Genetic management of small animal populations

Based on Chapter 1 in: Genetic management of small animal populations: the use of genome models in the estimation of genetic variation and the effects of social structures. PhD disseration 2 October 1998, F.P.G.. Princée

2 October 1998
by Frank Princée
EAZA/EEP Executive Office
POB 20164 1000 HD Amsterdam

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Introduction

Evolutionary history shows that extinction can be considered as a natural phenomenon that can happen to any species. Entire taxonomic groups such as ammonites, trilobites and dinosaurs are only known from fossil records. The range of numbers of species that may have existed at one time or another since the beginning of life varies from 5 to 50 *billion* species [Raup, 1991]. Some 3 to 30 *million* species of various life forms are assumed to be living at present [May, 1992]. These figures are rough estimates, as will be explained later. Nevertheless, they indicate the order of magnitude of extinction processes that have occurred in the past. The majority of current extinctions appear to be caused either directly or indirectly by human activities [Magin *et al.*, 1994]. Governments have expressed their concern about the present rate of decline of the earth's biodiversity due to activities such as habitat destruction, (over-)hunting and environmental pollution. Since the United Nations Conference on Environment and Development in May 1992 in Rio de Janeiro, Brazil, some 153 nations have signed the Convention on Biodiversity. This treaty promotes protection and sustainable use of biodiversity. Consequently, governments of these nations are expected to develop and implement measures that prevent (or minimize) further decline in biodiversity at both national and international level.

This paper on population genetics refers to conservation of, especially, animal species which are endangered with extinction. Given the large number of species that became extinct in the past one may question the relevance of human intervention in reducing (or minimizing) the extinction processes. The following sections will provide arguments for such an intervention by comparing differences in causes and rates of extinction in past and present.

Extinction: past, present and future

Palaeontologists recognize two major types of extinction: *background* extinction and *mass* extinction. The general characteristics of background extinction are continuous occurrence, gradual rates, and effects that are restricted to a few (local) species [Donovan, 1989]. Interspecies competition is considered as the major process in background extinction. Mass extinction refers to high rates of extinction during a restricted period of time that affects numerous species at a global scale [Donovan, 1989]. Five main mass extinctions which have occurred since the Precambrian (600 million years BP) are recognized: end Ordovician; Frasnian-Famennian boundary or Devonian; end Permian; late Triassic and end Cretaceous or K-T extinction [Donovan, 1989]. Fundamental alterations in the physical environment, such as long-term global cooling or warming, are assumed to be the main initiators of mass extinctions [see e.g. Upchurch, 1989]. The K-T mass extinction (65 million years BP) may have been initiated by an extraterrestrial event like a bolid [Alvarez *et al.*, 1980] that resulted in long-term global cooling and major marine regression [see Hoffman, 1989; Upchurch, 1989; Priem, 1997].

Estimation of past extinction rates are subject to error as only a small part of organisms have been fossilized. Aquatic organisms are more likely to be preserved than land organisms due to sediment deposition in lakes and oceans. Animals with hard mineralized skeletons fossilize more readily than soft-bodied organisms [Raup, 1991]. Consequently, the numbers of species that existed at one time or another need to be considered as a coarse estimate which is based on extrapolation of data on fossil records. Although the fossil record underestimates biodiversity that existed during a given period of time, they provide some indication of levels of extinction. For example, about 52 percent of the families of marine animals (then living) became extinct by the

end of the Permian period (250 million years ago). Interpolation of data on extinction of these families yielded an estimate of 77 to 96 percent extinction at the species level [Raup, 1991]. The severity of mass extinctions varies between taxonomic groups. For example, fossil records from the North American region indicate that 8 percent of the 26 freshwater vertebrate families and 51 percent of the 37 land vertebrate families went extinct during the K-T mass extinction [Upchurch, 1989].

The periods from Eocene to Oligocene (58 to 24 million years BP) and the Late Pleistocene (50,000 to 10,000 years BP) are also considered as two major episodes of extinctions [Donovan, 1989]. A 'new' cause of extinction was introduced during the Late Pleistocene: predation by man. Extinction of mammoths, horses and camels in North America coincidence with arrival of humans with an efficient hunting technology [Barnosky, 1989]. A more "documented" history of extinction of animal species due to human activities starts in the 17th century (AD) when West-Europeans started to explore and to colonize other continents. The dodo, *Raphus cucullatus*, which was discovered in 1669 became extinct during the 18th century due to slaughter, hunting and introduction of domestic animals like pigs and dogs. A total number of 491 described animal species for example the aurochs, *Bos primigenius primigenius*, the blaauwbok, *Hippotragus equinus leucophaeus*, the quagga, *Equus quagga quagga*, and the passenger pigeon, *Ectopistes migratorius*, are known to have become extinct since AD 1600 [Magin *et al.*, 1994]. Human activities are the major cause, as mentioned previously, of present extinctions. These activities could be classified as interspecies competition, which is a 'characteristic' of background extinction, however in this case the competition seems to be largely biased in favour of one species i.e. *Homo sapiens*.

The 491 animal species that are known to have become extinct in the last four centuries, as mentioned by Magin *et al.* [1994], may at first appear not to be impressive compared to the estimated billions of species that became extinct in the past [Raup, 1991]. Clearly, such a comparison is not correct, as even the relatively short episode (on the geological time-scale) of the K-T extinction may have lasted several millions of years [Raup, 1991]. It is more appropriate to compare current extinction rates with 'natural' or background extinction before human interference. Background extinction can be computed by dividing the number of living species by the average persistence time [Magin *et al.*, 1994]. Since average persistence time is computed from fossil records and also includes episodes of mass extinctions it needs to be considered as a coarse estimate [Raup, 1991]. Magin *et al.* [1994] estimated that background extinction (based on an average persistence time of four million years) is roughly 3 species per year for the 1.3 million described animal species. The rate of documented extinctions among described animal species since 1600 AD is estimated as 1.25 species per year. Magin *et al.* [1994] argue that this rate is likely to be an under-estimate. The majority of taxa that became extinct belong to the two scientifically best-known taxa, mammals and birds. Up to 18,000 described animal species may have become extinct since 1600 AD if rates of extinctions among mammals and birds are evenly extrapolated to other animal taxa [Magin *et al.*, 1994]. Thus, the extinction rate in the last 400 years may have been 45 animal species per year, which is 15 times higher than the estimated 'natural' or background extinction rate.

The extinction rates presented above refer to 1.3 million described animal species. The total number of described animal and plant species ranges from 1.4 to 1.8 million species, but the total number of living species may be within the range from 3 to 30 million species [May, 1992]. This discrepancy between numbers of described and undescribed species is due to the fact that temperate and boreal areas have been better studied than tropical forests. Furthermore, mammals, birds and vascular plants have gained more attention in taxonomic studies than other groups [May, 1992].

Various methods have been developed to estimate the earth's biodiversity. For example, taxonomic data on birds and mammals show that roughly two tropical species exist for each species in temperate and boreal areas [May, 1992]. Extrapolation of the

ratio tropical - temperate species, as observed in mammals and birds, to less-well studied groups such as insects, results in an estimate of 3 to 5 million living species at present [May, 1992]. Another method to estimate the number of living species is by extrapolation of the fraction of new species that have been discovered in a region that has previously not been studied [May, 1992]. Extrapolation from studies on the ratio of parasite to host species is another method to estimate biodiversity. The ratio of vascular plants to fungi in Northern Europe is about 1:6. Extrapolating this ratio to the 270 thousand species of vascular plants world-wide would yield a number of 1.6 million species of fungi while only 69,000 have been recorded [Hawksworth, 1992; May, 1992]. Studies on herbivorous beetle species in tropical forest canopies suggest a number of living species in the order of some 30 million [see Erwin, 1988]. This estimate is based on assumptions of the host specifity of herbivorous beetles and the number of tropical tree species and has been subject of discussion [see Gaston, 1991; May, 1992]. Nevertheless, it is generally agreed that biodiversity in tropical forests is tremendous.

Tropical forests are being cleared at rate of 1 to 2 percent per year [May, 1992]. This means that, if these trends continue, more than 85 percent of the current tropical forest will vanish in 100 years. Assuming a ‘worst case’ scenario in which the extinction of species is linearly related to loss in habitat given that some two-thirds of terrestrial species are living in tropical forests. This would imply that more than 50 percent of the current terrestrial species would be extinct in a 100 years time. Such an extinction rate would even exceed the mass extinctions of the past, especially if the extremely short time interval is taken into consideration. These estimates of extinction rates indicate the necessity of *conservation* of biodiversity.

Conservation

The European bison or wisent, *Bison bonasus*, is an early example of a species that has been saved from total extinction by human involvement. Although the lowland wisent, *B. b. bonasus*, in BialowieÏa Primeval Forest (Poland and Byelorussia) was legally protected from hunting since 1532, the last wild animal died in 1919. The primary reasons for their final extinction in the wild was competition with re-introduced red deer, *Cervus elaphus*, and introduced fallow deer, *Dama dama*, in this forest around 1890. The Caucasian subspecies *B. b. caucasus* which had suffered from severe hunting pressures managed to survive in the Caucasus until 1927 [Pucek, 1991]. Fortunately, captive (*ex situ*[1]) breeding of wisent in zoological gardens has prevented this species from total extinction. Captive-born wisent were released in the BialowieÏa Primeval Forest as early as 1929 [Pucek, 1991]. Other examples of species that have been saved from total extinction by human intervention are Northern elephant seals, *Mirounga angustirostris* (by banning hunting in 1890) and Przewalski’s horses, *Equus (ferus) przewalskii* (by *ex situ* breeding since 1900).

However, do these few historical examples of human involvement in preventing extinction and in conservation[2] of species reflect the reality of the “modern” world? The order of magnitude of the “modern” extinction process may lead to a pessimistic feeling

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[1]*Ex situ* breeding refers to breeding of species outside their natural habitat. The location can be a zoo but also a (semi)-reserve.

[2]The terms *conservation* and *preservation* are often used interchangeably to describe human activities in preventing species extinction. Frankel and Soulé [1981] consider conservation as “policies and programmes for long-term retention of natural communities which provide the potential for continuing evolution”. Preservation refers, according their opinion, to ”maintenance of individuals or groups but not for their evolutionary change”.

that conservation of the earth's biological diversity is almost an impossible task. Especially, as habitat destruction will continue as long as the human population is growing. Although it may be difficult to preserve all endangered species, establishment of efficient conservation programmes can at least result in the reduction of extinction rates. This approach is the essence of a new discipline in biology: *conservation biology*. This discipline can be characterized as a synthesis of various biological disciplines like ecology, ethology, evolutionary biology and population genetics. Development of methods to assess threats enable prioritization of conservation activities and development of management strategies for conservation, can be considered as important issues within conservation biology. The Mace-Lande criteria to assess threats of extinction in terms of probabilities [Mace and Lande, 1991] may be considered as one result of conservation biology. Various aspects, such as general biology of a taxon, its population dynamics and habitat fragmentation are considered in these criteria. The Mace-Lande criteria have been adopted - in a revised edition - by the World Conservation Organization IUCN[3] in their Red List categories [IUCN, 1994a].

Small populations at risk

Habitat protection and the elimination of hunting and poaching are obviously major premises in protection of endangered species. However, (imaginary) fences around wildlife reserves are not necessarily equivalent to the conservation of endangered species. Endangered species are characterized, as indicated by their status, by small population sizes and these populations are often fragmented into even smaller isolated demes. Viability and reproduction of individuals in small populations can be diminished due to the *Allee effect* i.e. the number of individuals or population density is below a threshold size or density [see Lande, 1988]. Allee effects can occur in species in which mating success is density dependent or in species that physically or chemically modify the environment by social interactions. Furthermore, populations are subject to various kinds of stochastic processes (i.e. processes in which a variable outcome is random or uncertain[4]) that affect population dynamics. Schaffer [1987] distinguished four broad classes of uncertainties:

- *Demographic uncertainty*: random events in the survival and reproduction of individuals, e.g. shifted sex-ratio at birth or mortality due to accidents.
- *Environmental uncertainty*: random - or unpredictable - changes in weather, food supply and populations of competitors, predators, parasites, etc.
- *Natural catastrophes*: e.g. floods, fires, droughts, etc., which may occur at random intervals.
- *Genetic uncertainty*: random changes in genetic make-up due to the founder effect, genetic drift, or inbreeding, which alter the survival and reproductive probabilities of individuals.

The concept of *minimum viable populations* or *MVPs* is used in conservation biology to determine critical population sizes where the risk of extinction within a given period of time is considered acceptable [see Soulé, 1987]. This criterion in general refers to

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[3]The abbreviation IUCN refers to International Union of Conservation of Nature and Natural Resources, the former name of the World Conservation Union.

[4]Schaffer[1987] prefers to use the term "uncertainty" rather than the term "stochastic process" in the context of population dynamics. He argues that there is little practical difference between a purely random event and the results of processes which, because they are not understood, remain unpredictable.

a probability of 95 percent of persistence of a population or species for 100 to 1000 years [see Soulé, 1987]. Stochastic processes can, by negatively affecting population dynamics, result in extinction of populations, and are likely to have a larger impact on small populations than on large populations. Schaffer [1987] compared effects of demographic uncertainty, environmental uncertainty and natural catastrophes on average *persistence time* based on studies of Goodman [1987], Belovsky [1987] and Ewens *et al.* [1987]. Demographic uncertainties especially affect persistence times of relatively small populations i.e. in the order of tens to hundreds of individuals [Schaffer, 1987; Goodman, 1987]. Combined effects of demographic and environmental uncertainties require population sizes in the order of hundreds to one million individuals to ensure long-term survival [Goodman, 1987; Belovsky, 1987; Schaffer, 1987]. The impact of these uncertainties on persistence time depends on population growth rates and the degree of environmental variability. Large populations are not free from risks of extinction. Natural catastrophes can result in extinction of these populations. Ewens *et al.* [1987] show that risks of extinction due to such catastrophes also affect large populations. Although catastrophes do not necessarily result in immediate extinction of large populations, they can reduce population sizes to levels that are vulnerable for environmental uncertainties. This means that risks of further decline of such populations increase. This chain of (combined) stochastic processes that results in a continuous population decline is called *extinction vortex* [Gilpin and Soulé, 1986].

Risks of extinction have been included, by adoption of the Mace-Lande criteria [Mace and Lande, 1991], as criteria in the IUCN Red List Categories [IUCN, 1994a]. For example, one criterion to consider a taxon as "Critically Endangered" (CR status) is based on quantitative analyses that show that the probability of extinction in the wild is at least 50 percent within 10 years or 3 generations. These quantitative analyses are conducted by, for example, the IUCN/SSC Conservation Breeding Specialist Group (CBSG)[5] in so called *Population and Habitat Viability Assessments* (PHVA's). The different types of uncertainties described above are incorporated in simulation models, such as VORTEX [Lacy, 1993], which are used in PHVA's. Furthermore, effects of re-introduction, removal of animals (due to poaching, hunting or culling) and decline in habitat are considered in a PHVA (and modelled in VORTEX). This not only allows estimation of the risks of extinction but also evaluates effectivity of possible management measures to preserve a taxon [Seal *et al.*, 1994].

PHVA models require data on populations, such as census size, age distribution, mortality and fertility rates; and data on the uncertainties discussed above. Environmental uncertainties can be extracted from data on annual fluctuations in births and deaths, while risks of catastrophes such as severe floods and hurricanes can be obtained from historical (weather and climatic) data. The quality of these data clearly determine predictive values of prognoses on survival chances of populations. Data on *in situ* populations are often missing or incomplete. For example, even census data for the largest living terrestrial species, the African elephant, *Loxondonta africana*, can be estimates [Jachmann,1991]. Furthermore, types of uncertainties (and associated risks and effects) are often unknown. This implies that various assumptions need to be made, from raw population data to risks and effects of uncertainties. Simulation models such as VORTEX [Lacy, 1993], can be used to determine the impact of assumptions by evaluating various scenarios [see for example Princée, 1995a, 1997].

Effects of genetic uncertainties on persistence time are more difficult to predict than the other three types of uncertainties. Firstly, it is difficult to quantify effects of loss in genetic variation directly in terms of increased mortality or reduced fertility. Secondly, the severity of the effects are not independent of previous events which occurred in the population. For example, inbreeding effects largely depend on the number and frequency

[5]SSC = Species Survival Commission of the World Conservation Union. The CBSG was previously named Caption Breeding Specialist Group.

of deleterious alleles in a population and, therefore, on the genetic structure and history of the population. Difficulties in quantifying the effects of genetic uncertainties may result in questioning the role of genetics in conservation [see for example Caro and Laurenson, 1994; Merola 1994]. In answer to this "opposition", Avise [1996] discusses the relevance of genetics to conservation. However, he refers to the contribution of tools provided by molecular genetics, rather than discussing the fundamental issue: the importance of genetic variation for survival of endangered species as predicted from evolutionary theory.

The current study is restricted to genetic processes (uncertainties) which affect genetic variation in small populations. The importance of genetic variation will be discussed in the next section.

The importance of genetic variation

Populations with low genetic variation are expected, according to evolutionary theory, to have lower adaptive potentials to cope with environmental changes than populations with high levels of genetic variation. The environment not only changes overtime due to, for example, climatic changes but also fluctuate annually, seasonally and even daily. Therefore, genetic variation in a population is important to adapt (or to be adapted) to both short-term fluctuations and long-term changes in the environment.

The cheetah (*Acinonyx jubatus*) has become a popular example among conservation biologists to stress the importance of genetic variation for long and short term survival. A feline infectious peritonitis virus (FIPV) epizootic in cheetahs in an Oregon wild animal park in 1982 and 1983 initiated an intensive genetic study in this species. O'Brien *et al.* [1985] studied 52 electrophoretic loci in cheetahs and did not find any variability in either captive and wild populations. Even the loci of the Major Histocompatibility Complex (MHC), which are generally highly polymorphic in mammals, are monomorphic in the cheetah. O'Brien *et al.* [1985] suggested that the high susceptibility of the cheetah to feline viral diseases, in comparision to other felids, is related to the lack of genetic variation at the MHC loci. Furthermore, the sperm abnormalities and high juvenile mortality, which have been observed in both zoo and wild populations, are also considered as effects of inbreeding depression [O'Brien *et al.*, 1985; O'Brien, 1994].

The study of O'Brien *et al.* [1985] is widely discussed. Merola [1994] questions the relevance of genetic variation to (future) survival of this species as cheetahs have survived for thousands of years with low levels of genetic variation. The rapid decline of wild populations during this century seems to be more related to loss of habitat. However, Merola's [1994] statement that "inbreeding depression is an artifact of captivity" ignores an important issue: cheetahs in zoological gardens live in modified environments. They are most likely to be exposed to a variety of "exotic" viral strains which are carried by other feline species which are found in zoological gardens, which do not naturally occur in the cheetah's habitat, like the jaguar, *Panthera onca*, the tiger, *Panthera tigris*, the puma, *Felis concolor*, and local feral cats, *Felis catus*. Disease transmission is expected to be higher under zoo than under natural conditions as the density of felids (cheetahs and other species is higher. The more or less solitary life-style of cheetahs may, as Merola [1994] indicates, compensate under natural conditions for lack of variability on the MHC loci. But, as cheetahs may be forced to live at high densities in managed wildlife reserves, the zoo example should be taken seriously. The precise cause of the low level of genetic variation in cheetahs is not known. O'Brien *et al.* [1985] suggest that genetic loss might be due to a series of severe declines in population size (*bottlenecks*) that occurred since the Pleistocene. Effects of bottlenecks, and other related processes, on genetic variation will be discussed in the following sections.

Measures of genetic variation

Various measures have been developed to describe genetic variation. One such measure is the proportion of loci with two or more alleles (P) that occur in the population:

$$P = \frac{r_p}{r}$$ **(1)** Hedrick *et al.* [1986]

where r is the total number of loci and r_p is the number of polymorphic loci. A common practice in population genetics is to consider those loci monomorphic where the most common allele has a frequency greater than 0.95 (or 0.99). Polymorphism is not indicative of the total number of alleles in a population.

$$\bar{n} = \frac{1}{r} \sum_{j=1}^{r} n_j$$ **(2)** Hedrick *et al.* [1986]

A second measure of genetic variation is the computation of the average number of alleles ($\hat{u}$) over r loci that are observed in a population. Polymorphism and (average) numbers of alleles do not provide complete information on the genetic variation of populations of diploid species as these measures do not consider the fact that an individual can be either heterozygous or homozygous at any given locus. Two different measures that reflect the level of *heterozygosity* in a population are used. The first measure, *observed heterozygosity* (H_o), refers to the proportion of heterozygous loci over all available locus sites in a population. This measure is computed as:

$$H_o = \frac{\sum_{x=1}^{N} h_x}{(N.r)}$$ **(3)**

where N is the number of individuals, r is the number of loci and h_x is the number of heterozygous loci of individual x.

The second measure, *expected heterozygosity* or *gene diversity* (H_e) refers to the expected proportion of heterozygous loci in a population. Gene diversity for a locus (h_e) is computed from the allele frequencies at a locus:

$$h_e = 1 - \sum_{i=1}^{n} p_i^2$$ **(4)** Nei [1975]

where n is the number of alleles and p_i is the frequency of allele i (i=1,.., n).

The *average gene diversity* over r loci (H_e) in a population is computed as:

$$H_e = \frac{\sum_{j=1}^{r} h_{ej}}{r}$$ **(5)** Nei [1975]

where h_{ej} is the gene diversity at locus j (j= 1, .., r).

Each of these measures has advantages and disadvantages as their statistical relevance depends on the sample size of both individuals and the number of loci that are studied [Hedrick et al, 1986; Princée, 1998].

Genetic variation in populations

Genomes of higher organisms contain somewhere between 4,000 and 50,000 structural loci [Nei, 1987]. Since it would be virtually impossible to examine all these loci for all individuals in a population, genetic variation needs to be determined on the basis of a random sample of loci and individuals. Various biochemical and molecular techniques have been developed to assess genetic variation [see for an overview: Avise *et al.*, 1995]. Often these techniques can be applied in different areas of genetics, varying from (sub-)species identification, genetic differences between populations, genetic variation within populations, to solving parentages. This section will describe the techniques that are applied to assess genetic variation within populations.

Table 1 Observed heterozygosity and polymorphism as observed in electrophoretic studies in major and higher taxa. Source: Nevo *et. al.* [1984]

	Observed heterozygosity			Polymorphism		
Taxa	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
Plants	56	0,075	0,069	75	0,295	0,251
Invertebrata	361	0,1	0,091	371	0,375	0,219
Vertebrata	551	0,054	0,059	596	0,226	0,146
Mammalia	184	0,041	0,035	181	0,191	0,137
Aves	46	0,051	0,029	56	0,302	0,143
Reptilia	75	0,083	0,119	84	0,256	0,148
Reptilia[a]	70	0,055	0,047	84	0,256	0,148
Amphibia	61	0,067	0,058	73	0,254	0,151
Pisces	183	0,051	0,035	200	0,209	0,137

[a] excluding parthogenetic species
N=number of species
SD= standard deviation

Protein electrophoresis

Although new techniques in the field of nuclear DNA analysis are evolving rapidly, most studies on genetic variation in populations refer to data that are obtained by *protein electrophoresis* [e.g. Nevo, 1978; Nevo *et al.*, 1984]. Results from these studies are generally presented in terms of proportion of polymorphic loci and average observed heterozygosity over the number of studied loci in a sample (see Equations 1 and 3, respectively). Nevo *et al.* [1984] analysed results from electrophoretic studies of 1111 animal and plant species. Table 1 presents mean observed heterozygosity and polymorphism in some major and higher taxa [Nevo *et al.*, 1984].

Heterozygosity and polymorphism is not uniformly distributed around the mean but follows a J-shape pattern among species within higher taxa. Especially heterozygosity in mammals shows a distinct skewed J-distribution [Nevo *et al.,* 1984]. Protein

electrophoresis has its limitations due to facts that only protein-coding loci are assayed and almost two thirds of nucleotide changes cannot be detected [Leberg, 1992; Avise *et al.*, 1995]. Furthermore, the availability of staining techniques for proteins determines which loci are included in genetic studies. Each protein, generally, requires a specific staining technique [see for example Meera Khan *et al.*, 1982]. However, it is assumed that loci which are examined by protein electrophoresis represent a random sample of the genome as availability of staining techniques is assumed to be a random factor [Hubby and Lewontin, 1966].

Nuclear DNA analysis

Nuclear DNA analysis generally involves the electrophoresis of DNA fragments and the detection of specific sequences within these fragments. This type of genetic analysis has three major advantages over protein electrophoresis. Firstly, analyses are not restricted to protein-coding loci but involve DNA sequences which are not necessarily part of functional genes. Secondly, small nucleotide changes in DNA fragments can, potentially, be detected, whereas protein electrophoresis only detects changes which result in different net charges. Thirdly, analyses of DNA fragments are not limited by the availability of staining techniques as a single technique, for example radioactive labelling, can be applied to reveal specific sequences in any DNA fragment.

Several methods have been developed in the field of nuclear DNA analysis during the last decade. Some of these are extensions or combinations of previously existing methods (and techniques). It is beyond the scope of this thesis to describe all available methods in detail. Methods that are particularly relevant for assessing genetic variation within populations will be described in this section. Readers are referred to Avise *et al.* [1995] for an overview of methods and key references.

A basic method in nuclear DNA analysis involves (1) using restriction enzymes (endonucleases) to split DNA into smaller fragments; (2) hybridizing radioactive labelled DNA probes - created using recombinant DNA techniques - with these DNA fragments (*Southern blotting*); and (3) development of an autoradiograph which reveals gel-banding patterns that represent the DNA fragments that have hybridized to the probe. These procedures form the basis of *Single-copy RFLP's (restriction fragment-length polymorphisms)* and *DNA fingerprinting*. Both methods differ in the part of DNA that is analysed i.e. gene regions and tandem-repetitive regions of DNA (minisatellites) in single-copy RFLP and DNA fingerprinting, respectively. Therefore, the interpretation of results differ between these methods.

The raw data yielded by single-copy RFLP's can be compared with those provided by protein electrophoresis as individuals can be described as homozygous and heterozygous. The advantage of this technique above protein electrophoresis is the large access of genetic variants through enzyme/probe combinations and detection of both silent and replacement nucleotide positions. However, single-copy RFLP's methods are time-consuming, particularly the construction of libraries with DNA probes. This method has not yet been widely applied in population genetics [Avise *et al.*, 1995].

DNA fingerprinting [Jeffreys *et al.*, 1985a,b] is based on Southern blotting procedures but uses DNA probes which are core sequences within tandem-repetitive regions of DNA (minisatellites). These minisatellites have been shown to be highly variable ('hypervariable'). Results of DNA fingerprinting consist of, often twenty or more, bands of varying intensity per individual. The variation in these bands is so great that virtually each individual is unique [Avise *et al.*, 1995]. Therefore, this technique is extremely valuable in solving parentages within populations.

DNA fingerprinting has been shown to be rather sensitive for detecting genetic variation in cases where none had been detected by protein electrophoresis as, for example, in the case of the population of harbour seal, *Phoca vitulina*, in the Dutch Wadden Sea [Kappe *et al.*, 1995]. However, Mendelian interpretations of gel patterns

of DNA fingerprints are difficult as it is generally unknown which bands belong to which locus, whether bands are linked and/or alternative “alleles” at a locus can be identified [Avise *et. al.*, 1995]. Stephens *et al.* [1992] developed a method to make a rough estimate of average heterozygosity in single-probe multilocus DNA fingerprint samples:

$$H = \frac{\sum s_k}{A - \sum \sqrt{(1-s_k)}} - 1$$

(6) Stephens *et al.* [1992]

where s_k is the frequency of the *k*th fragment in the sample, and A is the total number of fragments observed overall at polymorphic and monomorphic loci. The estimated values for average heterozygosities in the population of harbour seals in the Dutch Wadden Sea (sample size is 26) for Jeffreys human minisatellite probes 33.6 and 33.15 are 0.21 and 0.18, respectively [Kappe *et al.*, 1995]. A direct comparison with results from protein electrophoresis is not fully justified, given the unknown relationship between bands and loci in DNA fingerprints. However, to indicate the (potential) strength of DNA fingerprinting: heterozygosity in the Dutch Wadden Sea population of harbour seals is almost five times higher than the mean heterozygosity observed in protein electrophoretic studies in mammals (see Table 1). DNA fingerprinting studies on wolf-like canids even show values for expected heterozygosity as high as 0.68 [Wayne, 1996].

Genetic drift and the bottleneck effect

A random mating system, in which each individual has equal chances to mate, is assumed for ideal populations. This implies that individuals in such populations 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. This sampling error of gametes per generation is called *genetic drift*. The effect of genetic drift on genetic variation can be estimated by considering an ideal population of N individuals (of a diploid species) as a zygote pool of $2N$ gametes from which $2N$ gametes are sampled. Wright [1931] showed that the expected gene diversity at generation $t+1$ can be estimated by the following equation:

$$H_{e(t+1)} = (1 - \frac{1}{2N})\, H_{e(t)}$$

(7) Wright [1931]

where $H_{e(t)}$ refers to the gene diversity at generation t. This equation shows that populations are expected to lose gene diversity at a rate of $1/(2N)$ per generation even in the absence of selection.

Genetic drift is not the only type of sampling error of gametes that can occur in populations. A sudden decline (“crash”) in population size results in genome loss from individuals and, consequently, in a loss of genetic variation. This effect on genetic variation is called the *bottleneck effect* and can be considered as taking a random sample of N individuals (or $2N$ gametes) from a population. Mathematically there is no difference between genetic drift and the bottleneck effect [see Nei *et al.*, 1975]. This means that the gene diversity expected to be retained after a bottleneck can be estimated by equation 7 ($H_{e(t)}$ and $H_{e(t+1)}$ in this case refer to gene diversity before and after the bottleneck, respectively).

The term *founder effect* refers to genetic variation in populations that are founded by a random sample of N individuals (or $2N$ gametes) from a larger (ideal) population and can be considered as a type of bottleneck effect. Equation 7 can also be applied to

estimate the expected gene diversity in a founder population ($H_{e(t)}$ and $H_{e(t+1)}$ refer in this case to gene diversity in the source population and in the founder population, respectively).

The expected genetic loss due to genetic drift, the bottleneck effect or founder effect is determined by population size N (see Equation 7) and increases for smaller populations. For example, a population that is founded with 10 individuals is expected to have 95 percent of the original gene diversity retained and is expected to lose 5 percent gene diversity per generation if population size remains stable. These random genetic processes play a key role in conservation genetics as populations of endangered species are, almost by definition, small. Endangered species have not only lost genetic variation due to one or more recent bottlenecks but also continue to lose it through genetic drift. Furthermore, the founder effect occurs whenever *in situ* populations become fragmented in isolated demes or *ex situ* populations are established for captive propagation.

Effective population size

Genetic drift in equation 7 is a function of the population size (N). This relation is correct for populations that behave according the Hardy-Weinberg population model, i.e. *panmictic* (random mating) and no generation overlap. It will be obvious that genetic drift is larger in populations which breeding is restricted to a few individuals than in populations where each individual has an equal chance to reproduce. Entire genomes of non-breeding animals are simply lost to the next generation. Wright [1931] introduced the concept of *effective population size* (N_e) to estimate genetic drift in populations that deviate from the ideal population. The effective size of a population can be described as the number of individuals in an ideal population that would have the same genetic properties (in terms of genetic drift) as an actual population [Lande and Barrowclough, 1987]. Effective population size for an ideal population equals the population size N. Therefore, population size (N) in equation 7 is in general substituted by effective population size (N_e).

Various methods have been developed to estimate effective population size in non-ideal populations [Wright, 1938; Kimura and Crow, 1963a; Crow and Kimura, 1970; Felsenstein, 1971; Lande and Barrowclough, 1987]. These methods differ from each other in the level of complexity of actual populations that is assumed. For example, effective population size of a population that differs from an ideal population by its unequal sex-ratio can be determined by:

$$\frac{1}{N_e} = \frac{1}{4N_m} + \frac{1}{4N_f} \qquad \textbf{(8)} \text{ Wright [1938]}$$

where N_m and N_f are the numbers of males and females, respectively.

Variance in number of progeny can be incorporated in equation 8 by substituting N_m and N_f for the effective numbers of males (N_{em}) and females (N_{ef}), respectively. The male effective number is computed from the actual number of males (N_m), and the mean and variance in number of progeny that is produced by a male individual during its lifetime (k_m) using equation 9. Female effective number is computed by substituting the

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appropriate female parameters for the male parameters into this equation.

Estimation of effective population size becomes difficult when generations overlap.

$$N_{em} = \frac{(N_m\overline{k_m} - 1)}{(\overline{k_m} + (\sigma^2_{km}/\overline{k_m}) - 1)}$$

(9) Lande and Barrowclough [1987]

Lande and Barrowclough [1987] presented a method to estimate effective size per average generation time that is based on data from life table analyses [see for example Krebs, 1978] such as growth rates, age-specific survivorship rates and age-specific fertility rates:

$$N_e = 4(\overline{T}/N_{em}T_m + \overline{T}/N_{ef}T_f)^{-1}$$

(10) Lande and Barrowclough [1987]

where T_m and T_f are the generation times for males and females, respectively; T is the average generation time for both sexes and N_{em} and N_{ef} are the effective numbers for males and females per average generation. See Lande and Barrowclough [1987] for further details on this method.

Computation of effective population sizes using methods described previously require information ranging from numbers of males and females (Equation 8) to detailed information on breeding structure and life history characteristics (Equation 10). Such information is not always available for populations and, therefore, the possibility to compute effective population size is restricted. Alternatively, effective population size for such populations can be based on *N_e/N ratios* that have been observed in well-studied vertebrate populations. The N_e/N ratio ranges from 0.1 to 0.5 [Mace and Lande, 1991; Foose *et al.*, 1995].

Inbreeding

Parents which are related share alleles of one or more ancestors and, consequently, their offspring will frequently inherit the same ancestral alleles from both parents (*identical by descent*). The probability that two homologous alleles in an individual are identical by descent (*autozygous*) is called the *coefficient of inbreeding* or *inbreeding coefficient* (*f*) [Wright, 1922]. The inbreeding coefficient of each individual in a population can be computed from pedigree data. Figure 1 shows a pedigree for a *full-sib* mating (brother and sister mating). The ancestors male 1 and female 2 are assumed to be unrelated and have genotypes *A1A2* and *A3A4*, respectively. The probability that individual 5 is autozygous for allele *A1* is estimated as follows: the probability that either male 3 or female 4 inherits allele *A1* is 0.5. The probability that both male 3 and female 4 inherit allele *A1* is 0.5 x 0.5 = 0.25. The probability that individual 5

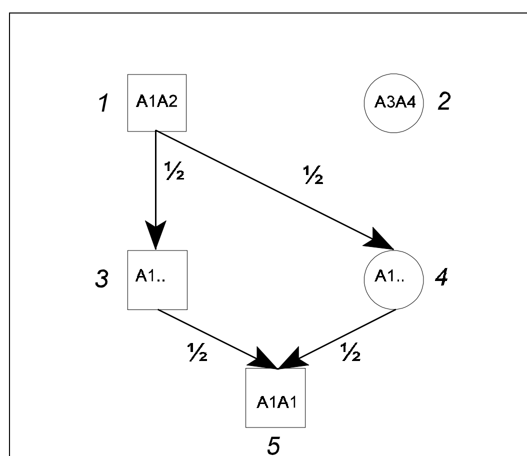

Figure 1 Pedigree of full-sib mating

inherits allele *A1* from both its parents is 0.5 x 0.5 = 0.25. Thus, the probability that individual 5 is homozygous for allele *A1* is 0.25 x 0.25 = 0.0625. The total probability that individual 5 is homozygous for one of the ancestral alleles at a locus, the inbreeding coefficient (*f*), is 4 x 0.0625 = 0.25 [see for other examples Crow and Kimura, 1970; Hedrick, 1985].

Computation of inbreeding coefficients, as illustrated in the previous example, can be a tedious and a time-consuming job for complex pedigrees. Various techniques like *path analysis* or *chain-counting technique* and the *additive relationship matrix method* have been developed to compute inbreeding coefficients of individuals in complex pedigrees [see for further details on these techniques Ballou, 1983; Hedrick, 1985]. Especially the additive relationship matrix method provides a straightforward method to compute inbreeding coefficients regardless the complexity of the pedigree. However, whenever pedigree data are computerized, it is much more efficient to compute inbreeding coefficients of individuals by computer. For example, Quaas [1976] and Henderson [1976] developed (computer) algorithms, based on the additive relationship matrix method.

The inbreeding coefficient as described above refers to the probability that an individual is homozygous by descent. This measure originates from the practice of livestock breeding in which it is considered important to determine the probability that an individual inherits recessive alleles (either deleterious or advantageous) from ancestors [see Wright, 1922]. However, individuals can also be homozygous for alleles which do not originate from the same ancestor (*homozygous by kind or state*). Increase in homozygosity in a population can be due to inbreeding and by genetic drift (fixation alleles). Inbreeding in this context is defined as matings between individuals who are more closely related than they would be if they had been chosen at random from a population [Crow and Kimura, 1970]. This means that inbreeding refers to the increase in homozygosity in the population relative to the random mating (Hardy-Weinberg) proportions.

The *inbreeding coefficient of the population* (*f*) can be expressed as the deviation from the Hardy-Weinberg proportions of genotypes. If no other factors like selection or assortative mating occur, *f* equals the *fixation index F* :

$$f = F = 1 - \frac{H_o}{H_e}$$ **(11)** Wright [1951]

where H_o is the observed heterozygosity (see equation 3) and H_e is the expected heterozygosity as computed by equations 4 and 5. No inbreeding occurs (f=0) when the observed heterozygosity equals the expected heterozygosity ($H_o = H_e$). A positive value for *f* indicates *inbreeding* in the population and a negative value indicates *outbreeding*, i.e. mates are less closely related than would be expected under random mating conditions. The maximum inbreeding coefficient is 1, indicating that the population is completely homozygous (H_o=0). The inbreeding coefficient of the population, as estimated from observed heterozygosity and expected heterozygosity, reflects the mean degree of inbreeding of individuals in the population (relative to random mating conditions).

Observed and expected heterozygosity are equal under random mating conditions. This means that the inbreeding coefficient (*f*) of a random mating population is 0 (see Equation 11). Yet, individuals in a random mating population can be *inbred* as relatives may mate by chance and/or when all individuals - especially in small finite random mating populations - are related. Roughgarden [1979] refers to inbreeding in the population as *inbreeding in the strict sense* and refers to matings between relatives (*consanguineous matings*) as *inbreeding in the broad sense*. This latter definition tends to be more commonly used in studies on populations for which pedigree data are

available such as livestock and zoo populations.

Inbreeding depression

As discussed previously inbreeding results in an increase in homozygosity in populations. This may result in reduced *fitness*, the relative ability of individuals to pass on gametes to the next generation. Individuals with a high proportion of homozygous loci often have lower fitness than heterozygotes and deleterious recessive alleles can be expressed due to consanguineous matings of homozygotes. The effects of inbreeding on fitness are referred to as *inbreeding depression* [Roughgarden, 1979]. Negative effects of consanguineous matings were known to breeders of domestic animal and plant species long before inheritance and its Mendelian laws[6] were common knowledge. For example, Darwin [1868] in his study on variation in domestic animal and plant species refers to the detrimental effects, such as reduced fecundity, due to mating between close relatives. Studies of inbreeding depression require information on genealogy and the life histories of individuals to determine survival rates and reproductive success. It is clear that most of the empirical data on inbreeding depression refer to studies on livestock [e.g. Lasly, 1978], laboratory species such as the fruitfly, *Drosophila melanogaster,* and zoo populations [e.g. Ralls and Ballou, 1983; Ralls *et al.*, 1988; Laikre and Ryman, 1991; Lacy *et al.*, 1993].

Inbreeding depression resulting from the expression of lethal alleles or obviously detrimental alleles is more likely to be detected than that due to alleles which only slightly reduce fitness. For example, blindness in wolves, *Canis lupus*, has a genetic basis and only occurs in inbred packs descending of specific founder lineages in the Scandinavian zoo population [Laikre and Ryman, 1991]. Comparative studies between groups of inbred and non-inbred individuals, using quantitative measures that reflect fitness, are required to detect slightly deleterious alleles. Ralls and Ballou [1983] compared infant mortality between groups of inbred and non-inbred mammals living in zoos. This study showed that infant mortality in inbred groups was generally higher than in non-inbred groups. Lacy *et al.* [1993] studied inbreeding depression in the zoo population of Goeldi's marmosets, *Callimico goeldii.* They suggest that this species is particularly sensitive to inbreeding as the survival rates of the female offspring of first cousin matings is only 25%, much lower than that of the offspring of unrelated parents (the observed survival rate in non-inbred progeny was 75%).

Reduced viability of juveniles is just one aspect of the decreased fitness that is due to inbreeding depression. Inbreeding depression also results in a reduced (reproductive) life-span and/or reduced fecundity. Life-table analyses, in which age-specific mortality and fertility rates are computed [see Krebs, 1978], can be used to compare fitness between inbred and non-inbred groups in a population. Differences in age-specific mortality rates and/or fertility rates between these groups may be small. Yet, the cumulative effect of these small differences may result in more clear differences in fitness than indicated by these single parameters. Princée [1992] used age-specific reproductive values in a preliminary study of inbreeding depression in the zoo population of Amur tigers, *Panthera tigris altaica*. The reproductive values express the number of offspring that an individual, belonging to a given age class, can be expected to produce during the rest of its life-time, i.e. the maximal observed age class in the population [see Krebs, 1978]. Male Amur tigers belonging to the inbred group have lower reproductive values per age class, and thus a lower fitness, than non-inbred males

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[6]Gregor Mendel published the results of his study on inheritance in 1866. However, this work remained unknown until 1900 when Hugo de Vries published the results of his study and referred in a footnote to Mendel's work. See Chapter 17. Flowering of Mendelian Genetics in Mayr [1982].

[Princée, 1992].

Since effects of inbreeding are more easily studied in *ex situ* populations it may wrongly lead to the assumption that inbreeding depression is an artifact of captivity, as for example stated by Merola [1994]. Inbreeding depression has also been documented in various natural populations [e.g. Frankham, 1995]. A high incidence of malocclusion, an anomaly which results in shortened mandibles, has been observed in an isolated population of the red fox, *Vulpes vulpes,* in the North Holland Dune Reserve, the Netherlands. This population descends from a few founders and genealogical studies suggest that this anomaly has a genetic basis [Bouwmeester *et al.*, 1989]. Other examples of inbreeding depression in natural populations include the great tit, *Parus major*, studied by Noordwijk and Scharloo [1981] and the Darwin's medium ground finch *Geospiza fortis* [Gibbs and Grant,1989].

Sensitivity to inbreeding depression may vary with species or even with population [Shields, 1987]. Ralls *et al.* [1988] observed a range of lethal equivalents for survivorship of offspring in first-degree matings between -1.4 and 30.3 (a median value of 3.1) in 40 captive mammalian populations[7]. Lethal equivalents can refer to single alleles at different loci or to combined lethal effects of several deleterious alleles at different loci. Differences in sensitivity to inbreeding depression between species or populations, as observed in the study of Ralls *et al.* [1988], may be the result of past natural inbreeding in these species and populations. For example, natural populations that frequently undergo (and survive) severe bottlenecks are expected to be less sensitive to inbreeding depression than large outbred populations. Frequencies of recessive deleterious alleles in such populations are thought to be reduced in subsequent generations by both the bottleneck and inbreeding, which expose recessive alleles to selective forces. Brewer *et al.* [1990] carried out breeding experiments with *Peromyscus* that were captured from insular, peninsular and mainland populations in Florida to test this hypothesis. Some of these populations such as the insular subspecies of beach mice, *Peromyscus polionotus leucocephalus*, occasionally suffer from bottlenecks due to hurricanes occurring in the Gulf Coast region. Linear relationships between the severity of inbreeding depression and inbreeding level were observed within populations. However, Brewer *et al.* [1990] did not find trends in the relationship between fitness of litters and the origin of the populations in response to inbreeding level. They suggest that depression in fitness of litters in *Peromyscus* is not wholly determined by the *genetic load* (the average number of deleterious recessive alleles per individual) but also by over-dominance or fitness-determining genes. The influence of (natural) bottlenecks and inbreeding on occurrence of inbreeding depression can be considered as unpredictable to a certain extent.

Purging inbreeding depression

Since inbreeding depression can endanger future survival of populations one would tend to recommend breeding strategies that focus on avoidance of inbreeding. However, the number of generations in which inbreeding can be avoided in small populations is limited. This means that inbreeding can only be minimized in subsequent generations and that small populations will be sooner or later at risk through inbreeding depression. Therefore, it is questionable whether minimizing inbreeding is an optimal strategy to maintain small *ex situ* populations [e.g. Backus *et al.*, 1995]. An alternative strategy involves intentional (moderate) inbreeding combined with rapid population growth, analogous to the bottleneck situation, to *purge* (eliminate) deleterious recessive alleles from populations [Templeton and Read, 1983,1984; Backus *et al.*, 1995; Lynch, 1995].

[7] A negative value for lethal equivalents implies that inbred offspring have a higher survival chance than non-inbred offspring.

This strategy was applied in the breeding management of the zoo population of Speke's gazelle, *Gazella spekei*, after a severe inbreeding depression was observed [Templeton and Read, 1983, 1984]. This population is often considered as a successful example of the purging strategy [e.g. Backus *et al.*, 1995]. Templeton and Read [1983], however, do not recommend purging as a general strategy for the management of endangered species if other alternatives are available. Willis and Wiese [1997] re-analysed studbook data of the Speke's gazelle and concluded that purging has not resulted in a significant decrease in inbreeding depression. Willis and Wiese [1997] do not advise purging to reduce the severity of inbreeding depression until empirical data show the effectiveness of such a strategy.

Purging primarily aims at maintaining populations under *ex situ* conditions. Deleterious alleles that are obviously detrimental under both *in situ* and *ex situ* conditions may be purged from *ex situ* populations. However the selection induced through purging may also result in elimination of alleles that are advantageous under *in situ* conditions but that are detrimental under *ex situ* conditions. The contrary, recessive alleles which are detrimental under *in situ* conditions but neutral or even advantageous under *ex situ* conditions may be favoured. These types of selection may even be enhanced through purging as this involves active selection by population managers for viable or most-fit individuals [Templeton, 1983, 1984; Backus *et al.*, 1995]. This means that purging may not be desirable for those populations that are maintained with the intention of (future) reintroduction. The study of Jiménez *et al.* [1994] on effects of inbreeding in white-footed mice, *Peromyscus leucopus noveboracenis*, illustrates this risk of purging. Inbreeding effects were not observed under *ex situ* (laboratory) conditions but became apparent after inbred and non-inbred specimens were released at the original capture site. Survival rates of released mice were estimated by recapture techniques. Inbred mice showed lower survival rates than non-inbred mice [Jiménez *et al.*, 1994].

Expression of lethal alleles and culling of less viable individuals under purging management result in decreased growth rates. If purging results in a (temporary) decline of population size, the population is then exposed to the risks of extinction e.g. through demographic uncertainty [see *Small populations at risk*, page 4]. Fertility rates need to be increased to compensate for increased culling and mortality rates. However, species-specific reproductive biology such as litter size, inter-birth interval and reproductive life-span are limiting factors in increasing overall fertility rates. This means that the intensity of purging, i.e. tolerated levels of inbreeding, needs to be balances with species-specific reproductive biology.

Mutation

Genetic variation in populations is lost in each generation due to genetic drift. This does not necessarily result in a depleted gene pool as new alleles are added to populations through mutation each generation. Although new alleles may be different from those that were lost due to genetic drift, the level of genetic variation is maintained whenever mutation and genetic drift are in equilibrium. Kimura and Crow [1964] developed the *infinite-allele model* in which each mutation is assumed to result in a unique allele. Genetic variation will be maintained at a stationary level once an equilibrium between genetic drift and the mutation rate (μ) of new types of neutral alleles has been established. In a population of stationary size N, the equilibrium gene diversity equals:

$$\overline{H_e} = \frac{4N\mu}{1 + 4N\mu}$$

(12) Kimura and Crow [1964]

The mutation rate is assumed to be of the order of 10^{-5} to 10^{-7} per locus per generation [Nei *et al.*, 1975; Nei, 1987]. This implies that a population size of around a quarter million individuals is required to achieve equilibrium between genetic drift and mutation.

The populations which have been through a bottleneck have lost alleles due to the bottleneck and continue to lose alleles as long as the population size is too small to establish an equilibrium between genetic drift and mutation. Recovery of the original level of gene diversity after a bottleneck or founder event requires that populations expand sufficiently to reduce genetic drift below the mutation rate. The population size required depends on the severity of the bottleneck and growth rate of the population. Nei *et al.* [1975] studied the effects of bottlenecks, growth rates and mutation on genetic variation. A time-span of 10^5 to 10^7 generations is required to restore the original level of gene diversity after a bottleneck.

These orders of magnitude in population size and recovery time indicate that mutation can generally be ignored in studies of population genetics in endangered species. Populations of such species range between less than 250 mature individuals for the "Critically Endangered" category to less than 10,000 mature individuals for the "Vulnerable" category [IUCN, 1994a]. Furthermore, recovery of genetic variation after a bottleneck requires rapid population growth [Nei *et al.*, 1975]. Since many populations have become endangered due to habitat loss, they can, consequently, not expand in size.

Population structure and gene flow

Population structure refers to populations which are subdivided into partially isolated smaller demes due to, for example, geographical or social structure. Such populations are not panmictic, though mating within demes can be random. Genetic drift can result in random differences in allele frequencies among demes, especially when these are small in size (see *Genetic drift and the bottleneck effect*). Gene flow through migration of gametes (or individuals) between demes can compensate for the effects of genetic drift and effectively result in one large panmictic population.

The simplest population structure is the *continent-island* model [Wright, 1931; see also Hedrick, 1985] in which migration from the main population (continent) to an island population occurs. The change in allele frequency of a given allele (A_2) in the island population () q) per generation is:

$$\Delta q = -m(q_0 - q_m)$$

(13) Wright [1931]

where *m* is the proportion of migrants, q_0 the frequency of allele A_2 before migration, and q_m the frequency of A_2 in the migrants. Equation 13 applies to an island population of infinite size. Wright [1940] showed that average allele frequencies in island populations of size *N* will be close to q_m if values for $4Nmq_m$ and $4Nm(1-q_m)$ are both much greater than one [see also Hedrick, 1985].

The *island model* [Wright, 1943] presupposes that a population is subdivided into a large number of subgroups (islands), each of size *N,* and migration rate *m* between each subgroup and a random pool of immigrants originating from the entire population per generation. At equilibrium the differentiation between these subgroups F_{ST} is:

$$F_{ST} = \frac{(1-m)^2}{2N-(2N-1)(1-m)^2}$$

(14) Wright [1943]

Population size *N* can be substituted by effective population size N_e [Lande and

Barrowclough, 1987]. The population can be treated as effectively panmictic for migration rates greater than about $1/(4N_e)$ [Crow and Kimura, 1970].

The continent-island and island models both consider relatively simple population structures. More complex population structures are considered in the *isolation by distance* [Wright, 1940,1943] and *stepping stone* models [Kimura, 1953; Kimura and Weiss, 1964]. The first of these considers the effects of the dispersal distances of individuals in continuously distributed populations, while the stepping stone model considers populations which are geographically distributed in subgroups and where migration occurs between adjacent subgroups.

Populations of endangered species are often fragmented due to habitat loss. These two models can be important in developing management strategies for conservation of such species.

Metapopulations

Frankel and Soulé [1981] recommend that the rate of inbreeding (i.e. loss of observed heterozygosity) in populations should not exceed 1% per generation. Frankel and Soulé [1981] refer to this "1% rule" as the *basic rule of conservation genetics*. This rule assumes that natural selection for performance and fertility can balance inbreeding depression if the rate of inbreeding per generation is less than 1% [Franklin,1980]. Consequently, effective population size must be at least 50 (see equation 7). It must be noted that the "1% rule" is derived from what Frankel and Soulé [1981] describe as "*an empirical rule of thumb used by animal breeders*" and should, therefore, be merely regarded as a rough guideline rather than a strict rule.

Genetic variation in populations with an effective size of 50 will loose genetic variation due genetic drift each generation. Therefore, the "1% rule" applies to short-term *preservation* of endangered species under *ex situ* conditions. *Conservation* of endangered species, however, needs to focus on long-term survival, i.e. these species must have the capacity for continuous adaptation to respond to environmental changes [Frankel and Soulé, 1981]. Mutation and genetic drift in these populations need to be balanced. This requires effective population sizes of the order of 10^4 or 10^5 [Lande and Barrowclough, 1987], a condition that can not be achieved when a species is endangered. Franklin [1980] and Soulé [1980] proposed effective sizes of around 500 to balance mutation and genetic drift for the genetic variants of quantitative characters i.e. on those which selection often acts. Lande [1995] argue that effective sizes as larger as 5,000 may be required to balance mutation and genetic drift in these quantitative characters. Effective population sizes of 500 to 5,000 refer to actual populations that range from 2,500 to 25,000 individuals using a conservative N_e/N ratio of 0.2.

Threatened species can generally be characterized by their small population sizes, ranging from 250 to 10,000 individuals[8] [IUCN, 1994a]. Furthermore, many populations of these species are divided into smaller and isolated populations due to habitat fragmentation. Each of these individual demes is subject to the uncertainties as described previously and, therefore, often has no reasonable chance to survive. Active management seems to be required to cope with the various risks of uncertainties. The viability of endangered species could be enhanced if small isolated sub-populations were managed as one population, known as the *metapopulation concept* [see Foose *et al.*, 1995].

Genetic management is an important component of metapopulation management.

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[8]Population sizes as mentioned in the (new) Red List categories [IUCN, 1994a] are based on categories of effective populations sizes and a N_e/N ratio of 0.2 as defined in the original Mace-Lande criteria [Mace and Lande, 1991].

This management aims at retention of genetic variation in the total population and in the (isolated) sub-populations. Small isolated sub- populations may be especially at risk from inbreeding depression or lack of genetic variation for potential future adaptation. This can endanger the future survival of the entire metapopulation. Management of metapopulations can be based on migration models as discussed in the previous section *Population structure and gene flow*. Important measures in genetic management of metapopulations involve the construction of corridors and/or the translocation of individuals between sub-populations to restore natural gene flow patterns. Furthermore, reproductive technologies such as artificial insemination and embryo transplantation may also facilitate the exchange of genetic material between the various demes within a metapopulation. Such techniques would be valuable in those cases where transmission of diseases by newly introduced individuals could have an adverse affect on the population [IUDZG/CBSG, 1993]. *Cryopreservation* techniques and the establishment of genome banks of frozen sperm and embryos are important in metapopulation management. Especially those species in which genetic drift and inbreeding are of primary concern may benefit from cryopreservation. However, the applicability of reproductive technology should not be over-estimated, at least on the short term, as each species may have its own specific problems to be solved [Holt, 1994].

The metapopulation strategy is not confined to *in situ* (i.e. wild) populations but may also include, and even can enforce, *ex situ* populations in zoological gardens and semi-reserves. Although the primary aim of metapopulation strategies is to maintain viable populations in their natural habitat, this aim is not always feasible. A number of species such as *Partula* tree snails and Przewalski's horses are extinct in the wild but still survive in *ex situ* populations. Other species, like the Black rhinoceros, *Diceros bicornis*, still face extinction as a consequence of continuous poaching. *Ex situ* populations of these species are a vital part of the metapopulation as they provide the basis for (future) reintroduction and restocking projects. Endangered species that are subject to severe natural catastrophes may also require to have *ex situ* populations established to minimize risks on total extinction by this category of uncertainties (see *Small populations at risk* on page 4).

The role of zoos in conservation

The previous section indicated the importance of *ex situ* populations in metapopulation management to maintain viable populations of endangered species. *Ex situ* is often considered as synonymous for *zoo populations*. However it also includes individuals released into semi-reserves outside the range of origin. The IUCN World Conservation Union has recognized the importance of captive programmes in a policy statement that recommends initiation of a captive propagation programme for any taxon whose wild population declines below one thousand [IUCN, 1987].

Zoological gardens started breeding programmes for European bison and Przewalski's horses at the beginning of this century (see also page 3). International studbooks were established for these species. These studbooks include data on individuals which were born in zoos and their wild ancestors. Management strategies of these early programmes were not developed in accordance with modern views of population genetics [Princée, 1990], but nevertheless resulted in preservation of both species. These studbooks, which include data as sex, parents, dates of birth and death, allow to evaluate the management in the past and to correct, whenever required and possible, short comings [e.g. Olech, 1987,1989; Princée, 1990; Ballou and Lacy, 1995].

Zoological gardens started on larger scale with captive propagation programmes in the 1960's. International studbooks were initiated by the International Union of Directors of Zoological Gardens (IUDZG) for species which were rare in captivity (and often endangered in the wild). They included the okapi, *Okapia johnstoni*, and various

subspecies of tigers, *Panthera tigris* ssp. The number of International studbooks continuous to grow as the devastating effects of human activities on wildlife became more apparent. Some 37 International studbooks were established before the Convention of International Trade in Endangered Plant and Animals Species (CITES) enetered into force in July 1975 [Anonymous, 1975; Wijnstekers, 1995].

Initially individual zoos used studbooks as sources of data and it was their responsibility to access unrelated specimens for breeding. However, as the importance of population genetics and demographics to the maintenance of zoo populations became more clear [e.g. Flesness, 1977; Foose, 1977], the IUDZG defined new management guidelines for studbook keepers [Anonymous, 1980]. These guidelines involved a more active approach of studbook keepers in conducting demographic and genetic analyses of the studbook population. A major step in the development of captive propagation programmes was the establishment of regional cooperative management programmes. The North American zoos established Species Survival Plans (SSPs) in 1981 for maintenance of populations of endangered species in their collections. These SSPs involve intensive demographic and genetic management (see following sections). Continental Europe followed in 1985 with the European Endangered Species Programmes (EEPs).

Zoological institutions have been actively involved in captive propagation and conservation for more than 25 years. However, the role that zoological gardens do, can and should play in conservation was not comprehensively documented until 1993, when *The World Zoo Conservation Strategy* [IUDZG/CBSG, 1993] was published. This clearly described the responsibilities, potentials and limitations of modern zoological gardens in conservation [see also de Boer, 1992]. According to this document zoo populations can play three roles in conservation:

- They serve as living ambassadors that can educate the public and can generate funds for *in situ* conservation.
- They are scientific resources and opportunities for research that provide information and technologies beneficial to protection and management of wild populations.
- They form a resource, reinforcing survival of taxa in the wild through re-stocking or re-introduction (the metapopulation concept).

The total number of species and sub-species held in zoological gardens worldwide is estimated of the order of 6,000, based on data that are in stored in the database at the International Species Information System (ISIS) [Flesness *et al.*, 1995]. Zoo populations include all individuals of taxa in all zoological gardens worldwide. Nevertheless, these populations can be characterized as relatively very small for most species. Princée [1994] reviewed population sizes of major vertebrate taxa in European zoo collections. Almost 70% numbered less than 100 specimens. Magin *et al.* [1994] observed that global zoo populations of 64% of the mammals which are classified as threatened by the IUCN are less than 100 specimens.

In the past many zoo populations died out as a result of genetic bottlenecks because they were founded on very small numbers of wild-born individuals (or *founders*). It should be clear that demographic and genetic uncertainties also apply to zoo populations and that population management is required to ensure their future survival. Global and regional cooperative management programmes have been established to minimize these risks by the implementation of demographic and genetic management.

About 300 regional breeding programmes which involve about 200 species have been established worldwide since 1981 [CBSG, 1992]. This number is still small compared to the 4452 species which are listed in the 1990 Red List [Magin *et al.*, 1994]. The direct role of zoological gardens in conservation by maintaining populations of endangered species for reintroduction purposes is, therefore, questioned by Magin *et al.* [1994].

These authors and Seal *et al.* [1994] suggest that zoos should increase the number of captive propagation programmes for endangered species and that these should also include more "less charismatic" species such as amphibians and small mammals. However, the question remains whether such an extension of captive propagation programmes feasible?

A major genetic goal in captive propagation programmes is based on recommendations of Soulé *et al.* [1986]. These recommendations involve the preservation of at least 90 percent of the genetic variation (gene diversity) originally present in the source (i.e. wild) population for a period of at least 200 years[9]. Soulé *et al.* [1986] also use a model based on ideal populations to estimate the population size required to maintain this 90 percent of the genetic variation (*target population*). In this model the effective number of founders, initial growth rate and generation length are varied.

The World Zoo Conservation Strategy [IUDZG/CBSG, 1993] use 250 to 500 individuals for the average target population size to estimate the number of (sub)species that can be maintained by zoos. This leads to an estimated 1,000 to 2,000 (sub)species for the 1,000 larger zoos worldwide. Thus, zoos could potentially maintain viable populations of 25 to 50 percent of the species which are currently considered endangered. However, this level of contribution also requires that non-endangered species to be replaced by species in the IUCN Red List categories. For example only 174 of the 507 mammals listed in the IUCN Red List, are represented in zoos [Magin *et al.*, 1994]. The number of (sub)species which can be maintained could be increased by reducing target populations of species that are less endangered. This could be achieved through limited import of wild individuals in each generation and/or the use of genome banks [Seal *et al.*, 1994]. Furthermore, the space available in zoos could also be more dynamically allocated for endangered species. Release programmes for the black-footed ferret, *Mustela nigripes*, e.g. started six years after the last 20 wild individuals had been captured [Miller *et al.*, 1994]. *Ex situ* populations for such species would not need to be maintained for a period as long as 200 years.

Zoos may have the potential to manage populations of a relatively large number of populations of endangered species for use in (future) reintroduction programmes. However, the number of 200 managed species is in sharp contrast with the number of 1,000 to 2,000 species as mentioned in *The World Zoo Conservation Strategy* [IUDZG/CBSG, 1993]. Increasing the number of regional management programmes is limited by manpower, zoo space and financial resources. The organizational structure of, for example, the European Endangered Species Programmes (EEP) of the European Association of Zoos and Aquaria (EAZA) requires that each programme is run by a coordinator and a committee composed of representatives from the holding institutions. In December 1996 there were 120 EEP programmes involving well over 150 different people from various European zoological gardens. Most of these people have indicated that time is a limiting factor in extending their activities [Princée and Rietkerk, 1996]. Therefore, a linear extrapolation of this number may be justified to estimate that active involvement of 1,500 to 3,000 people is required to manage 1,000 to 2,000 zoo populations in Europe. This may seriously limit the number of EEP programmes (and similar programmes in other regions) which can be established.

Coordinated breeding programmes are based on intensive genetic and demographic management which involves 'animal by animal recommendations' [e.g. Ballou and Lacy, 1995]. One solution to the problem of increasing the number of managed

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[9]The '90 percent level' as recommended by Soulé *et al.* [1986] refers to ideal populations. It is based on observations that inbreeding coefficients lower than 0.1 do, in general, not affect the viability of a population [Ballou, pers. comm]. Soulé *et al.* [1986] assume that new technologies for maintaining species will have been developed in 200 years time and replace captive breeding.

populations may be the development of *low-intensity* management models for preservation of genetic variation as these are less time consuming [Lacy *et al.*, 1995; Princée, 1995b; Starfield *et al.*, 1995]. The topic of low-intensity management is covered in this dissertation.

Risks in genetic management

As opposed to wildlife managers, zoo biologists are in the luxurious position of having complete historical pedigree data available through studbooks. Furthermore, most studbooks are recorded in computerized databases such as *Single Animal Record Keeping System* (*SPARKS*) [Scobie and Flesness, 1989] and the *Zooresearch Studbook Management Program* (*ZRBOOK*) [Princée, 1989a, 1991]. These database programmes also include the software for a variety of demographic and genetic analyses such as life table analysis and the computation of inbreeding coefficients. Additionally simulation models, based on Monte Carlo methods, have been developed to estimate various measures of genetic variation (and genetic loss) in studbook populations with complex genealogies. Examples of these simulation models are GENE DROP [MacCluer *et al.*, 1986], GENES [Lacy 1994] and GENEFLOW [Princée, 1988; *Chapter 2* of this study]. These tools enable the comparison of genetic processes in actual populations with theoretical models based on ideal populations. Studies on studbook populations such as that of the Nepalese red panda, *Ailurus fulgens fulgens*, [Princée, 1988, 1989b] have shown that extensive generation overlap and non-random matings violate the assumptions of the Hardy-Weinberg population models. Therefore, methods to compute effective population sizes are not necessarily valid for these populations.

Genetic loss in zoo populations is generally estimated by using simulation models or calculated by formulas for effective population size, what introduces some elements of risks in genetic management due to the stochastic nature of Mendelian segregation. These risks are associated with the use of *expected* values for genetic variation and genetic loss in management strategies. Most models which describe genetic drift deal with expected values of genetic variation. However, the nature of the drift process implies that the fate of any individual small population is unpredictable. Lacy [1987] illustrated the large variance between individual simulation runs in genetic loss at a single locus (with two alleles in equal frequencies in a hypothetical population of 120 individuals. Although management of a population may be optimal in the sense that expected rate of genetic loss is minimized, the actual genetic variation may be well below this expected value. The fact that highly endangered species are subject to population management would suggest that such a risk must be minimized by using empirical data on genetic variation. Biochemical and molecular techniques [see *Genetic variation in populations*, page 8] can be applied to obtain such data. However, the validity of these techniques largely depends on whether the studied variants are neutral or subject to selection and the number of polymorphic loci (in the source population). A large number of (endangered) taxa require management. Costs may limit the use of biochemical and molecular techniques for each population belonging to these taxa. Furthermore, obtaining blood and tissue samples of wild animals can be difficult. Handling of wild animals, especially when sedation is involved, can be associated with mortality risks. These risks are not necessarily associated with medical side-effects of sedation. For example, male impalas, *Aepyceros melampus*, which are affected by immobilization drugs are at risk of being killed by male con-specifics [Siefert, *pers. comm*; Princée, unpublished]. Clearly such risks to endangered species need to minimized. Another possible side effect of handling animals - which can mean temporary isolation from a social group - can have impact on hierarchy and therefore, reproductive success. For example, changes in social rank have been observed in packs of African wild dogs, *Lycaon pictus*, after individuals had been sedated [De Villiers,

pers. comm]. These practical limitations mean that genetic management will more often be based on probabilistic models rather than empirical data. Risk assessment is important considering the fact that many zoo populations are or will be the last resource of unique wild species.

Studies using GENEFLOW have shown that different levels of initial genetic variation result in different variances of mean gene diversity and observed heterozygosity [Princée, unpublished]. This means that it is important to pay attention to the genetic composition used in simulation models. Since genetic variation differs among taxa [see *Genetic variation in populations*, page 8] assumptions regarding genetic composition used in simulation models may need to be population-specific. Different measures of genetic variation also effect management strategies. Observed heterozygosity is more subject to variance than gene diversity [Princée, 1995b]. Average observed heterozygosity and gene diversity (or expected heterozygosity) do not differ under random mating conditions. As indicated previously, the assumption of random mating is violated in zoo populations by both the natural mating-structure and human interference. Levels of heterozygosity and gene diversity differ in populations that are managed as isolated demes [Princée, 1989b]. Furthermore, alleles with frequencies of less than five percent do not contribute substantially to gene diversity or (observed) heterozygosity, but do contribute, depending on arbitrary criteria, to the level of polymorphism, and thus to the adaptive potential. As all these measures provide different information on the genetic status of a population, they must be considered in population management.

Simulation models such as GENE DROP and GENEFLOW do not consider genome structure. Individual loci are treated as being independently transmitted from one generation to the next. However, in reality, genes are organized in chromosomes and linked loci tend to be co-transmitted. This means that linked blocks of genes rather than individual genes are being lost in the process of genetic drift. In "bean bag" models (i.e. models in which genes are independently inherited) the variance in loss depends on the number of independent loci among other factors. It is obvious that for any real organism, the genome structure, i.e. number of chromosomes, map length of chromosomes and distribution patterns of genes over chromosomes, will influence the variance in genetic loss. A large variety of taxa, each with its own genome structure and different genetic composition are, or need to be, managed. To date, genetic management in zoos does not consider these differences in genome complexity. This means that success of genetic management is subject to errors that are based on the species-specific genome structure and the population-specific genetic composition.

Low-intensity models do not presume complete pedigree data but are based on assumptions with respect to mating structures within the population. These assumptions could range from random mating, as in ideal populations, to species specific mating-structure. The use of assumptions regarding mating structure involves additional risks in genetic management. Firstly, inbreeding may occur in these populations at higher levels than would be considered acceptable as individuals may wrongly be assumed to be unrelated. Secondly, it is obvious that predictions of genetic loss in such populations, either estimated by simulation models or theoretical models, are unreliable when data on ancestries are missing and assumptions on parentages have been made.

The original idea behind development of low-intensity management as described in Princée [1995b] refers to various requirements of maintaining species in natural social units under *ex situ* conditions. Strategies to manage such populations may have a broader application value than originally intended. They could be applied in management of small *in situ* populations for which social structure is a natural fact and, consequently, pedigree data are not available. These strategies could also be applied to reduce the amount of time involved in animal by animal recommendations for, especially, larger zoo populations. *Low intensity* in this latter context refers to a more relaxed and less time-consuming genetic management of zoo populations.

Social structure

The privileged position of zoo biologists in studying population genetic processes in studbook populations with good pedigree data may be more limited than stated in the previous section. Different attitudes in the past with respect to (wild) animals and the relatively late implementation of population genetics in breeding management never forced zoos to keep records on ancestry of all their animals. It is likely that the availability of older data is restricted to zoos that were established by scientific zoological societies in the 19th century. Furthermore, these older data are likely to be restricted to rare (endangered) high profile species as for example okapi, *Okapia johnstoni*. Although (computerized) in-house registration of zoo collections has increased greatly at a global level [Flesness and Mace, 1988; Flesness *et al.*, 1995] incomplete data in pedigrees of populations of a number of species will always occur. This refers particularly to those species that live in groups with complex mating structures.

The composition of breeding groups can be manipulated under *ex situ* conditions. Housing species in separate breeding pairs is preferred above harems and colonies in terms of optimal genetic management. Housing in pairs results in a larger effective population size and, thus, minimizes genetic loss, compared to harem systems in which only a limited number of males is allowed to reproduce. Furthermore, parentage in the pair situation is unambiguous. Biochemical and molecular techniques are required to determine parentages in, especially, multi-male/female groups. However, there are various reasons why population managers are confronted with natural social structure, and consequently, poor pedigree data:

- Management of wildlife populations. The metapopulation concept involves interaction between fragmented *in situ* sub-populations and/or between *in situ* and *ex situ* populations. Although the *ex situ* population may be managed in a genetically optimal way (i.e. breeding pairs), the social structure under *in situ* conditions has to be accepted as a natural fact.
- Animals in captivity may not reproduce or do not raise their young if the breeding group does not reflect the species-specific social structure [e.g. Rijksen, 1981; Erwin, 1986; Tilson, 1986]. Raising youngsters in breeding groups that reflect natural social structure may be a necessary prerequisite for successful reintroduction of species.
- A changing attitude towards animals is reflected by studies on animal welfare. Guidelines for housing primates in zoos and other related institutions in the European Community, recommend group composition which resembles natural social units [Griede 1989].
- The educational role of modern zoos as indicated by the IUDZG [IUDZG/CBSG, 1993] is not limited to the display of various species. Visitors get more involved in the protection of species and natural habitat when they can watch natural behaviour rather than when they can only gaze at some external features of the exhibited species [van Hooff, 1986]. Therefore, the ambassador role of zoo animals requires a compromise between social structure and optimal genetic management.

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