Ecological Applications, 2(1), 1992, pp. 30–46

CONSERVATION GENETICS: TECHNIQUES AND FUNDAMENTALS[1]

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Abstract. Conservation genetics utilizes the tools and concepts of genetics and applies them to problems in conservation biology. For example, molecular genetic techniques, such as protein electrophoresis, and analysis of mitochondrial DNA and highly variable nuclear genes (including DNA fingerprinting), have been important in documenting the extent and pattern of genetic variation in endangered species. We review these techniques and their advantages and disadvantages, and give examples of their application to endangered species.

For captive animal populations, pedigree analysis has become the basic approach to evaluate breeding priority of particular individuals. Several pedigree analysis techniques are commonly used, but peeling and gene dropping give the most information. We compare these techniques and illustrate their value with applications to the Guam Rail, Przewalski's horse, and other endangered captive animals.

The rationale for much conservation genetic interpretation is based in evolutionary genetics. We discuss the avoidance of inbreeding depression and the maintenance of genetic variation—both primary conservation genetic goals—from this perspective. In addition, we suggest aspects of these factors that deserve greater attention in their overall application to conservation planning.

Finally, we briefly mention three evolutionary topics—the relationship of heterozygosity and fitness, population bottlenecks, and outbreeding depression—that have implications for conservation genetics. Although simple interpretation in these areas is appealing, we feel that because they are only generally understood and often quite controversial, their application to endangered-species management should be carefully evaluated and monitored.

Key words: additive genetic variation; bottlenecks; conservation biology; DNA fingerprinting; electrophoresis; evolution; extinction; heterozygosity; inbreeding depression; mtDNA; outbreeding depression; pedigrees.

INTRODUCTION

Because of the increase in the human population and the expansion of human-related activity in recent decades, many species have become extinct or are on the verge of extinction (e.g., Ehrlich and Ehrlich 1981). Increased human activity has led to dramatic habitat fragmentation and loss, particularly in the tropics; hence, extinction rates have arisen alarmingly over the last 15 yr (Wilson 1988). Although preservation of most endangered species depends primarily on protection of the species and maintenance of adequate natural habitat, in the past decade substantial attention has focused on the conservation genetics of endangered species and captive animal populations (e.g., Frankel and Soulé 1981, Schonewald-Cox et al. 1983, Ralls and Ballou 1986, Soulé, 1986, 1987, Soulé and Kohm 1989). Conservation genetics may become even more important because some species (and probably many more in the future) exist only in captive situations.

The immediate causes of extinction are often due to several factors, but human predation, introduction of exotic species, and habitat destruction appear to have been important in the extinction of nearly all mammal or bird species in the last three centuries (e.g., Ziswiller 1967). However, even when there appears to be adequate natural habitat and a species is protected, random factors may cause a species to become extinct (e.g., Shaffer 1981, Soulé and Simberloff 1986). These factors can be classified into extrinsic factors, which include variation in the influence of other species such as predators or pathogens (environmental uncertainty) and abiotic variation such as floods, fires, or droughts (natural catastrophe), or into factors intrinsic to the species such as demographic stochasticity or genetic deterioration (see discussion by Lande [1988]).

For each factor, we can in general relate the average persistence time of a population to its size for both natural and captive (or highly managed) populations (Fig. 1). Note that in natural populations of relatively large size, environmental uncertainty and natural catastrophes appear to be the most critical factors limiting persistence, while demographic or genetic factors are of relatively little concern. In small natural populations, however, all four factors may be important;

[1] Manuscript received 7 November 1990; revised 27 February 1991; accepted 7 March 1991.

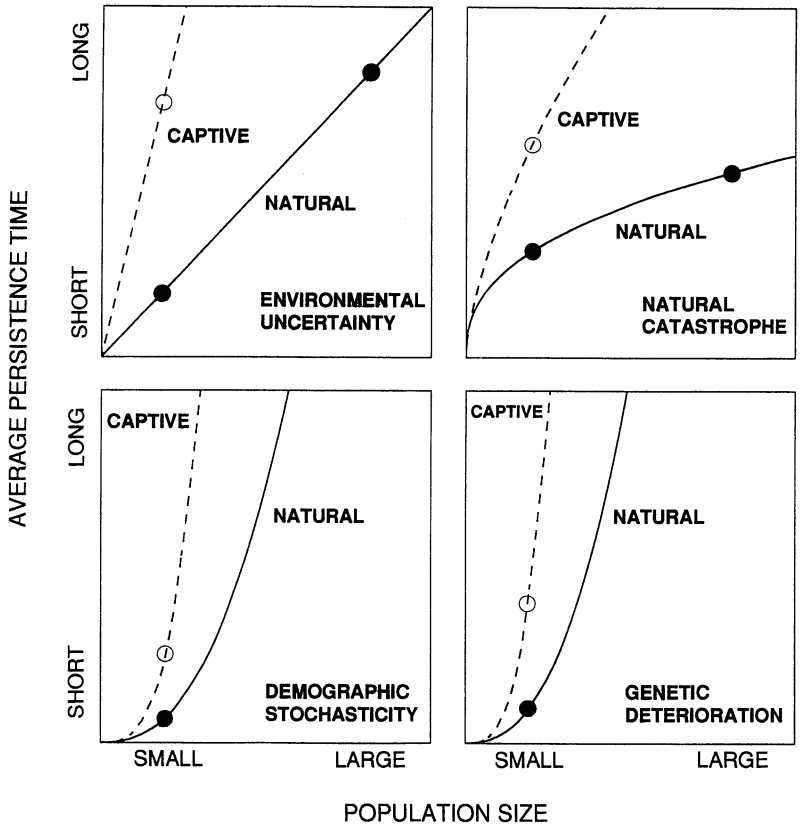

FIG. 1. The general expected relationship between population size and the average persistence time for four factors that influence extinction in captive and natural populations. The circles (○ and ● for captive and natural populations, respectively) indicate the effect on persistence time of a small or large population size.

for example, demographic factors such as sex ratio variation and genetic factors such as inbreeding depression may play pivotal roles in causing extinction. In captive populations of small size probably the most important factors are the intrinsic ones, demographic stochasticity and genetic deterioration, because population fluctuations caused by extrinsic factors, such as disease, predators, weather, etc., can often be avoided.

Because the factors influencing extinction may interact, an approach called population viability analysis (PVA) was introduced to consider the complex interdependence of these factors (Gilpin and Soulé 1986). For example, an environmental perturbation may reduce the population size and induce both detrimental demographic and genetic changes, which in turn can reduce the number of patches occupied by the population. Although in the following review we will discuss genetic factors, please keep in mind this general perspective, which involves a number of potentially interacting factors.

Techniques from molecular evolutionary genetics have recently been applied to various problems in conservation genetics. Because these applications have wide-ranging implications in many areas we will begin this review with an introduction to these techniques and their utility. Because theoretical ideas from population and quantitative genetics have been widely applied to conservation genetics, a major focus of the review is to evaluate and put in a reasonable perspective some of these ideas. First, we discuss developments in pedigree analysis, with emphasis on the techniques of gene-drop analysis and peeling. Second, we introduce some ideas about inbreeding depression and its relation to conservation genetics. Third, we discuss suggestions concerning the population size necessary to conserve a given amount of variation and other factors that may influence genetic variation. Finally, we discuss briefly several topics often mentioned in conservation genetics, namely, population bottlenecks and founder events, the relationship of heterozygosity and fitness, and outbreeding depression.

MOLECULAR CONSERVATION GENETICS

Molecular conservation genetics has received considerable attention in the last decade, and holds great promise as a tool to aid conservation biologists in implementing programs intended to preserve genetic diversity in both captive and natural populations. As a general note, we caution that these techniques only

sample the genetic variation in populations and that other, unsampled loci may have different patterns. In particular, molecular techniques probably do not survey most loci that are adaptively important. We will focus on the methodology and applications to conservation biology of four molecular techniques: protein electrophoresis, mitochondrial DNA analysis, analysis of highly variable nuclear DNA sequences including DNA fingerprinting, and amplification of DNA sequences by the polymerase chain reaction (PCR). Other laboratory techniques, such as blood typing (e.g., Bowling and Ryder 1987) or comparative chromosomal morphology (e.g., Seuanez et al. 1988, de Boer and de Bruijn 1990), are also useful in certain situations.

Protein electrophoresis

The most widely used technique for analyzing genetic variation in plants and animals is protein electrophoresis because it is relatively inexpensive, it allows screening of many individuals, and genetic variants are easily identified. We should mention that by altering the conditions for protein electrophoresis, e.g., temperature, buffer pH, or pore size of the gel, additional genetic variation has been identified in some species (e.g., Coyne 1976, Ramshaw et al. 1979). However, protein variants are often monomorphic in endangered species (see examples below) so that sometimes little information may result from these surveys.

For example, in the widely publicized surveys of cheetahs, *Acinonyx jubatus,* O'Brien and his colleagues (e.g., O'Brien et al. 1983, 1985, 1987*a*) found these animals to be virtually monomorphic at all loci studied. Several recent surveys of endangered plant species have found no genetic variation using protein electrophoresis (e.g., Waller et al. 1987, Lesica et al. 1988). At the other extreme, despite a recent bottleneck a survey in the greater one-horned rhinoceros, *Rhinoceros unicornis,* found a relatively high heterozygosity of 0.099 at 29 loci (Dinerstein and McCracken 1990).

Besides the utility of identifying levels of genetic variability in captive or natural populations of endangered species, electrophoretic studies can be used to help determine the taxonomic status of a given species or subspecies. For example, Daughtery et al. (1990) have found evidence using protein electrophoresis that the tuatara, *Sphenodon punctatus,* a unique reptile found in New Zealand, actually consists of two species. In this case, due to the previous taxonomic misjudgment, isolated populations of *Sphenodon* on the brink of extinction have not been given high priority for management intervention, and 25% of all tuatara populations have gone extinct in the last century. On the other hand, a survey of 23 loci in the Spotted Owl showed no variation within and no differentiation between two subspecies, the Northern Spotted Owl, *Strix occidentalis caurina,* and *S. occidentalis occidentalis,* found in California (Barrowclough and Gutiérrez 1990).

Electrophoresis can also be employed to help estimate the genetic "purity" of captive populations. For example, a survey by Davis et al. (1988) indicated hybridization between domestic cattle and the banteng (*Bos javanicus* d'Alton), but was also able to conclude that the probability of the survival of any domestic cattle genes in the captive banteng population was low. In contrast, O'Brien et al. (1987*b*) found significant evidence for introgression of African lion genes into the captive Asiatic lion (*Panthera leo persica*) population.

Mitochondrial DNA analyses

Although mitochondrial DNA (mtDNA) analysis costs more and requires more technical expertise than protein electrophoresis, it possesses two properties that make it particularly useful in the determination of genetic variation within and among animal and plant populations (e.g., Wilson et al. 1985). First, mtDNA is maternally inherited, thereby allowing the determination of maternal lineages of the population under study. Second, the rate of nucleotide substitution for mtDNA is generally five to ten times greater than in nuclear DNA. Because of this high rate of evolution, and because it is so highly polymorphic within species, mtDNA can be used to examine the geographic structure of populations of a given species or to investigate the differentiation of closely related species when electrophoretic surveys may not be informative. Of course, mtDNA codes for only a small proportion of the total DNA in an organism (mammalian mtDNA has only $\approx$ 16 000 bases), and as a result its lineage may differ greatly from that of nuclear genes (e.g., Ferris et al. 1983).

Analysis of mtDNA was used to examine genetic differentiation in the Dusky Seaside Sparrow (*Ammodramus maritimus nigrescens*), a subspecies that went extinct in 1987 (Avise and Nelson 1989). Comparison of mtDNA sequences between the Dusky and other extant subspecies of Seaside Sparrows revealed no evidence for a phylogenetic distinction between *A. maritimus nigrescens* and the other Atlantic Coast Seaside Sparrow populations, but did show distinct differences between Gulf Coast and Atlantic Coast populations. As a result, the Dusky Seaside Sparrow may not be as distinct as previously thought, leading Avise and Nelson to suggest that resources might have been better directed toward preservation of the two major phylogenetic subunits of *A. maritimus.* Ashley et al. (1990) examined mtDNA variability in two subspecies of the African black rhinoceros (*Diceros bicornis*). In a relatively small sample, they found only a 0.29% divergence between the two subspecies and suggested that the subspecies (*minor* and *michaeli*) might be considered single populations within political boundaries, an approach that would increase the breeding population size.

On the other hand, the hellbender, the largest North American salamander (*Crytobranchus alleganiensis*) is uniform throughout its range for protein variants (Merkle et al. 1977). However, mtDNA analysis showed

that the eastern populations and the northern and southern populations in the Ozark mountains all have nonoverlapping mtDNA types (Templeton et al. 1990), suggesting that the previous subspecies designations (the eastern and the northern Ozark populations as subspecies *C. alleganiensis alleganiensis* and southern Ozark populations as *C. alleganiensis bishopi*) may be incorrect.

Analysis of mtDNA variation can also be used in captive populations. For example, in the Speke's gazelle (*Gazella spekei*), out of 41 mtDNA restriction sites examined by Templeton et al. (1987), only three were polymorphic. Given that the entire captive population in the United States is derived from only three females, this low level of variability was consistent with expectations. Furthermore, tracing these mtDNA patterns through the pedigree revealed the presence of three distinct mtDNA restriction-site patterns for the three female founders, so that it appears that the three founding females were from different mothers. This type of information is important, as it provides evidence for maternal unrelatedness of the founders of a captive population, something that is only an assumption in most cases.

Highly variable nuclear DNA

The most widely used highly variable nuclear sequences are those that compose the variable number of tandem repeats (VNTR) loci used for DNA fingerprinting (Jeffreys et al. 1985*a, b*). Because a number of loci code for these sequences and each locus is quite variable, unrelated individuals generally have a unique genetic "fingerprint." For example, Ryder et al. (1989) found individual-specific DNA fingerprints in a captive population of the giant Galapagos tortoise, *Geochelone elephantopus.* Ely and Ferrell (1990) have used DNA fingerprinting to assess paternity in captive chimpanzees (*Pan troglodytes*). In all of the 21 cases they studied involving multiple potential sires, compatibility with a single sire has been confirmed using DNA fingerprinting. Gilbert et al. (1990) was able to show population differentiation using DNA fingerprints among animals from different islands in the Channel Island fox, *Urocyon litoralis.*

Although DNA fingerprinting can give definitive results, there are often a number of technical problems, including those associated with DNA preparation and interpretation of the gels (e.g., Lander 1989). Furthermore, one must be careful in using DNA fingerprints, realizing that they are phenotypes, not genotypes, and the precise cause of different bands is usually not known. In fact, similar-sized repeats may not be distinguishable from homozygotes (Devlin et al. 1990), and bands of the same size may be produced by alleles at different loci. As the genetic basis of DNA fingerprints becomes more clear, hopefully these ambiguities will be resolved. Recently, Lynch (1988*a,* 1990) has developed the appropriate statistical methods for determining DNA-fingerprint similarity between relatives and for use with other related topics (see also Brookfield 1989, Cohen 1990).

DNA from two other highly variable regions have been used to examine genetic variation: ribosomal DNA (e.g., Templeton et al. 1990) and the major histocompatibility complex (e.g., Gibbs et al. 1990, Yuhki and O'Brien 1990). Furthermore, Lyckegaard and Clark (1989) and Williams et al. (1987) have reported extensive variation on the Y chromosome, which obviously could be utilized to determine paternal lineages and as a complement to mtDNA (see Gibbons 1991).

Polymerase chain reaction (PCR)

A promising area of molecular conservation genetics is the application of the newly developed technique of in-vitro DNA amplification known as the polymerase chain reaction, or PCR. Using this method, a single copy of a nucleotide sequence of interest can be amplified over a million times in only a few hours, in striking contrast to the traditional gene cloning methods that take days to weeks (Saiki et al. 1988). The PCR technique is important in that it requires only a small sample of DNA (single human hairs have been used, Higuchi et al. 1988), making noninvasive sampling of live animals possible as well as the use of preserved or dried specimens from museums or other sources. PCR has tremendous potential to aid in the taxonomic classification of organisms through the direct comparison of a wide variety of specific DNA sequences (Arnheim et al. 1990).

Pedigree Analysis

The catastrophic decline in global biodiversity has led some conservation biologists to conclude that the last refuge for a number of organisms, primarily birds and mammals, may be zoological parks. As a result, research into conserving genetic diversity within captive populations has recently received significant scientific attention (e.g., Ralls and Ballou 1986, Ballou et al. 1991) and has resulted in the Species Survival Plan (SSP) program (AAZPA 1983). The SSP is a cooperative effort among zoos to strengthen and coordinate captive-breeding programs of a selected group of over 40 critically endangered species like the Siberian tiger (*Panthera tigris altaica*; Seal and Foose 1983) and orangutan (*Pongo pygmaeus* ssp.; Perkins and Maple 1990). One of the important tools available to those involved in the genetic management of captive populations is pedigree analysis. With detailed pedigrees it is possible to obtain genetic information, such as the level of inbreeding and the distribution of founder genes within the current members of the population. The identification of genetically important individuals (e.g., Geyer et al. 1989, and discussion below) within a captive population through pedigree analysis has become a vital component of any breeding program because lack of space for animals is a serious problem in zoos. In addition, minimization of inbreeding levels and,

consequently, minimization of inbreeding depression is a primary short-term goal in captive breeding.

Inbreeding levels

The inbreeding coefficient of individual X when lines of descent for that individual are known can be calculated as

$$f_X = \sum_{i=1}^{k} (1/2)^{n_i+m_i+1}(1 + f_{CA_i}), \quad (1)$$

where n_i and m_i are the number of generations from male and female parent, respectively, to a common ancestor CA_i, f_{CA_i} is the inbreeding coefficient of common ancestor i, and k is the number of common ancestors. One of the most common algorithms used to calculate f is the path-analysis technique (e.g., Stevens 1975, Boyce 1983) in which all distinct paths that ascend from one parent of the individual in question to the common ancestor and then descend to the same individual through the other parent are identified. The major stipulations are that no individual can be counted more than once (thereby constituting a "stem" in the path), only ascending links can be constructed while travelling from the first parent to the common ancestor, and once the descent to the other parent has begun, only descending links can be constructed. Another technique to calculate inbreeding levels is the numerator relationship method defined by Quaas (1976) and applied to endangered-species pedigrees through the use of the additive relationship matrix (Ballou 1983). The additive relationship matrix is that matrix with the ith diagonal element (equivalently, the ith individual) equal to $1 + f_i$ (for details about this procedure, see Quaas [1976], Ballou [1983]).

Both of these computational methods were used by MacCluer et al. (1983) in their study of the extent of inbreeding in North American Standardbred horses. In the pedigree for these horses a total of 5207 animals were represented, with paths of co-ancestry tracing back as far as 27 generations. It was determined that remote inbreeding occurring early in the pedigree contributed significantly to f in the current population, suggesting that the common practice of truncating the search for common ancestors after just a few generations may result in a serious underestimate of the true inbreeding coefficients. MacCluer et al. also found that the numerator relationship method of Quaas (1976) is significantly faster and easier to apply to complex extended pedigrees than is the path-analysis technique of Stevens (1975).

Founder allele representation

One of the primary goals of any captive-breeding program is the maximization of founder allele survival. As reintroduction of the species to its original habitat is a high priority, it is crucial to maintain the genetic representation of all the wild-caught founders. With information about genetically important individuals (or important groups of individuals) among the current population, subsequent breeding recommendations can be made with the short-term goal of equalizing founder contributions or achieving particular target founder contributions within the current population (for a discussion of these and other criteria, see Haig et al. [1990]).

One of the more direct ways to determine the genetic contribution of each founder to the current population is by way of the additive relationship matrix (Ballou 1983). Using this matrix, the coefficient of relationship between an individual and a particular founder can be calculated, and the summation of these values over all living descendants in the population gives the expected contribution for that particular founder. An example of the founder contribution of the 16 wild-caught Guam Rails (*Rallus owstoni*) to the current population is given in Fig. 2 (Haig et al. 1990). Five of the potential founders (8, 10, 32, 33, and 36) have not contributed at all. If all the birds had equal representation (parity), they would each have values of 0.0625. In fact, a number have much higher representation (1, 11, and 15), and several besides the ones that have not contributed at all have much lower representation (7, 9, 31, and 98). Unequal representation will lead to lower levels of genetic variation in the population relative to a descendent population derived from the same number of founders in which all have equal representation. Lacy (1989) has defined the number of founder equivalents, f_e, to be that number of founders required to produce the same level of genetic variation as in the pedigree population if all founders had contributed equally to each generation, or

$$f_e = \frac{1}{\Sigma p_i^2},$$

where p_i is the proportion of the alleles of the current population that can be traced to founder i. Calculations on the pedigrees of the okapi (*Okapia johnstoni*) and Goeldi's monkey (*Callimico goeldii*) show the number of founder equivalents to be 60–65% of the true number of founders (Lacy 1989).

Founder allele survival and extinction

An extension of the simple founder contribution calculations is the determination of probabilities of founder allele extinction or survival in a given pedigree. In other words, we can calculate the probability that particular founder alleles are still present in the descendent population. Such an analysis can not only reveal those founders that are underrepresented in the pedigree, but can identify founders whose alleles are probably in very low frequency in the descendent population and are therefore at high risk of subsequent loss. These calculations can either be done using computer simulation (gene dropping) or analytically (peeling).

Gene dropping (MacCluer et al. 1986) is a Monte Carlo simulation method that applies Mendelian seg-

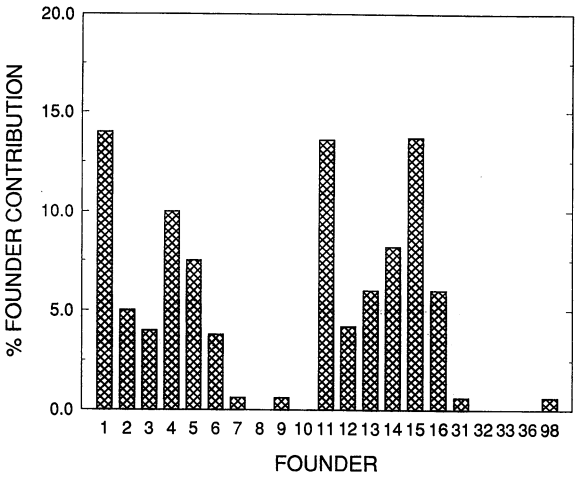

FIG. 2. The contributions of the 16 founders to the current captive Guam Rail population (Haig et al. 1990).

regation to a given pedigree. At the beginning of a simulation, each founder individual is assigned two unique alleles at a hypothetical locus. A pseudo-random number generator is used to determine which of each founder's alleles is passed down to each offspring, a process that continues for each generation through the pedigree. Table 1 shows the kind of information that can be obtained using this method for the Przewalski's horse (*Equus przewalskii*) pedigree (MacCluer et al. 1986). In the first data column is the contribution of each of the 13 founders to the current population, values quite different from parity, which in this case would be $1/13 = 0.077$. Two other measures, the estimated proportion of the genome lost and that at high risk of loss, are also given in Table 1. Note that although these measures are generally associated, there are some exceptions. For example, founders 39 and 40 have the third and fourth highest founder contributions but also the highest proportions of alleles at high risk. The straightforward, simple nature of gene dropping makes it an attractive tool for managing zoo populations.

TABLE 1. Founder contribution and loss of genetic variation in Przewalski's horses determined by the Monte Carlo simulation method "gene dropping" (MacCluer et al. 1986).

		Estimated proportion of	
Founder	Founder contribution	Founder genome lost	Surviving alleles at high risk of loss*
1	0.038	0.663	0.060
5	0.019	0.817	0.077
11	0.126	0.504	0.035
12	0.063	0.710	0.045
17	0.061	0.726	0.019
18	0.061	0.731	0.026
39	0.110	0.338	0.200
40	0.098	0.442	0.165
52	0.019	0.818	0.074
DOM†	0.063	0.713	0.040
211	0.067	0.751	0.010
212	0.201	0.377	0.008
231	0.074	0.123	0.053

* Proportion of simulation replicates in which each allele was present at a frequency of <0.01.
† DOM was a domestic mare.

The technique of *peeling* a pedigree (Cannings et al. 1978) gives exact probabilities for the possible genotypes in the set of current individuals in the pedigree given a particular distribution of founder genotypes. For example, consider a gene with alleles A_1 and A_2 and genotypes A_1A_1, A_1A_2, and A_2A_2. The random vectors $\boldsymbol{C}$ and $\boldsymbol{F}$ give the genotypes of the current population and the founders, respectively. Particular states for $\boldsymbol{C}$ and $\boldsymbol{F}$ are $\boldsymbol{c}$ and $\boldsymbol{f}$, such as $\boldsymbol{c} = \{A_1A_1, A_1A_1, A_1A_2, A_2A_2, \ldots\}$. The relevant conditional probability is then $P(\boldsymbol{C} = \boldsymbol{c} | \boldsymbol{F} = \boldsymbol{f})$ (Thomas 1990). In practice, starting with a distribution of all current individual genotypes, one can work backwards through the pedigree to evaluate the above expression for all possible founder numbers of A_1 and A_2 genotype combinations. When this is done, the joint extinction probabilities for all combinations of founder alleles are obtained, and the probability distribution for the number of distinct founder alleles can then be computed.

Such techniques have been used extensively on human pedigrees (e.g., Thompson 1978, 1981, 1983, Thomas and Thompson 1984) and are now being applied to captive populations of endangered species (Thompson 1986, Geyer and Thompson 1988, Geyer et al. 1989). Of particular interest is the work on the pedigree of Przewalski's horse, now extinct in the wild and the subject of a global captive-breeding program (Geyer and Thompson 1988, Geyer et al. 1989). Peeling techniques have shown that there is a complex dependency on founder allele survival as determined from evaluation of the joint probability distributions. Furthermore, peeling can readily identify genetically important individuals in the Przewalski's horse pedigree–those animals with high probabilities of possessing single-copy alleles (Geyer et al. 1989). Information like this is extremely important in captive-breeding programs where equalization of founder allelic representation is a high priority.

Thompson (1986) graphically illustrates the advantages of peeling (or gene dropping) for calculating founder contributions in a current population over the additive relationship matrix method with data from the human population on the island of Tristan da Cunha (Fig. 3). Ten of the 11 founders of the population could be divided into two genealogical groups, each contributing approximately equally to the descendent population; as determined by the method of Ballou (1983), group A contributes 33% and group B contributes 36%. However, peeling revealed founder group B contributed a significantly greater number of distinct alleles to the descendants (≈ 7) than did group A (≈ 5).

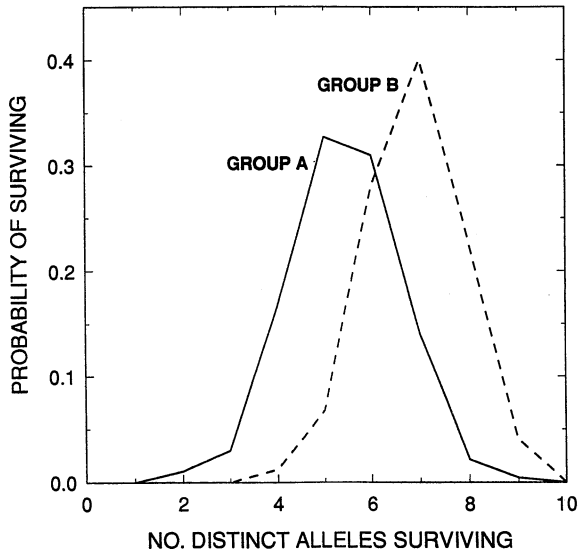

FIG. 3. Allele survival probabilities for two founder groups (each of five individuals) out of the 11 founders of the human Tristan da Cunha population (Thompson 1986).

Notice also that group B must have at least four distinct alleles surviving while group A may have as few as two. The basis for this difference in allele survival is that group A is dominated by multiple copies of two or three alleles from one founder couple.

How do the methods of gene dropping and peeling directly compare with one another regarding the calculation of founder allele representation? In general, one might expect that the calculation of exact probabilities of founder gene survival using the peeling procedure is preferable over approximations obtained through gene dropping. However, peeling is extremely intensive with respect to computer memory requirements; in fact, this requirement increases exponentially with the size of the pedigree, compared to a linear increase with pedigree size in the gene-dropping procedure. Consequently, gene dropping may be the preferred method of analysis on large, complex pedigrees, provided the resulting probability distribution is comparable to that produced by peeling.

A direct comparison of the two procedures is given in Fig. 4 for the analysis of the Oman oryx (*Oryx leucoryx*) pedigree (Thomas 1990). Obviously there is a very close correlation between the results obtained by peeling and by gene dropping, with the largest difference for a given number of alleles being 0.005. It should be noted, however, that in order to achieve the same level of precision as through peeling, 40 000 gene-dropping iterations were necessary, making gene dropping much more time intensive than peeling. The lack of precision associated with the gene-dropping technique with small numbers of replicates is its major weakness, particularly when events of very small probability are the subject of study. Thomas (1990) concluded that both techniques have their respective places in pedigree analysis; a practical mode of operation might be to attempt peeling on the pedigree of interest and, if this turns out to be prohibitively complex, gene dropping with a sufficient (but manageable) number of iterations can be substituted.

Inbreeding Depression

The detrimental effects of inbreeding on the fitness of a species have been long known and were even noted in the last century by Darwin (1868, 1876). Early in the development of Mendelian genetics, researchers realized that the increased homozygosity resulting from inbreeding caused a loss in fitness, a phenomenon termed inbreeding depression (see Lerner [1954] for an extensive discussion). There has been debate for

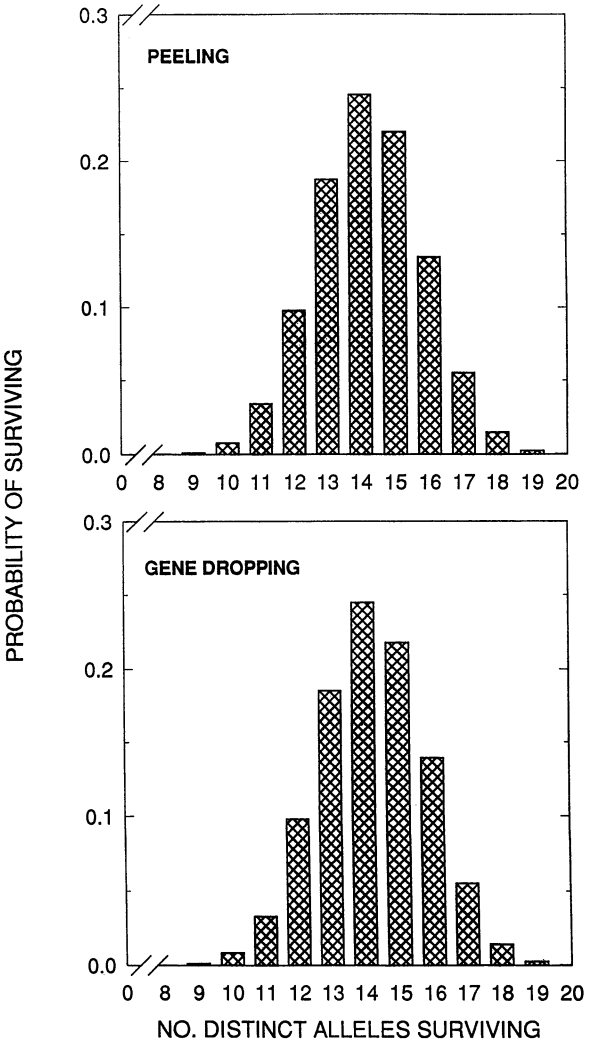

FIG. 4. The probability of the number of alleles surviving in the Oman oryx, as calculated by the techniques of peeling and gene dropping (Thomas 1990).

many decades over the genetic basis of inbreeding depression (and its complement, heterosis), some advocating a dominance explanation and others an overdominance (heterozygous advantage) hypothesis (e.g., Wright 1977). Current evidence generally indicates that homozygosity for unfavorable recessive alleles is the major cause of inbreeding depression (e.g., Charlesworth and Charlesworth 1987). More specifically, detailed studies in *Drosophila melanogaster* indicate that homozygosity of recessive lethals in inbred offspring accounts for approximately half of the observed inbreeding depression in viability (for reviews, see Simmons and Crow [1977] and Crow and Simmons [1983]). The majority of the remainder appears to be caused by homozygosity of detrimental alleles ranging from semi-lethals to mildly deleterious variants.

For a single locus, inbreeding depression can be defined simply as the difference in the mean fitness between an outbred and an inbred population, $\bar{w}$ and $\bar{w}_f$, respectively, or

$$\bar{w} - \bar{w}_f = sfpq\,(1 - 2h), \tag{3}$$

where f is the inbreeding coefficient, p and q are the frequencies of alleles A_1 and A_2 and 1, $1 - hs$, and $1 - s$ are the relative fitnesses of genotypes A_1A_1, A_1A_2, and A_2A_2, respectively, and h is a measure of dominance (e.g., Hedrick 1983). In other words, the inbreeding depression is expected to increase linearly with both inbreeding and the selection coefficient s and is highest for loci with intermediate allelic frequencies. Furthermore, for there to be inbreeding depression, the fitness of the heterozygote must be closer to that of the favorable homozygote than the deleterious one ($h < \frac{1}{2}$).

A model to describe the general impact of inbreeding (for all loci) on the fitness in a population was given by Morton et al. (1956) as

$$\bar{w}_f = e^{-(A+Bf)}, \tag{4}$$

where A indicates the influence of the environment and genetics on fitness in a random-mating population and B is the rate at which fitness declines with a change in inbreeding. Generally, Eq. 4 is applied to viability measures, such as juvenile survival or egg-to-adult survival. In this case, B is a fairly good measure of the number of lethal equivalents per gamete, i.e., the effective number of genes containing lethal alleles in a gamete (for discussion, see Crow and Kimura [1970] and Cavalli-Sforza and Bodmer [1971]).

This approach is useful when matings producing offspring with different inbreeding levels (f_i) are available. If we take the natural logarithms of Eq. 4 and assume that the inbreeding level f_i results in viability v_i, then

$$\ln(v_i) = -A - Bf_i, \tag{5}$$

and B becomes the regression coefficient in a linear regression equation that can be used to estimate the number of lethal equivalents (e.g., Templeton and Read 1984). (However, because these data are not independent, a standard least-squares approach may be useful [Lynch 1988*c*].) As an example of this approach, Table 2 gives the estimated number of lethal equivalents for the 10 species with the most extensive data from Ralls et al. (1988). The estimate of the number of lethal equivalents in this group ranges from 0.01 in the Sumatran tiger to a high of 9.17 in the brown lemur. Also given in Table 2 is the cost of inbreeding i (reduction in survival) for $f = 0.25$ (matings between first-degree relative such as full sibs), which is

$$i = 1 - e^{-0.25B}. \tag{6}$$

This value ranges from 0.003 (Sumatran tiger) to 0.90 (brown lemur), with an average of 0.31 (the maximum of i is 1.0). To give the cost of inbreeding some perspective, note that in this data set it is on average 1.24 times the inbreeding coefficient for this single aspect of fitness (juvenile survival). In fact, Soulé (1980) suggested as a general rule that a 10% reduction in a given fitness trait occurs with a 10% increase in the inbreeding coefficient, and that, when total fitness is considered, a 10% increase in f results in a 25% reduction in fitness. Obviously, from Table 2, some animals have much more inbreeding depression than this while other species have much less.

TABLE 2. The number of lethal equivalents and cost of inbreeding when the inbreeding coefficient $f = 0.25$ (i.e., full-sib mating or parent–offspring mating) for 10 species with sample sizes (N) greater than 100 (Ralls et al. 1988).

Species	N	B (no. lethal equivalents)	Cost of inbreeding, i (with $f = 0.25$)
Short bare-tailed opossum (*Monodelphis domestica*)	251	0.43*	0.10
Elephant shrew (*Elephantulus rufescens*)	218	2.12	0.41
Golden lion tamarin (*Leontopithecus r. rosalia*)	974	2.15*	0.42
Brown lemur (*Lemur fulvus*)	136	9.17*	0.90
Greater galago (*Galago c. crassicaudatus*)	251	1.69*	0.34
Gaur (*Bos gaurus*)	182	0.51	0.12
Dorcas gazelle (*Gazella dorcas*)	143	1.85*	0.37
Bush dog (*Speothos venaticus*)	176	0.24	0.06
Sumatran tiger (*Panthera tigris sumatrae*)	427	0.01	0.003
Pygmy hippopotamus (*Choeropsis liberiensis*)	419	1.59*	0.33

* Slope significant at .05 level.

Although inbreeding depression has been a topic of interest in animal breeding (Wright 1977), plant mating systems (Charlesworth and Charlesworth 1987), and determination of the genetic load of mutations (Crow and Simmons 1983), there are several aspects of inbreeding depression critical to conservation genetics that have not been adequately addressed. First, most studies of inbreeding depression have focused on aspects of viability. However, viability is only one component of fitness, with total fitness resulting from viability, fecundity, and mating components (e.g., Christiansen and Frydenberg 1973, Hedrick and Murray 1983). In fact, other aspects of fitness such as fecundity (Marinkovic 1967), male mating success (Pendlebury and Kidwell 1974, Sharp 1984), and seed yield (Cornelius and Dudley 1974, Levin and Bulinska-Radomska 1988, Karron 1989) may be greatly influenced by inbreeding. For example, using a competitive index to estimate fitness in *Drosophila,* Latter and Robertson (1962) estimated that for small inbreeding coefficients the reduction in overall fitness was 2.7 times the inbreeding coefficient.

An ingenious approach was used in *Drosophila* (Sved and Ayala 1970, Sved 1971) to determine the total (or net) fitness of chromosomes (see also Mackay 1985). For example, Sved (1971) estimated that the average relative viability of 24 individual isogenic (completely homozygous) chromosomes was 0.73. He then estimated the total fitness of the chromosome from their equilibrium frequency when competing with a mutant chromosome. The average total fitness value was 0.28. This large additional effect, 62.5% of the total inbreeding effect, he attributed to other components of fitness, specifically female fecundity and male mating success.

Second, the effect of inbreeding on components of fitness has generally been estimated for populations in a benign or optimal environment. For example, the captive animals analyzed for inbreeding depression by Ralls et al. (1988) were in situations in which there were no predators, no competitors, few pathogens, and no severe physical environmental factors such as extreme heat or cold. When examining isogenic chromosomes in *Drosophila,* optimum temperature and low densities are generally utilized. In other words, the effects of inbreeding on fitness may be much more severe than generally suggested if inbred individuals are more sensitive to stressful or extreme environments (e.g., Lerner 1954, Dudash 1990, Schmitt and Ehrhardt 1990).

Third, a simple analysis based on Eq. 3 implies that the amount of inbreeding depression is a linear function of the inbreeding coefficient, and that the extent of inbreeding depression is a static one. Given a certain level of inbreeding for a period of time, the frequency of lethal or detrimental alleles will decline from the level before inbreeding because inbreeding exposes more recessive lethal or detrimental alleles to selection in homozygotes, thereby causing a reduction in their frequency. Assuming that inbreeding continues at a given level for a long time, the new equilibrium frequency of allele A_2 is

$$q_e = \frac{-f + \left[f^2 + 4(1 - f)\frac{u}{s}\right]^{\frac{1}{2}}}{2(1 - f)} \qquad (7)$$

where u is the mutation rate to A_2 (Haldane 1940). In other words, Eq. 3 may not be sufficient because a noninbred and an inbred population generally have different allelic frequencies at selected loci.

Finally, the extent of inbreeding depression is expected to be greater in a small population than a large population for a given inbreeding coefficient because of the reduced effectiveness of selection relative to genetic drift (chance genetic changes due to finite population size). For example, the equilibrium mutation load is larger in smaller finite populations (Kimura et al. 1963). A genetic drift–selection–mutation equilibrium may not occur in very small populations, resulting in a rapid decline of fitness or "mutational meltdown" (Lynch and Gabriel 1990).

Proposals for viable population size and breeding plans for captive populations suggest that inbreeding depression can be minimized by various breeding strategies that may reduce the inbreeding depression. For example, Franklin (1980) states that "animal breeders accept inbreeding coefficients as high as a one percent increase per generation (i.e., an effective population size, N_e, of 50) in domestic animals without great concern" because such slow inbreeding allows selection to remove deleterious alleles without endangering the population (Soulé [1980] also made the same proposal). In fact, Tantawy and Reeve (1956) and Latter and Robertson (1962) demonstrated that a slow increase in inbreeding results in less inbreeding depression than a fast increase.

We should note that this number of 50, based on inbreeding depression, and that of 500, based on maintenance of genetic variation (see below) were only intended as general guideposts (for further discussion of N_e and these numbers, see Lande and Barrowclough 1987). For example, a number of endangered species with successful breeding programs have had quite low founder numbers, e.g., Przewalski's horse, 13 founders; Speke's gazelle, 4 founders; black-footed ferret (*Mustela nigripes*), 6 founders; red-ruffed lemur (*Varecia variegata variegata*), 7 founders; Guam Rail, 16 founders, etc. In other words, just because the founder number is low does not mean that all attempts should not be made to save the species. Eventually numbers may be high enough to overcome the detrimental effects of the initial population size restriction.

Templeton and Read (1983, 1984) have suggested that in certain situations a radical breeding program which rapidly increases the inbreeding coefficient will purge a population of lethals and avoid the effects of

inbreeding depression over many generations (see also Hollingsworth and Maynard Smith 1955). The success of a breeding scheme to purge lethals would depend upon the reproductive potential in the species, with the probability of success being much less in species with low intrinsic rates of increase. Furthermore, intentional inbreeding and selection may greatly reduce the genetic variation at other genes (non-lethal genes). In other words, the goal of reducing inbreeding depression by purging lethals may cause a reduction in the genetic variation necessary for future adaptation and successful reintroduction as well as possibly increase the short-term probability of extinction.

It has been suggested that concern over inbreeding depression is overstated, based on the observation that highly inbred lines of mice, rats, and *Drosophila* exist and that endangered species such as Pere David's deer (*Elaphurus davidianus*) and the European bison (*Bison bonatus*) both appear to have healthy populations even though they both were initiated from very small numbers. However, often the surviving inbred lines are very few (in a survey of seven studies in five species, Soulé [1980] found that between 0 and 14.3% of the initial lines survived intense inbreeding), allowing strong selection among lines to occur. In the two endangered species mentioned above, it is likely that many of the deleterious alleles were eliminated by a slow increase in inbreeding combined with selection (Frankel and Soulé 1981).

It therefore appears necessary to consider the joint dynamic effects of inbreeding and selection (as well as genetic drift) on fitness. Organisms in populations that have a long history of inbreeding appear to have lower inbreeding depression (e.g., Rao and Inbaraj 1977, Charlesworth and Charlesworth 1987, Hedrick 1987), although even highly self-fertilizing populations may still have some inbreeding depression (Charlesworth et al. 1990). Most endangered species are outbred and are only now with their present low numbers experiencing inbreeding and, therefore, generally have high inbreeding depression. Obviously, there will be some endangered species with low levels of inbreeding depression, as illustrated in Table 2.

Maintenance of Genetic Variation

One of the major long-term goals of conservation genetics is the retention of enough genetic variation so that future adaptation, successful expansion, or reestablishment in natural populations is possible. Franklin (1980) suggested that in general an effective population size of 500 would be necessary to retain the genetic variation at a level found in a natural population. This size estimate was obtained by determining the mutation–genetic drift equilibrium for a quantitative trait, i.e., the value at which the additive genetic variance is increased by mutation to the same extent it is reduced by genetic drift. Genetic drift acts through the effective population size N_e such that

$$V_{a(t+1)} = V_{a(t)}\left(1 - \frac{1}{2N_e}\right), \tag{8}$$

where $V_{a(t)}$ is the additive genetic variance in the tth generation. The same relationship between generations, reduction by a factor of $(1 - 1/[2N_e])$ is also true for heterozygosity (e.g., Crow and Kimura 1970, Hedrick 1983).

Soulé et al. (1986) expanded this initial framework to consider demographic differences that occur between species and to focus on more exact goals. They suggested that a principal aim of captive breeding should be the maintenance of 90% of the genetic variation in the source (wild) population over a period of 200 yr (or some similar target). In their treatment the actual population size necessary to achieve this level is primarily a function of the size of the initial founder population, the rate of population growth to the final population size, and the generation length.

The generation of additional additive genetic variance by mutation, V_m/V_e, where V_m is the additive genetic variation due to mutation and V_e is the environmental variance, has been estimated to be ≈ 0.001 per generation (Lande 1975, Lynch 1988*b*). For mutation to increase the genetic variance by the amount that genetic drift reduces it, then

$$\frac{V_m}{V_e} - V_a\left(\frac{1}{2N_e}\right) = 0 \tag{9}$$

(e.g., Clayton and Robertson 1955). In other words, if $V_m/V_e = 0.001$ (and $V_a = 1$), then $N_e = 500$. Although this number and the value of 50 related to inbreeding depression have been widely cited and somewhat abused, their original intent was to give general guideposts for the genetic management of endangered species (see discussion earlier in *Inbreeding depression*).

In order to focus on these problems, let us introduce a basic discrete time model that allows predictions about genetic variation with different population parameters (this follows the continuous time treatment of Lande 1991). Assume the population grows geometrically with discrete, non-overlapping generations so that, given the effective number of founders is $N_{e(0)}$, the effective number of individuals in generation t is

$$N_{e(t)} = N_{e(0)}R_0^t, \tag{10a}$$

where R_0 is the finite rate of growth per generation ($R_0 = e^r$ where r is the intrinsic rate of increase). If we are concerned with a seasonal breeder, then the effective population size in year y is

$$N_{e(y)} = N_{e(0)}\lambda^y, \tag{10b}$$

where λ is the finite rate of growth per year ($\lambda = e^{r/T}$ where T is the generation length). If we assume that V_a is reduced by genetic drift and increased by mutation, then the recursion relationship for V_a between generations is

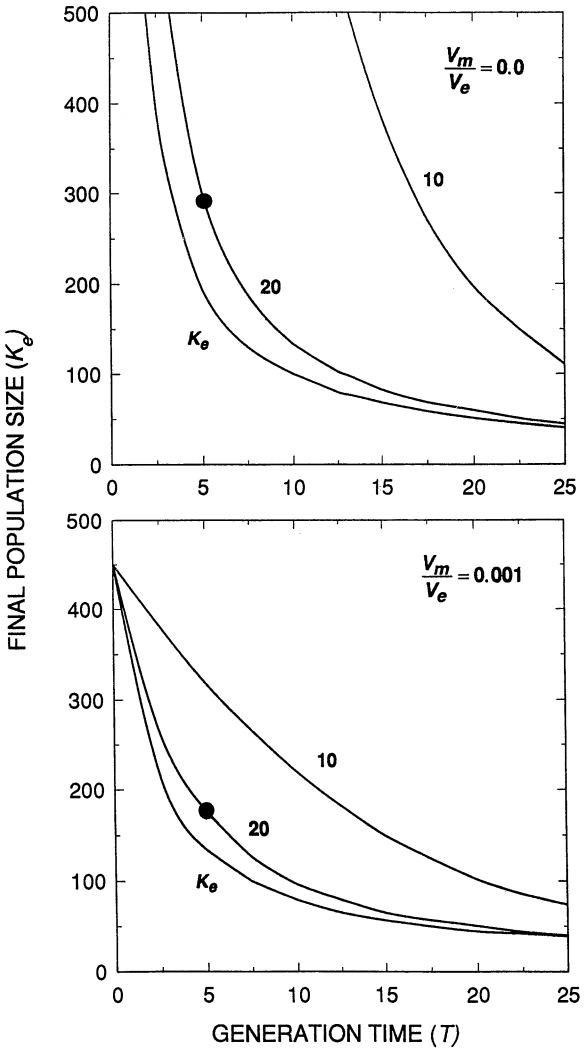

FIG. 5. The final effective population size (K_e) necessary to retain 90% of the additive genetic variation after 200 yr for organisms of different generation lengths when $r = 0.5$ and the number of founders ($N_{e(0)}$) is 10, 20, or K_e. The top part of the figure gives these values when there is no mutation, while the bottom gives the value when variance due to mutation $V_m/V_e = 0.001$ (Lande 1991). ● indicates the final population size necessary when $N_{e(0)} = 20$ and $T = 5$.

$$V_{a(t+1)} = V_{a(t)}\left[1 - \frac{1}{2N_{e(t)}}\right] + \frac{V_m}{V_e}, \qquad (11a)$$

and between years is

$$V_{a(y+1)} \approx V_{a(y)}\left[1 - \frac{1}{2TN_{e(t)}}\right] + \frac{V_m}{V_eT} \qquad (11b)$$

when the influence of genetic drift is small so that the effects are linear over one generation (following Hill 1979).

If we assume $V_m/V_e = 0$ and that $r = 0.5$, the final effective population size, K_e, necessary to retain 90% of the variation after 200 yr is given in the top half of Fig. 5 (after Lande 1991). For example, when the effective number of founders is 20 and the generation time is 5 yr, the final effective population size must be ≥ 280 to retain 90% of the variation (indicated by the solid circle). As also shown by Soulé et al. (1986) for heterozygosity (which has the same dynamics as V_a in this case), if the generation time is short, and even with a final effective size of 500, 90% of the variation cannot be retained in this case.

If mutation is allowed to contribute to the genetic variance, then the generation of new variation via mutation may overcome the loss of variation from genetic drift when the population size is relatively large. The bottom half of Fig. 5 illustrates that given $V_m/V_e = 0.001$ when, for example, the effective number of founders is 20 and the generation time is 5 yr, the final population size must be only 180 (solid circle). Mutation has the most substantial effect when the effective population is near the mutation–genetic drift balance. For example, if $V_m/V_e = 0.001$ and if $N_{e(0)} = K_e = 450$, 90% of V_a will be retained.

These findings when mutation is included are somewhat reassuring with higher N_e values. Although it is thought that generation of additive genetic variance by mutational input is generally independent of population size (e.g., Lynch 1988*b*), for heterozygosity when N_e is lower, mutation will have a much less significant influence in generating genetic variation (see also Lacy [1987]). However, the generation of variation by mutation is probably variable over time and may not occur at the regular rate suggested here. In addition, the new mutants have not gone through the sorting process of natural selection, and they may, for example, reduce fitness (e.g., Simmons and Crow [1977] find that most new mutations are deleterious and not completely recessive) as well as increase variance. Such concerns and other topics have been widely contested in the recent quantitative genetics literature (e.g., Turelli 1986), but because we are concerned with avoiding extinction of endangered species, relying on variation generated by mutation does indeed seem risky.

In addition, Lande (1991) has examined the impact of population subdivision on genetic variation. If a species is split into separate subpopulations with no gene flow among them, alternate alleles may be fixed in different subpopulations, permanently retaining them in the total population. However, extreme inbreeding depression may occur in these subpopulations, and the character of genetic variation may be irreversibly changed, e.g., from a natural degree of heterozygosity to a very low level within a subpopulation (see Hedrick et al. 1986).

If subpopulations go extinct and are recolonized, then the rate and pattern of loss of genetic variation may be quite different from that predicted by knowing only subpopulation size (e.g., Gilpin 1991). Maruyama and Kimura (1980) and Ewens (1989) analytically exam-

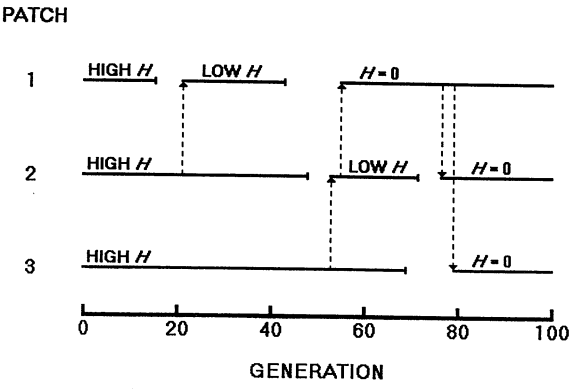

FIG. 6. The level of heterozygosity (H) over time in a simulation of a population existing in three patches (after Gilpin 1991). Short vertical bars of right-hand end of horizontal lines indicate extinctions in a patch, and the arrows indicate recolonization.

ined the loss of genetic variation in such metapopulations under certain conditions (see also Slatkin 1977, Wade and McCauley 1988). Gilpin (1991), on the other hand, using computer simulation, has attempted to evaluate the influence of metapopulation dynamics on genetic variation in a model that "emphasizes both ecological and genetic realism."

Investigating an array of extinction and recolonization rates of patches (subpopulations), Gilpin (1991) found a broad range of conditions in which patch coalescence occurs, i.e., all occupied patches of the metapopulation descend from individuals in a single patch in the past. As a result, even though the numbers in the metapopulation have always been and still are large, there is no genetic variation left. To illustrate this phenomenon, a diagrammatic representation of the results of a three-patch metapopulation simulation is given in Fig. 6 (after Gilpin 1991). Initially all subpopulations have high heterozygosity (they each have $N_e = 500$). The important sequence of events starts in generation 48 when patch 2 goes extinct and is recolonized from patch 3 with a consequent reduction in heterozygosity. Next patch 1 is colonized from patch 2 with a founder population having no genetic variation. When patch 2 goes extinct in generation 71, the metapopulation has no variation although there are still 500 individuals in patch 1. All of these individuals can be traced back to some individuals in patch 3 before generation 51.

Gilpin (1991) has estimated the general range of colonization and extinction probabilities in which patch coalescence occurs, i.e., the species (metapopulation) would not become extinct but all variation would be lost (see Fig. 7). Perhaps such a scenario could explain the low genetic variation in cheetahs; at least it may be a reasonable alternative to the bottleneck hypothesis suggested by O'Brien et al. (1985). In any case, the loss of genetic variation with these metapopulation dynamics is much greater than predicted from knowing the current population size. To make these findings relevant for quantitative traits, mutation would need to be included.

Other Topics

Relationship of heterozygosity and fitness

A number of biologists suggest that heterozygosity as measured by electrophoresis is a good measure of individual fitness or population variation in fitness, i.e., potential for inbreeding depression. Some studies have found a positive correlation between protein variation, either of particular alleles, genotypes, or heterozygosity, and traits related to individual fitness, although other studies have not (for different perspectives on these results, see Mitton and Grant [1984], Zouros and Foltz [1987]). These correlations suggest that some protein variants may have different intrinsic fitness values, or, perhaps more likely, that they are marking selectively important regions of the genome (see Hedrick et al. 1986). In carefully designed experiments in which the genetic background of a protein-coding locus was made isogenic (e.g., Dykjhuizen and Hartl 1980) or randomized (e.g., Yamazaki 1971, Houle 1989), there appear to be few detectable selective differences among individuals carrying different alleles at the locus in question.

In fact, the actual cause of an association between fitness and individual heterozygosity may not be due to any intrinsic properties of the loci (or associated linked region) examined. Such an association may stem, for example, from the composite nature of the sample, which may include some inbred individuals (see Ledig 1986). To illustrate, let us assume that selfed and random-mated progeny consist of 25% and 75% of a sam-

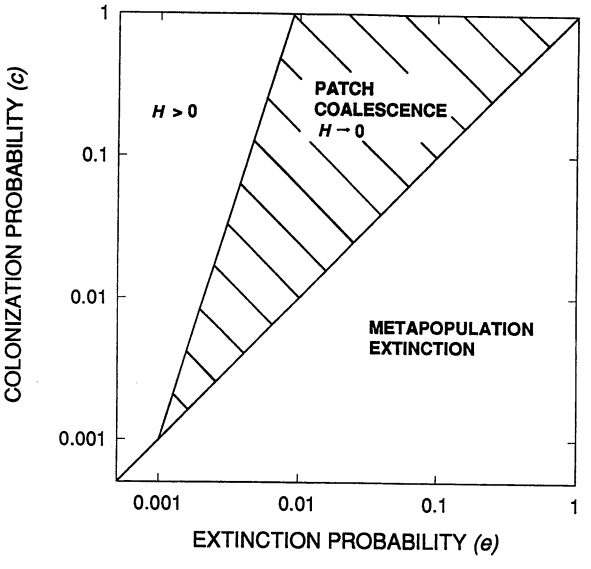

FIG. 7. The general combinations of colonization and extinction probabilities that result in heterozygosity (H) > 0, patch coalescence (H approaching 0), or extinction of the metapopulation (after Gilpin 1991).

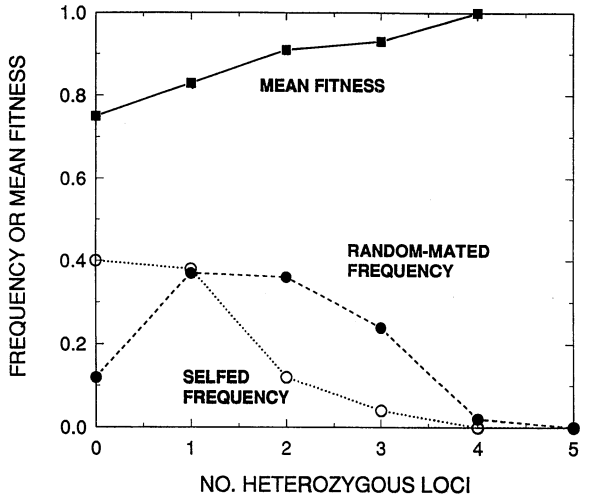

FIG. 8. The frequency of selfed (○) and random-mated (●) progeny having different heterozygosities, and the fitness (■) for different heterozygote classes when a sample is composed of 25% selfed progeny and 75% random-mated progeny, and the fitness of selfed progeny is half that of random-mated progeny.

ple, respectively, and that the selfed progeny have half the fitness of the random-mated proportion. The bottom two lines of Fig. 8 give the expected frequency of progeny with different numbers of heterozygous loci produced from random mating or from selfing (see Hedrick [1990] for details). Because the selfed progeny have both a lower individual heterozygosity and a lower fitness than the random-mated proportion, the composite sample shows a decreasing fitness (top line in Fig. 8) with decreasing individual heterozygosity. As a result, there needs to be an objective evaluation of the relationship of individual heterozygosity with fitness, as well as an effort to understand the causal mechanisms before such associations are used in conservation genetics.

A number of researchers have suggested that populations with higher fitness or higher adaptive potential may be identified by their level of heterozygosity (e.g., Soulé 1980, Quattro and Vrijenhoek 1989). Although a positive correlation between population fitness and heterozygosity is sometimes seen, inferring population fitness from the heterozygosity level is quite risky (e.g., Hedrick et al. 1986). For many endangered species, avoiding the reduction in fitness due to inbreeding depression is a high priority (see *Inbreeding depression,* above). If the heterozygosity level could be used to predict inbreeding depression, this would be an important conservation tool.

However, scenarios can be constructed in which a population may have any combination of heterozygosity and inbreeding depression (Table 3). First, these two measures of genetic variation may be positively correlated, as in large populations at equilibrium (both heterozygosity and inbreeding depression high) or a population after a severe bottleneck (both are low). However, they may also be negatively correlated, as in a population some time after a bottleneck in which there has been time for recovery of quantitative variation that causes inbreeding depression but not enough time for recovery of heterozygosity. Remember that the rate of input of additive genetic variance to quantitative traits from mutation is ≈ 0.001 per generation, while mutation rates for electrophoretic loci are approximately three orders of magnitude lower (e.g., Voelker et al. 1980). Finally, high heterozygosity and low inbreeding depression may occur when two populations have combined after bottlenecks that reduced inbreeding depression in both but differentially fixed them for electrophoretic variants. In fact, a recent extensive study in subspecies of the mouse genus *Peromyscus* found little association between levels of heterozygosity and inbreeding depression (Brewer et al. 1990).

Population bottlenecks

It is generally agreed that population bottlenecks should be avoided as much as possible in endangered species because the general effect of a bottleneck is to reduce genetic variation and increase the level of inbreeding. In recent years there have been several experimental studies that suggest that bottlenecks may sometimes increase the additive genetic variation (e.g., Bryant et al. 1986, Lopez-Fanjul and Villaverde 1989). However, in these studies the mean value was greatly reduced for fitness-related traits (viability was reduced 32% in Bryant et al. [1986] and 21% in Lopez-Fanjul and Villaverde [1989]). In other words, even if the potential or actual response to selection is sometimes increased by a bottleneck, first the lost ground due to a reduced mean fitness must be made up. As a result, purposely exposing an endangered species to a bottleneck (or not avoiding one at all costs) would be extremely shortsighted to say the least. Recently, Bryant et al. (1990) claim that much of the initial fitness loss in their small bottleneck lines disappeared over a series of bottlenecks. However, because there were only two replicates of each bottleneck size in their experiment,

TABLE 3. Situations that may result in various associations of population heterozygosity and inbreeding depression.

Heterozygosity	Inbreeding depression	Situation
High	High	Large population at equilibrium
Low	Low	Population shortly after severe bottleneck
Low	High	Population some time after severe bottleneck
High	Low	Mixture of two populations after severe bottlenecks, some level of inbreeding in every population

these conclusions should be carefully evaluated (see Lynch [1988*c*] for comments about Bryant et al. [1986] and discussion about the number of replicates necessary to document inbreeding depression).

Outbreeding depression

There has been some suggestion that outbreeding depression (reduced fitness in crosses between distantly related individuals) may be a problem in some endangered species (e.g., Templeton et al. 1986). Templeton and Read (1984) defined a hybridity coefficient to measure "the average proportion of the parent's genetic composition derived from different founding animals." (Lynch [1991] has suggested that a measurement of hybridity in the individual of concern, rather than its parents, may be more appropriate.) Ballou and Ralls (1991) have surveyed pedigrees from 20 species using the parental hybridity coefficient and found little evidence of outbreeding depression for juvenile survival. Obviously, in cases in which populations are strongly differentiated some effect may occur, but it would generally be much less of a problem than inbreeding depression.

Conclusions

Preventing further degradation of our environment, such as the prevention of the extinction of endangered plants and animals, should be of major concern for everyone. Although political, economic, and social factors may often be given major roles in decisions affecting endangered species, reasoned scientific information, such as the conservation genetic reasoning we have attempted to present here, could aid in preventing further extinctions.

In our discussion of conservation genetics we have attempted to be circumspect. To err in the application of genetics to an endangered species may result in its extinction. For example, we agree with Lande (1988) that demographic factors, not genetic ones, are most critical in the preservation of the Northern Spotted Owl. Furthermore, we caution that molecular genetic data generally give a picture of only a small part of the genetic variation, and in fact may not always be a good indicator of adaptive genetic differences. Only by thoroughly understanding molecular measures of genetic variation, pedigree analysis, inbreeding depression, maintenance of genetic variation, etc., can responsible application to endangered species be carried out. The simple genetic guideposts suggested a decade ago cannot be taken as absolute dogma and must be evaluated for each species in order for conservation genetics to gain the respectability it merits.

Acknowledgments

We appreciate the comments of Bob Lacy, Russ Lande, Mike Lynch, Mike Soulé, and Bob Vrijenhoek on the manuscript. This research was supported by NSF BSR-8923007.

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