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Preface and acknowledgements

I had great difficulty finding a title for this book. For long, the working title was *Genetic Variation and Extinction*. However, this title implies a causal and simple relationship between genetic variation and extinction. I do think that the study of genetic variation is extremely important for conservation biology but, as will become apparent while reading the text, I am not as sure that this relationship is as simple and straightforward as I thought when I began this voyage. I then started to think of alternatives and found two: *Evolutionary Conservation Biology* and *Conservation Biology and Evolution*. Of these, the first one has already been used for the volume edited by Ferriere *et al.* (2004) and I was not happy with the other one. This book is about conservation biology, so the first part is fine, but by using the word *Evolution* in the title I would have had to put more emphasis on the history of life on Earth and on how genetic diversity has evolved on the planet Earth. That is not a topic of this book and therefore I preferred to use the word *Evolutionary*, which implies that evolutionary theory and thinking in a more general sense are a large part of the book. One early morning and during the final stages of writing, I woke up and I decided that the title should be *Conservation and Evolutionary Biology*. However, conservation biology is more than what is covered by this book. What I have done in the following is an attempt to cover the evolutionary aspects of the genetic parts of conservation biology; there are no attempts to review the issues of, for example, habitat management, restoration projects, and the socioeconomic aspects conservation. The final decision on the title was therefore *Evolutionary Conservation Genetics*.

I am indebted to the many people who have helped and aided me while writing this book. My colleagues at the Evolutionary Biology Centre at Uppsala University are acknowledged for providing a world 'read in tooth and claw'. Dianna Steiner assisted in creating the reference list and in my understanding of the mysteries of software for handling references. She also compiled a summary on landscape genetics which was very helpful. Hans Höglund assisted in preparing all the figures in a suitable digital format and also assisted with the handling of references. Ian Sherman, Helen Eaton, and the rest of the staff at Oxford University Press provided much support and understanding during all stages of

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Jacob Höglund
June 2008

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1 *The extinction vortex, is genetic variation related to extinction?*

1.1 Introduction

Extinction is a fact. Ever since organic life first evolved on this planet, life forms have been changing. New species have arisen and old ones have gone extinct (Raup 1992). Speciation, the birth of new species, and extinction, the death of species, are as natural events in evolution as birth and death of individuals in demography. Seen over the entire history of organic life on Earth, biodiversity has generally increased. There has been a build up of life forms. However, five times in the evolutionary past of the planet have mass extinction events taken place. The so-called big five are periods when the rate of extinction of species has become vastly elevated and have outnumbered the level of new species forming (Raup 1994). It is now established that some of the elevated levels of mass extinction coincide with major celestial impacts on the Earth's surface and their climatic consequences, although some workers advocate more complex scenarios that include a number of factors that may explain mass extinction (Erwin 2006). Today we are witnessing a sixth major mass extinction event and this time celestial impact has nothing to do with it. It is beyond doubt that this event is caused by the activities of one of the species inhabiting the Earth: modern humans. I can think of no other scientific activity more important than trying to understand the causes and consequences of this contemporary mass extinction. This book is therefore concerned with a proposition put forward some years ago that extinction of species is somehow related to loss of genetic variation.

It has been suggested that genetic variation is crucial for the persistence of populations (Soulé 1980, 1986, 1987, Frankel and Soulé 1981, Gilpin and Soulé 1986). Two reasons have been given. In the short term, inbreeding and genetic drift leads to lower fitness of individuals and increased extinction risk of populations. In the long term, populations that lose genetic variation cannot evolve since evolution cannot proceed without genetic variation. In a world of rapid environmental change, any population that is unable to adapt to changing conditions will go extinct (Spielman *et al.* 2004).

After initial enthusiasm over this idea much scepticism has been raised. In 1988, Russell Lande wrote an influential paper (Lande 1988) in which he discussed the arguments for and against demographic versus genetic reasons for extinction of endangered populations: "Theory and empirical examples suggest that demography is usually of more immediate importance than population genetics in determining the minimum viable sizes of wild populations. The practical need in biological conservation for understanding the interaction of demographic and genetic factors in extinction may provide a focus for fundamental advances at the interface of ecology and evolution". He thus argued that demographic factors were more important than genetics in explaining why populations go extinct but that the interaction between demography and genetics should be a research focus. Unfortunately the paper has often been cited as an argument against genetic studies in conservation biology (e.g. Pimm 1991, Young 1991, Wilson 1992, Caro and Laurenson 1994, Caughley 1994, Holsinger *et al.* 1999, Elgar and Clode 2001). Recently, a perhaps more balanced view has emerged, in which both genetic and demographic factors are believed to be important in the study of endangered populations and species (Soulé and Mills 1998, Hedrick 2001, Oostermeijer *et al.* 2003). This chapter is a review of genetic studies and examples that suggest a link between genetic diversity and population persistence.

1.2 **The extinction vortex**

Theoretical considerations suggest that small—that is, endangered—populations are different from large ones in two important aspects. The level of inbreeding is increased and likewise the importance of genetic drift, the stochastic loss of alleles, in shaping a population's genetic architecture is increased. Both these processes ultimately lead to loss of genetic variation. Below I examine each of these arguments.

Inbreeding and its consequences on individual fitness will be covered in more detail later in this book. At this point it suffices to define inbreeding as matings between individuals that carry alleles identical by descent. In non-random mating populations, such as species that are fragmented into subpopulations with limited dispersal, the frequency of matings between individuals that carry alleles identical by descent (i.e. relatives) is increased. In diploid organisms this has the consequence that heterozygosity will be reduced. In a closed population of finite size, the rate at which inbreeding will increase as measured by the inbreeding coefficient is given by:

$$F_t = 1 - (1 - (1/2N))^t$$

where N is population size and t is the number of generations since the founding generation (Falconer and Mackay 1996). From this formula it can be seen that F will increase faster with small N and more slowly with large N (Fig. 1.1). It is important to note that inbreeding as such may not have any harmful effects. It is when inbreeding leads to inbreeding depression that endangered populations become severely impacted. I will come back to the issue of inbreeding and inbreeding depression in Chapter 3.

The random loss of alleles due to the stochastic processes of Mendelian segregation and sexual reproduction is more or less negligible in large populations. In large populations selection is the main cause for shaping allele frequencies. However, in small populations the importance of genetic drift becomes a far more important process. Assuming a biallelic locus subject to drift and selection, selection predominates when $4N_e s >> 1$ (where N_e is the effective population size and $1-s$ is the fitness of homozygotes relative to the heterozygote) and drift predominates when $4N_e s << 1$ (Kimura 1983). From these inequalities it is evident that for any given level of selection it is more likely that drift becomes more prominent when N is small.

In general, the proportion of selectively neutral genetic variation lost per generation is $1/(2N_e)$. Small populations (low N_e) thus lose genetic variation faster than larger ones (Wright 1969). In real populations the actual population size N is always higher than N_e due to variance in the number of breeders and family sizes, fluctuations in population size, and unequal sex ratios (Wright 1969). Frankham

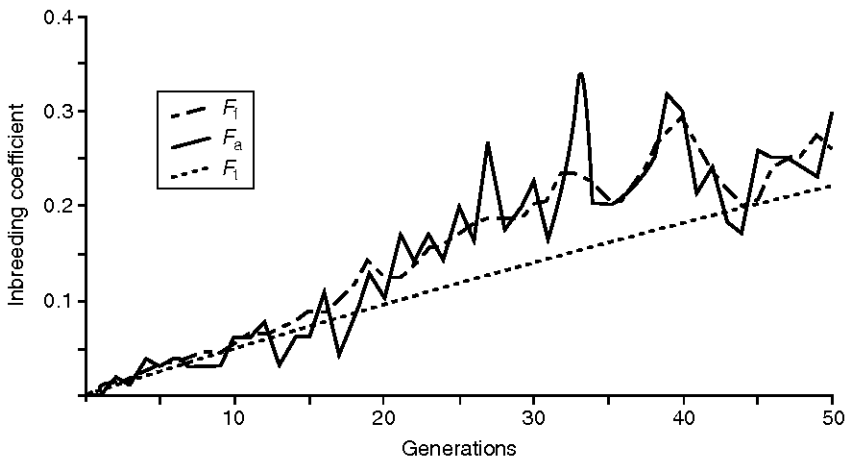

Figure 1.1 Inbreeding increases with time in a closed population. The line (F_t) is the theoretical expectation. The other trajectories (F_a and F_f) are based on stochastic simulation using Populus 5.3.

(1995) suggested that the ratio N_e/N in natural populations would typically be in the order of 0.1.

Large portions of the genome of any organism are selectively neutral, or at least nearly so at any given point in time. It may thus be argued that genetic variation is irrelevant for population survival. However, even if much of the standing genetic variation in an endangered population at any given point in time is selectively neutral, significant and important portions are not. Furthermore, standing genetic variation may be needed when and if conditions change. Alleles that are selectively neutral may become selectively advantageous in the future. Populations that have lost genetic variation have lost the ability to adapt to new conditions and consequently have become more prone to extinction.

To maintain levels of heritable variation in quantitative characters and ensure evolutionary viability, Franklin (1980) suggested a minimum effective populations size of $N_e = 500$. Taken together with the suggestion that a minimum population size of 50 is required to safeguard a population from extinction due to demographic stochastic reasons (Lande 1976), this has become known as the 50/500 'rule'. With $N_e/N = 0.1$ this would mean that the actual population size of any endangered population would need to be in the order of 5000 individuals. Clearly, many endangered populations typically harbour fewer individuals than this. Furthermore, it has been argued that since most genetic variation in quantitative characters in fact is harmful and maintained in the recessive state, only a fraction is quasi-neutral and potentially adaptive. This would increase the critical number to an N_e in the order of 5000 and the critical N to 50 000 (Lande 1995, 1999). If these theoretical considerations apply to real populations, genetic considerations are needed for many populations regardless of whether they are considered endangered or not.

Another harmful result of genetic drift is that drift may cause fixation of mildly deleterious mutations. Fixation of such mutations leads to a reduction in individual fitness which may negatively impact endangered populations. As shown above, drift is more potent in small populations and endangered populations tend to be small. Since accumulation of deleterious mutations speeds up as a population's size decreases, the population may be caught in a negative feedback loop towards extinction. This process has been termed mutational meltdown (Lynch *et al.* 1993). There is controversy over the significance of this process and its relevance to population persistence (see Gaggiotti 2003 for a review). The time scales involved when mildly deleterious mutations accumulate are in the order of hundreds of generations and their effect is only predicted to be severe in very small populations ($N < 100$; Lande 1999).

In empirical research it is often not possible to sort out the relative effects of inbreeding and drift since both processes work in the same direction, reducing genetic variation. A review of data from studies of plant species show that

small and isolated populations typically harbour less genetic variation than large populations within dispersal distance of other populations of the same species (Fig. 1.2).

Both reduction of individual fitness and population adaptability ultimately lead to lower reproduction and increased mortality, factors that further lower an already small population size. When populations are caught in this downward spiral they are said to be trapped in an extinction vortex (Fagan and Holmes 2006) (Fig. 1.3).

1.3 Evidence from wild populations of a link between low genetic diversity and extinction

The extinction vortex hypothesis makes a few clear predictions as to whether genetic factors are important in the extinction of endangered species. The first prediction is that small and endangered populations and species should harbour less genetic variation as compared with taxonomically related non-threatened taxa. This prediction has been tested in an extensive meta-analysis of 170 threatened taxa and their non-threatened sister taxa (Spielman *et al.* 2004). The analysis covered both plants (Angiosperms and Gymnosperms) and animals (vertebrates and invertebrates). Average heterozygosity was lower in threatened taxa in 77% of the comparisons, a result which is significantly different from the null hypothesis of no difference between threatened and non-threatened taxa. On average, heterozygosity was 35% lower in threatened taxa than in non-threatened taxa. These results indicate lowered evolutionary potential, compromised reproductive fitness, and elevated extinction risk for threatened taxa. From this study it is clear that most taxa are not driven to extinction before genetic factors affect them negatively and furthermore that genetic methods in most cases can be employed to diagnose threatened taxa, at least when there is taxon we can identify *a priori* as non-threatened for comparison. The second prediction is that known cases of extinction should commonly be preceded by a radical loss of genetic diversity.

For obvious reasons it is not very common for species and populations that go extinct to have been extensively surveyed for genetic variation prior to their extinction. An exceptional case is the now-extinct heath hen *Tympanuchus cupido cupido* which once inhabited grasslands and barrens along the mid-Atlantic coast of eastern North America. This species was once numerous throughout its former range but went extinct on the mainland by around 1870. The last bird was seen on the island Martha's Vineyard on the 11 March 1932 (Johnson and Dunn 2006). Extraction of DNA from museum skins and subsequent amplification of mitochondrial DNA (mtDNA) has revealed that 30 years prior to their extinction, heath hens on Martha's Vineyard had low levels of mtDNA variation as compared with

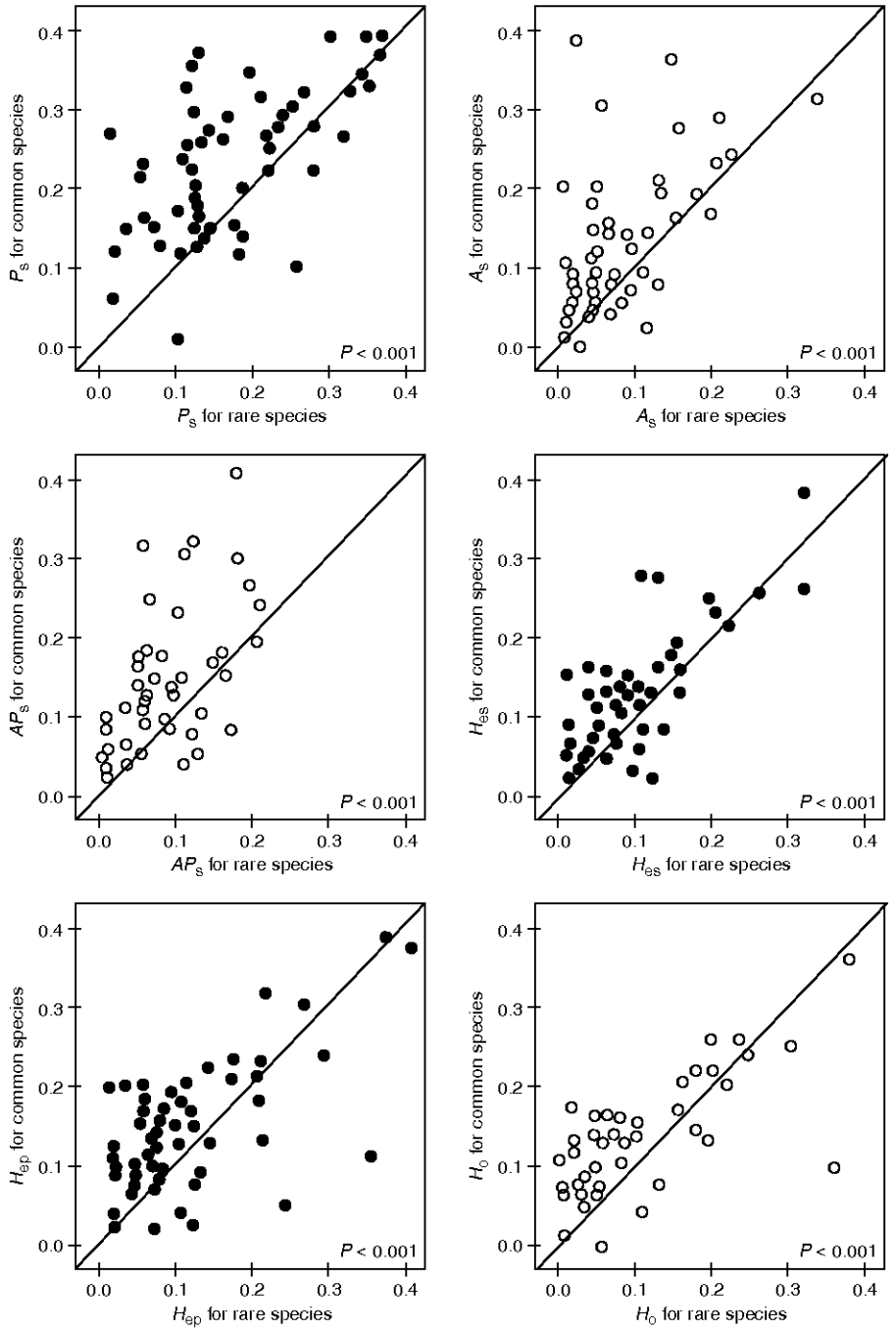

Figure 1.2 Levels of genetic (isozyme) variation in rare and common plant species. The line of equal expectation is drawn through each figure and *P* values are found in the right-hand corner of each graph. Subscript s indicates species-wide values, subscript p indicates the mean of population values. From top left to bottom right: *P*, percentage of polymorphic loci; *A*, alleles per locus; *AP*, alleles per polymorphic locus; H_e, expected heterozygosity; H_o, observed heterozygosity (from Cole 2003, reprinted with permission from the publisher).

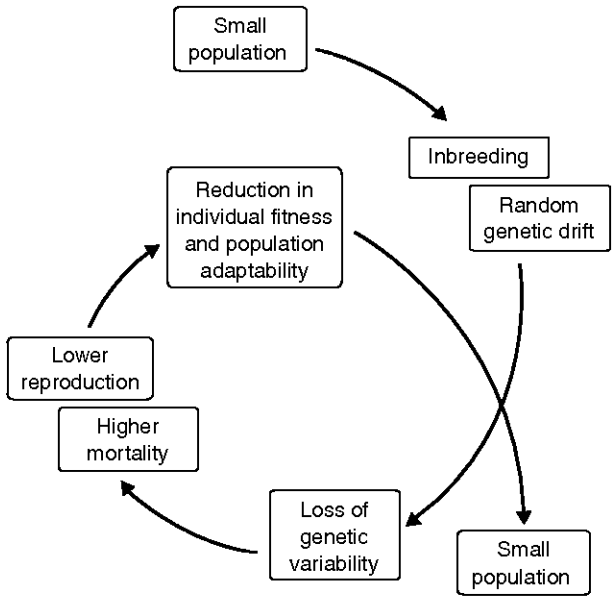

Figure 1.3 A schematic representation of the extinction vortex.

contemporary populations of prairie chickens (which are considered subspecies of heath hens, and all of which are considered presently endangered to varying degrees; Johnson and Dunn 2006).

The species extinction more or less coincided with the settlement of Europeans in North America. Approximately 200 years after the arrival of Europeans and colonization of the eastern United States, heath hens perished on the mainland. Thus it is more than likely that the extinction of heath hens were caused by human actions. Second, the heath hens on Martha's Vineyard indeed had exceptionally low genetic variation prior to their extinction (mitochondrial DNA haplotype diversity, $h = 0.363 + 0.029$; Johnson and Dunn 2006). Other endangered prairie chicken populations typically display a haplotype diversity in the region of 0.900. The only contemporary exception is the extremely endangered Attwater's prairie chicken *Tympanuchus cupido attwateri* which in museum samples from 1951 to 1954 had a haplotype diversity of 0.900, but presently (1998–2000) subpopulations lie in the range of 0.400–0.800, showing that the Attwater's prairie chicken is presently suffering loss of genetic diversity.

Habitat destruction, overexploitation by humans, disease, and poor reproductive success as a consequence of low genetic variation have all been cited as contributors to the decline and extinction of species including heath hens (Gross

1928, Simberloff 1998, Westemeier *et al.* 1998). Throughout this book I will argue that it is likely that all these factors contribute to the extinction of endangered populations: the argument for a role of genetics does not preclude other factors also being important. However, the reverse argument, that genetic factors may be considered less important, have indeed been put forward (Lande 1988, Caughley 1994, Elgar and Clode 2001). In the case of the heath hen I would personally bet on human overexploitation being the main reason for heath hen populations to become small and fragmented. This fragmentation ultimately led to a point when heath hen populations became vulnerable to loss of genetic variation. Whether or not the last heath hen population ultimately went extinct due to genetic effects we can never be certain. However, the last population did indeed show the diagnostics based on mtDNA data of being genetically impoverished. A prudent interpretation of these data is that a multitude of factors may contribute to the extinction of species. Very few, if any, numerous and widespread species go extinct without a period of range contraction, fragmentation, and severe contraction in numbers. A lot is gained in the preservation of biodiversity if populations can be diagnosed as threatened before genetic and demographic stochastic events lead to their extinction. Furthermore, if small and fragmented populations indeed commonly perish due to genetic reasons it is important to prevent this from happening by subjecting such populations to genetic restoration (Ingvarsson and Whitlock 2000, Ingvarsson 2002).

In the above example the ultimate reason for the extinction was unknown. Studies of populations that has nearly gone extinct but have been rescued may provide clues to the role of genetics in extinction. An example of such a species is the Scandinavian wolf. By the late twentieth century, the Scandinavian population of wolves *Canis lupus* had been almost driven to extinction. Only stray individuals persisted and there had been no successful reproduction reported for years. In Finland, however, a few reproducing packs remained. After many years without reproduction one pack in Sweden suddenly produced offspring in 1983, nearly 1000 km from the closest known packs in Finland and Russia (Liberg *et al.* 2005). The Swedish population has since been monitored closely but showed signs of inbreeding depression, such as hereditary blindness, known from captive populations (Laikre and Ryman 1991, Ellegren 1999). Detailed studies of a pedigreed population from 1983 to 2002 showed that the entire Scandinavian population was founded by only three individuals and that the inbreeding coefficient F varied between 0.00 and 0.41 for wolves born during the study period. First-winter survival of pups was strongly negatively correlated with their inbreeding coefficient ($r^2 = 0.39$, $P < 0.001$; Liberg *et al.* 2005). In 1991, the Scandinavian population started to increase and current numbers are now about 10–11 breeding packs annually, corresponding to about 100 wolves. It has been proposed that the sudden increase in numbers coincided with the immigration of a single successful

breeder of Finnish or Russian origin in 1991 (Vilà *et al.* 2003). Vilà *et al.* suggested that of 72 wolves born after 1993, 68 can trace at least part of their ancestry back to this immigrant male. Thus, if correct, the genetic restoration of the Scandinavian wolf population is to a large extent due to one individual. In this case it seems clear that genetic effects cannot be ignored in conservation efforts (Ingvarsson 2002).

Another possible example of genetic rescue is an isolated population of adders *Vipera berus* at the very southern tip of the Scandinavian peninsula. This population suffered from low reproductive rates, possibly caused by inbreeding depression. Following the experimental movement of individuals to this population, reproductive rates has increased (Madsen *et al.* 1999). This suggests that enforced or natural low levels of migration between individuals of endangered populations can restore genetic diversity and reduce the risk of extinction, especially if the cause is inbreeding depression.

Yet another detailed study of possible genetic rescue is the greater prairie chicken *Tympanuchus cupido pinnatus* in midwestern North America. This once widespread species is now split into several disjunct ranges (Bouzat *et al.* 1998a). Especially in the eastern part of the range, in Wisconsin and Illinois, populations have been severely contracted and reduced in numbers. In Wisconsin the estimated population size was 54 850 birds in 1930 (Gross 1930). Since the 1950s the estimate has been around 1500 birds, a number observed also in 2003 (Bellinger *et al.* 2003). In Illinois greater prairie chickens declined from over 25 000 birds in 1933 to about 2000 in 1962 and 46 birds in 1994 (Westemeier *et al.* 1998). In Wisconsin, microsatellite allelic diversity has been shown to have been lost in the contemporary population compared to the historic population sampled from museum skins (Bellinger *et al.* 2003). In Illinois similar observations were made while no loss of alleles could be observed in the larger populations in Kansas, Minnesota, and Nebraska (Bouzat *et al.* 1998a, 1998b). Data from Illinois show that, with the exception of a temporary peak in male numbers in the early 1970s, displaying male numbers have steadily declined since the start of observations in 1963. Corresponding to this decline is a decline in the percentage of eggs hatched in observed clutches. Hatchability went down from a usually observed value of about 90–95% to around 65% by 1990 (Fig. 1.4). Following the translocation of birds in 1992, hatching success was restored to the usual level of around 95% (Westemeier *et al.* 1998). These data suggest that hatching success was impaired due to inbreeding depression and that genetic considerations cannot be ignored while attempting to rescue these endangered populations.

The previous examples have been on animals but the above-cited principles about genetic variation and extinction risk should also apply to plants and other organisms. Yet many botanists have been strong advocates for the case that genetic variation is of minor importance when studying extinction of endangered