GENETIC VARIABILITY IN CAPTIVE STOCKS: ASSESSING PAST LOSS, PRESENT STATUS, AND FUTURE OUTLOOK

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Within a few decades, zoos will be able to display only those animals which they (or some other zoo) have propagated. Legal, financial, and ethical constraints will prevent zoos from obtaining further animals from the wild. Long-term captive propagation of all species we care to exhibit (not just the handful of species managed under Species Survival Plans) will become essential to the future of zoos and, in some cases, essential to the future of the species themselves.

Unfortunately for everyone other than geneticists looking for jobs, maintenance of a captive population over many generations requires that we manage genes as well as bodies. What, genetically, happens to a captive population, from the moment that wild-caught animals are brought into zoos through the generations of captive breeding? Principally, and most distressingly, they lose genetic variability -- that is, fewer genetic variants (alleles) persist in the population over time, each individual is less likely to be heterozygous (have two different alleles at its two copies of any gene), and individuals become more genetically similar to each other. Genetic variants are lost for the simple reason that only one of the two copies of each gene in an individual are transmitted to any one of its offspring. With just one offspring, half of the genetic make-up of a parent is lost, and even with more offspring it is possible that all would receive the same allele from a parent for a given gene. If those alleles not passed on to offspring do not exist elsewhere in the population, or are not passed on the future generations by any other breeder within the population, then the genetic variability of the population is diminished by that loss. In a population with few breeding individuals the chance that an allele present in one generation will not get passed on to the subsequent generation is much greater than it would be in a larger population with more breeding individuals potentially transmitting the allele.

Why is this inevitable downward fate of genetic variability a problem for captive, but not wild populations? The restoration of variability can occur in two ways: by mutation and by immigration from other populations that contain different genetic variants. In large, natural populations that exchange migrants with nearby populations the loss of variability is slow enough to be offset by the reintroduction of new variants by occasional mutation and immigration. In captive populations, or any very small population that is isolated from sources of immigrants, the random loss of variability is generally much greater than is the restoration of variability by mutation (typically, only one in a million individuals has a new mutation at any given gene each generation), and immigration (capture of new stock from wild populations) is often precluded.

Genetic Processes during the History of a Captive Population

To understand the genetic fate of a captive population, it is useful to consider what happens, genetically, during the history of a captive stock. The first phase (point A on Figure 1) is the capture of a small sample of a

wild population to become the founders of a captive stock. Through the next phase (B), the captive population is expanded from the initial wild-caught animals to the capacity of zoos wishing to hold that species. Finally, the population remains more-or-less stable at that "carrying capacity" (phase C). Clearly, this is a simplification, the course of any real population would be more erratic, with perhaps periods of population decline and unstable fluctuation around some average final capacity. The important point, however, is that genetic variability is lost during all three of these phases, and the techniques for minimizing those losses differ some as a population progresses from founders to an expanding population to a stable one.

At the point when animals are captured from a wild population to initiate a captive population, genetic variability is lost. Some of the genetic variants present in the wild are not present in any of those individuals that are captured. This loss of genetic variation, a result of the incomplete sampling of a source population, is referred to as the "founder effect" and is shown as phase A in Figure 2. The expected loss of variation that occurs when any random sample is taken is inversely proportional to the sample size:

$$V(\text{sample}) = V(\text{population}) \times (1 - 1/N) \qquad \text{Equation 1.}$$

Genetically, the mean heterozygosity (H) of a randomly breeding population is proportional to the genetic variance (binomial variance in allele frequencies) of the population -- which is one reason why heterozygosity is a convenient measure of gene diversity. The sample of alleles is twice the number of individuals (for diploid organisms). Thus:

$$H(\text{sample}) = H(\text{population}) \times (1 - 1/[2N]) \qquad \text{Equation 2.}$$

Equation 2 describes the loss of genetic variation from a sexually reproducing, diploid population whenever a random sample is taken: when animals are captured from the wild, when a stock is subdivided, or when one generation passes on a sample of its genes to the next generation.

Obviously, only those wild-caught animals that leave offspring are truly founders of the captive population; non-breeding wild-caught animals have no genetic or demographic impact on the captive stock. Similarly, in subsequent generations, only those animals that reproduce contribute any genes to those generations (i.e., are genetically effective), and it is important to assure that as many of the animals produce progeny each generation as is possible. Equation 2 applies to <u>randomly</u> breeding populations, but in most captive and wild populations many animals are precluded from breeding by social factors, poor health, or death. The "effective population size" of a population (symbolized N_e) is that size of a randomly breeding population which would lose genetic variation at the same rate as does the real population under study. Thus, for a population in which reproduction is not at random, the effective population size (N_e), not simply the population size (N), must be substituted into Eq. 2. Often, among those animals that do survive and breed, reproduction is approximately randomly distributed. As a result, the number of breeding individuals in a population usually closely approximates the genetically "effective population size". (If you want to know, approximately, the effective population size of a population that you manage, count the breeders and don't worry about complex mathematical formulas.) The founder effect can be minimized by starting with many founders, but the genetic advantage of additional founders is a matter of diminishing returns (1/[2N] rapidly

approaches 0 as N becomes large), with the probability of capturing an otherwise unsampled genetic variant less with each additional founder. Above 20 to 30 founders, it is unlikely that substantially more genetic variability will be obtained by the capture of further animals.

During the population growth phase (Figure 2, phase B), the loss of genetic variability each generation is still inversely proportional to the number of animals, but the population size is changing. The most effective way to minimize the loss during this "residual founder effect" is to increase the population size as quickly as possible, thereby also making this phase as short (in number of generations) as possible.

Even after a managed population reaches the carrying capacity of the participating zoos, genetic variability will likely continue to be lost (phase C). Few zoo populations, or even wild populations of conservation concern, are sufficiently large so that the restoration of variability by mutation offsets the random losses from incomplete sampling (Lacy 1987). As in earlier phases, the rate of loss each generation is given by Eq. 2, and the best management strategy is to maximize the effective population size within the constraints of the available space for captive animals. Moreover, because genetic variation is lost on a per generation basis, a lengthening of the generation time will also delay losses of genetic variation. For a species with high adult mortality, the deleterious loss of potential breeders (reduction in effective population size) that would occur if breeding were delayed may more than offset any genetic gains from a longer generation time; in species with very low adult mortality, a delay in breeding can postpone genetic losses with a minimal cost in pre-reproductive mortality. The best strategy for setting generation time in a population managed for genetic and demographic stability may be to delay breeding of each generation until natural mortality had reduced the potential breeders to the number desired (i.e., don't breed the offspring of an animal until natural mortality has removed all but two of those offspring from the pool of potential replacements for the breeder). Earlier designation of breeders and culling of excess adults unnecessarily speeds the losses of genetic diversity and creates problems disposing of surplus animals. (A caveat: animals reaching post-reproductive age must be considered as genetically dead. Breeding cannot be postponed until all animals are post-reproductive.)

Assessing Genetic Variability in a Captive Population

Most captive populations are presently somewhere in phase B, population growth, though a few have already saturated all identifiable zoo spaces, and managers are trying to stabilize the populations. A population manager cannot reverse earlier losses of genetic variability except by importation of unrelated stock from the wild or from overseas zoos (returning to phase A). To wisely manage a population, one needs to assess past, probably irreversible, losses and then to project future losses under various management scenarios. The first step in this assessment should be the identification of founder animals -- those wild-caught and presumably unrelated animals that have descendants in the living captive population. Some of the genes of those founders, however, would already have been lost from the captive population. The best approach to estimating the losses of genetic variability that have occurred during the past history of a captive

stock is to employ a computer simulation to model possible fates of founder genes as they descend through the pedigree (MacCluer, et al. 1986). Such "gene drop" simulations track the fates of the two alleles at a hypothetical gene that each founder potentially contributes to a captive population, letting the alleles "drop" through the pedigree by randomly selecting one allele from each parent to transmit to each offspring. Because each simulation traces only one of many possible outcomes for the founder alleles at one genetic locus, a thousand or more simulations should be run to obtain average results that predict what is likely to occur over the genome as a whole.

At the end of a gene drop simulation, the extant genetic variability can be expressed in several ways. The overall level of variability can be expressed as the fraction of heterozygosity (gene diversity) originally present in the founder population that still remains. As the simulations assume that each founder brings a unique pair of alleles into the captive population (founders are assumed to be totally unrelated) and is therefore heterozygous, enumeration of the proportion of the living descendant population that is heterozygous yields the fraction of the initial heterozygosity remaining. Moreover, because a descendant animal can only receive two identical copies of an allele from the parents if both parents received that allele from the same founder lineage, any descendant that is homozygous in some of the simulated populations must be inbred (have parents that are genetically related). The inbreeding coefficient of an individual (F, defined as the probability that an individual is homozygous because it received two identical copies of an allele present in an ancestor common to both parents) is thus the proportion of simulations in which that individual is homozygous. The fraction of homozygous descendants in the population is the average inbreeding coefficient of the population:

$H_t = H_0 \times (1 - F)$ and $1 - H_t = F$ Equation 3,

in which H_t is the mean heterozygosity (1 - homozygosity) at generation t, and the heterozygosity of the founders (H_0) is set to 1.

Another way to express the extant variability in a captive population is to determine how many wild-caught animals would be needed to obtain the amount of genetic variability present in the current captive population -- i.e., the total number of founder genomes that remain in the population. I have defined a measure, "founder equivalents" (symbolized f_e), to express the current genetic variability of a population as that number of founders that would have yielded the observed variability, if they had been managed so that they produced equal numbers of descendants (Lacy, in press):

$f_e = 1 / \sum p_i^2$ Equation 4,

in which p_i is the proportion of the genes of the living descendants that are derived from founder i and the summation is taken over all founders.

The complete history of a founder's genetic lineage (the number of descendants in each previous generation as well as the number of living descendants) is needed to specify precisely the likelihood that its genes still exist in a population (Thompson 1986). Founder alleles lost when a founder has few descendants in early generations of captive propagation cannot be recovered by later expanding that founder's contribution to the living population through focusing breeding efforts on those few descendants. I therefore defined a more computationally complex measure, "founder genome equivalents" (symbolized f_g), to express the extant

variability as the number of founders needed to obtain that diversity of genes, if no founder alleles had been lost (i.e., each founder had an infinite number of descendants in every generation) (Lacy, in press). These two measures, founder equivalents adjusting for unequal founder contributions to the present population and founder genome equivalents adjusting also for genetic losses during bottlenecks in earlier generations of the pedigree, provide one way to determine whether an existing population has a sufficient genetic base to be sustained through many generations of captive propagation. A common rule-of-thumb is that 20 or more founders, encompassing 97.5% of the genetic variability of the wild stock from which they were taken, should be used to start a captive breeding effort (Foose, et al. 1986). If genetic management of a population begins after several to many generations of rather haphazard captive breeding, then an effort should be made to obtain 20 or more founder equivalents (or, more conservatively, 20 founder genome equivalents). Additional founder equivalents may be obtained by breeding more offspring from still-living wild-caught animals that have contributed little to the captive population, or by obtaining additional wild-caught stock. (Adjusting founder representations of dead founders by propagation of descendants can increase the number of founder equivalents by increasingly the number of copies of previously rare founder alleles, but more importantly will slow further decay of the founder equivalents in subsequent generations.)

Because those founder genes lost from the pedigree cannot be recovered (unless the founder itself is still alive and capable of breeding), the optimal "equalization" of founder contributions should aim for each founder to be represented in the population in proportion to the fraction of its genes that are likely still to exist in at least one living descendant. The difference between such "target representations" and the current founder contributions can be used to derive measures of the genetic importance of breeding the descendants from each founder (Ballou, in press). Animals that descended from founders whose contributions to the living population are below their target representations should receive priority in breeding plans, because they contain some of the few copies remaining copies of those founders' genes. Animals descended from founders whose contributions are well above the target values should be bred relatively less, because they contain one of many copies of those founders' genes in the population. (Descendants from over-represented founders should produce some offspring, however; otherwise those founders would become under-represented in the following generation and their genes perhaps lost.)

To predict future genetic losses of a population, one needs to know the effective population size, N_e, each subsequent generation. During phase B, when management strategies are changing as more is learned about propagating the species and the population is expanding, estimates of effective population size can be difficult to make and are of little value in predicting the future genetic behavior of a population. After a population reaches a stable state (phase C), however, N_e can be reasonably estimated either as the number of breeding individuals or, more precisely, by applying standard population genetic formulas (Lande and Barrowclough 1987) to estimates of variance in lifetime reproductive success, sex ratio, and the variance in population size from generation to generation. The estimated effective population size can be used to calculate the future losses of heterozygosity per generation (by applying Eq. 2) or, equivalently, the

increase per generation in the mean inbreeding coefficient if the population were to mate randomly.

At any point during the management of a captive population, in fact at every point during wise management, the remaining variation should be assessed as the fraction of the original heterozygosity (e.g., Lacy and Clark, in press) and/or as the number of founder genome equivalents within the population. Somewhat problematically, although many SSP masterplans have a stated goal of retaining 90% of the genetic variation for 200 years, those plans generally do not state what population (the wild, the founders, or the captive stock at the time that the SSP master plan is developed) is considered the (100% variability) baseline, nor when the 200 years is considered to start. The number of founder genome equivalents is a measure of genetic variability relative to the wild population that served as a source of the captive stock, the percent heterozygosity remaining is usually taken as a percent of the variability in the founder animals themselves (which is less than the wild variability), and projections based on effective population sizes often are used to determine the future genetic variability relative to the present status of the population, in whichever phase it may be on Fig. 2. Clearly the baseline genetic population and starting year needs to be stated for a 90%/200 year goal to be meaningful.

Thus far, I have said little about the avoidance of inbreeding. That is not because inbreeding is not likely to be a problem in zoo populations: inbreeding probably is often a cause of increased mortality and decreased fecundity (Ralls, et al. 1979; Ralls and Ballou 1986). As a first approximation for the likely amount of inbreeding depression in a population, animal breeders have found that many aspects of fitness are inversely proportional to inbreeding coefficients. For example, an inbreeding coefficient of .25 often results in a 25% reduction of survival and reproductive rates. Thus, when SSP groups plan for the preservation of about 90% of the initial genetic diversity, they are implicitly accepting about a 10% loss in fitness of the stock due to genetic problems or defects.

The reason that I have not discussed inbreeding avoidance as an important part of a management strategy is because it will follow almost automatically from a careful management of founder representations. If a population contains a wide diversity of founder alleles, then close inbreeding, the mating of two animals with a substantial portion of their ancestries in common, will be rare or at least easy to avoid. On the other hand, if much of a captive population descends from one or a few founders, it will be difficult to avoid matings between closely related animals since most descendants share founder genes. Mathematically, the relationship between management of founder representations and inbreeding avoidance can be seen in that the percent loss of heterozygosity is equal both to the average inbreeding coefficient and to the inverse of twice the number of founder genome equivalents ($H_t/H_0 = 1 - F = 1 - 1/[2 * f_g]$; and see the Y-axis scales on Fig. 2). Measures of heterozygosity assess how well genetic variability has been retained in a population; inbreeding coefficients portend the magnitude of genetic problems resulting from lost variability; and founder equivalents reveal how difficult it will be (how many new founders would be required) to improve the genetics of a captive stock.

In summary, to manage a captive population genetically, one should:

1) set genetic goals for the captive management -- e.g., preservation of at least 90% of the genetic variability present in the wild population for 200 years from the point of capture of the founder stock,

2) determine the founder stock -- those wild-caught animals that have living descendants in the captive population,

3) determine the extant genetic variability of the population, expressed both as the proportion of genetic variability remaining and as the number of founder equivalents (and/or founder genome equivalents) in the stock,

4) obtain more founder stock, if necessary, to assure that the population has sufficient genetic resources to keep it viable well into the future and to meet genetic goals,

5) determine whether the available spaces for captive propagation are sufficient to maintain a population large enough to preserve much of the present variability (and obtain more captive space for the population if current carrying capacity is deemed insufficient),

6) increase the captive population up to the pre-determined carrying capacity as rapidly as possible so as to minimize genetic losses while the population is still very small,

7) place priority on breeding those few descendants carrying genes from founders that are poorly represented in the current population, and

8) maximize the proportion of the living population that is breeding and try to equalize the lifetime reproduction of breeders -- thereby maximizing the genetically effective population size.

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Figure 1. Growth of a hypothetical captive stock

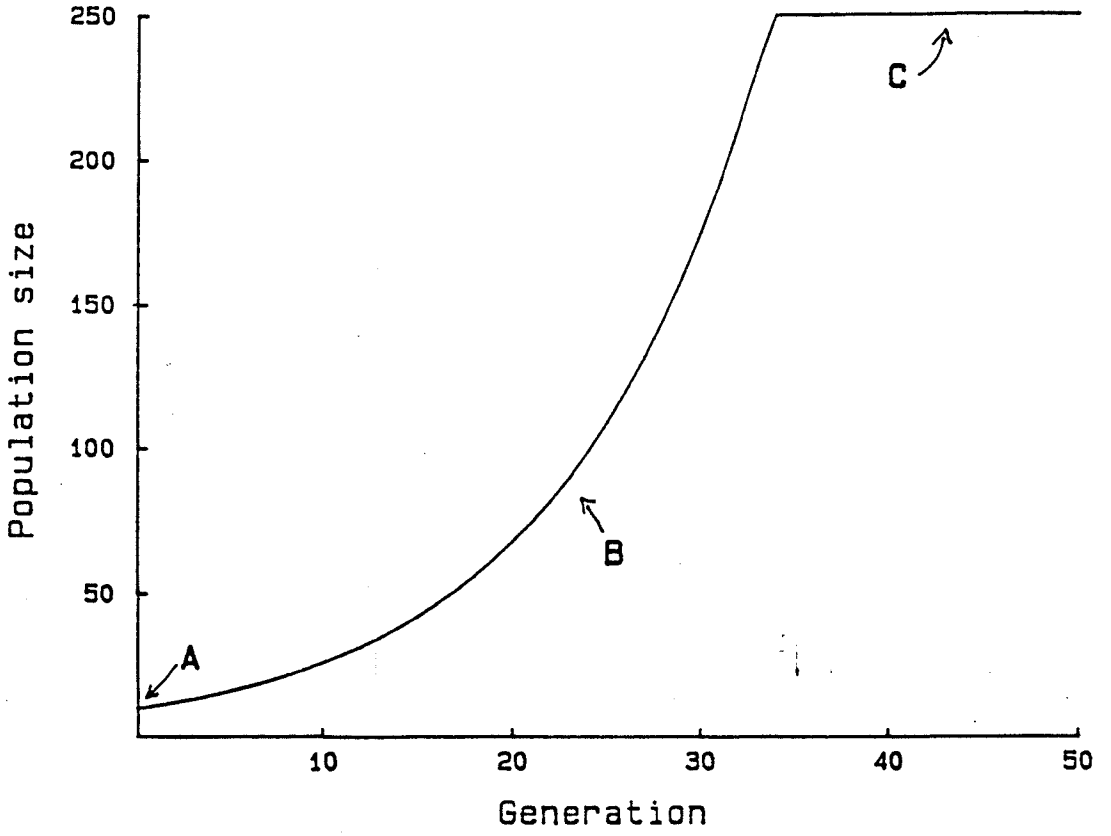

Figure 2. Loss of gene diversity from a captive stock