Vol.5, No.8, 903-913 (2013)
http://dx.doi.org/10.4236/ns.2013.58109

Natural Science

Conservation of endangered animals: From biotechnologies to digital preservation

Flavia Pizzi[1], Anna Maria Caroli[2], Martina Landini[3], Nadia Galluccio[3], Alessandra Mezzelani[3*], Luciano Milanesi[3]

[1]Istituto di Biologia e Biotecnologia Agraria (IBBA), CNR, Milano, Italy
[2]Dipartimento di Medicina Molecolare e Traslazionale (DMMT), Università degli Studi di Brescia, Brescia, Italy
[3]Institute for Biomedical Technologies, National Research Council, Segrate, Italy;
[*]Corresponding Author: alessandra.mezzelani@itb.cnr.it

Received 29 March 2013; revised 29 April 2013; accepted 7 May 2013

ABSTRACT

In the recent years, the number of endangered animals, both referred to livestock and wild species, has grown enormously. The "livestock" term refers to animals domesticated for producing commodities for man such as food, fiber and draught. Livestock biodiversity is integral to our culture, history, environment, and economy. Thousands of livestock breeds have evolved over time to suit particular environments and farming systems. Conservation and analyses of these genetic resources rely on demographic characterization and correct breeding schemes. In addition, molecular genetic studies allow to identify and monitor the genetic diversity within and across breeds and to reconstruct their evolution history. The conservation of livestock variability is also a crucial element in order to preserve and valorise specific nutritional and nutraceutical properties of animal products. Efficient *ex situ* and *in situ* conservation strategies, as well as the creation of bio-banks and specific biotechnological and bioinformatics tools for genetic analyses and digital preservation, are obligatory requirements in order to implement an appropriate action for the conservation of animal biodiversity. The main issues concerning different species are summarised, with particular reference to the livestock biodiversity still existing. Some examples of *ex situ* conservation strategies, which mainly refer to cryoconservation of semen, ova, embryos or tissues, developed in Italy, are presented, and the different actions in defense of Animal Genetic Resources (AnGR) developed within the European Community are illustrated. Interestingly, the same strategies for biological and digital analyses and preservation of livestock biodiversity can be exported to wild endangered animals in order to plan a correct conservation and repopulation of the species. Furthermore, the European Union has set up the guidelines to safeguard the biodiversity and to combat the extinction of animal species, and has made the protection of biodiversity and ecosystems one of the main objectives of the Sixth Environment Action Programme.

Keywords: Livestock Biodiversity; Wild Animals; Endangered Animals; *In Situ* Conservation; *Ex Situ* Conservation

1. INTRODUCTION

Domestic and wild animal species are undergoing a profound erosion process due to natural and genetic causes. Considering the role of livestock in producing food, energy, raw materials and fertilizers, great efforts in the conservation of these genetic resources are needed to prevent, stop and reverse this trend of thinning of animal biodiversity. In the second half of the 20th century, in fact, the increasing industrialization of agriculture and the genetic selection of only few, highly promoted breeds, have constantly reduced the number of breeds, leading, in some cases, to their extinction. Thus, the importance of maintaining livestock breeds and their genetic resources has become mandatory in the last decades, as it is was also well stated in the Convention on Biological Diversity (www.cbd.int/doc/legal/cbd-en.pdf). The most relevant reason for preserving genetic diversity is strictly associated to the breed's nutritional value and to its role in food production but, moreover, there are also socio-economical and cultural reasons that make livestock preservation so important. Local breeds, in fact, can play a key role in sustaining rural economies and marginal areas and, reflecting a long history of symbiosis with mankind, they often acquire historical and cultural values to be preserved too.

As for wild animals, while we tend to think of extinct species as animals that died out long ago, this isn't necessary the case. In fact, there have been several extinctions in the last years and, despite several conservation plans have been set for the most critically endangered animals around the world, a comprehensive strategy for the protection of wild animals, including both *in situ* and *ex situ*, is far to be proposed.

Considering all these elements, the aim of this overview is to highlight the main aspects for the conservation of endangered animals, with particular emphasis to the novel methodological tools, ranging from biotechnologies to digital preservation. On this regard, domestic animals will be considered as the starting point, and common strategies for preservation of both domestic and wild animal species will be discussed.

In this contest, conservation genetics can preserve endangered species in a number of ways and it provides the evaluation of genetic diversity by molecular characterization of breeds, by planning genetic breeding and by preserving germplasm or other biological resources.

Despite preserving species in their natural habitat is, especially for wild animals, the top priority, the relevance of gene banks in the safeguard of breeds in extinction is emerging as a key issue for planning the long-term conservation of biodiversity and for enduring its survival in the future. Because such a strategy becomes effective, the support of bio-banks as well as of bioinformatics tools for digital preservation, storage and analysis of the biological materials collected are required and they represent one of the main target for the next future.

2. ENDANGERED LIVESTOCK AND *IN SITU* CONSERVATION STRATEGIES

Food production and agriculture utilize only a few animal species, within which many breeds are available providing unique characteristics for mankind. This livestock biodiversity is essential for a sustainable and efficient production of food in order to satisfy the different needs of the wide variety of consumers, starting from the increasing demand for food and agriculture in the world.

Domestication of animals producing commodities for man such as food, fiber and draught started about 11 millennia ago in different parts of the world. Livestock domestication in the Mediterranean basin initiated in different parts of the Fertile Crescent, resulting in multiple domestic lineages for each species [1]. Thousands of livestock breeds have evolved to suit particular environments and farming systems, both as result of natural processes and human needs [2]. Very different morphological aspects and physiological peculiarities were obtained by selection within each domestic species. Genetic diversity is a prerequisite for genetic improvement and environmental adaptation. However, the diversity of Animal Genetic Resources (AnGR) has been in a continual state of decline as human population and economic pressures accelerate the rate of change in traditional agricultural systems. As a result, more and more breeds of domestic animals are in danger of becoming extinct. The main causes can be summarised in three groups: trends in the livestock sector; disasters and emergencies; animal disease epidemics and control measures [3]. The need for implementing safeguard strategies both at the national and international level was assessed since the seventies-eighties of last century [4,5].

The international framework for the sustainable use, development and conservation of AnGR that are of vital importance to agriculture, food production, rural development and to the environment is managed by the Global Plan of Action for Farm Animal Genetic Resources [6] and the Interlaken Declaration of Animal Genetic Resources. The Declaration was adopted by 109 States, the European Community and 42 Organizations, at the invitation of the Food and Agriculture Organization of the United Nations (FAO), through the International Technical Conference on Animal Genetic Resources for Food and Agriculture held in Interlaken, Switzerland, in 2007. The World Watch List for Domestic Animal Diversity (WWL-DAD), is an information system (IS) to identify and monitor domestic animal breeds all over the world aiming to reduce the loss of biodiversity due to genetic erosion. A total of 6379 livestock breeds were reported belonging to 30 different species in WWL-DAD 3rd edition [6]. Population size data was available for 4183 breeds of which 740 breeds were already extinct and 1335, or 32%, were classified at high risk of loss and were threatened by extinction.

The number of breeds reported constantly increased. However, in 2008, 48% of 10550 mammalian national breed populations and 53% of the 3450 avian national breed populations recorded in DAD-IS had no population data recorded; thus, the risk-status categories of approximately 64% of reported breeds were available in the DAD-IS [7].

Different actions in defense of AnGR are developed. As an example, 5 of the 17 EU actions for genetic resources within the Community Programme 2006-2011 are related to domestic animal resources: EuReCa (http://www.regionalcattlebreeds.eu/), Heritage Sheep (http://www.heritagesheep.eu/), ELBARN (http://www.save-foundation.net/ELBARN/index.htm), Globaldiv (http://www.globaldiv.eu/), and EFABIS (http://www.eaap.org/Content/EFABIS.htm).

One of the first steps for a safeguard action is the characterization of a breed population, which should be carried out at different levels, including zootechnical [8] and demographic level [9]. Moreover, the phenotypic characterization of AnGR refers to the process of identifying distinct breed populations and describing their external and production characteristics within a given production environment [10].

In addition, genetic studies are an important tool for characterizing livestock biodiversity [11-13]. Nowadays, analyses at the DNA level are mainly based on high-throughput assays and technological platforms detecting thousands of genetic polymorphisms simultaneously [14,15].

Molecular studies allow to identify and monitor the genetic diversity within and across breeds, to infer the population structure [16], to reconstruct evolution history [17,18], and to discover evidences on mankind history, as in the case of the novel clues found from *Bos taurus* mitochondrial DNA about the mystery of Etruscan origins [19]. The evidence collected in the last paper on the basis of mitochondrial DNA corroborated the hypothesis of a common past migration of humans and cattle, reaching Etruria from the Eastern Mediterranean area by sea.

Different sea and continental routes followed by cattle domestication were suggested by molecular studies [20]. Genomic data are increasing in an exponential way and could really give indication about which genes deserve more attention for safeguard. Data-bases including genetic information for the most common livestock species are available [12].

The conservation of livestock variability is also a crucial element in order to preserve and valorise specific nutritional and nutraceutical properties of animal products. The null alleles of particular casein genes, occurring at different frequencies depending on the goat breed, are an example of this important aspect, indicating that milk from goats carrying particular casein genotypes could be devoted to feeding children allergic to specific milk proteins, and that local breeds could be a reservoir of such genotypes [21].

An example of the nutraceutical properties linked to animal food concern biopeptides which can be released from animal protein by the action of proteolitic enzymes during human digestion. Milk proteins represent a reservoir for a wide variety of bioactive peptides, could affect milk nutritional value [22]. The activity of biopeptides might be affected by amino-acid exchanges or deletions resulting from gene mutations and thus linked to particular breeds [23-25]. Thus, the reason for preserving a particular breed can be related to the importance of conserving biological components of particular significance from a nutritional point of view.

A further example of the importance of genetic information for conservation strategies can be found in the coevolution proposed for the diversity of milk protein genes in cattle and the lactose tolerance in European human populations [26]. Milk from domestic cows has been an important food source for over 8000 years, especially in lactose-tolerant human societies. In fact, some individuals have the genetically determined ability to digest lactose by the action of persistent lactase enzyme in adulthood, thereby benefiting from the rich food resources occurring in cows' milk. Cow's milk protein genes and humane lactase genes underwent a geneculture coevolution in Europe resulting from the divergent use of milk (cheesemaking, direct consumption) in the different areas of Europe [26]. This is impressive proof of the nonrandom occurrence of milk protein genetic variation over the centuries.

Efficient *in situ* and *ex situ* conservation strategies are obligatory tools in order to implement an appropriate action for the conservation of livestock biodiversity.

"*In situ* conditions" were defined in the Convention on Biological Diversity as "conditions where genetic resources exist within ecosystems and natural habitats, and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties" [27]. The presence of a local breed on a farm together with more productive ones can be linked to the cultural affection of the farmers to the local breeds reared by their parents, like for the Italian Reggiana and Modenese cattle breeds kept by some Holstein farmers [28].

Conservation is not simply preservation of those breeds currently not in use. It also includes the monitoring, characterization, and utilization over time of the gene pool of each species. Efficient *in situ* conservation strategies are based on the real self-sustainability of a breed. Since we cannot preserve each breed or population for economic reasons, we must decide what to safeguard on the basis of the real value of a breed. An example comes from the Reggiana cattle breed, an Italian very rustic breed reared since the barbarian invasions. The milk of the Reggiana was used to produce the well-known Parmigiano Reggiano. In the last century the breed underwent a bottleneck due to the competition of the more productive Holstein Friesian breed, but in the eighties the creation of specific brands of Parmigiano Reggiano (the "Parmigiano Reggiano delle Vacche Rosse" and the "Parmigiano Reggiano di Reggiana") made only from the milk of this breed and appreciated by consumers stopped the bottleneck and led to a constant increase of the breed (http://www.regionalcattlebreeds.eu/breeds/Reggiana.html). Thus, the high profitability of the Reggiana breed is linked to the success of the specific Parmigiano Reggiano brands that are sold at a higher price [29]. The same situation occurs for the Modenese breed, with the production of a Parmigiano Reggiano brand made only from Modenese milk. Conservation activities started in the eighties including inbreeding control, cryoconservation of semen, development of a branded Modenese Parmigiano Reggiano cheese made with Modenese milk only (www.regionalcattlebreeds.eu/breeds/Modenese.html).

Thus, the link of a local breed to a typical product is a winning promotion tool for the breed. Further Italian examples are the Fatulì cheese made from raw milk of the Bionda dell'Adamello goat, the Cinta Senese ham and sausages produced from the meat of this local pig of the Tuscany region, some dairy products from the milk of the Rendena cows reared in the Italian Alps, the Fiorentina of the Chianina, the well-known Fontina cheese linked to the Valdostana breeds in Valle d'Aosta mountain region.

The cultural and historical values of a local breed are also important for its conservation [30]. The Valdostana cattle involved in the traditional Battle of the Queens (http://www.naturaosta.it/cultura.htm) is an example of the conservation of local livestock resulting from the traditional use of domestic animals.

Last but not least, the occurrence of local breeds in the art is a further valid reason for their safeguard. In the wonderful Nativity by Giotto (www.britannica.com/bps/media-view/106488/1/0/0) in Padua at the Cappella degli Scrovegni we can find the Amiata donkey, the Garfagnina sheep and goat, and the red ox from Reggiana breed. The Maremmana *Bos taurus* breed, the cattle with the long horns so useful to clean the typical Mediterranean flora ("*Macchia mediterranea*"), is illustrated in several paints by Giovanni Fattori (www.starrylink.it/portale/arte/arte284.html), often with the Maremmano horses ridden by the famous "Butteri" pockmarks (www.frammentiarte.it/dall'Impressionismo/Fattori.html).

3. *EX SITU* CONSERVATION STRATEGIES

As mention before conservation strategies of AnGR are categorized as *in situ* and *ex situ*. *Ex situ* conservation mainly refers to cryoconservation of semen, ova, embryos or tissues. The Convention on Biological Diversity emphasizes the importance of *in situ* conservation and considers *ex situ* conservation as an essential complementary strategy to *in situ* [27].

Conservation strategies differ in their capacity to achieve different objectives, therefore the choice between *in situ* and *ex situ* technique, or of a combination of techniques, depends upon the conservation aims [31]. Conservation of genetic diversity across and within farm animal breeds is an insurance for the future. A broad genetic base is crucial to deal with future changes in environment, in consumers demand for animal products and in animal production systems. A wide genetic variation is also a safeguard against sanitary outbreak and natural disasters, in the maintenance of agro-ecosystem diversity and conservation of rural cultural diversity. *In situ* conservation strategies are effective in reaching all these objectives except for the safeguard against emerging diseases, political instability and natural disaster. *Ex situ* is the method of choice to safeguard farm Animal Genetic Resources against disasters and is valuable option when socio-economic, cultural and ecological values linked to a breed are already missing or of no interest.

Genetic materials cryopreserved in gene banks can be used for different purposes: 1) to reconstruct a breed in case of extinction or loss of a substantial number of animals, 2) to create new lines or breeds, 3) as a back-up to quickly modify and/or reorient selection programmes, 4) to support populations conserved *in vivo* in cryoaided live schemes, or 5) as a genetic resource for research [32]. Gene banks can also be used also for storing alleles of a particular locus that are being eradicated through artificial selection programs [33]. Concerning the support to living population semen stored in gene banks for short-term storage can be distributed to farmers for using in breeding programs of local breeds, whereas long-term storage of semen, called genetic reserve, can be considered as back up to secure the breed [34].

Concerning genetic material semen and embryos have been both proposed for the creation of farm animal genetic resources cryobanks. These two type of germplasm have different potentialities and limitations: embryos carrying the entire genome, included the extra-nuclear genetic material contained in the mitochondria, are considered a valuable material for cryopreservation. This material would allow the complete recovery of the breed in case of extinction within one generation. In contrast by using only semen for the reconstruction of the breed, several generations of repeated backcrossing are needed, and consequently the required number of semen insemination doses is high. For this reason and in particular for species with a long generation interval, such as cattle, the storage of both semen and embryos is recommended [35]. In cattle, the costs for storing in gene banks embryos in combination with semen are not significantly different from those for storing only semen [36]. The choice of the type of germplasm to store in gene banks depends also on the availability of efficient cryopreservation procedures in the different species: semen cryopreservation is available at low cost for most of the livestock specie whereas embryos cryopreservation is routinely used mainly in cattle.

Animal gene banks development is limited by high costs of creation. The recovery and freezing of viable sperm recovered from the epididymes of slaughtered or castrated animals can be a cheap alternative for preserving male gametes. Nowadays concrete examples of application of *post-mortem* sperm recovery from the epididymis are available for conservation of endangered species [37] and in constructing semen banks [38-40]. Preservation of epididymal sperm from accidental dead or slaughtered animals has been reported in several domestic and wild species as boar [41], cattle [42,43], goat [44], ibex [45,46], red deer [47] and sheep [38,48]. However epididymal sperm extraction sometimes is not easily practicable in marginal areas, due to the lack of facilities and expertise near the farming areas of local breeds. To overcome this issue studies are in progress to determine the entity of decay of epididymal spermatozoa quality recovered *post-mortem* in different species and under several experimental conditions [49-51].

In order to rationalize cryo-storage of AnGR, some European countries have created national gene banks. These include: Austria (Austrian Gene Bank for Farm Animals), France (Cryobanque Nationale), Netherland (Center for Genetic Resources—Gene Bank) and Nordic Countries (Denmark, Finland, Iceland, Norway and Sweden: Viking Genetics, the Danish-Swedish-Finnish AI-centre).

Detailed guidelines for cryopreservation programmes have been developed in the European [32] and global context [52]. Within the EuReCa project (http://www.regionalcattlebreeds.eu/) a detailed survey was carried out comparing cryopreservation activities and policies in Finland, France, Italy and the Netherlands. The main purposes of the survey were the detection of similarities and differences between countries and the formulation of recommendations for initiating and strengthening of cryopreservation programmes [53].

According to the survey the most common cryopreserved genetic material of European local breeds is semen. The main conclusions of the survey were that countries organise cryopreservation programme differently depending on the role and responsibilities of different stakeholders. In the surveyed countries the close involvement of farmers of local breeds, breeders associations and artificial insemination (AI) centers in linking the cryopreservation schemes with routine AI operations is an important factor for the development of efficient cryopreservation programmes. A recommendation raised from this project is that European and national sanitary legislation should facilitate and not hamper cryopreservation of local breeds.

In Italy, in the last two decades, semen of local livestock breeds has been collected in the framework of different conservation programs by national breeders association, some regional administrations, Universities and research institutes. Due to the lack of coordination at the national level, the acquisition process has been most of the time opportunistic, without the search of specific animals within specific gene banking aims.

In this framework from 1999 the Institute of Agricultural Biology and Biotechnology of the National Research Council (IBBA CNR) started the collection of semen samples of 4 Italian pig breeds within the EU project "European Gene Banking Project for pig genetic resources" GENRES012, at the end of the project the "Giuseppe Rognoni" IBBA CNR Cryobank has been created. According to the indication of the FAO guidelines the genetic material has been divided in two storage sites. At present the collection includes genetic material of Casertana, Cinta Senese, Mora Romagnola and Nero Siciliano breeds (www.ibba.cnr.it/index.php/en/research-activities/225).

In 2008 the Lombardia Region and IBBA CNR started the creation of a regional cryobank for the Animal Genetic Resources (Lombardia Animal bank). Genetic material of Varzese cattle breed, Brianzola sheep breed and of 3 goat breeds (Frisa Valtellinese, Verzaschese, Orobica) has been collected. Storage includes cryopreserved semen, moreover blood and hair are stored for future DNA extraction. For security reason semen doses of each donor have been split into two liquid nitrogen tanks. In 2013 a total of 2497 doses collected from 46 donors were stored (http://www.ibba.cnr.it/index.php/en/research-activities/227). IBBA CNR Cryobank and Lombardia Animal bank are part of the Italian Network of Genetic Resources recently built up by the National Research Council (www.biogenres.it).

Being aware that conservation of animal genetic resources cannot only be planned at the regional level, the "Network of the Italian cryobanks of farm Animal Genetic Resources—CRIONET-IT" (www.genrescryonet.unimi.it) has been created [54]. The aims of CRIONET-IT includes the promotion of collaboration among collections, sharing information among collections and Institutions working in farm Animal Genetic Resources conservation, development of cryoconservation programmes, as indicated by the Strategic Priority 8 of the Global Plan of Action for Animal Genetic Resources, adopted by Interlaken Declaration. Moreover CRIONET-IT can be considered as the first step toward the development of a national gene banking system. CRIONET-IT is made of two major components. The first is a website, open to the public, where the aims of the Network are illustrated, Partners and Collaborators are given, and a summary of the material stored by all partners is presented. The second is the CryoWEB reserved area, in which Partners can manage their own data by using a CryoWEB software [55]. CryoWEB allows storing information and documentation of national gene banks of cryopreserved domestic animals.

4. WILD ANIMAL ENDANGERED THREATS AND CONSERVATION STRATEGIES

As for wildlife animals, their ability to survive and reproduce has been greatly threatened in the last decades. The major causes and threats to their extinction are mainly due to habitat destruction, both for loss and degradation, leading to changes in the physical environment such as atmospheric conditions, moisture and temperature changes; environmental toxic chemical and pollutants (*i.e.* pesticides, acid rains, CO_2 emissions, the climate warning); human overpopulation, leading to an over-exploitation of natural resources; unregulated hunting, indiscriminate poaching and natural phenomena. Over the last few years, in fact, ecosystems have been impacted by all these new stressors, which have greatly affected their integrity and the survival of wildlife animals [56]. Moreover, introduction of new non-native species often leaves endemic species unable to compete or causes genetic pollution. Genetic pollution (*i.e.* uncontrolled hybridization, introgression and genetic swamping), is a further threat that causes homogenization or replacement of wild genomes by numerical and/or fitness advantages of an introduced species leading to the loss of genetic diversity [57] and the resulting loss of biological diversity or "biodiversity".

Biodiversity means "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (Article 2: Convention on Biological Diversity) [27]. In this scenario, the wildlife conservation strategies are part of the so called "conservation biology" that investigates the possible causes of global biodiversity declines and plans strategies to protect biodiversity [58].

On this regard, as any strategy for the preservation of biology becomes effective, information on the distribution and abundance of wildlife species, including low and declining populations, should be considered as a good indication of biological diversity in a given environment. Plans for monitoring endangered species and their habitats are also required. For every species at risk, a population vulnerability analysis should be performed, involving the assessment of the population biology, with particular regards to the population structure and fitness as well as its natural environment [56]. On this regard, to preserve biodiversity, ecosystem integrity has been saved too. Despite there are no estimates of how much an ecosystem is needed to retain its integrity and sustainability, ecosystems and natural habitat preservation is mandatory as long term strategy in endangered species preservation [56].

These strategies are supported by regional and global regional policy commitments, such as the Convention on Biological Diversity (article 8-f) [27] and the Commission of the European Community. The Ecological Restoration can be undertaken at different scales including the local and habitat-specific actions and regional levels. Many authors recognize the urgent need to greatly expand the scale of ecosystem conservation and restoration [59]. Moreover, to safeguard the biodiversity and to counteract the extinction of animal species, also of the plants, the European Union has set up a vast network of protected sites (the Natura 2000 network; http://ec.europa.eu/environment/nature/natura2000/) [60] and made the protection of biodiversity one of the key objectives of the Sixth Environment Action Programme (http://eur-lex.europa.eu/en/dossier/dossier_34.htm#1) [61]. Action plans engage public policy, local and governmental authorities and non-governmental organizations to protect and recover genetic, species and ecosystem diversity thus employing both natural and market capital.

The importance to invest in ecosystem diversity is that it strictly correlates to ecosystem services, the benefits people obtain from ecosystems. These include "provisioning services such as genetic resource, food, fiber and water; regulating services such as regulation of climate, flood and disease control; cultural services such as spiritual, recreational, aesthetic experience and cultural benefits; and supporting services such as nutrient and water cycling, biomass production, production of atmospheric oxygen, soil formation and retention, and provisioning of habitat that maintain the conditions for life on Earth". (http://www.millenniumassessment.org/documents/document.776.aspx.pdf) [62].

The major protection and restoration techniques for wild animal conservation consist first in the identification of species candidates for reintroduction using databases such as the Encyclopedia of Life (http://eol.org/), the Global Biodiversity Information Facility (http://www.gbif.org/), International Barcode of Life (http://www.ibol.org/), and Digital Automated Identification System (Daisy) (www.tumblingdice.co.uk/daisy).

Furthermore, the reintroduction of banked species or animals in captivity to the ecosystem, and the removal of invasive species, that are often recognized as a primary driver of native species endangerment, are also potential strategy for preserving species in danger of extinction. A careful consideration of the order in which species are reintroduced or removed from an ecosystem must be taken in order to avoid unexpected consequences [63]. For instance, in the mid-1990s, an overpopulation of *Aquila chrysaetos* was causing the extinction of three subspecies of fox *Urocyon littoralis* on the northern California

Channel Islands. The eagles were attracted in the island by an abundant supply of ferine pigs *Sus scrofa* that, in turn, competed with the foxes. Thus both eagles and pigs must be removed from the island to contain the fox extinction, but capturing lives eagles was difficult and the lethal ones politically distasteful. On the other hand removing pig first induced eagles to hunt the remaining fox [63].

Moreover, despite the use of captive breeding in endangered species recovery has strongly increased in the last years, genetic and phenotypic changes, occurring frequently in captivity, as well as the high costs and the risk in disease outbreaks have limited its application only for those animal species at high rate of extinction and for short term conservation strategy [64]. However, because any recovery efforts become effective, integrative *in situ* strategies have been also required. Comprehensive strategies in maintaining or restoring population in their wild habitat as well as the protection of natural ecosystems and the role of zoological institution should be taken into consideration for improving *in situ* conservation programs [64]. On this regard, many zoological institutions directly support both field studies and educational programs with the purpose to preserve the overall biodiversity of wild species [64].

Furthermore, the creation of bio-banks for the collection and characterization of specimens and genetic material has been improved in the last years as a potential strategy for *ex situ* and long term preservation of wild endangered species or at high risk of extinction.

The application of the most recent biotechnological tools, such as high throughput genotyping, allows not only the cryoconservation but also the genetic characterization of the animal genomes, leading to perform taxonomic and evolutive studies and plans for animal selection and breeding. In this context, recent development of bioinformatics has coupled biotechnological tools with data storage and analyses systems that allow to interpret and use genotyping data and to manage bio-bank archives.

Digital preservation is also one of the most important and recent key point bioinformatics aim to address.

5. BIO-BANKS AND DIGITAL PRESERVATION

Bio-banks are repository to safeguard our most valuable biodiversity in order to improve or maintain the heritage of endangered animals, plants and ecosystems.

In the past, the bio-banks were defined like the collections of biological materials maintained by a cryogenic facility associated with information on animal. Nowadays, it is associated with databases that contain the gene sequences from the organic samples of a specific animal.

As bio-banks play a crucial role in germplasm and biodiversity preservation they must have a number of structural and organizational requirements to protect samples from any kind of damages or losses, both accidental and intentional (**Table 1**). The registration of each sample entering and exiting the bio-bank is centrally controlled and managed by a software that must be backed up frequently [65] The labeling, registration and physical location of each sample, as well as its identifycation data, quantity, type of resource are link to a bar-

Table 1. Tissue and germplasm banks.

Bio-bank	Web-site
Austrian Gene Bank for Endangered Farm Animal Races	www.raumberg-gumpenstein.at
Banco Português de Germoplasma Animal (*BPGA*)—*Instituto Nacional de Recursos Biológicos*	http://horta.0catch.com/bpga/bpga.html
Centre for genetic resources the Netherlands	www.wageningenur.nl
CRIONET-IT network—Italy	www.genrescryonet.unimi.it
Conservation Genome Resource Bank for Korean Wildlife	www.cgrb.org/index_e.htm
Cryobank Nationale—France	www.cryobanque.org
European Regional Focal Point for Animal Genetic Resources (ERFP)	www.rfp-europe.org
FABISnet database (*cooperation with* 17 *partners*)	http://efabis.tzv.fal.de
Italian Network of Genetic Resources—Italy	www.biogenres.cnr.it
NordGen Farm Animals—Norway	www.nordgen.org
ViaGen-Texas	www.viagen.com
Viking Genetics-Sweden, Denmark, Finland	www.vikinggenetics.com

code and barcode reader system to allow the rapid tracking and position and quantity of the specimen. As for biological point of view, optimization of protocols for cryoconservation, definition of best practice and standard operating procedures, bio-safety, and a temperature system control must be defined.

Furthermore the European Commission indicates in its recommendations on access to and preservation of scientific information [66] the importance of preserving, reuse and access openly the scientific information developing a dedicate infrastructure through a multi stakeholder dialogue at national, European and international level. One of the main objectives refers to the construction of technological solutions and innovative methods to maintain the digital data available and usable over time. The data preservation for the conservation of livestock and endangered animal biodiversity is emerging as one of the key issues in order to plan a correct safeguard and repopulation of the species and resources to ensure their survival for future generation. The situation is particularly acute for genetics data and phenotype information since these are the heritage and the cornerstone for the future memory of the biological diversity and the ecosystem knowledge conservation. The fast change in the technological landscape makes long-term data access problematic. The storage media may degrade, and the technological developments make system obsolete. The information related to the endangered animals can be rendered inaccessible by the changes in encoding the formats and the obsolescence of the program used for the analysis. The development of a framework to characterize and store information, together with services to enable the data to be quarried, browsed and retrieved based on modern the semantic web technology will be the key to be able the data preservation for the future. In this prospective the data preservation is driven by the definition of the data decoding keys within the "Preservation Network Model" to allow the information about the digital record content and knowledge to be always available even in the far future when the present platform might not exploitable since obsolete. The theoretical frame used is the "Open Archival Information System" (OAIS) [67]. The Long Term Data Preservation will be particularly important within the European Framework Programmed "HORIZON 2020".

6. CONCLUSIONS

Species biodiversity is integral to our culture, environment, history and economy and is strictly correlated to the benefits people obtain from ecosystems, the so called "ecosystem services", that provide genetic resource, food, fiber and water, regulate the clime and offer suitable habitats to maintain the conditions for life on the Earth.

The protection and preservation of biodiversity and ecosystems is one of the main objectives of the Sixth Environment Action Programme and requires economic and scientific efforts that span from biotechnologies for germplasm conservation and genotyping to bio-banking creation and to bioinformatics tool development for the data analysis and digital preservation. However, the impressive increase of the technological tools as well as the valuable work of researchers, bioinformatics and other scientists, represents one of the major hopes for the preservation of biodiversity in the future [68].

7. ACKNOWLEDGEMENTS

This work was supported by the Italian Ministry of Education, University and Research MIUR Flagship InterOmics Project (cod. PB05), HIRMA (RBAP11YS7K), PIDES, PON01_01297 and the European "MIMOMICS" projects.

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