

# Genetic aspects of *ex situ* conservation

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## Abstract

More than 50 percent of all vertebrate species are classified as threatened today and this has become a central concern for people all over the world. There are several ways of dealing with preservation of species and one of the techniques receiving most attention is *ex situ* conservation. In *ex situ* conservation animals are bred in captivity in order to eventually be reintroduced into the wild when their habitats are safer. This is often called conservation breeding. Since threatened species usually have small and/or declining populations, the effect of small population size is a major concern in conservation breeding. The aim of the present paper is to review genetic aspects of *ex situ* conservation and to discuss how populations should be managed in captivity in order to be successful in a reintroduction situation. Populations in captivity may deteriorate due to loss of genetic diversity, inbreeding depression, genetic adaptations to captivity and accumulation of deleterious alleles. These factors could seriously jeopardize the successfulness of *ex situ* conservation and need to be investigated thoroughly in order to optimize conservation breeding programs.

*Keywords:* Genetics; *Ex situ* conservation; Conservation breeding

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## Introduction

It has been estimated that there are between 3 and 30 million species of various life living today (May, 1992). This can be compared to the 5 to 50 billion species that may have existed at some point since the beginning of life (Raup, 1991). Such comparisons indicate the magnitude of extinctions that have occurred in the past. What is important to remember is that some degree of extinction is an entirely natural occurrence. Magin et al. (1994) estimated that roughly three described animal species became extinct each year before the threat of human influence. However, the rate of

which extinction occurs today is at least partly due to human influences. It is possible that up to 18 000 species have become extinct since 1600AD (Magin et al., 1994). This means that the extinction rate in the last 400 years may have been 45 animals per year if rates of extinctions among mammals and birds are evenly extrapolated to other taxa. This rate is 15 times higher than the estimated natural extinction rate and conservation of species has now become a central concern for people all over the world.

There are several ways of preserving species and the focus of this paper is on *ex situ* conservation of animals. In *ex situ*

conservation animals are bred in captivity for an eventual reintroduction to the wild. This is called conservation breeding and is one of the species preservation techniques receiving most attention.

Today, over 50 percent of all vertebrate species are classified as threatened (Frankham et al. 2002). The first convention on biodiversity took place in Rio de Janeiro in 1992 and the zoos' response to this was *The World Zoo Conservation Strategy* (Wheater et al., 1993) with the aim to help conserve the earth's fast-disappearing wildlife and biodiversity. Many species require conservation breeding to save them from extinction as they may not be capable of surviving in the hostile natural environment (Ralls and Ballou, 1983). Soulé et al. (1986) estimated that approximately 2000 species of terrestrial

vertebrates may have to be bred in captivity in order to survive. To preserve all threatened species is practically an impossible mission but, hopefully, the establishment of conservation programs can at least reduce the extinction rates.

Species in need for conservation actions are categorized into different groups; *critically endangered*, *endangered* and *vulnerable* species (IUCN, 2001). Additionally, there are some species that are completely *extinct in the wild* and survives only in cultivation, in captivity or as a naturalized population outside the past range. Threatened species are found in all taxonomic groups and the number of species in each category is shown in table 1. The survival of many of these species depends upon well-designed *ex situ* conservation actions.

<b>Taxonomic group</b>	<b>EW</b>	<b>CR</b>	<b>EN</b>	<b>VU</b>
Mammals	4	184	337	609
Birds	3	182	331	681
Reptiles	1	57	78	158
Amphibians	0	30	37	90

Table 1. Number of species extinct in the wild (EW), critically endangered (CR), endangered (EN) and vulnerable (VU) in four different taxonomic groups. (After IUCN, 2003)

The ultimate goal of *ex situ* conservation is to provide support for the survival of species in the natural environment (e.g. Wheater et al., 1993). Hence, conservation breeding programs are not an alternative for, but rather a complement to, *in situ* conservation, i.e. conservation through biotope protection and preservation. This can be accomplished through captive propagation of rare species

to prevent immediate extinction as well as re-establishment and support of natural populations to ensure ultimate survival of species in their natural environment. Conservation breeding programs must be complemented with for example habitat preservation and elimination of the original threats in order to give the reintroduced animals a fair chance to survive.

Reintroduction is the intentional movement of captive-born animals into the wild. Animals can be moved into (or near) the species' historical range either to re-establish a new population or to boost a wild population. According to Beck et al. (1994) only 11 percent (16 of 145) vertebrate reintroductions where captive-born individuals were released into the wild can be considered successful. Causes of failure or problems in reintroduction of captive-bred animals vary from case to case but a common cause is behavioural deficiencies in released animals (e.g. Fleming and Gross, 1993; Kleiman, 1989; Shepherdson, 1994). Still, few data are available to explain why reintroductions fail and despite guidelines stating the need for evaluation, the results of reintroductions are rarely published (Beck et al., 1994; Kleiman et al., 1994). For conservation breeding to be a successful technique to save species from extinction, more knowledge is needed about what happens with behaviour and genetics in small captive populations bred in captivity.

### **Aim**

The aim of the present paper is to review genetic aspects of *ex situ* conservation and to discuss how populations should be managed in captivity in order to be successful in a reintroduction situation.

### **Genetic considerations of *ex situ* conservation**

Given that threatened species usually have small and/or declining populations, the effect of small population size is a major concern in *ex situ* conservation. According to Woodworth et al. (2002), populations in captivity deteriorate due to *loss of genetic diversity*, *inbreeding depression* and *genetic adaptations to captivity* that are deleterious in the wild. *Ex situ* conservation may also lead to genetic changes due to *accumulation*

*of deleterious alleles* (Couvet and Ronfort 1994). All these factors could seriously jeopardize the successfulness of a conservation breeding program and despite a certain overlap; they will be discussed one by one.

### ***Loss of genetic diversity***

From a genetic point of view, the most critical point of *ex situ* conservation is to keep high levels of genetic diversity so that the population's ability to face new environmental challenges is not at risk and to prevent adaptation to captive conditions (Fernández and Caballero, 2001). Genetic diversity is reflected in the differences among individuals, including coat colour, behavioural patterns and DNA sequences. Maintenance of genetic diversity must be in focus in conservation breeding because environmental change is a continuous process and genetic diversity is required for evolving to adapt to such change. Furthermore, loss of genetic diversity is often associated with inbreeding and reduction in reproductive fitness. According to Tegelström and Sjöberg (1995), high levels of homozygosity and loss of allelic variation may affect a population negatively in at least three ways. First, homozygous genotypes may be more susceptible to environmental variation (Beardmore, 1983). Secondly, if individuals share genes by descent, deleterious genes expressed in the homozygous condition result in abnormalities, low viability and death (Chambers, 1983; Bensch et al., 1994). Thirdly, a low degree of allelic variation limits the ability of a population to respond to temporal and spatial environmental variation (Beardmore, 1983).

Most threatened species and populations have lower genetic diversity than related, non-threatened species with larger population sizes (Frankham et al., 2002). According to evolutionary theory,

populations with low genetic variation are expected to have lower adaptive potentials to cope with environmental changes than populations with higher levels of genetic variation. The environment changes annually, seasonally and daily but also over time due to, for example, climatic changes. Genetic variation in a population is therefore important to be adapted to both short-term fluctuations and long-term environmental changes. Some of the heritable variation will of course disappear slowly if the average pattern of selective forces remains constant for a very long time but usually this occurs at approximately the same rate as the foundation of new genetic material through mutations. Because of the constantly present and self-rejuvenating genetic variation, a population in the wild can adapt both to sudden fluctuations and a gradually changing environment.

Many different methods have been proposed in the literature to minimize or delay the loss of genetic diversity and the appearance of inbreeding. Several studies have also theoretically and experimentally investigated the best strategies to manage small populations in captivity in order to retain the highest possible genetic information. The majority of them conclude that the best way to control the loss of genetic diversity is minimizing the group coancestry or average mean coancestry (e.g. Ballou and Lacy, 1995; Caballero and Toro, 2002).

In order to make the right decisions about management the genetic diversity of a population must be known. There are many different ways of describing genetic variation. One way is to measure the proportion of loci with two or more alleles ( $P$ ) that occur in the population.

$$P = \frac{r_p}{r} \quad (\text{Hedrick et al., 1986})$$

where  $r$  is the total number of loci and  $r_p$  is the number of polymorphic loci.

The genetic composition of a population can also be described by allele frequencies. The allele frequency or the frequency of the  $F$  allele ( $p$ ) in a population is the proportion of all alleles examined which are  $F$ . The number of each homozygote and the total is doubled when dealing with diploid animals. Similar procedures can be applied to obtain allele frequencies when there are more than two alleles at a locus, as found for many microsatellite loci.

$$p = \frac{(2 \times FF) + FS}{2 \times \text{Total}} \quad (\text{Frankham et al., 2002})$$

where  $S$  is an allele other than  $F$ .

Genetic diversity at a single locus is commonly characterized by *observed heterozygosity*, *expected heterozygosity* and *allelic diversity*. These different measurements will be discussed one by one.

**Observed heterozygosity**,  $H_o$ , is simply analyzed as the number of heterozygotes at a locus divided by the total number of individuals sampled (Frankham et al., 2002).

$$H_o = \frac{\text{No. of heterozygotes at a locus}}{\text{Total no. of individuals}}$$

Gene diversity or **expected heterozygosity**,  $H_e$ , refers to the expected proportion of heterozygous loci in a population. At a single locus with two alleles with frequencies  $p$  and  $q$ , the expected heterozygosity is

$$H_e = 1 - \sum_{i=1}^{\text{No. of alleles}} p_i^2 \quad (\text{Frankham et al., 2002})$$

where  $p_i$  is the frequency of allele  $i$ . This formula can be better understood for the case of two alleles at a locus where the

expected genotype frequencies are  $p^2$ ,  $2pq$  and  $q^2$ . Since  $p^2 + 2pq + q^2 = 1$ ,  $2pq = 1 - p^2 - q^2$  which corresponds to the last equation  $H_e$  is usually preferred over observed heterozygosity as it is less affected by sampling.

In conservation genetics changes in levels of genetic diversity over time is often a central concern, as loss of genetic diversity is an indication of inbreeding and loss of evolutionary potential. Heterozygosity is therefore often expressed as the proportion of heterozygosity retained over time, i.e.  $H_t/H$  where  $H_t$  is the level of heterozygosity at generation  $t$  and  $H$  the level at some earlier time. For example,  $H$  may be the heterozygosity before a bottleneck and  $H_t$  after the bottleneck. Then  $1 - H_t/H$  would reflect the proportion of heterozygosity lost as a result of the bottleneck (Frankham et al., 2002).

**Allelic diversity** is the average number of alleles per locus and this is another way to characterize genetic diversity. When there is more than one locus, allelic diversity,  $A$ , is the average number of alleles across loci (Frankham et al., 2002):

$$A = \frac{\text{total number of alleles over all loci}}{\text{number of loci}}$$

The expected genetic loss due to bottleneck effect, founder effect or genetic drift is determined by population size and it increases for smaller populations. Such random genetic processes play key roles in conservation genetics since populations of endangered species usually are small. Not only have endangered species often lost genetic variation due to bottlenecks but they also continue to lose it through genetic drift. Furthermore, founder effects occur whenever *in situ* populations become fragmented or *ex situ* populations are established for captive propagation. The

term founder effect refers to genetic variation in populations that are founded by a random sample of  $N$  individuals from a larger population and it can be considered as a type of bottleneck effect.

Ideal populations are assumed to have random mating system where each individual has equal chances to mate. In such populations each individual also have an equal chance that their genes are not passed on to the next generation. Allele frequencies in the offspring generation may therefore differ from the parental generation and this is called genetic drift. Genetic drift is often the most powerful evolutionary force acting on small populations so it is very important that management really takes notice of it (Lacy, 1987). However, loss of genetic variation due to genetic drift does not necessarily result in an empty gene pool since new alleles are added through mutations each generation. The level of genetic variation is maintained whenever mutation and genetic drift are in equilibrium although new alleles may be different from those that were lost due to genetic drift. Populations that have been through a bottleneck have lost alleles and continue to lose alleles as long as the population size is too small to establish such equilibrium (Princée, 1998).

### ***Inbreeding depression***

Inbreeding ( $f$ ) in a population can be expressed as

$$f = 1 - \frac{H_0}{H_e} \quad (\text{Frankham et al., 2002})$$

Consequently, no inbreeding occurs ( $f=0$ ) when the observed heterozygosity equals the expected heterozygosity ( $H_0=H_e$ ). Positive value for  $f$  indicates that there is inbreeding in the population and negative value indicates outbreeding which means that

mates are less closely related than would be expected under random mating conditions.

Inbreeding results in an increase in homozygosity in populations and this may result in reduced fitness. Individuals with high proportion of homozygous loci often have lower fitness than heterozygous. Furthermore, deleterious recessive alleles can be expressed due to mating of homozygotes.

Effects of inbreeding on fitness are referred to as inbreeding depression (Roughgarden, 1979). Inbreeding depression that results from expression of lethal alleles or evidently harmful alleles is more likely to be detected than inbreeding depression due to alleles which only slightly reduce fitness. One obvious inbreeding depression is blindness in wolves, *Canis lupus*. This has genetic basis and occurs exclusively in inbred packs descending of specific founder lineages in the Scandinavian zoo population (Laikre and Ryman, 1991). In order to detect slightly deleterious alleles, comparative studies between groups of inbred and non-inbred animals are usually required. For example, Ralls and Ballou (1983) compared infant mortality between groups of inbred and non-bred zoo mammals and their study showed that infant mortality in inbred groups was generally higher than in non-bred groups.

### ***Genetic adaptations to captivity***

It has long been known that animals adapt to their local environment conditions.

When comparing populations in different environments, animals have the highest fitness in their own environment and perform least well in others. Hence, genetic adaptations to captivity will increase fitness in the captive environment, but may be deleterious when animals are returned to their natural environment (Arnold, 1995; Frankham, 1995). Genetic adaptation to captivity has for example been described in several species of fruit flies and it is likely to occur in all species (Frankham and Loebel, 1992; Arnold, 1995). When wild animals are brought into captivity the selection pressures change. Captive populations are naturally or unintentionally selected for their ability to reproduce in the captive environment and selection for tameness may be favoured by keepers.

While genetic adaptation to captivity or domestication has been recognized since Darwin's time, it has, until recently, been considered to be a minor problem in conservation breeding. However, now there is convincing evidence that it can be a major threat to the success of reintroductions (Frankham et al., 2002). Domestication is an evolutionary process which involves genotypic adaptation to the captive environment (Price and King, 1968). When we move an animal from the wild into captivity, it is inevitable that it becomes domesticated to some degree. The genetic changes associated to domestication are the result of three phenomena: *genetic drift*, *inbreeding* and *selection* (Fig. 1).

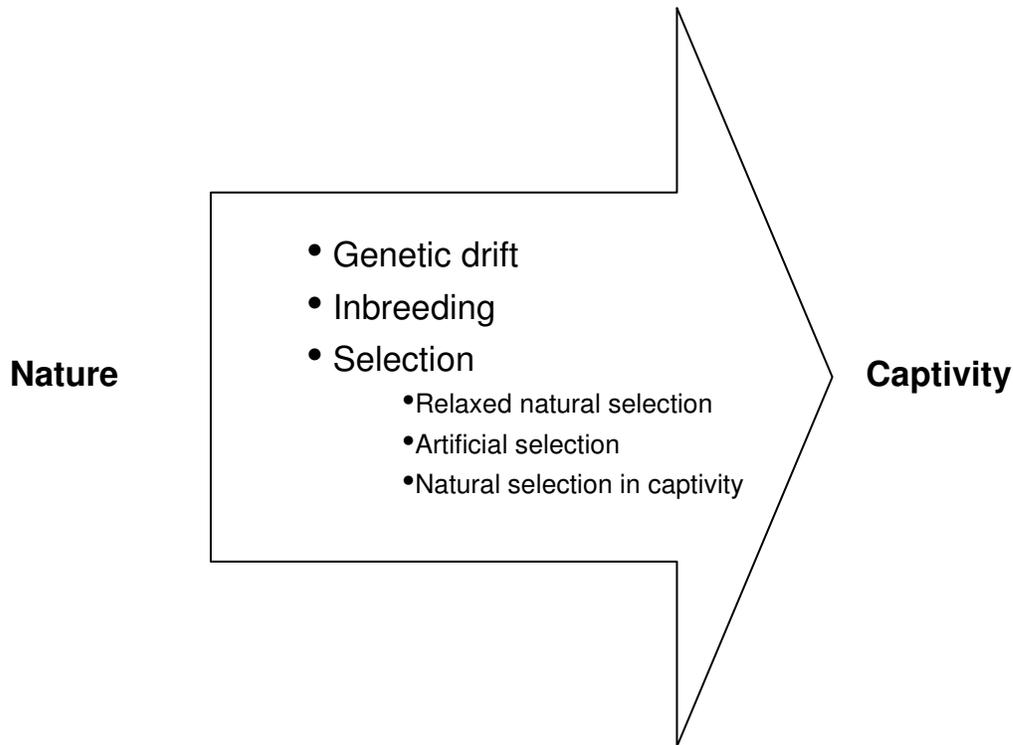


Figure 1. Genetic influences during domestication, the adaptation to physical and biological environment. (After Price and King, 1968)

As shown in Figure 1 there are basically three selection pressures operating on populations in captivity; relaxed natural selection; artificial selection and natural selection in captivity.

*Relaxation of natural selection* is a natural consequence of the environmental changes associated with provision of food, shelter and protection from predators in captivity (Price and King, 1968). Relaxation of natural selection takes place when captive conditions allow certain behavioural traits, which would have been selected against under wild conditions, to remain in the population. In captivity, animals are provided with food and protection against predators and therefore, certain behaviours that are important for survival in nature lose much of their adaptive significance (Price, 1984). Predators and parasites are usually controlled or absent, food is provided by humans and carnivores are no longer

selected for their ability to catch prey. Furthermore, in captivity, there is usually no competition with other species and limited competition for mates within species. Natural selection on such characters will be relaxed or they may even be selected against if there are trade-offs with other aspects of reproductive fitness (Bryant and Reed, 1999). Hence, selection on reproductive fitness is likely to maximize reproductive fitness in the captive environment rather than the animals' natural environment. Furthermore, unconscious selection may take place when animals of different tolerance to captivity and different reproductive fitness in captivity are mixed together in a breeding stock and this may alter the gene pool of captive populations considerably (Frankham et al., 1986). Hence, there is no doubt that functions essential to survival in the wild, like predator avoidance, ability to catch live prey

etc., will eventually be lost in captive populations unless they are subject to selection.

*Artificial selection* is selection for biological traits desired by humans. Artificial selection is avoided as much as possible when animals are bred in captivity for conservation purposes. However, there is still a risk that individuals that are easy to handle and cope well with the situation are preferred simply because of maintenance issues. Selection for traits such as tameness can be very strong in captivity. An example of how quick major changes can occur is Belyaev's (1979) domesticated silver foxes (*Vulpes fulva*), which after only a few (less than 20) generations of selection for tameness exhibited dog-like behavioural traits.

*Natural selection in captivity* is another selection adapting the animals to the captive environment. This usually starts early in the domestication process because individuals differ in their ability to cope with the captive environment. According to Price and King (1968) this selection acts primarily to eliminate those individuals that are biologically and/or psychologically incapable of producing offspring in captivity. In the absence of human interference, the individuals best able to cope with captive conditions will have the highest reproductive success.

#### ***Accumulation of deleterious mutations***

Deleterious alleles which are harmful under both in the wild and in captivity may be eliminated from captive populations. However, this kind of selection may also result in elimination of alleles that are advantageous in the wild but not in captivity. The risk is also that recessive alleles that are detrimental in the wild but neutral or beneficial in captivity may be favoured. The accumulation of deleterious mutations could be enhanced in captivity

both due to the small population size and the relaxed selection (Theodorou and Couvet 2004). The benign conditions in captivity, e.g. absence of predators, provision of food, medical treatment, may result in a relaxation of selection pressures against harmful alleles that would otherwise be kept in low frequencies in natural populations. This may increase the genetic load and the risk of extinction of natural populations (Lynch and O'Hely, 2001; Theodorou, and Couvet, 2004). Consequently, populations of endangered species may be subjected to introductions of individuals from an alternative setting where selection may be relaxed or in a direction opposite the natural selection and this may increase the risk of extinction. Woodworth et al. (2002) found that captive populations of *Drosophila* showed fast genetic deterioration in reproductive fitness when translocated to "wild" conditions and they concluded that the changes were due to genetic adaptation to captivity and inbreeding depression. Genetic adaptation was more important in large populations while inbreeding depression had most impact in smaller populations (Woodworth et al., 2002).

#### **Management of captive populations**

As Earnhardt et al. (2001) point out, self-sustaining captive populations are important because many captive populations represent insurance strategies against extinction or act as ambassadors for conservation of wild populations. Given the importance of captive populations for conservation and the restricted space available in captivity, captive management programs should use the scarce space optimally and preserve as many species as possible (Conway, 1986; Soulé et al. 1986; Hutchins et al. 1995). However, the question of how to best manage captive populations is not an easy one to answer. There are many

aspects to take into account and different analyses may result in different recommendations. More knowledge about management of small captive populations is needed and there are several improvements that require attention for the captive breeding efforts to be fully successful.

Margan et al. (1998) and Woodworth et al. (2002) recommend that captive populations of endangered species should be fragmented in order to minimize genetic adaptation to captivity but since small populations often have problems with inbreeding, occasional translocations will be required to prevent serious inbreeding depression and extinctions. Other advantages with fragmented populations in captivity are maintenance of more genetic diversity than a single population of the same total size (Margan et al., 1998), and reduction of movements between institutions which will reduce costs and disease transfers (Woodworth et al., 2002). In a comparison of the effect of loss of genetic variation in captive populations on average heterozygosity and the number of alleles at a locus, Allendorf (1986) concluded that the loss of both allelic diversity and heterozygosity should be considered in the design of breeding schemes for captive populations. Furthermore, Caballero and Toro (2002) showed that conservation decisions using genetic distance methods can be completely misleading when applied to subpopulations of a given metapopulation. They argue that the global diversity of the metapopulation should be considered and hence, both within and between subpopulation variability should be included in conservation decisions. Lacy (1987), on the other hand, suggests that simulations can help to define the effects that different management strategies will have on the genetic constitution of a population. With such knowledge, management plans can be planned so that

they better achieve the long-term genetic goals of captive propagation.

Frankham et al. (1986) suggest different managing techniques for three different categories of captive breeding; 1) long-term conservation of endangered species, 2) captive breeding for release back into the wild, and 3) rare species not yet capable of self-sustaining reproduction in captivity.

1) *Long-term conservation of endangered species.* This category involves species whose wild habitat may be lost and whose future lie in captive maintenance. The main considerations for this group are to maintain viable populations and to preserve as much of the original genetic diversity as possible. In such cases it may be impossible to recollect individuals from the wild so it is crucial to do the best of the situation. There may be conflicts between culling of outliers and maintaining equal representation of founder individuals when the number of founders is small. In such a case Frankham et al (1986) suggest that it is better to delay the culling of outliers until the population is larger and the genetic loss is not greater than the gain. In the long term this kind of population is destined to lose functions that are essential to survival in the wild.

2) *Captive breeding for release back into the wild.* This is according to Frankham et al. (1986), a short-term approach in which population size is the main concern. The aim is fast increase in numbers for instant release into the wild. Such populations usually spend little time in captivity and therefore, the relaxation of natural selection will not be serious. Selection against outliers is a concern as well as preservation of the genetic variation. The captive environment should be as similar as possible to the wild in order to ensure that the animals are returned to the wild with the required immunities, tolerances and physiological adaptations.

3) *Rare species not yet capable of self-sustaining reproduction in captivity.* Unsuccessful conservation breeding efforts in this group is very worrying because it critically endangers the survival of a species. In many cases the reason for failure of self-sustaining captive breeding is motivation or money. However, other issues like stress management, behavioural and environmental aspects are also important as well as genetics. According to Frankham et al. (1986), successful conservation breeding of many species requires such intensive selection for adaptation to the captive conditions that only a small proportion of males and females contribute to the gene pool. It must then be recognized that this is a step in the direction of domestication, although it is beneficial for breeding success.

Since genetic variation is so important in the wild, it is of course crucial to keep the original variation as intact as possible in *ex situ* populations. As mentioned before, captive populations generally differ from natural populations in three ways. They are much smaller and therefore susceptible to random processes that lead to a loss of genetic variability. They are also often divided into many small sub-populations which lead to a risk of inbreeding if there is no exchange between the sub-populations. Furthermore, the animals in *ex situ* populations live in environments that are different from their natural habitats and thus there is a risk of unnatural selective pressures. Fortunately, deleterious processes that can occur in small, fragmented populations have been intensively studied in recent years and on account of the insight gained so far a number of guidelines have been established (Wheater et al., 1993). These are summarized below.

1. The founder group should consist of at least several dozen animals.

2. The founder group must be increased as quickly as possible to the target population size.
3. The minimum size of the target population should generally include 250 to 500 animals.
4. The sex ratio of the reproductive animals must remain as close as possible to 1:1.
5. Inbreeding must be avoided as much as possible.
6. To slow down the potential rate of genetic change, the generation time should be extended so that animals will reproduce at later ages, as soon as the target population size is reached.
7. Continual efforts must be made to avoid unnatural selection pressures.
8. If possible, a small number of animals unrelated to the population should be added to it each generation.

The practical implementation of these recommendations and suggestions must be based on thorough information about the population in question. Keeping records on all animals involved is of course essential to all forms of population management. Good management is impossible without reliable information on aspects such as the number of animals in the population, location, kin relationships, descendants, sex, and age. The zoo world has three levels for the recording and reporting of individual data. First of all, each individual zoo registers their animal data including e.g. place of origin, birth, parents, offspring, diet, feeding habits, health and medical treatment. Secondly, there is a worldwide database for zoo animal information. The third level of animal data is the studbooks for endangered species. These are based on the previously mentioned database and also take into account zoos that have not yet registered their animals in the

database. Each international studbook is maintained by assigned studbook keepers and contains information on all individuals of one particular species that live or have ever lived in a zoo throughout the world.

As has been discussed in this paper, optimal conservation breeding is not an easy task for conservation biologists. More knowledge is needed about factors influencing populations in captivity and the interactions between those factors. Without

this knowledge, numerous reintroductions are predestined to fail just like in the past. Only with the right knowledge decision-makers have the chance to make accurate decisions about management techniques. However, as Rahbek (1993) points out, conservation breeding must not on any level result in misallocation of available resources or become an excuse to avoid dealing with preservation of habitats.

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