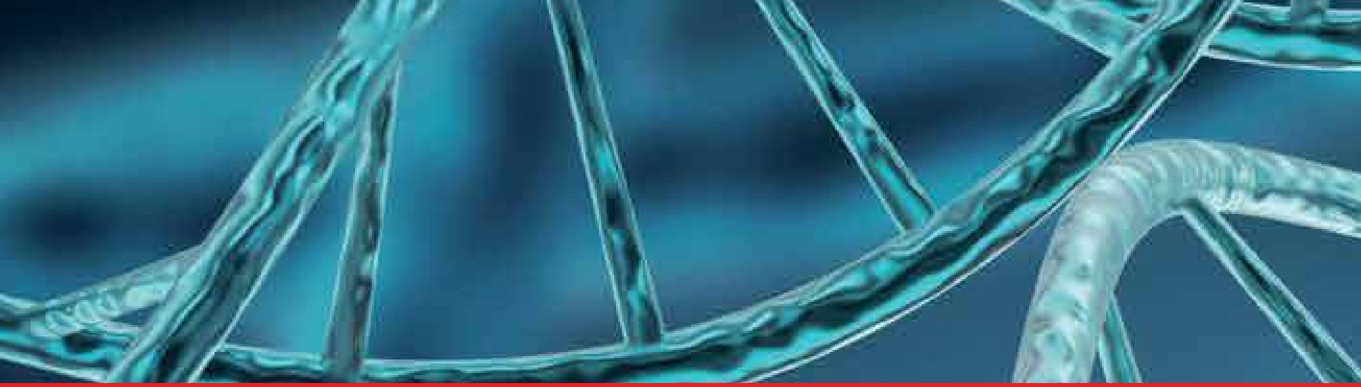

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Genetic diversity is the entire amount of genes and genotypes in a group of organisms and is of vital importance for their adaptation to different living conditions. If, for example, all humans were identical, the extinction of the entire kind could happen very fast. Let us care and nourish differences! The goal of this book is to present some of the contemporary thoughts on understandings of the genetic diversity patterns and their altering in a changing world. The book is aimed to the ones inspired to study and contemplate genetic diversity and to the audience beyond any frames.

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