CAPTIVE BREEDING AND GENE BANKS

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Summary

Today, our planet is in a state of crisis and many of its natural resources have been destroyed by human activities. One of the principle aims of conservation biology is to promote concrete management programs to limit any further increase in the number of extinct and threatened species and ecosystems. Different approaches may be followed to maintain the world's biodiversity: the *in situ* approach and the *ex situ* approach. The former takes into account the preservation of natural resources in the wild, while the latter consists of removal of samples of endangered species and of their genes from their origin sites for conservation elsewhere under institutional protection.

Ex situ procedures include zoological gardens, aquaria, botanical gardens and arboretums, where the species are maintained alive; but also techniques such as cryopreservation of semen and tissues, and seed banks, are included in this category.

It is generally recognized that the preferred method of conservation is the in-site approach, but off-site procedures play a fundamental role for all species at high risk of extinction. The importance of *ex situ* conservation has to be evaluated from a long-term view, since its ultimate goal is the eventual re-establishment of new populations of species which have become extinct in the wild. Moreover, *ex situ* facilities, and in particular zoological and botanical gardens, may have an active role in promoting research on animals and plants, as well as influencing and enhancing the awareness of public opinion regarding the necessity for prompt and general application of a conservation strategy.

Modern zoological and botanical gardens are able to successfully maintain captive breeding populations, principally thanks to the increase in knowledge on the biology of plants and animals, and population genetics. From this point of view, the management of *ex situ* populations is a delicate process that must be performed carefully; in fact, it has to solve the same genetic problems that arise when a wild population reaches a very small population size—the loss of genetic variability and onset of inbreeding depression.

1. Conservation and management of living natural resources

Conservation biology is a multidisciplinary science developed in response to the increasing loss of biodiversity throughout the world with the main aim of guiding real procedures to protect and restore wild resources (biological diversity).

Conservation biology sums the knowledge of many basis sciences (such as ecology, zoology, genetics, evolutionary biology, and population biology) and social science (such as anthropology, sociology, law, and economy). These latter sciences provide insight into how people can be educated to respect and protect natural resources since the principle reason for the biodiversity crisis is human pressures.

One of the principles of conservation biology is the preservation of biological diversity. But what is **biological diversity**? Following the definition given by the World Wildlife Fund (1989) biodiversity is “the wealth of life on earth, the millions of animals, plants, and micro-organisms, the genes they contain, and the intricate ecosystem they help build into the living environment”. Thus biodiversity must be considered at three different level: 1) **ecosystem level**, referred to the variation of biological communities and of ecosystems; 2) **species level**, the wholeness of organisms, from bacteria to higher animals and plants (Table 1 summarizes species diversity for the principle taxonomic groups. At this time, the estimate of the total number of existing species is over 2 million, but this number is subject to change since every year about 10 000 new species are discovered and most of these are insects or other micro-animals living in the tropics); 3) **gene level**, this includes intraspecific genetic variation, both between geographically separated populations and between individuals in a single population.

Taxon		Described species	Extinct species	Threatened species
Virus		1000	No data	No data
Procariota		4800	No data	No data
Fungi		48 000	No data	No data
Algae		27 000	No data	No data
Protozoa		31 000	No data	No data
Animalia				
Annelida		12 000	0	6
Mollusca		50 000	314	960
Arthropoda				
	Crustacea	56 000	9	428

	Insecta	751 000	73	589
	Other arthropods	67 000	0	11
Echinoidea		6700	0	0
Other invertebrates		47 000	4	6
Tunicata (urochordata)		1250	0	0
Cephalochordata		23	0	0
Agnata		63	1	3
Chondroichthyes		843	0	49
Osteichthyes		21 000	93	754
Amphibia		4200	5	151
Reptilia		6300	25	339
Aves		9000	131	1183
Mammalia		4500	117	1552
Plantae				
Gymnosperma		807	5	478
Angiosperma				
	Dicotolydonae	170 000	556	25 524
	Monocotolydonae	63 610	168	6646
Other plants		10 400	22	770
Total		**1393 496**	**1523**	**39 449**

Table 1. Number of described species divided into the main taxonomic groups, along with the percentage extinct (i.e., Red List categories *extinct* and *extinct in wild*) and threatened (i.e., Red List categories *critically endangered*, *endangered*, and *vulnerable*) species in accordance to the IUCN 2000 Red Lists
Data of the number of described species come from multiple sources. The percentage of threatened species, especially for invertebrates and vertebrate minor taxa, must be considered greatly underestimated.

Here, a brief remark on the concept of **species** is needed. A universally accepted point is that the species is the fundamental natural evolutionary unit, but, conversely, biologists have difficulty in agreeing on the definition of this taxonomic category.

The taxonomists generally define a species as a group of individuals that look similar (to some unspecified degree) to each other and, on the other hand, different from another set of individuals (*phenetic species concept,* PhSC). More formally, it would be specified the level of similarity/dissimilarity at which a group of individuals may be considered belonging to the same species or not. To be scientifically valid, a phenetic species must be defined on the basis of several features. This definition is criticized on the basis that the choice of features can be based on arbitrary and subjective decisions.

Another definition of species is: a group of individuals that can potentially breed among themselves and, conversely, is reproductively isolated from other such groups of individuals. This is the *biological species concept* (BSC) and it is the most widely accepted today, at least among zoologists. It is an historically important concept, introduced by Darwin, and supported by several founders of the modern synthesis, such as Dobzhansky, Mayr, and Huxley.

A group of interbreeding individuals is, in genetic terms, a gene pool. Starting from this concept, it has been formulated into a modern definition of biological species: a population or a series of populations among whose individuals a free movement of genes (called *gene flow)* can occur under natural conditions. The sharing of genetic materials, however, does not encompass individuals belonging to different species. In other words, a species differs from another species because the genes of each species are confined to their own specific gene pool. Even if this latter definition is the more accepted in the scientific world, it has been a subject of heated argument. A central question is that for some animals and plants hybridization is so common that hybrids and their parent species, applying the concept of biological species, would need to be considered as a unique species.

Besides, there is no sense in applying the BSC for prokaryotes because it fails in one of the basic statements, the interbreeding discrimination. This is due to the fact that genetic exchange in prokaryotes is less frequent but more promiscuous than that in eukaryotes. In eukaryotes with sexual reproduction, populations that are separated by only 2% sequence divergence are frequently unable to exchange genes. Prokaryotic genomes, in contrast, may undergo homologous recombination with related species that are up to 25% (and possibly more) divergent in the sequences of homologous genes. Thus, the definition of species in the bacterial world is much more difficult than in eukaryotes. Indeed there is no official definition of a species in microbiology. However, from a microbiologist's point of view "*a microbial species is a concept represented by a group of strains, that contains freshly isolated strains, stock strains maintained in vitro for varying periods of time, and their variants (strains not identical with their parents in all characteristics), which have in common a set or pattern of correlating stable properties that separates the group from other groups of strains*". This definition only applies to prokaryotes which have been isolated in pure culture (essential for the classification of new prokaryotic species), and excludes uncultured organisms which constitute the largest proportion of living prokaryotes.

It is very difficult to compare species concepts for prokaryotes and eukaryotes; the basic notions about what constitutes a species would be necessarily different for, say, vertebrates and bacteria. Comparisons among the different species units of living organisms can only be made after a universal species concept has been devised that applies to all organisms. This is, however, not easy and the discussion on the concept of species is far from over: at least 22 different concepts have been developed to accommodate species, but for a more extensive treatment of this subject we refer to some specific texts.

Conservation biology must operate to preserve biodiversity at all the above-mentioned levels and for this it needs insights from different sciences: for example, conservation at species and gene level requires information from genetics. The introduction of genetics into nature conservation is only two decades old.

Thus, the lowest level of conservation is the DNA level and it must be recognized that it plays a fundamental role in conservation programs. If a species is to enjoy long-term survival in the wild, it must evolve in relation to environmental changes: the more genetically variable a species is, the more successful will be such adaptive changes. This is a simple principle, but it must be kept well in mind!

Without doubt, global biodiversity has reached a state of crisis and human activities are continuing to destroy biological resources. We can get an idea of the scale of this crisis by consulting the Red List of Threatened Species compiled by IUCN (the World Conservation Union), the most solid documentation providing insight about the status of wild animals and plants, updated in 2000. Recently, IUCN has also produced a Red List of Threatened Fungi.

IUCN is a union of sovereign states, government agencies and non-government institutions that has basically three main goals: to secure the conservation of nature, and especially of biological diversity; to ensure that natural resources are used in a wise, equitable and sustainable way, and to guide humanity towards a style of life that will be of good quality and in harmony with the other components of nature. IUCN is organized into a series of commissions and offices that operate in the name of specific purposes. For conservation of biodiversity by mean of development of specific programs to study and then restore species and their natural habitats, there is the *Species Survival Commission* (SSC), the largest commission of IUCN.

The Red List classifies species or populations into eight categories according to their risk of extinction as reported in the following list:

- Extinct (EX): a taxon is considered extinct when all its individuals have died without any doubt;
- Extinct in the Wild (EW): a taxon that is extinct in the wild but it is maintained in captivity;
- Critically Endangered (CR): a taxon that is facing an extremely high risk of extinction in the wild in the immediate future;
- Endangered (EN): a taxon not definable as critically endangered, but that is near to becoming extinct in the wild in the near future;
- Vulnerable (VU): a taxon that could become endangered in the near future since it is facing a high risk of extinction in the wild in the medium term future;
- Lower Risk (LR): a taxon that does not satisfy any criteria for the categories CR, EN, VU. This categories is divided into three sub categories, Conservation dependent (cd), Near Threatened (nt) and Least Concern (lc).
- Data Deficient (DD): a taxon is defined as data deficient when there is no adequate information on which to base direct or indirect assessment of its risk of extinction. More information is clearly required for appropriate management of taxa included in this category;
- Not Evaluated (NE): a taxon is included in this category when it has not yet been assessed for being included or excluded from the Red List.

A species is classed as Threatened if it falls in the Critically Endangered, Endangered and Vulnerable categories. Definitions are taken from the original source.

According to the 2000 IUCN Red List of Threatened Species, populations of many species, including reptiles and mammals, are dramatically declining (see Table 1) and this estimation has increased with respect to the previous assessment in 1996 (for example, in just four years, Critically Endangered primates increased from 13 to 19, and freshwater turtles went from 10 to 24 Critically Endangered species). A total of about 40 000 species of plants and animals are threatened (see Table 1) and in almost all cases as a consequence of anthropogenic pressures. Human activity, in fact, has destroyed or degraded several natural habitats, as well as exploited natural resources for economic reasons. In the last 500 years, moreover, human activity has forced about 1500 species into extinction. Unfortunately, the number of at-risk species is greatly underestimated: for example, the Red List includes as threatened more than 30 000 plants, but only a part of the world's plant species have so far been evaluated.

In the light of the above, it becomes clear that conservation biology must work promptly and continuously to avoid exploitation and loss of natural resources. Conservation biology, moreover, has to guide the world's governments towards **sustainable development**. Such a goal, considered utopian by most people, would be coupled to economic interests with respect to natural resources.

Today, the necessity of conserving biodiversity is globally accepted, but there is disagreement on how to conserve it, and in particular, how to prioritize conservation measures. A key question is whether the core of conservation biology should be the ecosystem or the population; as well as whether it should concentrate on endangered and keystone species or have a wider habitat-based approach. Regarding this controversy, Soulé and Mills presented a wise and critical essay: they believe that the single-species approach and the ecosystem approach are not a true dichotomy but two essential elements of the same task.

2. Species Preservation

Conservation must be directed towards the conservation of biological diversity and biomass. Conservation of biological diversity means the conservation of species and of biological communities.

Two antithetic approaches can be followed for species preservation: an *in situ* (in site) approach and an *ex situ* (off site) one. In simple terms, the former concerns the conservation of resources in the wild, while the latter involves removal of samples of endangered species and of their genes from their original sites for conservation elsewhere under institutional protection.

The best approach for the protection of natural resources is the preservation of natural habitats and, thus, of communities and species in the wild. Many species cannot survive away from communities in which they are naturally found, but, unfortunately, for many rare and endangered species *in situ* strategies cannot be applied (e.g. when the wild population is too small to survive, or in other words, when it does not reach the

minimum viable population size). In such circumstances, the only way to preserve a species from extinction is to maintain individuals in artificial conditions under human supervision.

The term *minimum viable population* (MVP) has come into fashion in the late seventies, even if the concept had been introduced a decade before. This term implies that there is a threshold or a set of thresholds for the number of individuals that will guarantee that a population can persist for a certain span of time in a viable state. Around this concept, it is born the *population vulnerability analysis* (PVA), i.e. the whole procedure for the estimation of the minimum viable population size. The first studies were based on a demographic approach, considering the birth and death rates of a population and establishing the size under which the population itself would become extinct. The successive studies have been focused on the genetic aspects coupled with the extinction of a population, such as the loss of genetic variation necessary for responding to the environmental changes and the inbreeding depression processes.

An accurate analysis of the minimum viable population size is a long, complex as well as expensive process that requires a demographic, an environmental and a genetic study: this study may require months or even years of research. Moreover, since the probability of extinction of a population cannot be related to the population size alone, but it depends on its life history, on spatial and temporal parameters as well as on its level of genetic variability, no single value for the MVP can be applicable to all species. Being different for different species, and being sometimes impossible to conduct a PVA (no time, no money or whatever), biologists have tried to draw out a general rule to assist planners and land managers in their process of species conservation: for this, it has been suggested, for vertebrates, that protecting at least from 500 to 5000 individuals would guarantee the survival of the population since this number seems adequate to maintain good level of genetic variability.

Actually, there is an ongoing development for integrating *in-situ* and *ex-situ* conservation programs, for example using off-site populations to support wild relict in-site populations.

3. *Ex situ* Species Preservation

Ex situ programs are regarded by some authors as crisis responses to the threat of extinction, but they play several other important roles in conservation, both from a short- and a long-term point of view.

Ex situ strategies include zoos, aquaria, botanical gardens and arboretums, seed and embryo banks, and captive breeding programs. Also, a micro-organisms store may be considered as *ex situ* care to preserve bacterial and fungal biodiversity: this matter will be discussed later. The basic techniques of this kind of approach are: capture, translocation, storage, breeding, and replacement. In the last decade, biotechnology has become an integrated support to maintenance of biodiversity in captive conditions: cryopreservation of tissues, seeds, sperms and oocytes, embryo storage, molecular probes, DNA and gene libraries, and cloning are just some aspects.

An intermediate strategy between in-site and off-site approaches is the preservation of semi-wild populations in small protected areas, where human intervention may be used only in case of strong population declines and not as a matter of course.

There is little disagreement on the active role of *ex situ* care structures in conservation efforts. First, they can supply individuals for maintaining critical natural populations and for reintroduction programs. Second, they must have an active role in research collaboration with laboratories and universities: studies on captive populations can provide insight into the basic knowledge of a species and guide correct management policies. Third, they can influence and enhance the sensitivity of broad public opinion and education programs. The third point is particularly evident for botanical and zoological gardens, as defended by many authors.

Finally, the ultimate goal of *ex situ* conservation is the re-establishment of new populations of species which have become extinct in the wild: at present, unfortunately, many animal and plant species survive exclusively in captivity.

3.1 *Ex situ* Animal Preservation

3.1.1 Zoos and Aquaria

First, we would like to clarify the meaning of the word "zoo", since, due to the enormous variation among the institutions that are known by this term, there is no uniform definition of the word. The term "zoo" covers all the institutions that possess and manage collections of wild (non-domesticated) animals. These animals are housed in a way that makes them easier to see and study than they are in nature. Moreover, they are displayed to the public for at least a part of the year.

Collections of live animals have existed since antiquity, and the first zoos was established in the late eighteenth and early nineteenth century (in Vienna, Paris, and London) with the primary aim of forming collections of exotic animals. Today, the main purpose of zoos is significantly different, being in the first place a tool for animal conservation.

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Biographical Sketches

Dr. Sara Fratini graduated in Biology at the Department of Animal Biology and Genetics of the University of Florence with a master thesis on social insects. She obtained her PhD in Animal Biology (Etholgy) in the same department with a thesis on the feeding ecology of mangrove macrobenthos. For some years, she has been collaborating with the Natural Museum *La specola* of the University of Florence and she is involved in population genetic and systematics research, as well as in eco-ethological studies. She is editor of various papers in international journals and she has participated in several national and international congresses.

Dr. Renato Fani graduated in Biology at the Department of Animal Biology and Genetics (DBAG) of the University of Florence with a thesis concerning the mechanism of genetic recombination in bacteria. He obtained his PhD in Genetic Sciences (Genetics and Molecular Biology) at the Genetics Department of the University of Pavia, with a thesis on genetics and molecular biology of diazotrophs.

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