

PREFACE

Genetic diversity plays a very important role in survival and adaptability of a species because when a species's environment changes, slight gene variations are necessary to produce changes in the organisms' anatomy that enables it to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the most fit alleles. In this book, the authors discuss topics that include genetic diversity of human and animal papilloma viruses; conservation genetics of the lion; genetic diversity for quality traits in wild and ancient wheats; the genetic history of Morocco; different approaches to assess structure and genetic diversity in forest species; new genetic provisions for the reinstatement of ginseng natural populations; genetic and epigenetic variations in disease and drug response; genetic diversity and genetic rescue within wildlife populations; and genetic variability and advances in taxonomy.

Chapter 1 - The papillomaviruses form a highly diverse group that infects mammals, birds and reptiles. There is a huge veterinary and public health importance associated with these viruses. Because of this, human and bovine papillomaviruses are the most studied viruses of this group. Infection with human papillomavirus (HPV) represents the most important risk factor in the genesis of cervical cancer. Bovine papillomavirus (BPV) infects basal epithelial cells leading to the formation of benign and malignant tumors, when in synergism with action of environmental co-factors. Brazil is a country with continental dimensions; therefore the understanding of the genetic variability and the distribution of these viruses is essential for an active surveillance program and the design of more efficient vaccines and diagnostic methods. In what concerns the association of HPV and cervical cancer, HPV-16 is the most frequent type worldwide, with the exception of Indonesia where the HPV-18 happens to be the most common type. Although the HPV-16 is the most frequent, the second most important type differs between different regions in the world. So, this difference in the incidence of HPV genotypes needs to be elucidated, along with the genetic variability of these isolates that could lead to distinct oncogenic potential among different variants and subtypes. Oncogenic variants of HPV-31 were found and some of the mutations were observed in regions involved in host immune recognition. Changes in amino acid sequences between variants from different geographical regions evidence the possibility of functional divergence between them, suggesting that the variants detected may have different mitogenic fitness. On the other hand, twelve BPV types are known so far, but only three of them are associated with cancer. There is a great diversity of BPV types among cattle and sheep. Infection was found. The presence of infection with multiple viral types could lead to

suppression of the immunological system making the clinical state of the animals worse. So, further molecular epidemiological investigations on the incidence and diversity of papillomavirus infection will assist in establishing a more accurate view of the distribution of these viruses. This could help the development of accurate and specific diagnostics, prophylactics and therapeutic requirements.

Chapter 2 - Maize (*Zea mays* subsp. *mays*) is a member of the grass family *Poaceae* (*Gramineae*), together with many other important agricultural crops. The wild species commonly known as teosintes are the closest relatives of maize. Teosintes are wild grasses with a native distribution area from Mexico to Nicaragua (Mesoamerican region) and are an important genetic resource for maize improvement. Teosinte from Nicaragua (*Zea nicaraguensis*) is to a large extent an unutilized genetic resource and its properties in terms of desirable traits, such as water-lodging adaptation and disease resistance have to be phenotypically and genetically characterized. This chapter includes results from cytological, genetic, morphological and phylogenetic studies. Cytogenetic studies were based on C-banding techniques. Microsatellite (SSR) markers and DNA sequences of cpDNA regions were used for genetic diversity and phylogenetic studies respectively, whereas morphological studies involved various quantitative morphological traits. The C-banding pattern revealed that *Z. nicaraguensis* is more similar to *Z. luxurians* than other teosintes and cultivated maize. Among the Meso-American teosintes, *Z. diploperennis*, *Z. perennis* and *Z. nicaraguensis* showed the highest values in the number of rare and unique alleles and the results also indicated that the gene flow between *Z. nicaraguensis* and *Z. luxurians* has been more frequent than between other teosintes in the Mesoamerican region. The morphological characterization revealed that the traits of the number of lateral branch nodes bearing ears, the glume width, the number of tiller nodes bearing ears and the number of tillers per plant were the most important in discriminating between taxa and the principal component analysis grouped many traits around the *Z. nicaraguensis*, *Z. luxurians* and *Z. mays* subsp. *huehuetenangensis*. Finally, the variation among cpDNA sequences need more analysis to give a definitive phylogenetic resolution among the five *Zea* species, but even so the results support the idea that *Z. nicaraguensis* could be treated as a separate species distinct from *Z. luxurians*.

Chapter 3 - As an ecological and cultural umbrella species, the lion has drawn a lot of attention from researchers from a variety of disciplines. Due to the decrease in suitable habitat and increasing pressure by a growing human population, many lion populations show a declining trend. When it comes to species conservation, initiatives often follow existing taxonomy. This implies that taxonomic distinction reflects the diversity of a taxon sufficiently to preserve it on the long term. In the view of this, it is remarkable that all lion populations in Africa are considered to be the same subspecies, while for other large mammals with a similar geographical distribution, often several subspecies are being distinguished. The following chapter will review the published literature dealing with the genetic diversity of the lion and illustrate how upcoming molecular techniques can contribute to the field of species conservation.

Because the lion populations from West and Central Africa were only rarely included in previous studies on genetic diversity, the position of these populations within the species is still unclear. Recent phylogenetic research showed that the West and Central African lion is genetically distinct from their conspecifics in East and southern Africa. Because of geographic barriers and the climatic history of this region, these populations have formed a

unique genetic make-up. This provides a strong argument to increase conservation efforts in West and Central Africa. In this region in particular, anthropogenic pressures have led to small, fragmented, and therefore vulnerable populations.

This chapter will also describe how different types of genetic markers can be combined to create a complete picture and come to a well-designed management plan. With the current development in next generation sequencing and genotyping techniques, these approaches come into reach for conservation studies. The resulting data can be used for a wide variety of research questions, for example on taxonomic uncertainties, defining management units, the design of (captive) breeding programs, forensics, and the genetic basis of morphological differences or disease vulnerability.

Despite the fact that the focus will be on the case of the lion, the resulting conclusions are also applicable to other species. In species conservation one of the main targets is to halt or to minimize the loss of genetic variation, in order to preserve the species as a dynamic entity with enough evolutionary potential to survive on the long term. Therefore it is of utmost importance to have a detailed picture of the present genetic diversity, both on the phylogenetic level, as on the population genetics level, to properly manage the species under consideration.

Chapter 4 - Global Change, a result of human action on ecosystems, is one of the major problems to face humanity at the present time. For crops, this means that there will be a need to find or create new cultivars able to adapt to a changing environment, without a reduction in nutritional standards or industrial quality. The quality characteristics of wheat depend on many factors, including gluten levels, starch levels and hardness, which are affected by seed storage proteins, starch synthases and puroindolines, respectively. There is little diversity among modern cultivars for the abovementioned characters, mainly due to the strong pressure exerted by wheat breeding programmes to adapt cultivars to different climatic conditions. Consequently, the search for allelic variants of these genes in local cultivars or closely related species could serve as a basis to increase the genetic pool of these components in modern wheat. In this context, a large programme has been developed to firstly, evaluate and characterize ancient wheats and some close relatives that could be used in wheat breeding and secondly, to improve wheats in general within the context of sustainable agriculture.

Chapter 5 - Morocco is a country located in northwest Africa. It has a long coastline on the Atlantic Ocean that reaches past the Strait of Gibraltar into the Mediterranean Sea. This layered country has a large history that left a mark on its current makeup. It's spread initially from Carthaginians and Romans to the coming of Islam that introduced the dominant religion and language, which are still both religious and political issues. This chapter reflects that layering through population genetic studies. A wide range of molecular markers have been used to study the Moroccan population as well as its three ethnical groups (Arabic, Berbers and Sahrawis) and to compare it to other populations, in order to well understand and reconstitute the history of this challenging area.

The results lead to an important conclusion about the origins of the Moroccan population. Thus the male gene pool, studied by means of the Y chromosomal STR and SNP, seems to have a Middle East provenance while the feminine lineage studied through the mitochondrial DNA present a European and North African kinship. Those results were supported by some studies on the X chromosome which spends two thirds of its life time in females. The differentiated contribution of males and females to the genetic landscape of the Moroccan population was observed with a higher migration rate in males than in females.

The autosomal markers shows a Middle East and North African predominance in the genetic pool of the Moroccan population, which reflect the importance of the Gibraltar Strait as a genetic Barrier. In the other hand, the results show that there is a small difference between Arabs, Berbers and Sahrawis. The Arabic population is closer to Middle East and North-African populations than Berber, the autochthonous population, which is closest to Europeans.

Chapter 6 - Forest systems provide timber, food, non-food goods and externalities, being one of them the contribution to the genetic diversity safeguard. Conservation of forest genetic resources is essential for sustaining the environmental and productive values of forests. The knowledge of such diversity is particularly relevant in the case of woody species, which have been named "ecosystem engineers". The objective of the study is to show the way to establish the safeguard of genetic diversity through landscape genetics discipline. This chapter present how the results obtained in some forest species using different approaches of this discipline has provided a better understanding on the species genetic resources.

Chapter 7 - Ginseng (*Panax ginseng* C.A. Meyer) belongs to the family Araliaceae and is one of the most important medicinal plants in East Asia. There are almost no wild ginseng plants left in China and Korea, and Russia is the only country where *P. ginseng* is still growing in its natural habitats. All natural populations of *P. ginseng* are now mostly exhausted; therefore, reintroduction is essential to restore this plant to its former range. One hundred sixty-seven (167) *P. ginseng* individuals were collected from the ten administrative areas of Primorsky Krai and studied using AFLP method. A high level of intraspecific genetic diversity ($P = 55.68\%$, $H_S = 0.1891$ at population level; $P = 99.65\%$, $H_t = 0.2857$ at species level) and significant population-genetic differentiation ($\theta_B = 0.363$ from the Bayesian full model; $F_{ST} = 0.360$ from the non-hierarchical AMOVA) were detected for fluorescent AFLPs. The populations with the highest and lowest levels of genetic diversity were determined, and these populations should be conserved for the first time. Two divergent population groups were identified with hierarchical AMOVA, by the UPGMA method, by principal coordinates analysis (PCoA), in minimum spanning tree (MST) and using the STRUCTURE program. These two groups should be recognized as management units (MUs) because the low level of gene flow among them indicates contemporary demographic independence. Using an MST analysis and assignment test, the content of *P. ginseng* populations was verified to differentiate the true population plants from the plants that are not inherent to the population under study. This differentiation can identify the "pure" plants that could be chosen to serve as a stock material for reintroduction.

Chapter 8 - DNA sequence variations (0.1%) between two randomly selected individuals are considered the major factors that determine genetic diversity among individuals, disease susceptibility and response to medication. The most common form of genetic variation is called single nucleotide polymorphism (SNP). Because of their frequency of distribution, stability and simplicity they represent unique genetic markers. They can be used to identify and map complex, common diseases such as high blood pressure and diabetes. SNP markers are also employed to identify drug response, ecology, evolutionary biology, diagnostic test and heterogeneity testing.

Recent progress in genetic technology has also identified a new form of genetic variation known as copy number variation (CNV). They are known to be associated with population diversity and several complex and common diseases including nervous system disorders. Because of their location and nature, many CNVs have functional consequences such as gene

dosage alteration, disruption of genes and modulation of activities of other genes. Therefore, these genetic variations will have an influence on phenotypes, susceptibility of an individual to disease, drug response and human genome evolution.

Recently, whole-genome association studies also suggest that epigenetic variation along with nucleotide variation may account for all the heritable phenotypic variation in health and disease. Epigenetics refers to the study of inheritable changes in gene expression without any changes in DNA sequence. These heritable changes include DNA methylation, histone modifications and microRNA expression. Epigenetic control mechanisms may differ among tissues and individuals and they may also change in time during aging. Many genes including enzymes, drug transporters receptors and drug targets are under epigenetic control. It is because of this reason, the importance of epigenetics in clinical medicine has been recently recognized but little is known regarding the role of epigenetics in the pathogenesis of diseases. In the near future, many additional rare polymorphisms, structural variants and tissues specific epigenetic features of the human genome may become significant contributors to disease pathogenesis and medicinal drug response.

Chapter 9 - Rhizobia form nitrogen fixing nodules in legumes but they are not the unique microbial nodular inhabitants. In the last years the endophytic bacteria of different legume nodules have been studied and the results revealed a high genetic diversity. Members of Proteobacteria, Firmicutes and Actinobacteria have been identified in the inner of nodules from different legumes. Moreover several of these endophytes are classified into novel genera and species of bacteria, revealing nodules as an important source of diversity that should be further studied, with special emphasis in their role in the formation and functionality of legume nodules.

Chapter 10 - The importance of genetic diversity within populations has been debated since the study of genetics began. This debate was brought to a head when O'Brien et al., 1985 suggested that population viability problems within the cheetah (*Acinonyx jubatus*) could be linked to low genetic diversity thus supporting the balanced school. Since then there has been a significant increase in conservation genetics studies within threatened and endangered species focusing on genetic variability and it's importance in wildlife conservation. Populations that experience bottlenecks through extirpation, isolation or fragmentation are thought to lose genetic diversity through genetic drift and inbreeding. Small population sizes may lead to inbreeding where related individuals produce offspring. Inbreeding may lead to the buildup of deleterious recessive genes, termed inbreeding depression, that may cause decreased fecundity, increased mortality, slowed growth, developmental defects, increased susceptibility to disease, decreased ability to withstand stress, and decreased ability to compete. Genetic load is the longer term loss of population fitness over time due to an increase in detrimental mutations becoming fixed in small populations through inbreeding. Genetic load may be documented as low estimates of fitness related variables when compared to other populations that have not experience bottlenecks or have no evidence of loss of fitness associated with loss of genetic diversity. Genetic rescue is a management technique that has been used to manage critically endangered populations with high genetic load. This strategy uses unrelated individuals from one population that are introduced into the population with apparent low fitness. This introduction of new genetic material acts to reduce genetic load by lowering or eliminating the frequency of detrimental gene variants in the population. This management technique has been used successfully within both captive and wild critically endangered populations. Due to the rapid increase in

the study of genetic variation within wildlife populations there is a growing body of evidence linking fitness variables with genetic methods, suggesting meaningful trends and management practices like genetic rescue that may affect long-term population survival and viability. In this chapter we highlight case studies that describe the consequences of low genetic diversity, inbreeding, genetic load and genetic rescue within several endangered wildlife populations.

Chapter 11 - Several native species are used in South American folk medicine justifying the scientific surveys and earning increasing attention from pharmacological and cosmetic industries among others. *Cunila* is an important aromatic and medicinal genus with increasing researches regarding chemical and genetic variability due its interesting essential oil composition. Along with the increasing scope of chemical studies, concerns about the maintenance of natural populations and its genetic variability have raised toward to the sustainable and rational use of these plants. The current chapter encompasses an actual review about genetic variability among South American *Cunila* species and an introduction to the genetic relationship among South and North American species.

Chapter 12 - Japanese stock enhancement has been carried out since the early sixties by releasing artificially bred seed of several economically important fish and shellfish species. Even though the aim of stocking is to increase the amount of resources in the wild, breeding part of the native population in a captive environment, to achieve this goal also implies the reduction of the genetic variability even in first generation offspring, due to the small genetic contribution of just a few breeders. Consequently, stocking may comprise a potential risk to the wild population if no action is taken with respect to the loss of the genetic variability of the hatchery stock. Hence, in the last two decades, when referring to conservation programs of rare and endangered species, strong attention has been paid on conservation genetics of the captive population, in addition to the primary protection of the species and the maintenance of an adequate natural habitat.

Specifically, in the case of Japanese marine finfish stocked species, such as barfin flounder, spotted halibut or red sea bream -which are migratory species, and thus not easily traceable- it is especially complicated to obtain feasible genetic population data to establish the genetic variability of the native population. Consequently, Japanese marine finfish stock enhancement becomes a novel model for marine finfish genetic diversity conservation programs.

For this purpose, computational modelling simulation of selective breeding scenarios is being applied in Japan in order to minimize genetic diversity loss of especially rare and endangered finfish species and thus to conserve the genetic variability of the native population.