ABSTRACT

Title of Dissertation: GENETIC MANAGEMENT, INBREEDING DEPRESSION AND OUTBREEDING DEPRESSION IN CAPTIVE POPULATIONS

Jonathan D. Ballou, Doctor of Philosophy, 1995

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The patterns and severity of inbreeding and outbreeding depression in organisms have been used to address fundamental questions relating to basic evolutionary issues of population genetic structure and dynamics. In addition, the genetic structure of populations, and the effect it has on the severity of inbreeding and outbreeding depression, play a critical role in the conservation of small populations. This dissertation examines three issues relating to populations genetics of small populations. The first chapter presents a method, based on the concept of mean kinship, for managing pedigreed populations for maximum retention of genetic diversity. Using Monte Carlo simulations, the mean kinship strategy is compared to and found more effective at maintaining gene diversity than other strategies recommended for this purpose. The second chapter investigates the potentially confounding effects of inbreeding and outbreeding depression in captive populations. It has been hypothesized that outbreeding, not inbreeding, is responsible for the observed depression often

documented in captive populations. Several models for detecting joint effects of inbreeding and outbreeding depression are presented and applied to data from five populations. While the data structure of four of the populations complicate the analyses, inbreeding effects are present in all populations and there is no evidence of outbreeding depression. Recognizing that inbreeding depression is a common problem in small populations, the last chapter addresses the question: can inbreeding depression be eliminated through selection against deleterious and lethal alleles (i.e. purging)? Selection for healthy, inbred animals (who are less likely to carry deleterious alleles than non-inbred individuals) theoretically can purge a population of deleterious or lethal alleles. This chapter presents an analysis of 24 captive populations of mammals for evidence of purging by comparing inbreeding depression in descendants of inbred animals to that in descendants of non-inbred individuals. The results suggest that while the unintentional purging that has already taken place in populations may reduce inbreeding depression to some extent, it has not been sufficient to eliminate depression.

GENETIC MANAGEMENT, INBREEDING DEPRESSION AND OUTBREEDING DEPRESSION IN CAPTIVE POPULATIONS

by

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FOREWORD

The second chapter, "Identification of Genetically Important Individuals for Management of Genetic Diversity in Pedigreed Populations," is co-authored by Robert C. Lacy. The Dissertation Committee acknowledges that Jonathan D. Ballou made a substantial contribution to this work, and approves of its inclusion in the dissertation.

DEDICATION

To Katherine Ralls

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INTRODUCTION

The patterns and severity of inbreeding depression (the reduction of fitness in organisms whose parents are related) and outbreeding depression (the reduction in fitness of hybridized organisms) has been used to address fundamental questions relating to basic evolutionary issues of population genetic structure and dynamics (Wright 1977; Templeton 1987). For example, the relative cost of inbreeding tips the balance between the evolution of inbreeding vs. outcrossing mating systems (Bengtsson 1978; May, 1979; Shields 1982; Charlesworth and Charlesworth 1987). Population structure can also be inferred from the severity of observed inbreeding and outbreeding depression (Templeton 1987). In addition, the genetic structure of populations, and the effect it has on the severity of inbreeding and outbreeding depression, play a critical role in conservation strategies for threatened species (Frankel and Soulé 1981), particularly in small captive populations. It is the latter that is the primary focus of this dissertation.

Conway (1987) estimated that approximately 75% of the 1100 or so mammalian species bred in zoos have populations with fewer than 25 individuals. The small size of these populations compromise the

potential contribution of captive propagation to threatened and endangered species recovery programs. Among the challenges facing the long-term viability of these populations are genetic risks associated with small population size. These include the loss of genetic diversity due to genetic drift and inbreeding, and the undesirable selection for adaptation to the captive environment (Arnold, in press).

This dissertation addresses three issues germane to genetic problems of small populations. Loss of genetic diversity and increased inbreeding are often associated with reduced fitness (increased mortality and decreased reproduction; Wright 1977; Allendorf and Leary 1986; Thornhill 1993), and evidence of inbreeding depression in captive populations is particularly strong (Ralls et al 1979; Ralls et al, 1988; Lacy et al 1993). Evidence of the undesirable effects of selection is less well documented (Frankham and Loebel 1992; Arnold, In press), but prolonged selection for adaptation to the usually benign captive environment is likely to result in some traits maladapted for a natural environment (Frankham and Loebel 1992).

Management strategies that maximize retention of genetic diversity will act to minimize rates of inbreeding and selection (Lacy et al. in press; Foose et al. 1986). In the first chapter, I (with coauthor R. Lacy) present and evaluate a breeding strategy for maintaining genetic diversity in pedigree populations based on the con-

cept of mean kinship. This strategy uses detailed pedigree information to identify the genetically most important animals to breed for maintenance of gene diversity (expected heterozygosity). Using Monte Carlo simulations in hypothetical pedigrees, the mean kinship strategy is then compared to and found more effective than other strategies recommended for this purpose.

The mean kinship breeding strategy is basically a strategy that promotes optimal outbreeding based on pedigree information. When populations are founded by individuals from different sources (e.g., populations in different geographical areas), using an outbreeding strategy raises the concern of outbreeding depression, or the reduction in fitness due to crossing individuals from populations adapted to different environments (Shields 1982; Templeton et al 1986). In captive populations, inbreeding and outbreeding effects can be confounded (Templeton and Read 1984) and Shields hypothesized that outbreeding, not inbreeding, is potentially responsible for the observed depression often documented in captive populations (Shields, pers comm; Templeton and Read 1984). Which, then, is the larger concern, inbreeding or outbreeding depression? This issue is addressed in the second chapter (coauthored by L. Chao), which presents an analysis of the joint effects of inbreeding and outbreeding in five populations of captive mammals. Several different models for detecting outbreeding depression are presented. The chapter focuses on the types of problems outbreeding analyses are likely to encounter when applied to captive populations. The results, and a review of

the literature, suggest that for vertebrate species outbreeding depression is not as pervasive as inbreeding depression, and that concerns over outbreeding depression should be confined to those cases where substantial genetic differences exist between taxa (Templeton et al. 1986; Templeton 1986).

Even in closely managed populations inbreeding, and inbreeding depression, can be substantial and threaten the survival of the population if the population is sufficiently small (Ralls and Ballou 1983; Hedrick 1994). When the threat is this severe, can inbreeding depression be reduced or eliminated by purging the population of deleterious or lethal alleles? Templeton and Read (1983, 1984) applied a purging management strategy to eliminate the severe inbreeding depression in the captive population of Speke's gazelle (*Gazella spekei*), and recommended that this strategy be used to purge other populations also suffering from debilitating inbreeding depression.

The third chapter of the dissertation examines the issue of purging to eliminate inbreeding depression. Templeton and Read's purging strategy is based primarily on selecting healthy, inbred animals as breeders, these individual being less likely to carry deleterious alleles than non-inbred individuals (Templeton and Read 1983). After this strategy was applied to the Speke's gazelle, inbreeding depression in offspring of inbred parents was less than in offspring of non-inbred parents (but see Willis and Wiese, submitted). Chapter three presents an analysis of 24 captive popula-

tions of mammals for similar evidence of purging by comparing inbreeding depression in descendants of inbred animals to that in descendants of non-inbred individuals. The results suggest that while the unintentional purging that has already taken place in populations may reduce inbreeding depression to some extent, it has not been sufficient to eliminate depression.

In summary, the three chapters address how small populations can be managed to retain genetic diversity, and two issues affecting the fitness of small populations: outbreeding depression (is it a concern?) and inbreeding depression (can it be eliminated through purging?). While these issues are of extreme relevance to the management of endangered and threatened species in captivity, their significance extends beyond the field of conservation. Questions of the significance of inbreeding and outbreeding depression are relevant to fundamental questions in evolutionary biology and population genetics. It is hoped that the work presented here also provides some insight into the genetic consequences of mammalian population structure.

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CHAPTER I

IDENTIFYING GENETICALLY IMPORTANT INDIVIDUALS FOR MANAGEMENT OF GENETIC VARIATION IN PEDIGREED POPULATIONS

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INTRODUCTION

Captive populations are a valuable contribution to the conservation of threatened and endangered species (Foose 1983, Foose et al. In press). Captive populations, by nature, however, are small, fragmented, and often dispersed among many zoos distributed over a wide geographic range. The capacity of any single institution to hold a large number of individuals of any one species is limited. Cooperative breeding programs are needed to insure that zoo collections are managed jointly under the goal of the species' long-term

conservation. Ideally, these programs should be part of a comprehensive and integrated conservation strategy for the species that includes protection and management of wild populations and habitat as well as the captive component (Jones 1990). The primary role of the captive population in such an integrated program is, if necessary, to provide animals for reinforcing or reestablishing wild populations.

The principal objective for cooperative breeding programs is to establish demographically secure and self-sustaining populations capable of maintaining high levels of genetic variation (Foose 1983). The genetic challenges that confront small populations in the zoo environment are extensive. Loss of genetic variation through genetic drift (Nei et al. 1975), inbreeding depression (Ralls et al. 1988; Lacy et al. 1993), and selection for the captive environment (Arnold, In press) all affect the species' short- and long-term fitness. Genetic management attempts to mitigate these problems by implementing breeding strategies that retain genetic variation. This approach minimizes changes in the population's gene pool thereby retaining, as much as possible, the genetic characteristics of the original founders of the population. Genetic management is most effectively accomplished under conditions that allow intensive management and in populations with completely known pedigrees. This permits explicit decisions to be made about who breeds, how often, with whom, and when.

This chapter addresses the issue of how breeding recommendations are made. In particular, it discusses several analytical methods, based on pedigree analyses, for identifying genetically "important" animals -- those individuals whose reproduction is most critical for the retention of genetic diversity. These individuals are then given the highest breeding priority. We present the concept of genetic importance as defined by mean kinship and kinship value, and present the results of computer models that evaluate how well various breeding strategies based on different measures of genetic importance maintain genetic diversity in a variety of pedigrees. Methods to calculate these measures when pedigree data are only partially known are also presented.

GENETIC CHARACTERISTICS OF CAPTIVE POPULATIONS

One basis for developing genetic management recommendations is to minimize loss of genetic variation through maximizing a population's effective size (N_e). The concept of an effective size of a population was originally introduced by Wright (1931) as the number of individuals which, if there were random union of gametes, would lose heterozygosity at the rate observed in the real population. However, loss of heterozygosity is just one consequence of genetic drift. Effective size has also been applied to the number of individuals in a population with random union of gametes that would drift at the rate of the studied population, with the rate of genetic drift being measured as the sampling variance of gene frequen-

cies from parental to offspring generations (the "variance effective number"), instead of the rate of change of heterozygosity or inbreeding (the "inbreeding effective number"). In a population of constant size, the inbreeding and variance effective sizes will be the same, but in a population that is changing in size, the consequences of genetic drift (loss of heterozygosity and variance in allele frequencies) can occur at somewhat different times. The inbreeding effective size depends primarily on the size of the parental generation, while the variance effective size is more dependent on the number of offspring (Crow and Kimura 1970). Heterozygosity is retained through maximizing the inbreeding effective size, while allelic diversity is retained through maximizing the variance effective size. Both effective numbers are functions of sex ratio, number of breeders, and the mean and variance in numbers of offspring they produce (Harris and Allendorf 1989; Lande and Barrowclough 1987; Crow and Kimura 1970). A general strategy for maintaining genetic diversity would be to maximize the number of breeders, equalize family size, equalize the sex ratio of breeders, and reduce fluctuations in population size over time (Foose et al. 1986).

Maximizing N_e, however, might not be the most effective strategy for maintaining genetic diversity in populations with known pedigrees. Pedigree analysis should allow the population manager to target individuals and lineages for preferential breeding. Quite possibly, a strategy that utilizes all the information contained within a pedigree could preserve genetic variation better than one,

based on maximization of N_e, that ignores the ancestry of each individual. Two factors make this an interesting and difficult problem. The first is that there has been no strong theoretical development of the concepts of breeding strategies based on pedigree analyses, and the second is the particular nature of the pedigrees of many captive populations.

Captive populations are generally characterized by few founders and relatively small population sizes (Hutchins et al. 1991). Few captive populations have had the benefit of genetic management throughout their history. In unmanaged populations, reproduction is often highly skewed in favor of tractable, easily handled animals. Consequently, a large proportion of the gene pool may descend from only a small proportion of the founders. Furthermore, a large proportion of the founders' alleles may have already been lost due to genetic drift, and inbreeding levels may also be high. Preferential breeding usually will have resulted in a high family-size variance and the likelihood of strong selection for the captive environment. In addition, sex ratios of breeders are highly skewed in many species managed as herds with a single breeding male. These characteristics combine to result in populations with historically small effective sizes and extremely complex pedigrees (figure 1).

The genetics of the population may be further complicated by population subdivision. Populations in zoos in different regions are likely to be founded by unrelated individuals. When migration bet-

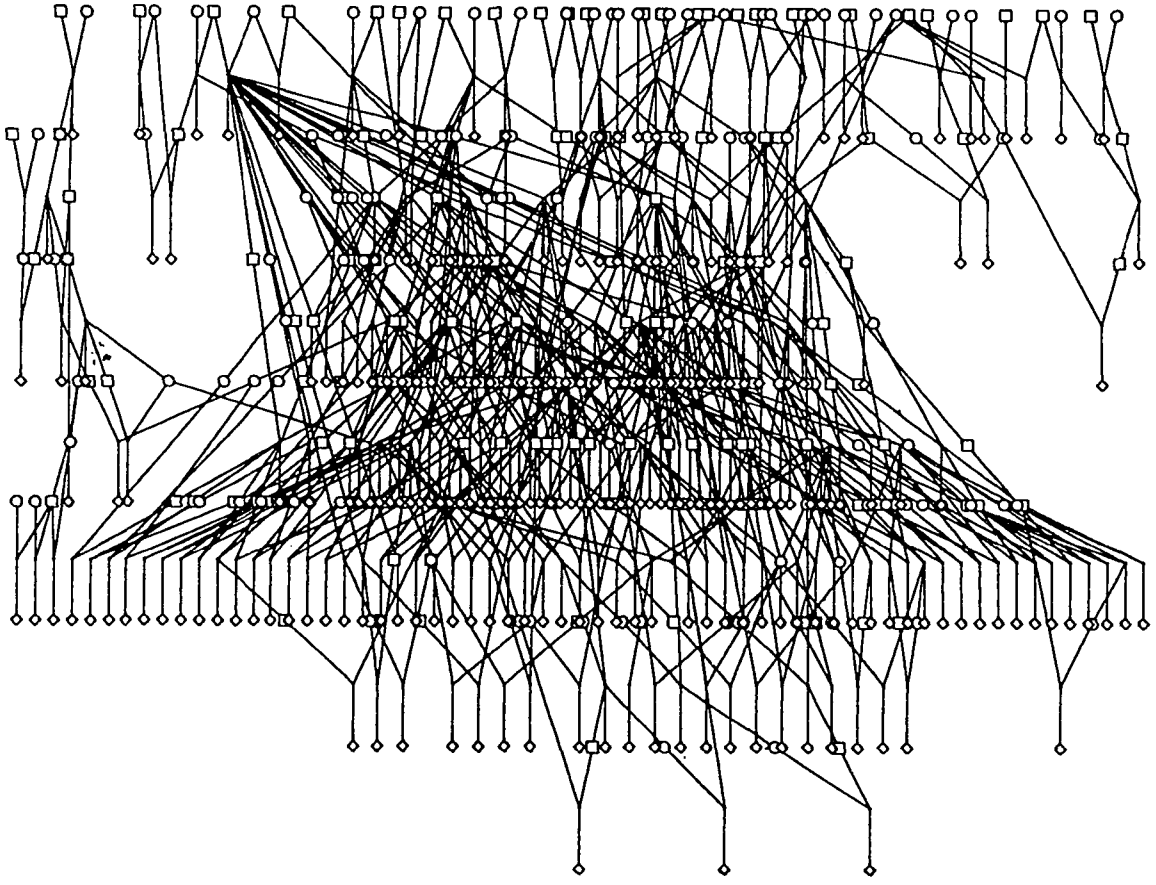

Figure 1. Marriage node pedigree of the 1990 golden lion tamarin (*Leontopithecus rosalia*) captive population (Ballou 1989). Pedigree drawn using PEDPACK (Thomas 1991).

ween regions is limited or non-existent (due to either logistic difficulties or lack of management), separate lineages are established and perpetuated, resulting in highly inbred lines descended from different founder stocks.

When genetic management is applied to a population, the population manager must attempt not only to compensate for these past management deficiencies but also to minimize further loss of genetic variation. Simply applying a general strategy to maximize the effective population size may not be the most appropriate strategy. Compensation will require preferential breeding of some individuals rather than breeding to equalize family size. Formulating genetic management recommendations, therefore, is a process of identifying, through pedigree analyses, the genetically most important animals in the population.

GENETIC MANAGEMENT AT THE POPULATION LEVEL

The goal of genetic management is the preservation of the genetic variation of the population from which the founders were drawn (Lacy, In press). Since the source population's genetic variation is represented by the gene pool of the founders, genetic management therefore strives to minimize loss of the founders' genetic variation.

The status of the founders' genetic variation in the extant population can be described using two concepts. The first is the genetic contribution of the founders to the extant gene pool. In accord with the rules of Mendelian segregation, the "founder contribution" ($p_{i.}$) is the expected proportion of the population's gene pool that has descended from founder i (Lacy 1989). The second is the loss of founder alleles due to genetic drift (Thompson 1986). Allele "retention" (r_i) is defined as the expected proportion of founder i's alleles that have survived to the extant population (Lacy 1989). Information on founder contribution and retention can be combined and summarized by "founder genome equivalents" (f_g, Lacy 1989). Founder genome equivalent is the expected number of founders that would be required to provide the level of genetic diversity observed in the living population if the founders were all equally represented and had lost no alleles (100% retention) (Lacy 1989). The value f_g can be estimated by:

$$f_g = \frac{1}{\sum_{j=1}^{N_f} (p_{j.}^2 / r_j)} \qquad \text{Equation 1}$$

in which N_f is the number of founders. Both skewed founder contribution and low retention result in a decrease in founder genome equivalents.

As pointed out by Lacy (1989), founder genome equivalents is directly related to loss of gene diversity (*GD*, the heterozygosity

expected if the population were in Hardy-Weinberg equilibrium). The gene diversity of the descendants of a randomly mating population, as a proportion of the gene diversity of the population from which the founders were randomly sampled, is $1 - 1/[2f_g]$. Therefore, genetic management strategies that maximize founder genome equivalents also maximize gene diversity. As can be noted from equation 1, this is not simply a matter of equalizing founder contributions ($p_{i.} = p_{j.}$). The upper limit to founder genome equivalents for any population is the retention totaled over all founders (Σr_i), a value Lacy (1989) terms the number of founder genomes surviving (f_s). It can be seen by substitution in equation 1 that f_g is maximized when

$$p_{j.} = \frac{r_j}{f_s} \qquad \text{Equation 2}$$

Thus, to maximize founder genome equivalents, and hence gene diversity, the contribution of each founder should be proportional to the number of founder genomes surviving. This is defined as the "target founder contribution" (Ballou and Foose, In press). Management for maintaining genetic diversity within the population could therefore strive to adjust the observed founder contributions to match the target founder contributions by preferentially breeding individuals descended from founders whose contributions currently fall below their targets. This achieves equalization, not of the proportion of the gene pool contributed by each founder, but of the frequencies of those founder alleles that are still retained within the population.

MEASURES OF GENETIC IMPORTANCE

While target founder contribution can provide goals for genetic management, identifying individuals that achieve this result is problematic. Pedigree analyses provide data on founder contribution, allele probability distributions, and level of inbreeding for each individual in the population. The amount of information to consider can be formidable in large populations with many founders. In addition, complex pedigrees result in individuals which are descended from numerous founders and related to each other via multiple comman ancestors. For example, individuals that are descendants of "under-represented" founders may also carry alleles from "over-represented" founders. These complexities have led to the development of a number of strategies to identify, or rank, animals by their genetic importance.

Founder Importance Coefficient (*fic*)

The first measure of genetic importance used in captive breeding programs was based on the goal of equalizing the genetic contribution of founders to the gene pool (Foose 1983), ignoring the complications (above) of loss of some founder alleles. Equal representation of founders assures that the genetic variation present in each founder is not excluded from the gene pool, and also assures that the gene pool is not dominated by genes from a few founders. Under this strategy, genetic importance is assigned to descendants

of under-represented founders; they should be given breeding preference.

The degree to which an individual (*i*) is descended from under- or over-represented founders can be summarized by its *founder importance coefficient* (fic_i):

$$fic_i = \sum_{j=1}^{N_f} (p_{j.} \times p_{ji}) \qquad \text{Equation 3}$$

where $p_{j.}$ is the founder contribution of founder *j* to the population's gene pool; p_{ji} is the contribution of founder *j* to individual *i*; and N_f is the number of founders contributing descendants to the population (Ballou and Foose, In press). The value fic_i is the weighted average of founder *i*'s contribution with each founders' contribution to the total population acting as the weights. Individuals with high *fic* values are descended from an over-represented founder(s). The *fic* values range from a low of $\min(p_{j.})$ (the $p_{j.}$ of the most under-represented founder if it is still alive) to $\max(p_{j.})$ if the most over-represented founder is alive. Ranking individuals by their *fic* provides a simple method of identifying genetically important animals as defined by founder contribution.

The problem with *fic* is that it does not consider loss of founder alleles. Equalizing founder contribution will not maximize gene diversity because it results in over-representation of alleles from founders that have low allelic retention. For this reason, it

is no longer used for population management. It was, however, used for the Species Survival Plan (SSP) program of the American Association of Zoological Parks and Aquariums (AAZPA) to identify genetically important animals during the late 1980s before other measures of genetic importance were developed (see below).

Genome Uniqueness (*gu*)

Genome uniqueness provides another method for measuring genetic importance. Genome uniqueness of an individual is the probability that an allele chosen at random from that individual is unique within the living population (i.e., the selected allele is identical by descent to no alleles in any other living animal). Under the assumption that loci are independent, genome uniqueness, by extension, is the proportion of an individual's genome that is unique in the population. Genome uniqueness is used to identify individuals carrying alleles at high risk of being lost (not passed on to the next generation; MacCluer et al. 1986).

While genome uniqueness can be calculated exactly (i.e., using peeling algorithms, Cannings et al. 1978; Thomas 1991), the methods are computationally intensive, even for moderate-sized pedigrees. An alternative is to use a "gene-drop" analysis, which simulates the transmission of founder alleles (each founder is assigned 2 uniquely identifiable alleles) through the pedigree to the living population (MacCluer *et al* 1986; Lacy et al. In press). The frequency and dis-

tribution of alleles in the living population is inferred from multiple simulations. Genome uniqueness is calculated as the proportion of simulations in which an individual receives the only copy of a founder allele:

$$gu_i = \frac{\sum_{j=1}^{NSIM} a_j}{2 \times NSIM} \qquad \text{Equation 4}$$

where a_j is the number of individual i's alleles that are present in no other living animal in simulation j (a_j = 0, 1 or 2) and *NSIM* is the number of simulations. Individuals with high *gu* should be given breeding priority in order to ensure that their unique alleles are maintained in the population.

The primary problem with genome uniqueness is that it measures only alleles that are unique and does not consider other alleles that are at high risk of being lost, for example, alleles that have only two copies in the population. While genome uniqueness as discussed here refers to the uniqueness of an individual's genome, the concept has been extended to the uniqueness of the gene pool of predefined groups of individuals (e.g., families or animals within a geographic region; Geyer et al. 1989; Thompson, In press).

Mean Kinship (*mk*)

We propose a third method of identifying genetically important individuals, based on the concept of kinship. Genetic importance can be defined using the average relationship of each individual to the population as a whole. Conceptually, genetic importance is related to the number and degree of relatives an individual has in the population. Individuals with many living, close relatives carry alleles that are more common in the population, and therefore are less important than individuals with few relatives. This can be quantified by mean kinship *(mk)*.

The relationship between a pair of individuals can be measured with the kinship coefficient (f_{ij}), which is defined as the probability that two alleles drawn randomly from homologous loci in each of two individuals (*i* and *j*) are identical by descent (Falconer 1981). *Mean kinship* of individual *i* (mk_i) is then defined as the average of the kinship coefficients between that individual and all living individuals (including itself):

$$mk_i = \frac{\sum_{j=1}^{N} f_{ij}}{N} \qquad \text{Equation 5}$$

where *N* is number of living animals in the population. Individuals with low mean kinship represent genetically important animals.

Mean kinship is an intuitively appealing method for ranking individuals in terms of their genetic importance. It also relates directly to maximizing founder genome equivalents and gene diversity. The mean kinship of an animal is the expected (in the statistical sense) inbreeding coefficient of progeny of this individual if it were mated at random in the population (regardless of the age or sex of itself or its mate and including possibly mating with itself). By extension, the average mean kinship ($\overline{mk}$) of the population is the expected mean inbreeding coefficient of all progeny if mating were at random. It therefore is equal to the proportional loss of gene diversity of the descendant population. The relationship between average mean kinship, founder genome equivalents, and proportional gene diversity of the descendants (*GD*) is given by:

$$\overline{mk} = \frac{1}{2f_g} = 1\text{-}GD \qquad \text{Equation 6}$$

A strategy that minimizes average *mk* therefore maximizes gene diversity. Mean kinship is easily calculated using the additive relationship matrix (Ballou 1983).

Relationship between Mean Kinship and Genome Uniqueness

Managing by mean kinship maximizes gene diversity, while management by genome uniqueness aims for retention of allelic diversity, or the number of unique alleles in the population. Both allelic and gene diversity are important for population fitness (Allendorf 1986) and, in general, strategies to retain gene diversity

will also retain allelic diversity (Allendorf 1986; Lacy et al. In press). This is also true for management strategies based on *mk* and *gu* because both are measures of the "rareness" of an individual's alleles. In fact, both are functions of the frequency distribution of an individual's alleles in the population. Such a frequency distribution can be calculated as the proportion of simulations (i.e., loci) in a gene drop analysis in which the individual's alleles were present at different frequencies in the population's gene pool. Figure 2 shows such an allele frequency distribution for the golden lion tamarin (GLT) Studbook # 1142. Mean kinship is a function of the mean of this distribution while genome uniqueness is a function of its lower limit: it is the probability that the individual's alleles have a frequency of zero in other individuals (figure 2). As an individual's alleles become more frequent, the distribution shifts towards the right, increasing *mk* and decreasing *gu*. For example, about 1% of 1142's alleles have a frequency of .04 in the population.

Genome uniqueness and mean kinship are therefore expected to be negatively correlated, as is seen when mean kinship is plotted against *gu* (figure 3). Individuals that are ranked highly by genome uniqueness often have low mean kinship. It is possible, however, for an animal to have many relatives (resulting in moderately high mean kinship), but still to carry unique alleles. This can occur, for example, in an animal who has one parent descended from a common lineage and the other descended from a rare lineage (e.g., GLT 1142

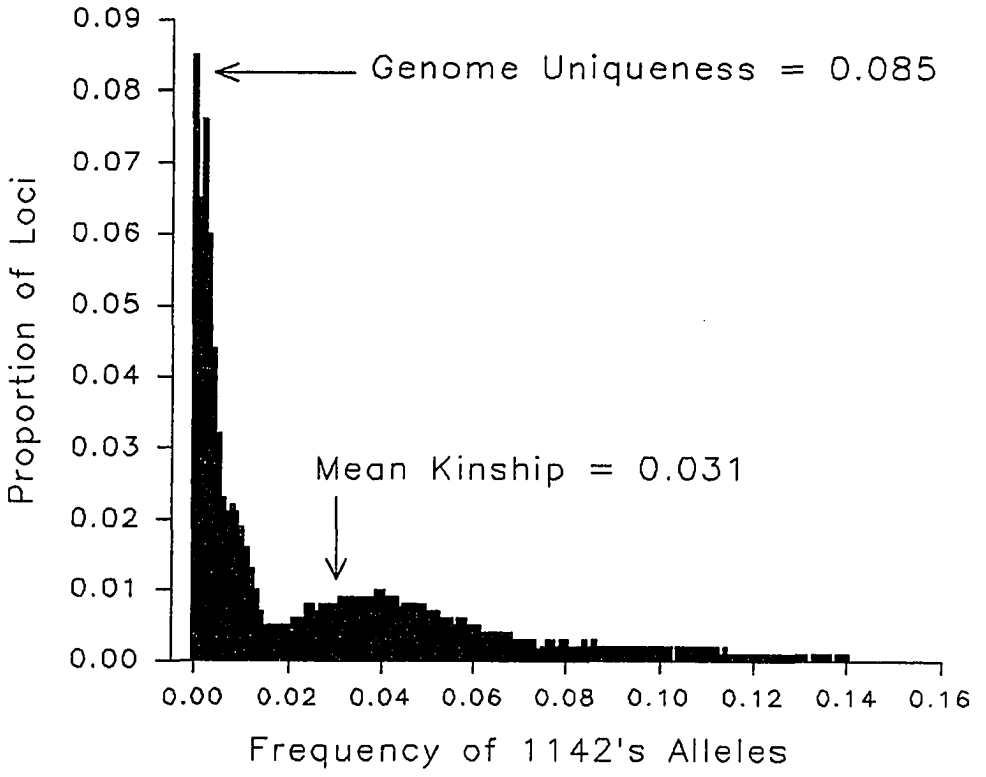

Figure 2. Frequency of golden lion tamarin #1142's alleles in the living golden lion tamarin population. Mean kinship is a function of the mean of this distribution while genome uniqueness is a function of its lower tail.

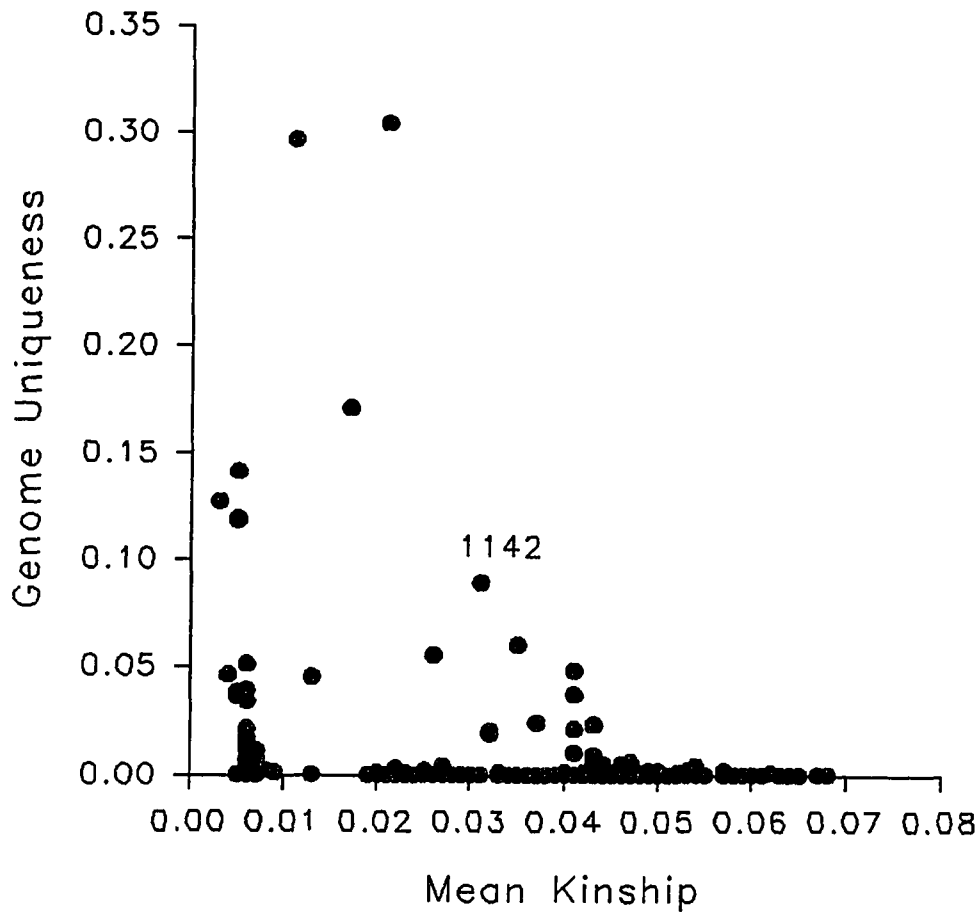

Figure 3. Genome uniqueness plotted against mean kinship for the 1991 captive population of golden lion tamarins (Ballou 1990). The values for GLT 1142 are shown.

in figure 3). The resulting offspring have a combination of both rare and common alleles; their allele frequency distribution will be bi-modal. Likewise, animals with low mean kinship may not have any unique genes (figure 3). For example, if both parents of any animal are alive, that individual will have no unique alleles, regardless of how few other relatives it may have.

Mean Kinship Under Demographic Constraints: Kinship Value (*kv*)

Mean kinship is blind to the age-structure of the population. It is calculated relative to the total gene pool. This may include post-reproductive animals, who can make no further genetic contribution to future generations. Including these animals when calculating mean kinship may underestimate an individual's genetic importance to the future genetic variation of the population. At the extreme, an individual whose relatives consist only of post-reproductive animals might have a moderate mean kinship, and therefore not be recognized as genetically important, even though it is the only individual in the population with the ability to perpetuate the genes it carries. The utility of mean kinship (as well as genome uniqueness, and any genetic metric that ignores the potential of individuals for future reproduction) is constrained by the demographic properties of an age-structured population.

This problem can be dealt with by taking into consideration the future reproductive potential of animals when calculating mean

kinships. We define the *kinship value* of an individual (kv_i) as a weighted mean of the kinship coefficients between individual i and all members of the population (including itself):

$$kv_i = \frac{\sum_{j=1}^{N} f_{ij} V_{xj}}{\sum_{j=1}^{N} V_{xj}} \qquad \text{Equation 7}$$

in which the weight (V_{xj}) is the reproductive value (Fisher 1930) for the age class (x) of which individual j is a member. The reproductive value is a measure of the extent to which an individual age x contributes to the ancestry of future generations (Crow and Kimura 1970). It is defined as:

$$V_x = \frac{\sum_{y=x}^{\infty} e^{-ry} l_y m_y}{e^{-rx} l_x} \qquad \text{Equation 8}$$

in which r is the intrinsic rate of increase, and l_x and m_x are the age specific survival and fecundity rates (Caughley 1977).

Kinship values will be lower than mean kinships (suggesting greater genetic value of that animal) if most of the kin of an animal are post-reproductive or nearly so. Kinship values will be greater (worse) than mean kinships if most of the kin are at a good breeding age. The kinship value of an individual is the expected inbreeding coefficient of progeny if it were mated at random and reproduced according to the V_x of its mate. Whereas the average mean

kinship is the genetic diversity of the descendant population if all individuals in the population were to be randomly bred, the average kinship value weighted by V_x is the expected gene diversity of the descendant population if the current population is paired at random but reproduces according to their life-table expectations.

Note that when calculating *kv*, the genetic importance of individuals will not be obscured by their own reproductive limitations. The reproductive value of the individual under consideration acts *only* as a weight applied to its kinship to itself, as do the reproductive values of the other members of the population. A post-reproductive individual can, in fact, have a non-zero *kv*. Consequently, a genetically important animal just entering reproductive senescence can still be identified as important and considered as a candidate for exceptional treatment (e.g., reproductive stimulation or surgical harvest of gametes).

Kinship value enables genetic importance to be calculated on the basis of the future expected genetic characteristics of the population, not solely on the basis of the current population's genetic status (as with *mk*). The implications of managing on the basis of the current population's genetic status can be easily seen in species with extremely short generation times. If genetic importance was determined relative to the current population, the importance could be heavily weighted by animals who would soon be dead. The problems associated with genetic importance based on current vs.

future population structure are mitigated in species with longer generation lengths, when the degree to which the population turns over each breeding cycle is reduced. Long generation times are more typical of populations now being managed. In such cases mean kinship and kinship value will be similar.

COMPARISON OF GENETIC MANAGEMENT STRATEGIES

Each of the measures of genetic importance defined above have been or currently are being used to develop breeding strategies for endangered species. The strategies differ in how they define genetic importance and could have different effects on the maintenance of genetic diversity.

This concern was addressed by developing computer simulation models to compare how well different breeding strategies maintained genetic variation in complex pedigrees. Breeding strategies based on *fic*, *mk*, and *gu* were compared to strategies based on maximum avoidance of inbreeding (*MAI*; Lesley 1978) and random breeding. The *MAI* strategy was used to represent a strategy that maximized inbreeding effective population size. The *mk* was used rather than *kv* because the model lacked age-structure (see below). Each of the strategies was used to select breeding animals in five simulated populations with different genetic characteristics. These populations were "managed" under each strategy for 20 generations and the strategies were

evaluated on how well they maintained alleles and gene diversity. The model is an extension of that presented by Ballou (1991).

The Model

The characteristics of the model were as follows:

1) The model considered a population of 30 sexually reproducing individuals (all reproductively capable) in non-overlapping generations. Population sizes were maintained at 30 individuals. Sexes were assigned randomly to individuals at the beginning of each generation; a 50/50 sex ratio was maintained.

2) The model was provided with 5 "seed" populations that had already undergone several generations (2 to 4, depending on the population) of unmanaged breeding. These populations had fairly complex pedigrees, with different characteristics (see below).

3) Each of the breeding strategies was used to "manage" the seed populations for 20 generations by selecting, each generation, the parents to produce the 30 offspring in the next generation. For the *fic*, *mk* and *gu* strategies, parents were selected using the following iterative approach:

   a) the genetic importance values (*fic*, *mk*, or *gu*) of the 30 parents in the last generation were calculated;

b) the male and female with highest genetic importance were selected;

c) an offspring from this pair was added to the next generation;

d) the genetic importance values of all parents were re-calculated *relative to only the gene pool of the offspring generation*;

e) the highest ranking male and female breeders were again selected to produce the next offspring, which was then added to the next generation. In the case of a tie (equal genetic value), the animal having produced the fewest number of offspring was selected;

f) steps d and e were repeated until 30 offspring were produced.

Since the genetic importance of the parental generation changed as offspring were produced, this iterative approach allowed genetically valuable individuals to continue to be selected as the breeders until their declining genetic importance values caused them to be replaced at the top of the list.

Founder contributions and mean kinship were calculated using an additive relationship matrix (Ballou 1983). Genome uniqueness was calculated using Monte Carlo simulations of 100 independent loci. Each founder was given 2 uniquely identifiable

alleles (numbered from 1 to 2N = 60) per locus and alleles were transmitted from parents to offspring by randomly selecting one allele each from the mother and father for each locus. Genome uniqueness for an individual was defined as the total number of unique alleles held by that individual (summed across all 100 loci) divided by 200.

For the *MAI* strategy, the approach was somewhat different since genetic importance calculations were not used. This strategy utilizes a recursive mating strategy that assigns equal genetic value to all individuals (Lesley 1978; Flesness 1977; Senner 1980; Princeé In press). Individuals in the first generation to be managed were sorted randomly and assigned numbers 1 through 30; odd numbers given to males, even to females. Female x was paired with male x-1 to produce male x/2 and female 15+(x/2) in the next generation. Thus, each pair produced two offspring and all pairs bred, maximizing the population's inbreeding effective size.

Under the random breeding strategy, the process of selecting a male and female randomly, with replacement, to produce one offspring was repeated until 30 offspring were "born."

4) The five breeding strategies being tested were each evaluated on their ability to: a) retain gene diversity; b) retain allelic diversity; and c) minimize inbreeding. Proportional gene di-

versity is defined as the percent of original population's expected heterozygosity retained and can be calculated as 1 - $\bar{mk}$. Allelic diversity is defined as the average number of unique founder alleles surviving each generation. It is calculated here as the number of different founder alleles per locus in the population averaged across the 100 loci.

5) Each complete simulation, starting with the complex pedigree provided and selecting breeders (according to the genetic importance measure being tested) until the 20th generation, was repeated 50 times. The breeding strategies were evaluated by averaging the results over the 50 simulations.

The Five "Seed" Pedigrees

The five populations used to seed the model were created to resemble populations that had already undergone several (2 to 4) generations of unmanaged breeding and therefore represented a variety of genetic characteristics typical of captive populations. The overall genetic diversity of the pedigreed population can be described in terms of gene and allelic diversity. However, it is also useful to consider individual differences within these pedigrees. Regardless of the level of genetic diversity present in the population, if all individuals are of similar genetic value, then the problem of selecting whom to breed is irrelevant. The complexity, and need for guidance, increases when there are significant dif-

ferences in genetic value among individuals. Figure 4a shows the level of gene diversity vs. allelic diversity in the test pedigrees, while figure 4b shows the variation of these measures at the individual level. The five pedigrees span a range of population levels of genetic diversity as well as a range of inter-individual variances in genetic diversity.

Model Simulation Results and Discussion

The genetic diversity retained after 20 generations in each of the five test pedigrees using the different breeding strategies is given in table 1. Figures 5 and 6 show the changes in genetic diversity over the 20 generations for two of the test pedigrees: pedigree "1", which started with the a high level of genetic diversity, and pedigree "4", which started with the lowest.

The mean kinship strategy retained the highest levels of gene and allelic diversity in all five pedigrees. It did not always minimize inbreeding. This is not unexpected since the strategy, as used by the model, does not preclude mating among sibs. Often the two individuals with the lowest mean kinships are related (e.g., full sibs). In practice, the mean kinship strategy can be constrained to preclude mating among highly related individuals.

While three of four breeding strategies performed substantially better than simple random breeding, the strategy to equalize

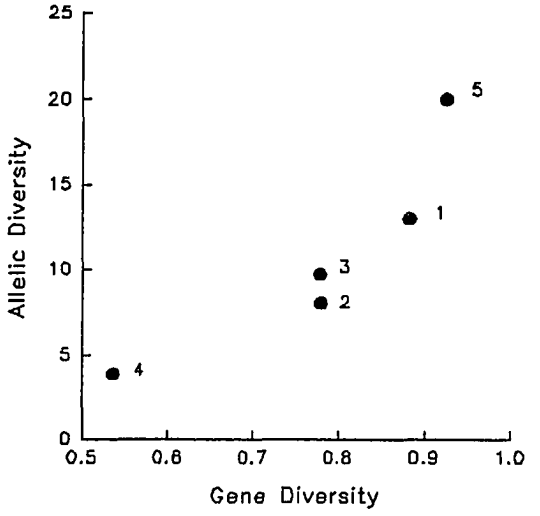

Figure 4a. Allelic diversity (# surviving founder alleles) vs. gene diversity in the last generation of the five "seed" pedigrees used in the computer simulation model.

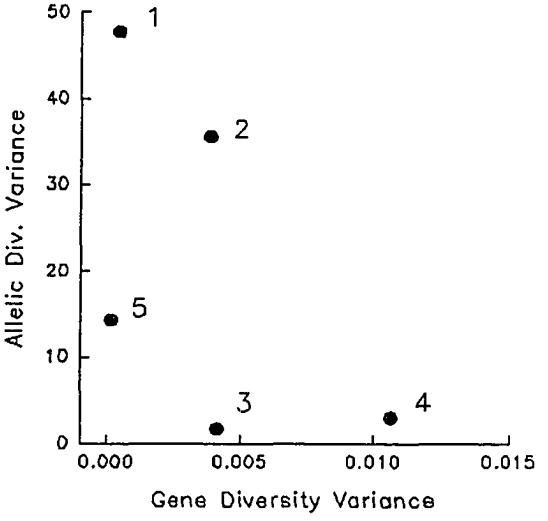

Figure 4b. The variance in mean kinship and allelic diversity (# surviving founder alleles) among individual in the last generation of the 5 "seed" pedigrees.

founder contribution using *fic* performed the least well compared to the other strategies, and in some cases was worse than random breeding. This is because the *fic* strategy cannot discriminate between individuals with the same founder contributions (e.g., siblings). Thus, changes in genetic importance will be highly correlated among individuals from the same sibship or lineage, often leading to pairings between individuals descended from a common set of founders. Depending on the starting pedigree configuration, this can lead to population sub-division and line-breeding, resulting in a rapid loss of gene diversity and high inbreeding. This occurred in test population #1 (figure 5a and 5b), in which levels of gene and allelic diversity dropped rapidly as the population was subdivided. Average allelic diversity converged towards preservation of only 2.6 alleles as alleles became fixed within the population sub-divisions.

Note that the *mk* and *gu* strategies can increase gene diversity (figure 6a), as they adjust for past mismanagement or lack of management. These strategies preferentially breed individuals who are carriers of rare genotypes, while the *MAI* and RANDOM strategies can only decrease gene diversity at rates dependent on the effective population size.

Figure 5 and 6 also show that after a few generations of management the rate of loss of genetic diversity is often similar under the *MAI*, *mk*, and *gu* breeding strategies. It does not take long for

Table 1. Levels of gene diversity retained in the 5 "seed" pedigrees after 20 generations of breeding under the 5 breeding strategies. Means and standard errors (SEM) are calculated over 50 simulations.

PEDIGREE "1"						
Breeding Strategy	Gene Diversity		# Founder Alleles		Inbreeding	
	Mean	SEM	Mean	SEM	Mean	SEM
mk	0.784	0.001	7.397	0.169	0.219	0.014
GU	0.759	0.002	7.042	0.123	0.223	0.008
MAI	0.770	0.002	7.007	0.122	0.211	0.015
FIC	0.606	0.022	3.604	0.334	0.376	0.017
RANDOM	0.679	0.012	5.091	0.195	0.298	0.013

PEDIGREE "2"						
Breeding Strategy	Gene Diversity		# Founder Alleles		Inbreeding	
	Mean	SEM	Mean	SEM	Mean	SEM
mk	0.729	0.001	5.818	0.121	0.275	0.011
GU	0.697	0.003	5.547	0.102	0.287	0.007
MAI	0.679	0.001	5.230	0.101	0.305	0.011
FIC	0.652	0.012	4.324	0.190	0.453	0.037
RANDOM	0.598	0.018	4.181	0.201	0.383	0.020

PEDIGREE "3"						
Breeding Strategy	Gene Diversity		# Founder Alleles		Inbreeding	
	Mean	SEM	Mean	SEM	Mean	SEM
mk	0.760	0.003	6.494	0.111	0.244	0.014
GU	0.728	0.003	6.184	0.102	0.255	0.007
MAI	0.667	0.002	4.965	0.114	0.322	0.019
FIC	0.726	0.010	5.362	0.211	0.550	0.058
RANDOM	0.580	0.026	3.840	0.252	0.400	0.028

Table 1 (Continued).

PEDIGREE "4"						
Breeding Strategy	Gene Diversity		# Founder Alleles		Inbreeding	
	Mean	SEM	Mean	SEM	Mean	SEM
mk	0.545	0.005	3.266	0.058	0.458	0.011
GU	0.505	0.007	3.195	0.067	0.482	0.008
MAI	0.468	0.001	2.893	0.075	0.525	0.015
FIC	0.455	0.008	2.614	0.082	0.528	0.009
RANDOM	0.411	0.019	2.544	0.106	0.575	0.020

PEDIGREE "5"						
Breeding Strategy	Gene Diversity		# Founder Alleles		Inbreeding	
	Mean	SEM	Mean	SEM	Mean	SEM
mk	0.814	0.001	8.600	0.129	0.193	0.017
GU	0.796	0.002	8.234	0.148	0.187	0.008
MAI	0.808	0.002	8.356	0.156	0.175	0.016
FIC	0.789	0.012	6.556	0.308	0.651	0.068
RANDOM	0.709	0.013	5.711	0.238	0.267	0.015

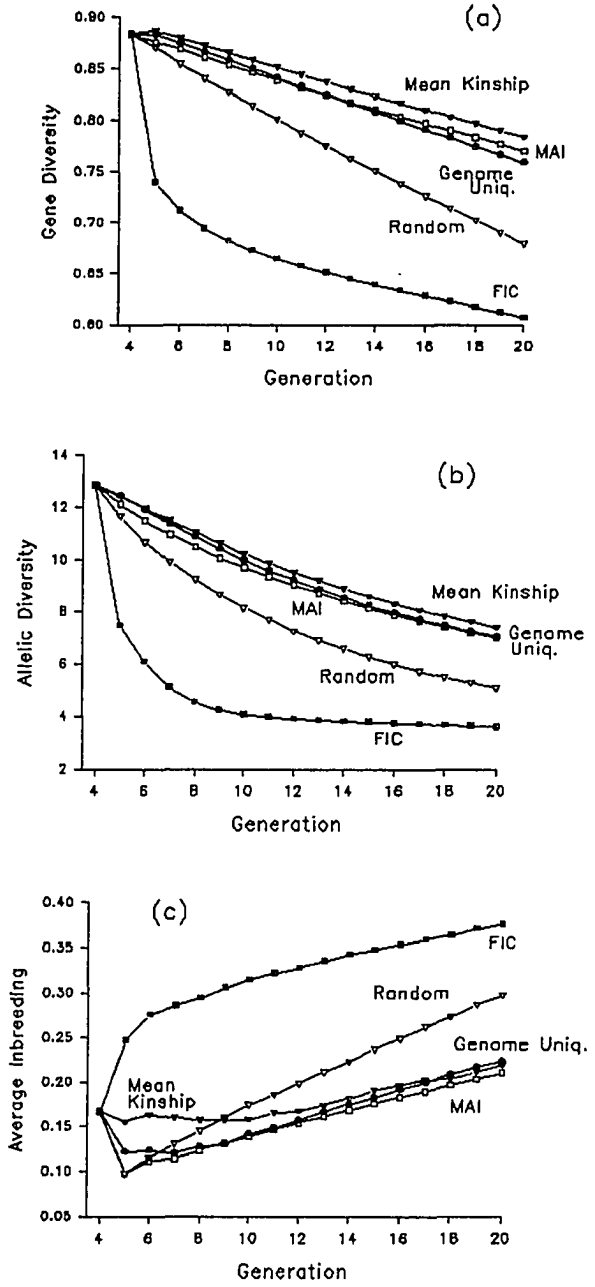

Figure 5. Effect of different breeding strategies on levels of gene diversity (a), allelic diversity (b) and inbreeding (c) in population "1" over 20 generations.

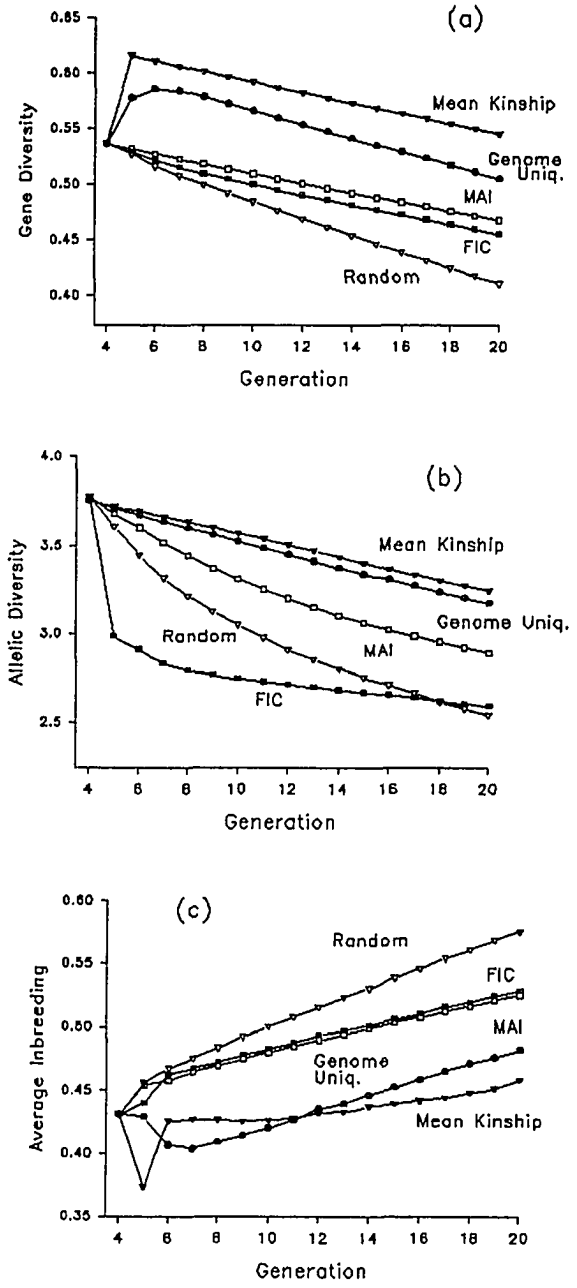

Figure 6. Effect of different breeding strategies on levels of gene diversity (a), allelic diversity (b) and inbreeding (c) in population "4" over 20 generations.

these breeding strategies to compensate for the initial complexities of pedigrees. Once this happens, all individuals have equal genetic importance and each of the breeding strategies result in each animal producing two offspring.

Over the long term, gene diversity declined slightly more rapidly under the *gu* strategy than under *mk* and *MAI*, but this was because of sampling error in the simulation model. The probability of an allele being unique is based on a sample of 100 loci, which will have a sampling error large enough to affect the results when estimating small probabilities. Small non-zero probabilities of allele uniqueness are usually estimated as zero probabilities, giving incorrect breeding priority and increasing the variance in family size (in accord with the sampling variance of the simulation). When the number of loci is increased to 200, the rate of gene diversity loss more closely approximates that of the *mk* and *MAI* strategies. In practice, estimates of genome uniqueness should be based on many thousands of simulations so that sampling problems do not measurably affect the results (Thomas 1990).

Despite its name, the *MAI* strategy does not always minimize inbreeding (table 1; figure 5 and 6). This is because it does not take into consideration the pedigree of the initial population, but simply breeds animals according to the recursive *MAI* strategy. By chance, some initially related animals will be paired. *MAI* does

minimize inbreeding (Crow and Kimura 1970) when applied to a population of initially unrelated individuals (e.g., founders).

In summary, mean kinship performed significantly better than all other strategies for all pedigrees provided. Both *MAI* and *gu* did fairly well, in general out-performing both *fic* and random breeding. Management to equalize founder contribution using *fics* is not recommended. Strategies that minimize loss of gene diversity (expected heterozygosity) generally also minimize loss of allelic variation.

EFFECT OF UNKNOWN PARENTAGE ON MEASURES OF GENETIC IMPORTANCE

Calculations of kinships, inbreeding coefficients, and founder allele survival are critically dependent on complete knowledge of the pedigree. Unfortunately, however, many pedigrees of interest have some individuals with one or both parents unknown. Molecular genetic information can resolve some of these uncertainties (Morin and Ryder 1991; Haig et al. 1994; Avise et al., In press), or alternative methods of population analysis and management can be applied (Lacy et al. In press.). Traditionally, such gaps in the pedigree have been bypassed in pedigree analysis by assuming that animals with unknown parents are founders, unrelated to all non-descendant animals within the pedigree. Minimum estimates of kinships, inbreeding, and rates of loss of genetic diversity can then be obtained. In cases in which the unknown parents are likely to be unrelated to other animals in the pedigree (for example, when unknowns came from

zoos on another continent), this optimistic assumption may be appropriate. In other cases, however, it may be known that the unknown parents are within the pedigree, such as when paternity cannot be assigned with certainty to any one of several males in a multi-male, multi-female social group. In such cases, the assignment of founder status to unknown parents can lead to substantial errors in estimating genetic parameters, often assigning high genetic importance to those animals descended from the unknown "founders."

On approach to the problem is to exclude individuals with unknown ancestry from the breeding program. This is likely to reduce levels of genetic diversity (Willis 1993). An alternative is to consider only that part of these individuals' genomes that is known. Unbiased estimates of kinships and inbreeding coefficients for the proportion of the genome that is known would omit from consideration those parts of genomes that descend from unknown parents. In a gene drop analysis, the exclusion of genes that cannot be traced to properly classified founders is accomplished simply: statistics on genetic variation within the population and on sharing of genes among individuals can be calculated after exclusion of any "founder" alleles that are derived from unknown animals that had been treated as founders. The gene drop analysis within the program GENES (Lacy 1992) calculates all parameters with and without any such unknown alleles.

Precise calculations of kinships (and therefore mean kinship and kinship values) and inbreeding coefficients can also be made from partially known pedigree data, after exclusion of those parts of genomes that cannot be traced to the pedigree founders. Conceptually, the task is to estimate the probability that two alleles drawn randomly from two individuals are identical by descent under the condition that those alleles have traceable ancestries. To derive the appropriate equations, let k_i be the proportion of the genome of animal i that can be traced to known founders. The k_i will be 1 for animals with completely known ancestry, 1/2 for animals with one unknown parent, and 0 for those with two unknown parents. For any other animal, $k_i = (k_m + k_p)/2$, the mean of the proportions known of the parents (m and p). Let f'_{ij} be the kinship between animals i and j relative only to those parts of the genomes that are traceable to known founders. Let F' be the inbreeding coefficient of an individual defined as the probability of identity by descent of a maternal allele and a paternal allele drawn at random from among those that are traceable. As in the case of a completely known pedigree, $F' = f'_{mp}$, in which m and p are the parents.

The kinship, f'_{ij}, between two individuals will be the probability that an allele sampled from the traceable (known) genome in j is identical by descent to an allele sampled from among the known maternal alleles in i, multiplied by the probability that a known allele sampled from i is maternally derived, plus the probability that the allele sampled from j is identical by descent to an allele

sampled from among the paternal alleles in i multiplied by the probability that an (known) allele sampled from i is paternally derived. Thus,

$$f'_{ij} = \frac{f'_{mj}k_m}{(k_m+k_p)} + \frac{f'_{pj}k_p}{(k_m+k_p)}$$

$$= \frac{(f'_{mj}k_m+f'_{pj}k_p)}{2k_i} \qquad \text{Equation 9}$$

in which: the subscripts m and p refer to the parents of i; i is not an ancestor of j; $f'_{ij} = 0$ when i,j are founders; and $k = 1$ for founders. Using the above formula, f'_{ij} can be calculated for all i,j combinations provided i is not a founder (simply reverse i and j in this case to allow the calculation to proceed using the above formula) and provided that values for ancestors are calculated before their descendants. The kinship is undefined if an animal's ancestry is unknown. This formula is particularly apropos for calculating f'_{ij} using a modified additive relationship matrix approach.

The kinship of an animal to itself, f'_{ii}, will be the probability that, when two alleles are drawn at random from the known portion of the genome, the first allele drawn is re-sampled (both alleles are the maternally derived allele or both are the paternal allele) or that the allele is not re-sampled (one paternal allele sampled, one maternal allele sampled) but that the two alleles are identical by descent nonetheless. The probability that the maternal allele is sampled twice is $[k_m/(k_m + k_p)]^2$, and analogously for the

probability that the paternal allele is sampled twice. The probability that one maternal allele and one paternal allele are sampled is $2[k_m / (k_m + k_p)] * [k_p / (k_m + k_p)]$. The probability that they are identical by descent is, by definition, f'_{mp}. Hence,

$$f'_{ii} = \left[\frac{k_m}{(k_m+k_p)}\right]^2+\left[\frac{k_p}{(k_m+k_p)}\right]^2+2f'_{mp}\left[\frac{k_m}{(k_m+k_p)}\right]\left[\frac{k_p}{(k_m+k_p)}\right] = \frac{k_m^2+k_p^2+2k_mk_pf'_{mp}}{(4k_i^2)} \quad \text{Equation 10}$$

Figure 7 shows a simple pedigree in which one animal is unknown. Table 2 shows the matrix of kinships of those animals when unknown genes are omitted as described in equations 9 and 10. Omitting unknown genes, $f'_{BD} = 1/2$, because all genes known in **D** come from **B**, but only half of the genes in **B** were transmitted to **D**. Note that $f'_{DD} = 1$, because only the maternal genes in **D** are known (**D** is haploid with respect to known genes.) The inbreeding coefficient of **E** is 1/4 ($= f'_{CD}$), when unknown genes are omitted.

The kinship and inbreeding coefficients calculated with unknown genes may be less than or greater than the values that would be calculated if the pedigree were fully known. In the above example, if the unknown animal is unrelated to **A** and to **B**, then animal **E** is the product of a half-sib mating and has an inbreeding coefficient of 1/8. If the unknown is the same animal as **A**, then **E** is the product of a full-sib mating and has an inbreeding coefficient of 1/4. If the unknown is the same as animal **C**, then **E** is the product of two generations of parent-offspring matings and has an inbreeding

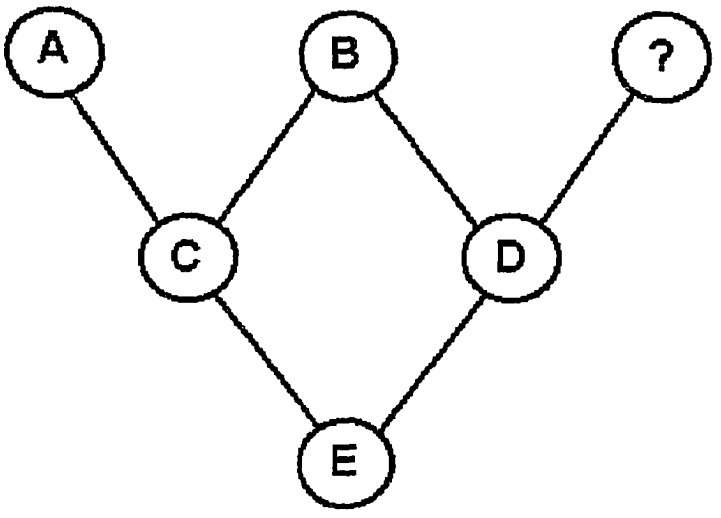

Figure 7. Hypothetical pedigree containing one unknown parent.

Table 2. Matrix of kinships among animals in the pedigree shown in figure 7 calculated by excluding the portions of individuals' genomes that are unknown.

	A	B	C	D	E
A	1/2	0	1/4	0	1/6
B	0	1/2	1/4	1/2	1/3
C	1/4	1/4	1/2	1/4	5/12
D	0	1/2	1/4	1	1/2
E	1/6	1/3	5/12	1/2	2/3

coefficient of 3/8. The inbreeding coefficient calculated after omitting the unknown genes (1/4) is intermediate to the extreme possibilities.

Mean Kinships When Unknown Genes Are Excluded

Mean kinship can be calculated from the above statistics when pedigrees contain unknown animals. This can give an unbiased estimate of the gene diversity when excluding the portion of the gene pool that has descended from unknown animals. The mean kinship of individual i should be a weighted average of f'_{ij}'s, with the weights being the probability that individual j's alleles are chosen from the gene pool. Because some animals are only partly known ($k < 1$), and therefore contribute only partially to the gene pool, the probability that j's known alleles are chosen when the gene pool is sampled randomly will be proportional to k_j. Thus,

$$mk'_i = \frac{\sum_{j=1}^{N} (k_j f'_{ij})}{\sum_{j=1}^{N} k_j} \qquad \text{Equation 11}$$

For the population, the average mean kinship (the expected loss of gene diversity in the descendants if all animals were mated at random) must be weighted by the probability that alleles from each pair of animals are selected from the gene pool. Thus,

$$\overline{mk}' = \frac{\sum_{i=1}^{N}\sum_{j=1}^{N}(k_i k_j f'_{ij})}{\sum_{i=1}^{N}\sum_{j=1}^{N}(k_j k_i)} \qquad \text{Equation 12}$$

The accuracy of these methods for correcting mean kinship (and, therefore, estimates of losses of gene diversity) when there are unknown parents within a pedigree has been confirmed by comparing gene diversities calculated by equation 12 to those generated by a gene drop simulation (in which unknown alleles were excluded from the calculations).

When a large proportion of the pedigree is unknown, detailed pedigree analyses are not appropriate; estimates of *mk* based on the procedures outlined above will apply only to a small part of the gene pool. However, the results from the *MAI* breeding strategy in the computer modeling discussed earlier have interesting implications for managing populations with unknown pedigrees. Despite lack of pedigree information, high levels of genetic variation can be maintained using a maximum avoidance of inbreeding strategy which does not rely on (nor make use of) prior pedigree information: populations with unknown pedigrees need not be excluded from genetic management (e.g barasinga, *Cervus duvaceli duvaceli;* Killmar 1991). The cost of not knowing the pedigree, however, is the potential for high levels of inbreeding (and the deleterious consequences of inbreeding depression) early in the breeding program resulting from

unknowingly pairing closely related animals. The use of *MAI* strategies in these cases is discussed further by Princée (In press).

CONCLUSIONS AND RECOMMENDATIONS

Theory and model results support the conclusion that management by mean kinship provides an efficient and relatively easy strategy for maintenance of both expected heterozygosity and alleles in populations with complex pedigrees. In the Guam rail (*Rallus owstoni*), Haig et al. (1990) also found that a mate selection based on maximizing founder genome equivalents performed better than strategies based on prior reproductive success, allozyme data, equalizing founder representation or simple random selection. As has been shown here, a breeding strategy that minimizes mean kinship is equivalent to strategies that maximize founder genome equivalents and gene diversity and relates directly to the previously recommended strategy of managing by target founder contributions.

In practice, we recommend that both kinship value and mean kinship be used to identify genetically important individuals. It is not recommended that kinship value be used alone since the reproductive values used in its calculation are based on life-table statistics summarizing average population trends. As is often the case for small captive populations, these may be based on poor data, particularly in the older age classes (see Taylor and Barlow, In press). Expert knowledge of the reproductive potential of specific individu-

als is obscured. Examining both mean kinship and kinship value rankings provides a dual approach to identifying genetic importance both with and without demographic constraints. We recommend also that genome uniqueness be calculated as a secondary measure of genetic importance to assure that individuals with high levels of genome uniqueness are provided with breeding opportunities. As was discussed above, it is possible for individuals with moderate levels of mean kinship to have high levels of genome uniqueness, and ranking by *mk* alone may not identify these individuals.

One approach to developing breeding recommendations is to select pairs from the top of sorted lists of mean kinship, kinship value, or genome uniqueness, excluding combinations involving highly related mates. The definition of "highly related" will be relative to the overall level of inbreeding in the population. Therefore, a useful rule of thumb is to select as mates animals whose kinships do not greatly exceed the average mean kinship (Ballou and Foose 1994). This will keep inbreeding coefficients near or below the mean expected if the population were randomly bred that generation. In avoiding breeding related animals, attempts should be made to pair mates with similar levels of *mk*. Mating a low *mk* to a high *mk* animal will result in mixing rare and common alleles, as in GLT 1142 (figure 2); thereafter, the number of copies of the rare alleles cannot be increased without also increasing the over-represented alleles.

A second approach to developing breeding recommendations is to select breeding pairs by iteratively re-calculating the *mk* rankings as breeding pairs are selected (as was done in the computer model). Each pair, once selected, is assumed to produce one offspring (or several offspring, depending on the life history of the species), which is added to the population. *mk* and *kv* are then re-calculated to provide a revised ranking of individuals based on pairs already selected to breed (but re-calculating *gu* is problematic since it requires a time-consuming gene drop analysis). It is possible, by using this method, to determine the set of matings and the number of offspring from each that will maximize gene diversity in the population.

The efficiency of any theoretically based breeding strategy is limited by demographic constraints that are imposed by life-history and management considerations. Breeding strategies must recognize these constraints. One obvious constraint is that there is a limit to the number of young any one pair (or individual) can produce over its lifetime. The maximum number of young desired from a pair for genetic management may exceed their reproductive limits. In this case, optimal genetic management may not be possible.

Application of the mean kinship concept can be applied to problems other than mate selection. Calculating *mk* values of animals in one population relative to another population allows one to access the genetic effects of transferring animals between popula-

tions. Both the effect of removing individual from one population and adding to another can be determined. This approach can easily be used to manage sub-divided population but can, and has been, applied in such diverse cases as selecting animals for reintroduction (in the California condor, *Gymnogyps californianus* and golden-lion tamarin) and for identifying males to donate sperm to a genome resource bank (Johnston and Lacy, In press). Haig et al. (1994) also applied the mean kinship concept to an analysis of DNA fingerprinting data in Guam rails. They were able to show that mean DNA profile similarity (calculated as the average profile similarity between an individual and the rest of the population) correlated significantly with mean kinship. A large variance in the data precluded accurate predictions of genetic importance at the individual level strictly from the band sharing data.

The concept is easily extended to the value of groups of individuals and can be applied to identify groups of priority breeders, groups (e.g. families) for reintroduction (Tonkyn 1993), or differences in genetic value of population sub-divisions (Geyer et al. 1989; Thompson, In press).

Computer software is available for calculating inbreeding coefficients, mean kinship, genome uniqueness, and kinship value from pedigrees. The Single Population Animal Record Keeping System (SPARKS; ISIS 1991), an IBM compatible software system for population management, is distributed with the genetic analysis program

GENES (Lacy 1993) and the demographic analysis program DEMOG (Bingaman and Ballou 1986) to provide an integrated system which combines the demographic and genetic calculations discussed above. This software may be obtained from ISIS, 12101 Johnny Cake Ridge Road, Apple Valley, MN 55124, USA.

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CHAPTER II

OUTBREEDING DEPRESSION: ANALYSIS OF DATA FROM CAPTIVE MAMMAL POPULATIONS

INTRODUCTION

The genetic structure of populations is of great interest to both evolutionary and conservation biologists as it can provide information on the evolutionary history, breeding structure and dispersal strategies of populations as well as guide conservation strategies for populations threatened with extinction. Two areas of particular interest are the effects of inbreeding and outbreeding on fitness.

Inbreeding depression is the decrease in fitness of offspring born to mates that are related. Inbreeding results in an increase in homozygosity in offspring and causes fitness depression by a combination of a decrease in the benefits from overdominant loci, and/-or an increase in the expression of detrimental effects of deleterious recessive alleles (Charlesworth and Charlesworth 1987). The deleterious effects of inbreeding on various fitness components has been documented extensively (Charlesworth and Charlesworth 1987,

Thornhill 1993). In mammals, inbreeding depression has been recorded in numerous laboratory (Lynch 1977, Wright 1977, Brewer et al. 1990)), domestic (Lasley 1978), zoo (Ralls et al. 1979; Ballou and Ralls 1982; Ralls et al. 1988), and, but to a much lesser extent, wild populations (Packer 1979; Bulmer 1973; Baker and Dietz *in prep*; Jiménez et al. 1994; Stockley et al. 1993). Most of these studies use survival as the fitness component of interest but recognize that this may only be a small component of total fitness.

The decline in offspring fitness resulting from crossing mates with different genetic backgrounds is referred to as outbreeding depression. Outbreeding depression usually refers to reduced fitness caused by "close" outbreeding (crosses of individuals between or even within populations) as opposed to "wide" outcrossing (between subspecies or species). Outbreeding depression has been described as resulting from two mechanisms (Price and Waser 1979; Waser 1993). The genetic, or "intrinsic" (Templeton 1986) mechanism requires that different populations evolve different intra-genomic coadaptations (e.g., coadapted gene complexes): chromosomes, loci and/or genes within genomes are coadapted to function together. Crossing individuals from different coadapted populations disrupts favorable coadaptations, reducing fitness. Outbreeding depression within the F_1 generation results from the breakdown of between-chromosome or within-locus coadaptations, while more complex crosses like the F_2, backcross or even later generations can experience outbreeding depression when recombination disrupts between-locus (epistatic) coadap-

tations (also known as F_2 breakdown or recombinational loss; Falconer, 1981). The "ecological" or "extrinsic" mechanism of outbreeding depression results from adaptations developing in response to different local biotic or abiotic environments. Crosses may produce progeny less suited to either environment (Templeton et al. 1986, Shields 1982; Templeton 1986).

Analyses of pedigrees from captive populations provide one means of evaluating the effects of inbreeding and outbreeding on fitness (Ralls et al. 1988; Templeton and Read 1984; Lacy et al. 1993). These studies use pedigrees of captive populations, which usually include a variety of generational types, to estimate the relationship between some component of fitness, usually survival, and outbreeding or inbreeding as measured from the pedigree. As pointed out by Shields (pers. comm., Templeton 1986), however, one complication in analyzing pedigrees from captive populations for inbreeding and outbreeding effects is that outbreeding effects may be confounded with inbreeding effects if the captive populations have been founded by individuals collected from differently adapted source populations (Templeton 1987). This possibility exists because both inbreeding and recombination, which can disrupt coadapted gene complexes, first occur in the F_2 or backcross generations (figure 8). Thus, the degree of recombination between the genomes of founde rs from different sources may be correlated with the degree of inbreeding. Because of this confounding effect, Shields (Templeton 1986 and pers. comm.) has argued that previous studies showing

evidence of inbreeding depression in captive populations may actually be due to outbreeding depression.

Here we address the concern that outbreeding depression, rather than inbreeding depression, might be responsible for the decreased fitness observed in a large variety of captive mammals. We present several models, none of which is ideal for all data sets, and use them to analyze the joint effects of inbreeding and outbreeding on survival in five captive mammalian populations that exemplify the best available data. We discuss the relative frequency and magnitude of deleterious inbreeding and outbreeding effects in these populations and the limitations of the available data and analyses.

Models of Outbreeding Depression

Numerous models have been proposed for the analysis of outbreeding/outcrossing effects (Kempthorne 1957; Eberhart and Gardener 1966; Dickerson 1969; Cockerham 1980; Kinghorn 1980, 1982; Sheriden 1981; Hill 1982; Mather and Jinks 1982; Templeton and Read 1984; Lynch 1991). The models used here are described below.

Templeton and Read Model

Templeton and Read (1984) proposed a model specifically for use in measuring the epistatic effects of outbreeding depression

(i.e., those associated with disruption of coadapted gene complexes) from complex pedigree data. They defined the "hybridity coefficient" (h_i) of individual i as the average proportion of its parents' genome descended from founders from different sources:

$$h_i = \frac{H_d+H_s}{2} \qquad \text{(Eq. 1)}$$

where H_d and H_s are the probabilities of the dam and sire of individual i being heterozygous for two alleles from different sources. Values of h_i range from 0 (individuals that have inherited intact founder chromosomes, i.e., chromosomes which have not had the opportunity to be recombined with homologous chromosomes from other founders - as in the F_1 individuals and "pure line" individuals) to 1.0. Hybridity coefficients for individuals in a simple pedigree are shown in figure 8.

While Templeton and Read originally intended hybridity coefficients to be calculated under the assumption that all founders of a captive population originated from different source populations (and thus potentially carried different coadapted gene systems), the computations can be modified easily to account for several founders originating from the same source population by assigning these founders a common pair of parents.

The Templeton and Read model suffers from several limitations. First, the hybridity coefficient, although intended as a measure of

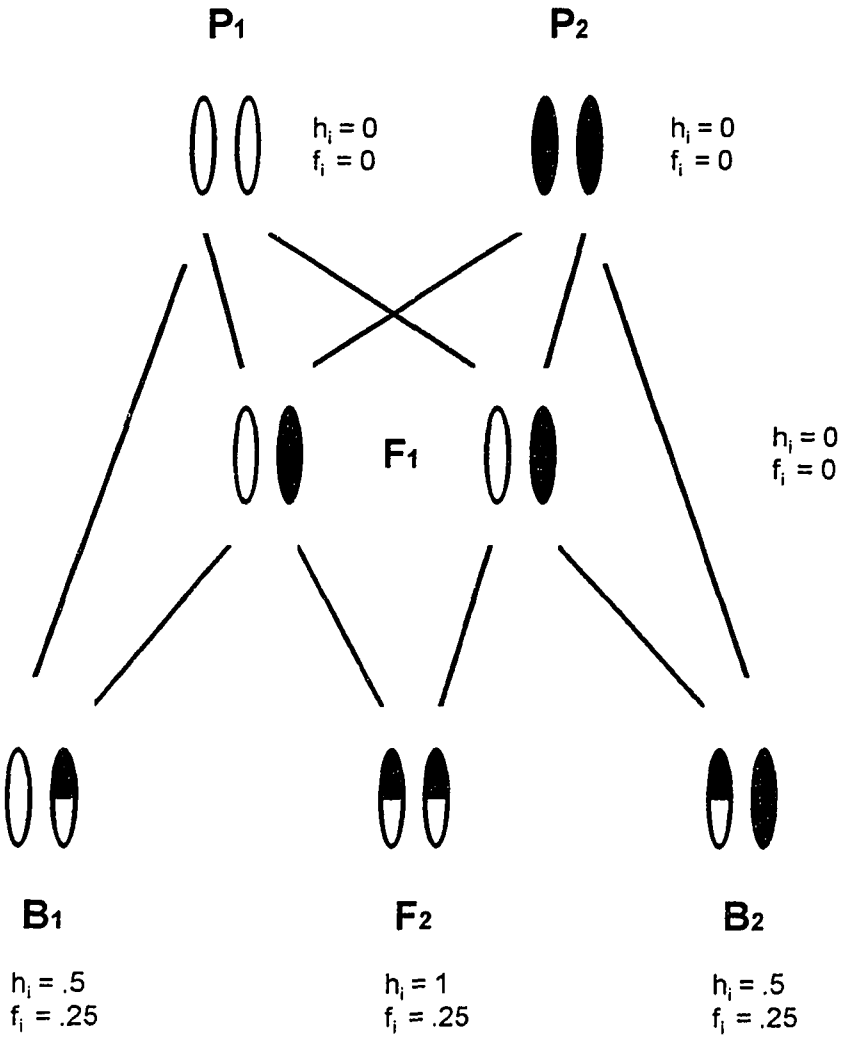

Figure 8. Templeton's hybridity coefficients (h_i) and inbreeding coefficients (f_i) calculated for individuals in a simple pedigree. Homologous chromosomes are shown for each individual. Shared shading patterns indicates sections of chromosomes derived from the same populations. (Figure modified from Templeton and Read 1984).

epistasis, is calculated solely as a function of allelic interactions *within* loci rather than between loci. Disruption of coadapted gene complexes, however, are disruptions of epistatic effects involving interactions *between* loci. Because of this, the hybridity coefficient does not adequately model the disruption of coadapted gene complexes in complex pedigrees with extensive inbreeding. The problem can be illustrated by considering a highly inbred population's pedigree. In this situation, the alleles at any given locus are identical by descent (f=1) but all loci involved in a coadapted gene complex will not necessarily be fixed for alleles from the same source. Coadapted gene complexes can still be disrupted in highly inbred individuals if they are fixed for different alleles at the loci involved. However, in these individuals, H_d and H_s approach zero and h approaches 0, implying no hybridity. Since the hybridity coefficient does not consider inter-locus interactions, it can underestimate levels of outbreeding in some individuals (Templeton, pers comm).

Secondly, the Templeton model does not include expressions to detect additive, or dominance (e.g., heterosis or F_1) effects. Inclusion of additive and domincance effects in these models is important because they may be confounded with outbreeding epistatic effects when pedigree data are unbalanced with respect to representation of generation types. For example, consider a case when fitness is strictly additive. Figure 9 shows the fitness plotted against Templeton's hybridity coefficient for several generational

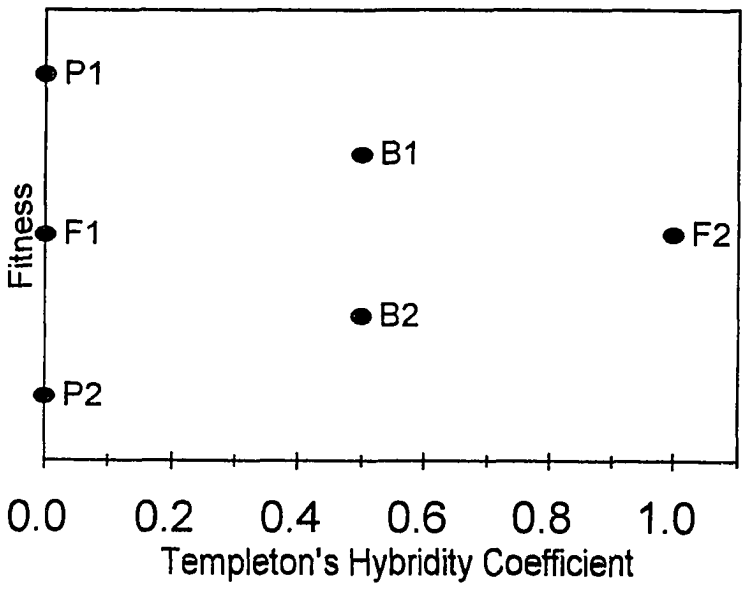

Figure 9. Templeton's hybridity coefficients plotted against fitness for individuals in pedigree shown in figure 8 when fitness is assumed to be additive and fitness $P_1 > P_2$.

types. A completely balanced data set (with all generations shown in figure 9 represented) would correctly show no correlation between hybridity and fitness. However, a negative correlation would be observed if the data consisted primarily of P_1, B_1 and F_2 individuals. This would erroneously lead to the interpretation that outbreeding depression (epistatic effects) as measured by Templeton's hybridity coefficient was responsible for reduced fitness, rather than the true additive effects. The same problem can arise if fitness is dominance in nature. Thus, additive and dominance effects should be incorporated in the model to control for the possible confounding of additive, dominance and epistatic effects in unbalanced data.

In addition, since a major effect of outbreeding may be negative heterosis or breakdown in the F_1 crosses between source populations (in addition to whatever other effects occur in later generations) the inclusion of parameters to detect such effects would be a useful component of any outbreeding depression model. These problems have been addressed in this study by adding to the Templeton model inbreeding, additive and dominance effect parameters.

Kinghorn Models

Kinghorn (1987) presented seven models of two-locus epistasis for the analysis of crossbreeding effects. These were based on seven hypothesized biochemical interpretations of possible gene actions

involving alleles from 2 loci. For example, Kinghorn's first model describes one-to-one interaction between gene products from two-loci. The combined product (e.g., a dimeric enzyme) is only considered effective if generated by gene products produced by alleles from the same source population (i.e., they form a coadapted gene complex); when produced by alleles from different source populations, the product is ineffective (the coadapted gene complex is disrupted). The probability of this occurring is the probability of choosing two different source alleles when randomly selecting one allele from each of two loci. This is a additive by additive model of epistasis. Kinghorn combined these epistatic effects with additive and dominance effects in a generalized multivariate regression model to evaluate the role of these effects on various components of fitness (Kinghorn 1980; 1987). Kinghorn (1980, 1987) did not present the mathematical formulas for his models, but provided model parameters for specific generational types (e.g., F_1, F_2, and backcrosses). To apply the Kinghorn models to data from more complex pedigrees, expressions were derived for each of the models (K_1 to K_7) in terms of parameters easily calculated from any arbitrary pedigree (table 3). Model K_3 was originally proposed by Sheridan as a "parental epistasis" model (Sheridan 1981). However, Kinghorn's presentation contained an error in interpretation of Sheridan's original model (Kinghorn 1987; Sheridan 1981; Kinghorn, pers. comm). This error is corrected here. In addition, K_4 was originally proposed by Andresen and Christensen (1981).

Kinghorn (1987) used data from S. Wright's guinea pig studies (Wright 1922) to evaluate the fit of these models for eleven fitness traits. Although Kinghorn concluded that model K_1 (the additive by additive model) gave the best general fit over all traits studied, this model frequently was not the best fitting model, and for one fitness trait (rate of weight gain) failed to detect an epistatic effect which was detected by another model (K_5). Because no one model was able to detect all epistatic effects in the data, we considered all seven models for our analyses.

Lynch Model

Lynch (1991) proposed a more explicit model of outbreeding (and inbreeding) depression based on earlier work by Cockerham (1980), Mather and Jinks (1982) and Hill (1982). Lynch's model is derived from a long history of models used to evaluate cross-breeding or line-crossing effects in the agricultural sciences, and is based on a quantitative genetics approach. This model, however, was developed exclusively for populations derived from two sources. Outbreeding is interpreted in terms of the effect on fitness of the additive, dominance and epistatic effects of genes derived from the two different source populations. Additive effects account for the linear effect of the source population on fitness (i.e., founder contributions), dominance accounts for the within-locus interaction of alleles from different sources (e.g., heterosis/hybrid vigor effects in the F_1 generation), and the two-locus between-loci inter-

actions (epistasis) are defined as additive-by-additive, additive-by-dominance, and dominance-by-dominance interactions. Higher order interactions (e.g., three-locus interactions) are similarly expressed as additive x additive x additive, additive x additive x dominance, etc, effects. Loci are assumed to be unlinked, and effects are interpreted as average effects over all loci (Lynch 1991).

METHODS

The Templeton, Kinghorn and Lynch models provide a variety of methods for the analysis of outbreeding effects on fitness. They are, however, not without their limitations. Lynch's model provides a computationally easy method for simultaneously evaluating outbreeding and inbreeding effects in pedigreed populations. A major advantage of this model is that it defines outbreeding effects in quantitative genetic terms and allows for examination of the specific genetic mechanisms of outbreeding. Its major disadvantage is that it can be applied only to populations derived from two sources. Neither the Templeton nor the Kinghorn models are limited by the number of source populations, making them more applicable for populations derived from multiple sources. When there are only two sources, however, the seven Kinghorn models can be shown to be simple linear functions of the genetic parameters used in the Lynch model (table 3). This is not the case with the Templeton model. Furthermore, under two sources, the K_3 model is equivalent to the K_6 model. Therefore, when analyzing populations derived from only two

Table 3. Description of Kinghorn's seven 2-locus models of epistasis (Kinghorn 1987). Both the general expression and the expression when there are only 2 source populations [expressed terms of the parameters used by Lynch (1991)] are shown.

Model	General Expression	Under 2 Sources
K_1	$1-\sum p_i^2$	$\frac{1}{2}(1-\theta_s^2)$
K_2	$\sum p_i(1-2p_i+f_{ii})$	$\frac{1}{4}(1-\theta_H-2\theta_s^2)$
K_3	$1-\frac{1}{N_s}\sum(1-f_{ii}-H_{i.})^2$	$\frac{1}{16}(7-6\theta_H-\theta_H^2-4\theta_s^2)$
K_4	$\sum\frac{2}{3}H_{i.}(f_{ii}+H_{i.})$	$\frac{1}{12}(5+6\theta_H+\theta_H^2)$
K_5	$2\sum_i\sum_j f_{ii}f_{jj}+2\sum_i\sum_j\sum_k f_{ii}H_{jk}+$ $2\sum_i\sum_j\sum_k\sum_l H_{ij}H_{kl},\quad i\neq j\neq k\neq l$	$\frac{1}{8}(1-2\theta_H+\theta_H^2-4\theta_s^2)$
K_6	$1-\sum(2p_i-f_{ii})^2$	$-\frac{1}{8}(1+6\theta_H+\theta_H^2+4\theta_s^2)$
K_7	$1-\frac{1}{2}\sum(p_i+f_{ii})^2$	$\frac{1}{32}(23+6\theta_H-\theta_H^2-16\theta_s^2)$

where: p_i = probability allele selected randomly from any locus is derived from source population i;
f_{ii} = probability both alleles present at locus derived from source population i;
$H_{i.}$ = probability only one allele at locus is derived from population i;
H_{ij} = prob. alleles from source i and j present;
N_s = number of different source populations.
θ_H = hybridity index; θ_s = source index;

sources, the Lynch model is sufficient to explain all of the epistatic effects described by the Kinghorn models.

In the analysis here, we used modified forms of the three types of outbreeding models described above. Two additional parameters were added to each model. To control for changes in husbandry over time, we included a time parameter, year of birth (*YOB*). We also included maternal inbreeding (f_d; inbreeding coefficient of dam) to examine the potential effects of inbreeding of the dam on survival of her offspring, which is known to affect survival in some populations (Ralls et al. 1980).

Modified Lynch Model (Model A):

Lynch's complete model of outbreeding and inbreeding effects, ignoring three-locus and higher order interactions, involves 15 parameters (equation 3, Lynch 1991), making it somewhat unwieldy for most data sets. We have thus chosen to use a modified version of the model which excludes the higher-order (three and higher) epistatic effects and quadratic effects of inbreeding and does not discriminate between inbreeding effects of the two sources. In this model, the fitness of an individual, u, is expressed as:

$$u = \mu_0 + tYOB + Ff + F_d f_d + \alpha_1\theta_S + \delta_1\theta_H + \alpha_2\theta_S^2 + \delta_2\theta_H^2 + (\alpha_1\delta_1)\theta_S\theta_H \qquad \text{(Eq. 2)}$$

where:

u_0 is the mean fitness;

t is the regression coefficient associated with time (*YOB*);

F is the regression coefficient associated with inbreeding (f = inbreeding coefficient, Wright 1922);

F_d is the regression coefficient associated with maternal inbreeding (f_d);

α_1, δ_1, α_2, δ_2 and $\alpha_1\delta_1$ are the regression coefficients associated with, respectively, the additive, dominance, additive x additive, dominance x dominance and additive x dominance effects of alleles from the two different source populations (Lynch 1991);

θ_s is the "source index" and is calculated as $2p_1$-1 where p_1 is the expected proportion of an individual's genome descended from source population 1 (one population is chosen arbitrarily as source 1; Lynch 1991); and

θ_H is the "hybridity index" and is calculated as $2H$-1 where H is the probability that an individual is heterozygous for different source alleles at any given locus (Lynch 1991).

The parameters f, f_d, θ_s, θ_H, (and their functions θ_s^2, θ_H^2 and $\theta_s\theta_H$) are calculated for each individual from the pedigree and the coefficients α_1, δ_1, α_2, δ_2, $\alpha_1\delta_1$ and F_d are estimated using, e.g.,

Table 4. Values for the parameters in the Lynch model for individuals in a simple pedigree (figure 9).

Generation	Lynch/Hill Parameters (from equation 2)				
	Additive (θ_s)	Dominance (θ_H)	Additive x Additive (θ_s^2)	Dominance x Dominance (θ_H^2)	Additive x Dominance ($\theta_s\theta_H$)
P_1	1	-1	1	1	-1
P_2	-1	-1	1	1	1
F_1	0	1	0	1	0
F_2	0	0	0	0	0
B_1	.5	0	.25	0	0
B_2	-.5	0	.25	0	0

regression analysis. Table 4 shows the θ_s and θ_H values for different generational types in a simple pedigree (figure 8). Note that the Lynch model uses the F_2 generation as the reference generation (i.e., all effects are defined as 0 so that the fitness of the F_2 = u_0). Therefore, care must be taken in interpreting the sign of coefficients associated with the genetic effects. For example, $\alpha_2 > 0$ implies disruption of coadapted additive x additive gene complexes while $\alpha_2 < 0$ suggest favorable additive x additive epistasis *between* genes from different source populations (Lynch 1991).

Modified Kinghorn Models (Model B)

The seven Kinghorn models take the form:

$$u = \mu_0 + tYOB + \sum \alpha_{1i} p_i + Ff + F_d f_d + \gamma H + k_j K_j \qquad \text{(Eq. 3)}$$

where α_{1i} is the regression coefficient of the additive effect of source i (i = 1 to number of sources -1);

p_i is the proportion of an individual's genome descended from source i;

γ is the regression coefficient associated with the source heterozygosity parameter (H, defined as the probability that a locus is heterozygous for two alleles from any two different source populations); and

the k_j are the regression coefficients associated with the seven K_j Kinghorn epistatic effects (j = 1 to 7).

Therefore, there are seven models associated with Model B, one for each of the seven Kinghorn epistasis models (table 3).

Modified Templeton Model (Model C):

$$u = \mu_0 + tYOB + \sum \alpha_{1i} p_i + Ff + F_d f_d + \gamma H + \tau h \qquad \text{(Eq. 4)}$$

where the effects are the same as in equation 3, but h is Templeton's hybridity coefficient and τ is its associated regression coefficient.

Models B and C are similar in form and differ only in their epistasis terms. In both models, the number of additive effects is the number of source populations less one. Here H is defined as the probability that a locus is heterozygous for two alleles from any two different source populations. Note that no effort is made to distinguish between dominance effects involving alleles from different pairs of source populations (e.g., dominance effects of alleles from source 1 with 2 are not distinguished from effects of alleles from source 1 with 3). To do so would increase the number of parameters beyond what would be reasonable for most data sets involving more than two sources.

A computer program was written to calculate from pedigree data all the parameters needed for these analyses. Individual and maternal inbreeding coefficients (f and f_d) as well as the additive effects (α_{1i}, founder contributions) were calculated as describe by Ballou (1983).

Data

The five populations we analyzed were chosen because: a) each represents a case of known or potential hybridization between individuals of different species, subspecies or geographic areas; b) pedigree and survivorship data for each of these populations have been routinely maintained in standardized and computerized studbook form (**SPARKS**, ISIS 1991); and c) these data are among the largest and most comprehensive available from captive populations. Details on each of these data sets are provided below.

Orang utan (*Pongo pygmaeus*)

The captive population of orang utans consists of both the Bornean (*P. p. pygmaeus*) and Sumatran (*P. p. abelii*) subspecies, as well as hybrids. Data were obtained from the International Orang Utan Studbook and were complete through January 1, 1993 (Perkins 1994). Subspecies status of individuals was determined by karyology and pedigree evaluation and recorded in the studbook for each individual. Additive effects were measured relative to the Sumatran

subspecies (i.e., Sumatran subspecies was defined as source 1 so that $\theta_s = 1$ for individuals of pure Sumatran descent). There was insufficient distribution of f_d values to conduct an analysis of this effect in this species. Age of weaning is 2 years, and 1128 individuals were included in the analysis.

Przewalski's Horse (*Equus przewalskii*)

The Przewalski's horse (or Mongolian wild ass) is extinct in the wild and has survived through captive propagation since the late 1800s. The entire captive population has descended from 13 founders, one of which was a domestic mare (*Equus caballus*) who was bred to a Przewalski's stallion in 1906 (Bouman and Bouman 1994). A large proportion of the subsequent population had genes from this domestic mare. Therefore, we assumed that the population was derived from two sources: pure Przewalski's horse and domestic horse. Additive effects were measured relative to the pure Przewalski's horse line. To date, there has been no analysis of the effects of this introgression on survival rates in the population. Data used in this study were obtained from O. Ryder and the International Studbook Keeper (J. Volf, Prague, Czechoslovakia) and are complete through December 31, 1992 (Volf 1991). Weaning age is 10 months and the analysis includes 1838 individuals. Excluded from the analysis were all individuals born at Askania Nova, Russia, (uncertain parentage) and those sent to China for the reintroduction program (O. Ryder, pers. comm.).

Gaur (*Bos gaurus*)

The gaur is a species of wild cattle ranging from India to Southeast Asia. The captive population is descended from two sources: eight founders were from the Indian subspecies (*B. g. gaurus*), and one from the Thai subspecies (*B. g. readei*) (Klös 1992). The Studbook data were obtained from D. Morris (Species Coordinator, Henry Doorly Zoo) and the International Studbook (Klös 1992). They are current through August 29, 1991. Additive effects were measured relative to the Indian subspecies. Weaning age is 9 months and 505 individuals were included in the analysis.

Asiatic lion (*Panthera leo persica*)

While once distributed throughout Asia Minor, Iran and central India, the Asiatic lion is currently limited to the Gir Forest Sanctuary in northwest India (O'Brien et al. 1987). The International Studbook traces the captive population back to 16 original founders, the earliest entering the population in the early 1960s (Fouraker et al. 1993). In 1985, O'Brien et al. (1987) found that four of the original founders were very likely African lions (*Panthera leo leo*) (based on electrophoretic and morphological data) and that a large proportion of the captive population was Asiatic x African hybrid. Since then, breeding of hybrids has been discouraged although the studbook has continued to maintain data on all hybrids, as well as pure Asiatic lions, in captivity (Fouraker et al. 1993). Because of

the potential for selection against undesirable hybrids at an early age (e.g., failure to provide veterinary assistance to sickly newborns), all births since 1985 were excluded from the analysis. In addition, lions born at Indian zoos were excluded as infant mortality data were not routinely collected by Indian zoos prior to 1981 (Fouraker et al. 1993). The studbook data were provided by M. Fouraker and were complete through August, 1993. Additive effects were measured relative to the Asiatic subspecies. Weaning age is 6 months and the analysis was based on 120 litters.

Golden-lion tamarin (*Leontopithecus rosalia*)

The International Studbook for this species traces the captive population back to the late 1950s (Ballou 1993). Unlike the other four populations analyzed in this study, this population is not the result of species or subspecies hybridization. While the exact origin of the founders is not known, most of them were brought into captivity when the wild population was already small and fragmented and it is possible that the founders originated from diverse geographic origins within the species' range along the Atlantic coastal rainforest of eastern Brazil. The captive population has descended from 51 different imports of animals since the 1950s, the latest occurring in 1991. An analysis of the genetic contribution of the founders in these 51 groups indicate that founders from only 13 imports produced sufficient numbers of descendants to be included in this study. Therefore, the analysis was limited only to descendants

of the founders from these 13 imports. We assumed that each of these imports was from a different source population. The data from the Studbook are complete through December 31, 1992 (Ballou 1993). Weaning age is 12 weeks and the analysis is based on 679 litters.

Models A and C were used in populations derived from two sources (i.e., orang utan, Przewalski's horse, gaur and Asiatic lion), while models B and C were applied to the population derived from multiple (> 2) sources (i.e., the golden lion tamarin).

For each population we analyzed survival to age of weaning. This age was chosen because survival to earlier ages (i.e., neonatal survival) would not include delayed deaths of interest (many inbred animals survive birth but die shortly thereafter; Ralls et al. 1980). While analyzing survival rates to older ages (e.g., age of sexual maturity) may be a better measure of total fitness than early survival rates, doing so results in losing animals to follow-up if they are sent outside the population surveyed by the studbook keeper before they reach the cutoff age. Excluding animals lost to follow-up may result in both substantial loss of data and possible bias in survival rates.

For the Przewalski's horse, gaur and orang utan, each individual was coded as either surviving to or dying before age of weaning. To control for litter effect in the lion and golden lion tamarin (survival of litter mates is not independent), we analyzed survival

of litters rather than individuals. A litter was coded as surviving if average survivorship of litter mates to age of weaning was at or above the average weaning age survivorship in the population. Animals with unknown death dates or ancestry were excluded, as were any animals born within weaning age of the cutoff date of the studbook data.

Statistical Analysis

Multiple logistic regression was used to estimate parameter coefficients in models A, B and C from the survival data.

The multivariate logistic regression takes the form:

$$s = \frac{\exp\{-x\}}{1 + \exp\{-x\}} \qquad \text{(Eq. 5)}$$

where s is the probability of surviving to age of weaning. For Model A,

$$x = \mu_0 + tYOB + Ff + F_d f_d + \alpha_1\theta_s + \delta_1\theta_H + \alpha_2\theta_s^2 + \delta_2\theta_H^2 + (\alpha_1\delta_1)\theta_s\theta_H. \qquad \text{(Eq. 6)}$$

The form is similar for models B and C; x is simply replaced by the right side of equations 3 and 4. The SAS LOGISTIC procedure was used to fit the data to the logistic regression (SAS 1991). Coefficients are estimated using maximum likelihood procedures and their statistical significance tested by a likelihood ratio test (Hosmer and Lemeshow 1989).

Parameter coefficients obtained from logistic regression can be interpreted in terms of their odds ratio. The odds ratio associated with a coefficient (e.g., α_1 in model A) is defined as $\exp(\alpha_1)$ and is a measure of the relative change in survival associated with a unit change in that coefficient's parameter value (Fleiss 1981, Hosmer and Lemeshow 1989). We used odds ratios to evaluate the relative effects of different model parameters on survival.

Examination of collinearity among variables was conducted using the SAS REG procedure invoking the COLLINOINT option (SAS 1991). Evaluation of model fit was based on the Akaike Information Criterion (*AIC*; SAS 1991). Lack of fit tests were performed on all analyses using the Hosmer-Lemeshow Tests (Hosmer and Lemeshow 1989; SAS 1991).

RESULTS

Limitations in Data Structure

Simultaneous analysis of outbreeding and inbreeding effects using the models presented above requires data from multiple generations with multiple levels of inbreeding. For the 2-source model (Model A), the data structure sufficient for estimation of the outbreeding parameters requires parental (P_1 and P_2), hybrid (F_1, F_2), and both backcross (B_1 and B_2) generations. This data structure can

be visualized by plotting θ_s against θ_H for these generational types (figure 10a). More complex pedigrees fill the data space more completely (figure 10b). Simultaneous estimation of all parameter coefficients may not be possible if data on one or more generational types is absent. Likewise, limitations in data structure may result in confounding of genetic effects (see figure 9). The data structures (shown as a θ_s-by-θ_H plot) of the four two-source populations are shown in figure 12a-d.

The data for the orang utan consists of P_1, P_2, F_1, F_2 and both backcross generations as well as some more complicated backcross types. This allowed simultaneous evaluation of all coefficients in Model A (figure 11a).

The pedigree structures of the Przewalski's horse, gaur and Asiatic lion populations (figure 11b-d) are limited by their early history. In the Przewalski's horse population, the domestic mare produced only one F_1 offspring which backcrossed to the pure Przewalski's horse lineage. All subsequent crosses were various kinds of backcrosses to the pure lineage (figure 11b). The same is true, but to a lesser extent, in both the gaur and Asiatic lion populations: early F_1 individuals backcrossed to individuals only from one of the pure subspecies (figures 11c and 11d). These limitations place severe restrictions on the extent to which the outbreeding models can be applied. In the Przewalski's horse (figure 11b), lack of F_2

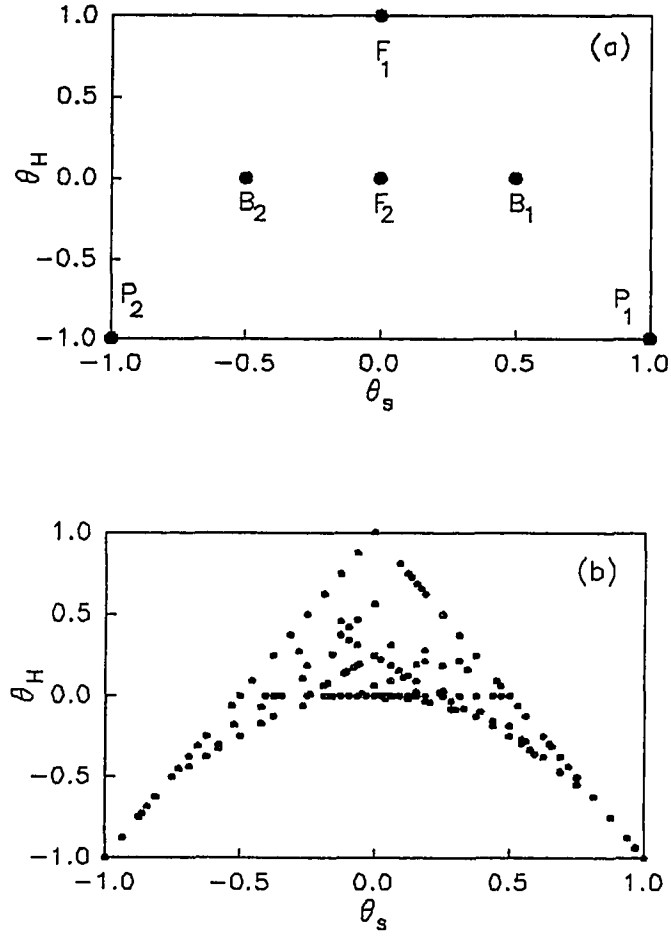

Figure 10. (a) The minimum data structure required for the analysis of the outbreeding effects presented in Model A (for populations derived from 2 sources). P_1 = animals from source population 1; P_2 = animals from source population 2; F_1 = F_1 crosses; F_2 = F_2 crosses; B_1 and B_2 are F_1 individuals backcrossed to the parental lineages 1 and 2, respectively. (b) The data structure provided by a more complex pedigree. This data structure is generated by a randomly breeding population of 200 individuals derived from 2 sources.

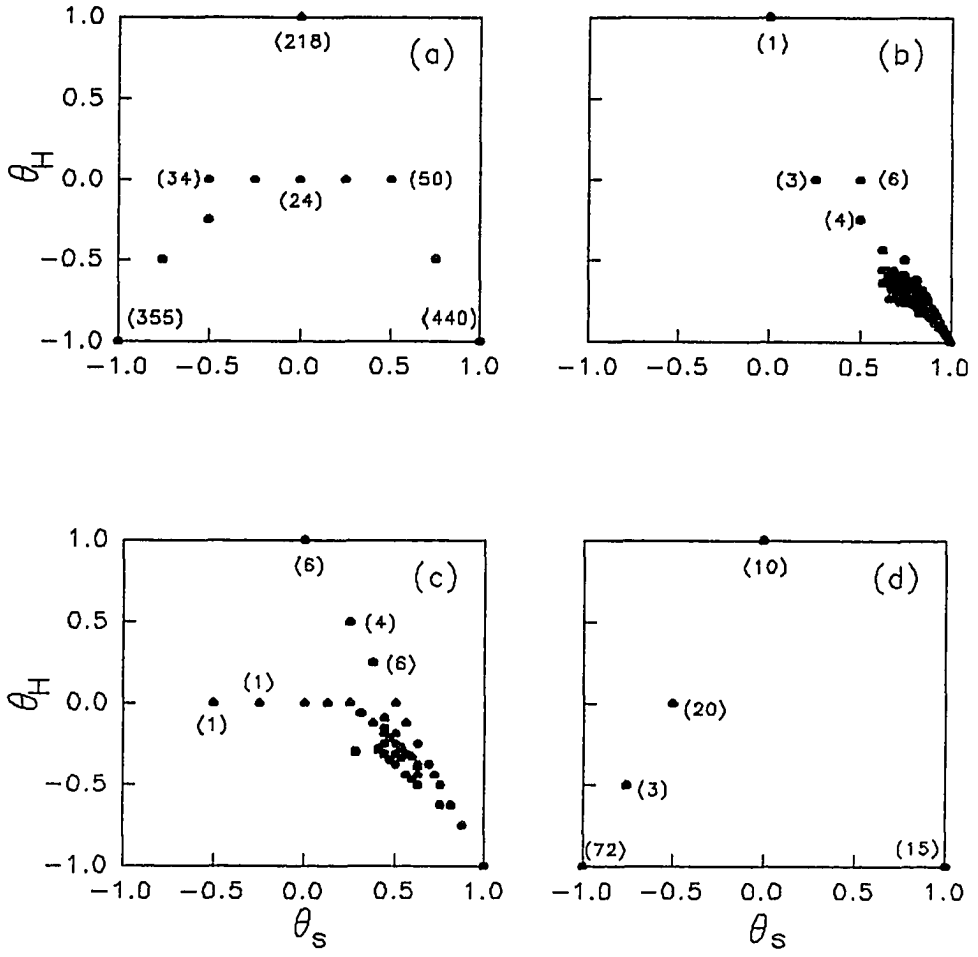

Figure 11. Data structure of orang utan (a), Przewalski's horse (b), gaur (c) and Asiatic lion (d) plotted as θ_s against θ_H. Sources arbitrarily assigned as S = 1 are the Sumatran subspecies of orang utan, pure Przewalski's horse, the Indian subspecies of gaur, and pure Asiatic lion. Numbers in parentheses represent the number of individuals of that generations type (θ_s x θ_H combination).

and F_1 crossbred generations as well as backcrosses to the domestic horse lineage cause strong correlations between θ_s, θ_H and the outbreeding interaction terms (θ_s^2, θ_H^2, and $\theta_s\theta_H$), confounding the interpretation of the effects of these variables. The interaction parameters were removed from the regression model and the analysis of this data set was limited to the *YOB*, f, f_d, α_1 and δ_1 parameters for Model A, recognizing that the α_1 and δ_1 effects were confounded with the epistatic outbreeding effects. The full Model C could be applied to these data. For both models, the four outlying individuals with $\theta_s < 0.5$ were excluded from the analysis.

Multicollinearity in the gaur and Asiatic lion was limited to strong correlations (gaur) and linear dependencies (Asiatic lion) among the epistatic terms. Simultaneous estimation of these parameter coefficients was not possible in either of these populations. In the gaur, each epistatic term could be evaluated in the absence of the others. Thus the analysis using Model A was restricted to the parameters *YOB*, f, f_d, α_1, δ_1 with each of the epistatic effects by itself (α_2, δ_2, and $\alpha_1\delta_1$) in turn. The full Model C could be used with these data. For all models, the two outlying individuals with $\theta_s < 0$ were excluded from the analysis.

In the lion, the data structure resulted in linear dependency among the epistatic, dominance and additive terms (e.g., $\theta_H^2 = 2\theta_H + 4\theta_s^2 - 1$). As in the gaur, the analysis using Model A was restricted to the parameters *YOB*, f, f_d, α_1, δ_1 and each of the epistatic ef-

fects by itself (α_2, δ_2, and $\alpha_1\delta_1$) in turn. Because of the linear dependencies, however, these three models did not provide different fits to the data: the genetic effects are simply re-partitioned among the parameters in the model. Model C can be applied in full.

While a θ_s-by-θ_H plot is not possible for the golden lion tamarin population since it is derived from multiple sources, collinearity analysis of the parameters showed that the model using K_4 could not be analyzed because of strong multicollinearity among the parameters f, f_d and H. Furthermore, in an attempt to reduce the number of parameters being considered (there are 13 additive terms in model B and C for this species), a univariate analysis of the additive terms was first performed. As suggested by Hosmer and Lemeshow (1989), the six variables whose univariate p values were greater than 0.25 were removed from further consideration. The remaining 7 additive effects are from founder groups "32" (consisting of founders 99, 100 and 101), group "36" (founder 112), group "40" (founder 123), group "50" (founders 134 and 135), group "53" (founders 190-197), group "61" (founders 209-212), and group "SD" (San Diego Zoo lineage).

Logistic Regression Analysis

The estimated model coefficients and their standard errors are shown in tables 5 and 6. Outbreeding depression effects are manifested by $\alpha_2 > 0$, $\delta_2 > 0$, $\delta_1 < 0$, $\alpha_1\delta_1 \neq 0$ for Model A; $k_1 < 0$ and

Table 5. Estimates of parameter coefficients and their standard errors for the 2-source (Model A) populations.

SPECIES	u_0	Time t	Inbreeding F	Maternal Inbreeding F_d	Additive α_1	Dominance δ_1	Epistatic Effects α_2	δ_2	$\alpha_1\delta_1$	τ
Prz. horse	3.05 ± 1.24	-0.01 ± 0.01	-0.43 ± 0.75	-0.25 ± 0.46	-1.60 ± 3.14	-0.94 ± 2.19	-----	-----	-----	-----
	-3.74 ± 3.64	-0.01 ± 0.01	-1.89± 0.80**	0.56 ± 0.62	2.88 ± 3.960	-3.22 ± 2.50	-----	-----	-----	10.03* ± 5.11
Gaur	6.38 ± 2.30	-0.05 ± 0.03	-2.92 ± 1.06**	0.73 ± 0.93	-2.44 ± 2.35	-0.39 ± 1.27	1.31 ± 2.26	-----	-----	-----
	6.49 ± 2.30	-0.05 ± 0.03	-2.98 ± 1.06**	0.77 ± 0.92	-1.55 ± 1.54	-0.61 ± 1.11	-----	0.33 ± 0.70	-----	-----
	6.35 ± 2.33	-0.05 ± 0.03	-2.96 ± 1.06**	0.75 ± 0.92	-1.63 ± 1.56	-0.17 ± 1.52	-----	-----	-0.82 ± 1.39	-----
	6.84 ± 2.19	-0.06 ± 0.02	-2.96 ± 1.06**	0.83 ± 0.94	-1.10 ± 2.21	-0.72 ± 1.08	-----	-----	-----	0.397 ± 1.49
Asiatic lion	15.73 ± 10.68	-0.16 ± 0.12	-3.22 ± 2.64	-7.97± 3.19**	-1.62 ± 0.59**	-0.14 ± 1.69	-0.93 ± 2.92	-----	-----	-----
	15.50 ± 10.31	-0.16 ± 0.13	-3.22 ± 2.64	-7.97± 3.19**	-1.62 ± 0.59**	0.32 ± 0.58	-----	-0.23 ± 0.73	-----	-----
	15.26 ± 9.99	-0.16 ± 0.13	-3.22 ± 2.64	-7.97± 3.19**	-2.09 ± 1.65	0.32 ± 0.58	-----	-----	-0.47 ± 1.46	-----
	14.93 ± 9.35	-0.16 ± 0.13	-3.23 ± 2.64	-7.99± 3.20*	-1.61 ± 0.58**	0.33 ± 0.59	-----	-----	-----	0.39 ± 1.49

Continued...

Table 5 (continued). Estimates of parameter coefficients and their standard errors for the 2-source (Model A) populations.

SPECIES	Time	Inbreeding	Maternal Inbreeding	Additive	Dominance	Epistatic Effects			
	u_0	t	F	F_m	α_1	δ_1	α_2	δ_2	$\alpha_1\delta_1$	τ
Orang utan	0.18 ± 0.87	0.02 ± 0.01*	-3.22± 1.14**	-----	-0.21 ± 0.48	-1.07 ± 1.13	-2.19 ± 2.25	0.57 ± 0.71	-0.29 ± 0.49	-----
	-0.31 ± 0.62	0.02 ± 0.01*	-3.35± 1.13**	-----	-0.08 ± 0.08	0.01 ± 0.09	-----	-----	-----	0.29 ± 0.39

* = significant at the 0.05 level; ** = significant at the 0.01 level; *** = significant at the .001 level.

Table 6. Estimates of joint inbreeding and outbreeding effects (+/- SE) from multivariate logistic regression analysis for the multi-source (Model B) golden lion tamarin population. Coefficient estimates are shown for each of the seven Kinghorn models (except model K_4) and the Templeton model.

	Kinghorn Models												Templeton	
	K_1		K_2		K_3		K_4		K_6		K_7		h	
Effect	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE	Est	SE
u_0	-2.85	2.01	-2.12	1.95	-2.93	1.93	-0.63	1.82	-1.94	1.91	-3.22	1.66	-2.29	1.65
t	0.04	0.03	0.03	0.03	0.04	0.03	0.01	0.03	0.02	0.03	0.04	0.02	0.03	0.02
$\alpha_{1,32}$	-1.25	0.64	-1.24	0.64	-1.26*	0.64	-1.17	0.64	-1.23	0.64	-1.16	0.63	-1.25	0.64
$\alpha_{1,36}$	-4.01**	1.50	-4.23**	1.49	-3.93**	1.51	-4.36**	1.43	-4.27**	1.49	-3.80*	1.49	-4.15**	1.48
$\alpha_{1,40}$	2.75**	0.89	2.68**	0.88	2.67**	0.88	2.68**	0.87	2.68**	0.88	3.15***	0.95	2.74**	0.89
$\alpha_{1,50}$	1.22*	0.61	1.28*	0.61	1.18	0.61	1.43*	0.61	1.30*	0.62	1.26*	0.59	1.27*	0.60
$\alpha_{1,53}$	-0.25	0.49	-0.22	0.50	-0.22	0.48	-0.02	0.51	-0.20	0.50	-0.12	0.48	-0.22	0.48
$\alpha_{1,61}$	-0.24	0.56	-0.26	0.56	-0.20	0.56	-0.18	0.57	-0.26	0.56	-0.15	0.56	-0.23	0.56
$\alpha_{1,SD}$	-0.34	0.59	-0.36	0.59	-0.35	0.59	-0.40	0.60	-0.36	0.59	-0.29	0.60	-0.34	0.59
γ	0.49	0.69	0.11	0.40	0.11	0.40	0.13	0.40	0.09	0.51	1.97	1.35	0.12	0.40
F	-6.09**	1.99	-6.32***	1.98	-6.25**	1.96	-6.03**	1.98	-6.32**	1.99	-4.88*	2.14	-6.16**	1.99
F_d	2.71	2.38	2.80	2.38	2.75	2.37	3.01	2.39	2.82	2.38	2.56	2.35	2.70	2.39
k_i	-0.82	1.20	-0.13	0.65	-0.37	0.47	0.86	0.87	-0.03	0.33	-2.35	1.63	-0.17	0.35

* = significant at the 0.05 level; ** = significant at the 0.01 level; *** = significant at the .001 level.

$\gamma < 0$ for Model B; and $\delta < 0$ and $\gamma < 0$ for Model C. Inbreeding depression effects will show f and $f_d < 0$ and $t > 0$ will indicate improved survival over time. Additive effects $(\alpha_1) < 0$ denote positive association between fitness and genes from source 1.

The only epistatic effect that showed significance was Templeton's hybridity coefficient in the Przewalski's horse. However, this effect was in the wrong direction. None of the epistatic effects were significant for any other species. There was no obvious trend in the signs of the epistatic coefficients across species. In the gaur, the additive x additive and dominance x dominance effects were negatively associated with survival, while the additive x dominance effect and Templeton's hybridity showed a positive association. In the Asiatic lion, all epistatic effects were positively associated with survival. The orang utan also showed a mixed result: the additive x additive, additive x dominance, and Templeton effects were positively associated with survival, while the dominance x dominance effect had a negative association. The epistatic parameters in the golden lion tamarin (k_1) were all positively associated with survival except in the K_5 model.

None of the analyses showed a statistically significant dominance effect, nor were there any trends in direction of the sign of dominance effects across species. In the Asiatic lion and orang utan, the sign of the dominance effect differed among the different models examined.

Significant additive effects were present in both the Asiatic lion (table 5) and golden lion tamarin (table 6). For the Asiatic lion, in the additive x additive and dominance x dominance models and Templeton Model, there was a significant negative effect of pure Asiatic lion genes on survival (p=0.006 in all models). The odds ratio (OR) of hybrids (50% Asiatic genes) relative to pure Asiatic lions is 5.3, indicating a 5-fold difference in the odds of survival of hybrid over pures. This effect was not significant in the additive x dominance model ($p = 0.207$). In the golden lion tamarin (table 6), decreased survival was associated with founder group "36" (founder 112; $p < 0.01$; OR = 0.02 for animals with 50% genome from founder 112 relative to 0% descent), but positively associated with survival for group "40" (founder 123; $p < 0.01$; OR = 15.8 for animals with 50% genome from founder 123 relative to 0% descent) and group "50" ($p < 0.05$; OR = 3.6).

Statistically significant inbreeding depression was detected in the orang utan (p=0.006; OR = 0.44 for $f = 0.25$ compared to $f = 0$), Przewalski's horse (Templeton model $p = 0.02$; OR = 0.62), gaur (p=0.006; OR = 0.48), and golden lion tamarin (p=0.001; OR = 0.22). Inbreeding effects were non-significantly negative in the Asiatic lion. A significant negative effect of maternal inbreeding was also detected in the Asiatic lion (p=0.01; OR = 0.14 for $f_d = 0.25$ compared to $f_d = 0$).

Significant time (t) effects were detected in the orang utan and gaur (in the Templeton model). In the orang utan, there was a statistically significant improvement in survival over time ($p=0.03$; OR = 1.18/decade) while in the gaur, survival declined over time ($p=0.01$; OR = 0.61/decade).

The results discussed above present the effects within the context of the full regression models. A backwards elimination model reduction strategy was performed on all models to remove non-statistically significant parameters and thereby gain a better estimate of the biologically relevant effects. Models with non-significant parameters removed provide higher power for estimation of remaining parameters. The BACKWARD model selection option in PROC LOGISTIC was used (SAS 1991). The parameter estimates (and their standard errors) in the reduced models are shown in table 7.

All effects that were significant in the full model were also present in the reduced models. However, inbreeding became significant in the reduced model in the Asiatic lion ($p < 0.05$) and time effects became significant in the gaur and golden lion tamarin. The parameter estimates changed little between the full and reduced models, which indicates that the parameters were independent of those that were excluded. These results clearly demonstrate that inbreeding effects are independent of outbreeding effects in these species.

Table 7. Reduced model results: estimates and standard errors of parameters remaining in models after backwards selection procedure.

Species	Intercept u_0	Time t	Inbreeding F	Maternal Inbreeding F_d	Additive Effects α_i		
Orang utan	-0.284 ±0.603	0.0163 ±0.008*	-2.963 ±1.067**	----		----	
Przewalski's horse	1.746 ±0.122	----	-1.177 ±0.480*	----		----	
Asiatic lion	2.666 ±0.609	----	-4.218 ±1.898*	-8.221 ±2.572**		-1.167 ±0.442**	
Gaur	5.996 ±0.051	-0.051 ±0.022*	-2.549 ±0.905**	----		----	
Golden lion tamarin	-2.827 ±1.105	0.034 ±0.014*	-6.145 ±1.715***	----	-3.822[1] ±1.378**	3.021[2] ±0.731***	1.288[3] ±0.535*

* = significant at the 0.05 level; ** = significant at the 0.01 level; *** = significant at the .001 level.

Golden lion tamarin additive effects are for founders groups 36 (1), 40 (2) and 50 (3).

In general, there were only minor differences in the fit of different models according to the Akaike Information Criterion (AIC) values for each analysis. No model tended to fit better than the others.

DISCUSSION

There are few pedigree analyses comparing the effects of inbreeding and outbreeding in captive populations in the literature. Templeton and Read (1984) examined the joint effects of inbreeding and outbreeding in the Speke's gazelle. They found that increased inbreeding, but not increased outbreeding, was associated with decreased juvenile survival. Lacy et al. (1993) examined the effects of inbreeding and outbreeding in the Goeldii's monkey and found that both were related to lower survival, although inbreeding depression was much more severe than outbreeding depression. Although the reason for the outbreeding depression was not known, it was hypothesized that founders could have originated from different geographic sources. In both of these studies, outbreeding was measured using Templeton and Read's hybridity coefficient (Templeton and Read 1984). However, neither the additive nor the dominance effects, both potentially confounding the epistatic effects, were considered in either study.

We have extended these analyses by applying a variety of different and expanded outbreeding depression models to five captive

populations whose history suggests at least the possibility of outbreeding depression. Our results show that while inbreeding depression was statistically significant in four of the five populations examined, outbreeding depression, defined both in terms of disruption of coadapted gene complexes (i.e., epistatic effects involving alleles from different source populations) and in terms of negative heterosis (i.e., F_1) effects, was not present. The only outbreeding effect observed was in the Przewalski's horse, which showed a significant increase (rather than a decrease as predicted by the outbreeding depression hypothesis) in survival associated with Templeton's hybridity coefficient (table 5). It is possible that there exists favorable epistatic interactions between Przewalski's and domestic horse genes which are expressed by recombination events between chromosomes from the two species (Lynch 1991).

Additive effects were significant in the Asiatic lion and the golden lion tamarin (table 6). In Asiatic lions, the estimated survival odds for lion litters with 50% Asiatic genes (e.g., F_1 hybrids) relative to the odds for pure African lions is 18%. Wildt et al. (1987) showed that Asiatic lions exhibit extremely low levels of genetic diversity and high percentages of abnormal sperm. The authors suggested that both are a result of extensive inbreeding: Asiatic lions have been restricted to a relatively small and closed population in the Gir forest in northwestern India for at least the last 100 years. The detrimental effect of Asiatic lion genes on survival in the captive population may be the result of deleterious

Asiatic lion alleles that have become fixed through inbreeding and drift.

Additive effects were significant in four of the seven golden lion tamarin founder groups (table 6). Genes from groups "40" and "50" were positively associated with survival, while groups "32" and "36" had the opposite effect. The strongest effects were seen in groups "36" and "40". The wild-caught origins of the founders in these groups in unknown, so we can draw no conclusions as to the relationship between these founders and the other founders entering the population. It is unlikely that these effects are due to differences in the environment in which these founders and their descendants were raised because descendants from lineages 36 and 40 were housed primarily at the National Zoological Park, while descendants from group 32 and 50 were kept at the Oklahoma City Zoo. The effects are also unlikely to be due to different treatments these groups received within each site because care and husbandry of golden lion tamarins is well established and standardized as much as possible, particularly in institutions within the United States.

The lack of outbreeding depression found in this study is interesting because four of the five populations examined are cases of known species or subspecies (i.e., wide) hybridization. In general, the larger the genetic distance between populations, the more likely outbreeding is considered a potential problem (Knowlton and Jackson 1993; Templeton et al. 1986, Ehiobu and Goddard 1990b,

but see Ehiobu et al. 1990). The genetic distances among the taxa analyzed here vary considerably. The average genetic distance (Nei's D based on 18 allozyme loci) between various breeds of domestic and Przewalski's horse is about 0.3 (Bowling and Ryder 1987) and the karyotype of the two species differs by a Robertsonian fusion (Ryder et al. 1978). However, the rate of genetic divergence has likely been reduced due to the possibility of continuous introgression of genes from local domestic horse populations into the wild Przewalski's horse population before it was driven to extinction in the wild (Boumann and Boumann 1994). The genetic distance (Nei's D based on 46 allozyme loci) between Asiatic lions and their African subspecific counterpart is small (0.006 - 0.009), with a divergence time estimated at about 10,000 years (O'Brien et al. 1987).

Differences between the orang utan subspecies are greater than those of the other species examined. While phenotypic differences between the two subspecies of orang utan's are subtle (MacKinnon 1975; Courtenay et al. 1988), significant genetic differences exist. Groves et al. (1992), on the basis of morphological analysis, suggest that differences may even exist within the Borneo subspecies. Janczewski et al. (1990) estimate that the orang utan subspecies have been geographically separated for 10,000 years, but genetic distances between the two subspecies (Nei's distance of 0.019 to 0.025, based on 44 isozyme loci) suggest that they might have been genetically isolated for as long as 730,000 to 1.5 million years (Janczewski et al. 1990; Ryder and Chemnick 1993). The two sub-

species differ with regard to a pericentric inversion in chromosome 2 and show distinctly different mtDNA haplotypes (Ryder and Chemnick 1993). Despite these differences, this analysis failed to find any signs of outbreeding depression associated with survival to weaning.

Genetic differences between the gaur subspecies are unknown (but the molecular work is underway, G. McCraken, pers. comm). Likewise, no information is available on genetic differences among fragmented populations of golden lion tamarins or among individual founders to the captive population. However, Forman et al. (1986) found very little differences between the three *Leontopithecus* subspecies (Nei's genetic distance ranged from 0.007 to 0.01).

While genetic distances between hybridized taxa in this study ranged from probably less than 0.007 in the golden lion tamarin to 0.3 in the Przewalski horse, there was no apparent relationship between genetic distance and magnitude of the outbreeding parameters in the models.

Kinghorn (1987) shows disruption of coadapted gene complexes can result through a variety of biochemical mechanisms, which can not be represented by any one model alone. In this study we used nine different models to detect outbreeding depression. Our results failed to show any differences in the ability of different models to detect outbreeding effects (since no effects were significant) and only minor differences in their fit of the data (table 6). The

Templeton model was the only model used in all species, but was ranked as the best fitting model according to the AIC criteriion in only two of the five species. In the golden lion tamarin, model K_7 fit the data most closely. Kinghorn (1987) also identified model K_7 as the model best fitting the data on survival in Guinea pigs. More analyses are needed to determine if model K_7 is the most appropriate model for analyzing survival data in general.

Since we have no results indicating a definite preference for one model over another, we recommend analyzing pedigree data using a variety of outbreeding models, as we have done here. A useful by-product of this study is the development of software (**OUTBREED**) that calculates a number of outbreeding models from arbitrary pedigrees. However, as Kinghorn and Vercoe (1989) point out, conclusions regarding the presence or absence of outbreeding depression will usually be robust with respect to differences in models used.

Despite the lack of outbreeding depression found here, it is nevertheless useful to ask the question: under what conditions might one expect to find outbreeding depression? The conventional view is that naturally small, isolated populations, with limited dispersal, high rates of inbreeding, and selection pressures which are different among populations, are more likely to evolve different genetic coadaptations between local populations than large outbreeding populations (Shields 1982; Endler 1986; Templeton 1986; Ehiobu and Goddard 1990a and b). These authors argue that naturally "in-

breeding" species are less susceptible to inbreeding depression (because mutational load has been purged from the population), but more susceptible to outbreeding depression because of the evolution of different coadapted gene complexes within the local populations. While outcrossing may initially enhance fitness in the F_1 (i.e., cause hybrid vigor), fitness is reduced in the F_2 and later generations as coadapted gene complexes are disrupted by recombination (i.e., hybrid breakdown; Vetukhiv 1953; Ehiobu and Goddard 1990a).

Likewise, Templeton (1987) and Shields (1982) argue that wide-ranging "outcrossing" species with large dispersal distances and low rates of inbreeding are less likely to have evolved local adaptations. Thus, they are less susceptible to outbreeding depression and more likely to exhibit inbreeding depression because mutational load has not been purged. Outbreeding depression would be expected only when individuals from widely separated geographic sources or taxa, showing significant genetic divergence, are crossbred (Ehiobu and Goddard 1990a). These individuals would likely be morphologically and/or chromosomally distinct races or subspecies (Ryder 1986).

Small populations of naturally inbreeding species must, however, be distinguished from populations of normally "outbreeding" species that have been more recently reduced in size, fragmented and inbred due to habitat destruction or other human activities. Among such latter populations, genetic differentiation might be the result of random drift and not involve local adaptations. Additionally,

deleterious recessive genes may not have been purged or may be fixed due to genetic drift. In these cases, outbreeding is likely to benefit, rather than decrease, fitness since it may restore heterozygosity and mask deleterious alleles (Templeton et al. 1986). The restored vigor upon crossing inbred lines is an example of this effect (Ehiobu and Goddard 1990a, Ehiobu et al. 1990; Jaquish 1994).

Empirical data, in general, support the conventional view with respect to outbreeding depression. Evidence for outbreeding depression comes primarily from: (a) organisms with extremely limited dispersal (copopods in tidal pools, Burton 1987, Brown 1991; *Ipomopsis*, Waser and Price 1989; scale insects, Alstad and Edmunds 1983; *Delphinium*, Price and Waser 1979); (b) crosses between individuals from vastly different geographic sources (*D. pseudoobscura*, Dobzhansky 1948, 1950, Vetukhiv 1953, Wallace and Vetukhiv 1955, Brncic 1954; *D. melanogaster*, Ehiobu and Goddard 1990a and b, but see McFarquhar and Robertson 1963; ibex, Turcek 1951); or (c) crosses between individuals with significant genetic (e.g., chromosomal) differences (owl monkey, Cicmane and Campbell 1977, Elliott et al. 1976; dik-dik, Ryder et al. 1989). However, in the last case, there are potential concerns about the taxonomic classification of the individuals crossed (based on karyotypic differences) and the outbreeding depression may be the predicted result of wide, interspecific, outcrossing. In the owl monkey, taxonomic identification of individuals was revised after outbreeding depression in the form of F_1 sterility alerted managers to the potential of taxonomic mis-

identification. Karyotypic analyses and information on the geographic origins of the animals subsequently verified that specimens were of different subspecies (Cicmane and Campbell 1977).

One interesting caveat, however, is that the evolution of different coadapted gene complexes need not require different selective pressures or long periods of time. Outbreeding depression can also occur in populations exposed to similar selection pressures and over relatively short time periods. King (1955) was able to select for DDT resistance in different lines of *D. melanogaster* in a relatively short time (12 generations). When resistant inbred lines were crossed, the resistance broke down in the F_2 generation, presumably because different lines had evolved different epistatic genetic mechanisms for the resistance, which were disrupted by recombination in the F_2 individuals. Hagger (1989) was able to show that different coadapted gene complexes can evolve under the same selection pressure by showing F_2 breakdown between crosses of inbred chicken lines selected for the same traits (weight maintenance, egg production and feed efficiency).

In general, outbreeding depression is more unlikely in most large vertebrates (which tend to fit the "outbreeding" species model) than in sessile animals and many plants (which tend to fit the "inbreeding" species model and where local adaptation can evolve on a small scale due to extreme population substructure; Waser 1993). This generalization is consistent with evidence for inbreed-

ing depression in a large number of vertebrates (Ralls et al. 1988, Lacy et al. 1993) and the view that the breeding structure of many vertebrate populations tends towards outbreeding as opposed to inbreeding (Templeton 1987; Ralls et al. 1985). Furthermore, a growing number of studies have failed to find outbreeding depression in mammalian species. Lacy et al. (pers. comm.) failed to find any evidence of outbreeding depression in extensive crossbreeding experiments among *Peromyscus polionotis* subspecies. Likewise Smith et al. (1987) observed no adverse effects of crossing rhesus macaques from China and India. Jaquish (1994) compared survival rates of four pure subspecies of saddle-back tamarin (*Saguinus fuscicollis*), their F_1, F_2 and backcross hybrids. There was no evidence for outbreeding depression effects and progeny of crosses between one pair of subspecies exhibited higher survival than their parental lines. However, there are a few exceptions where outbreeding depression has been documented in mammalian species (ibex, Turcek 1951, Templeton 1985; *Callimico*, Lacy et al 1993; humans, Bresler 1970), and these exceptions caution us that outbreeding depression should not be ignored.

The lack of outbreeding depression observed in this study may be due to a number of limitations in the data. These include failure to analyze the appropriate fitness variables, lack of data from appropriate generational types and lack of statistical power. These issues are addressed below as they are likely to be problems common to most outbreeding depression analyses.

The few analyses of inbreeding and outbreeding effects in captive populations that have been conducted evaluate the effects of these factors on survival rates (Ralls et al. 1988; Lacy et al. 1993; Templeton and Read 1984). This is primarily because these data are readily available from studbooks and zoo records. Survival to a particular age, however, represents only one, possibly minor, component of total individual fitness (Shields 1982; Templeton et al. 1986). Data from laboratory and domestic populations of mammals indicate that inbreeding effects may be as strong, or even stronger, in reproductive components of fitness (i.e., fertility and fecundity; Wright 1977, Miller 1994). The same is likely to be true for outbreeding depression. For example, some of the strongest documented effects of outbreeding depression are reproductive failures (e.g., sterility and fetal loss) in the F_1 due to abnormal meiosis resulting from chromosomal incompatibilities (Elliott et al. 1976; Cicmanec and Campbell 1977; Ryder et al. 1978; de Boer 1982, Coyne and Orr 1989). Crosses between individuals with different chromosomal structures may be particularly susceptible to reproductive forms of outbreeding depression for this reason (although differences in karyotypes are not always associated with outbreeding depression). Despite the different number of chromosomes, there is no apparent problem with meiosis in Przewalski's x domestic horse hybrids, although crosses between Przewalski's horse and other equids are sterile (Short et al. 1974). While the chromosomal differences between subspecies of orang utans appear not to affect survival, it is not known if there is any effect on reproduction. Hybrids are cer-

tainly fertile, as evidenced by the data available for this study. Unfortunately, data on reproductive rates of captive orang utans, like all species, are strongly biased, as only reproductive successes (births) are routinely documented in institutional records and studbooks. Without data on reproductive failure (breeding opportunities not resulting in parturition), full evaluation of the reproductive components of fitness is not possible. Failure to measure other components of fitness (e.g., survival to different ages) may also fail to detect outbreeding depression in those variables. Data from studies on crossing different strains of livestock suggest that variables such as litter size, weight at different life-history stages, and growth rate (Sheridan 1981, Kinghorn 1980) may be important indicators of outbreeding depression.

The ability of outbreeding analyses to identify outbreeding depression may also be limited by the structure of the pedigree. As has been demonstrated in this study, the early breeding history of a population plays a significant role in shaping future pedigree structure. Lack of data from multiple generations or poor sample sizes can result in insufficient data to estimate all the parameters in the model, or cause significant multicollinearity problems resulting in the confounding of effects. In addition, lack of sufficient numbers of founders from each of the source populations makes it impossible to distinguish between true source effects or simple differences between individuals. In both the Przewalski's horse and gaur, one of the sources was represented by only one founder. These

types of problems will be inherent in most post-hoc analyses of captive populations using historical pedigree data.

Ideally, data for outbreeding analyses should at least be available from both pure lineages (P_1 and P_2), F_1, F_2, and both backcross generations to detect all the different kinds of outbreeding effects present in the Lynch model (Lynch 1991). When data are limited, effects are confounded (table 8). Perhaps the most useful "limited" data set consists of both parental sources, F_1 and F_2 generations. This provides estimates of additive, dominance, additive x additive, and dominance x dominance effects. While the last two effects will be confounded, the data will be sufficient to evaluate the population for F_1 heterosis as well as F_2 breakdown. Data from backcross generations are required to statistically separate the additive x additive and dominance x dominance effects (Mather and Jinks 1982).

While planned breeding under controlled conditions, based on specific experimental designs, will produce the kinds of data required for these analyses (Sölkner and James 1990), such breeding studies will not produce results within a practical time frame for most endangered or threatened species' captive breeding programs. Decisions to group or keep separate founders or lineages from different geographic areas need to be made early in the development of captive breeding programs. Decisions will often have to be made on the basis of the genetic and demographic history of the source pop

Table 8. Outbreeding effects that can be estimated under various conditions of data limitations. Effects enclosed in parenthesis are confounded.

Data Available:	**Estimatable Effects**
P_1 and P_2	(α_1 and $\alpha_1\delta_1$)
P_1, P_2, and F_1	(δ_1 and α_2) and (α_1 and $\alpha_1\delta_1$)
P_1, P_2, F_1 and F_2	α_1, δ_1, and (α_2, δ_2)
F_1 and F_2	(δ_1 and δ_2)
F_1 and B_1 (or B_2)	(α_1, δ_1, α_2 and $\alpha_1\delta_1$)
F_2 and B_1 (or B_2)	(α_1 and α_2)
P_1, P_2, F_1, F_2, B_1 and B_2	α_1, δ_1, α_2 and $\alpha_1\delta_1$

ulations, molecular genetic data, and the taxonomic differences between populations. If decisions are made to breed individuals from different sources, the population should be monitored for potential outbreeding effects.

A problem related to poor data structure is that of statistical power. Failure to reject the null hypothesis (no outbreeding depression present) can result either from the absence of outbreeding depression, or the inability of the analysis to detect an outbreeding depression that actually does exists, i.e., lack of statistical power (Cohen 1988). It is important to evaluate the statistical power of any analysis which fails to reject a null hypothesis when, in fact, the observed differences are large from a biological consideration. This is particularly true in the field of conservation biology, where acceptance or rejection of an hypothesis can drive management strategies for endangered species (Taylor and Gerrodette 1993).

We used the data from the orang utan to examine the statistical power of outbreeding depression analyses. Power analysis of multivariate logistic regression is currently unavailable. Therefore, the data were summarized in contingency table form (table 9). Mortality rates are very similar among generational types, and overall there is no significant difference between generations (X^2 = 0.24, df 3, $p >> 0.05$). Such small differences are unlikely to affect the success of a captive breeding program. However, from an

Table 9. Survival rates to age of weaning (2 years) in different generational types of orang utans.

	Pure Sumatran	Pure Bornean	F_1	Recombinant[1]
# Survived	317	252	157	82
# Died	123	105	61	33
% Mortality	27.9	29.4	28.0	28.7

1. Individuals in which recombination between Sumatran and Bornean chromosomes could have occurred. This includes all generational types except pure and F_1 generations.

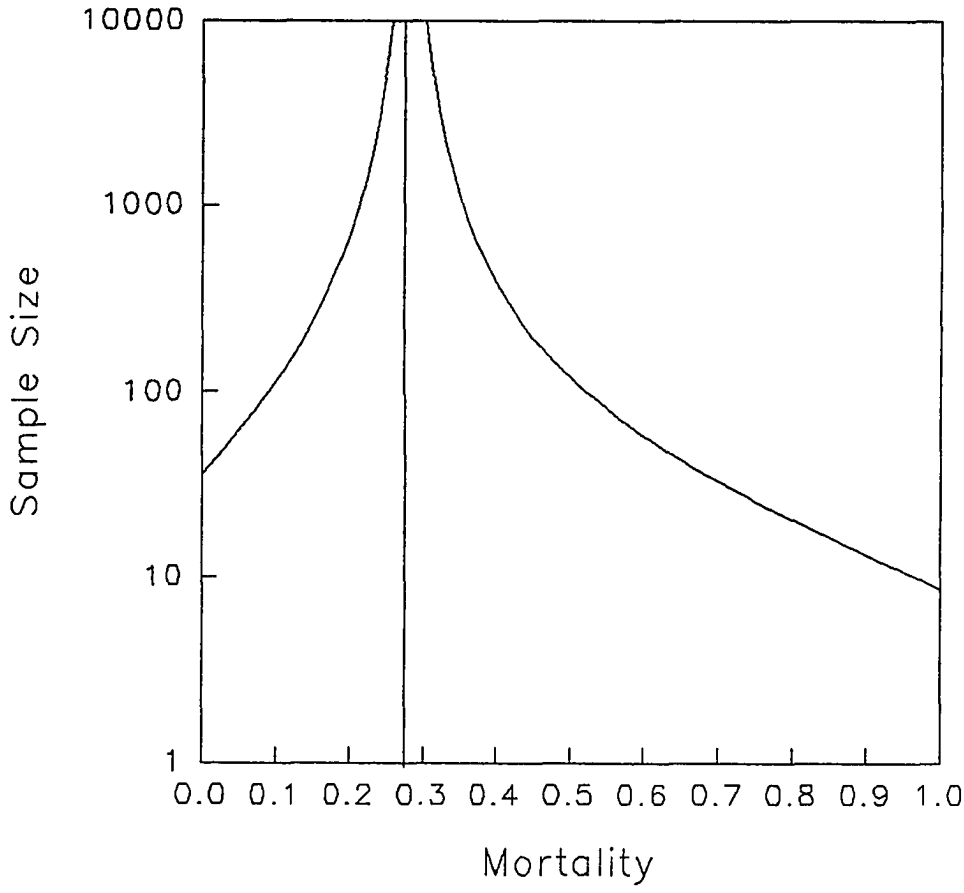

Figure 12. Sample sizes required to detect various differences from 28% mortality with statistical power of 80% and an type I error level (α level) of 0.05. For example, a sample size of approximately 1000 is needed to detect a significant difference between mortality rates of .28 and .20 with power of .8 Calculations based on Lachin (1981).

evolutionary perspective, mortality differences on the order of 1-2% could be selectively important. Thus it may be desirable to detect such differences as they can provide insight into the evolutionary divergence between populations. Unfortunately, sample size requirements to detect such differences with any reasonable level of statistical power far exceed those available for most captive breeding programs (figure 12).

Although outbreeding depression does not appear to be a problem in orang utans, it is of interest to determine at what level depression could be detected using multivariate logistic regression in a data set like the orang utans. This was accomplished by iteratively and randomly increasing the level of outbreeding depression in the population until it reached a level of statistical significance. Starting with the original orang utan data, additional mortality was randomly imposed in 2% increments to individuals with $\theta_s^2 > 0$. At each increment the logistic analysis was performed, and the p values of the coefficient associated with the θ_s^2 parameter recorded. Mortality was randomly imposed each increment by assigning a random number between 0 and 1 to each living animal with $\theta_s^2 > 0$ then recoding the animal as dead if the random number was less than the additional mortality rate being imposed during that particular iteration (i.e., if the iteration was imposing an additional 15% mortality, a living individual with a random number ≤ 0.15 was recorded as dead). Mortality was increased until an additional 24% mortality rate was imposed on $\theta_s^2 > 0$ individuals. This entire

process was repeated 10 times to obtain a distribution of the relationship between increased outbreeding related mortality and statistical significance (figure 13). Not until mortality was increased 15 to 20% above current levels was additive x additive outbreeding depression statistically significant.

The results of these simulations suggest that even with our best data (here represented by the orang utan), outbreeding depression effects probably can not be detected until they are fairly high. While these tests will be useful for detecting outbreeding depression at a level that jeopardizes the demographic security of the population, they are not capable, with the type of data analyzed here, of detecting less severe, but potentially evolutionarily significant, outbreeding effects.

CONCLUSIONS

Conservation biologists concerned about possible deleterious effects of outbreeding in captive breeding programs have been advised to carry out pedigree analyses using inbreeding and Templeton's hybridity coefficients (Templeton et al. 1986; Templeton 1987; Lacy et al. 1993; Simberloff 1988; Ballou 1989). However, this recommendation, strictly applied, has a limited ability to detect outbreeding effects.

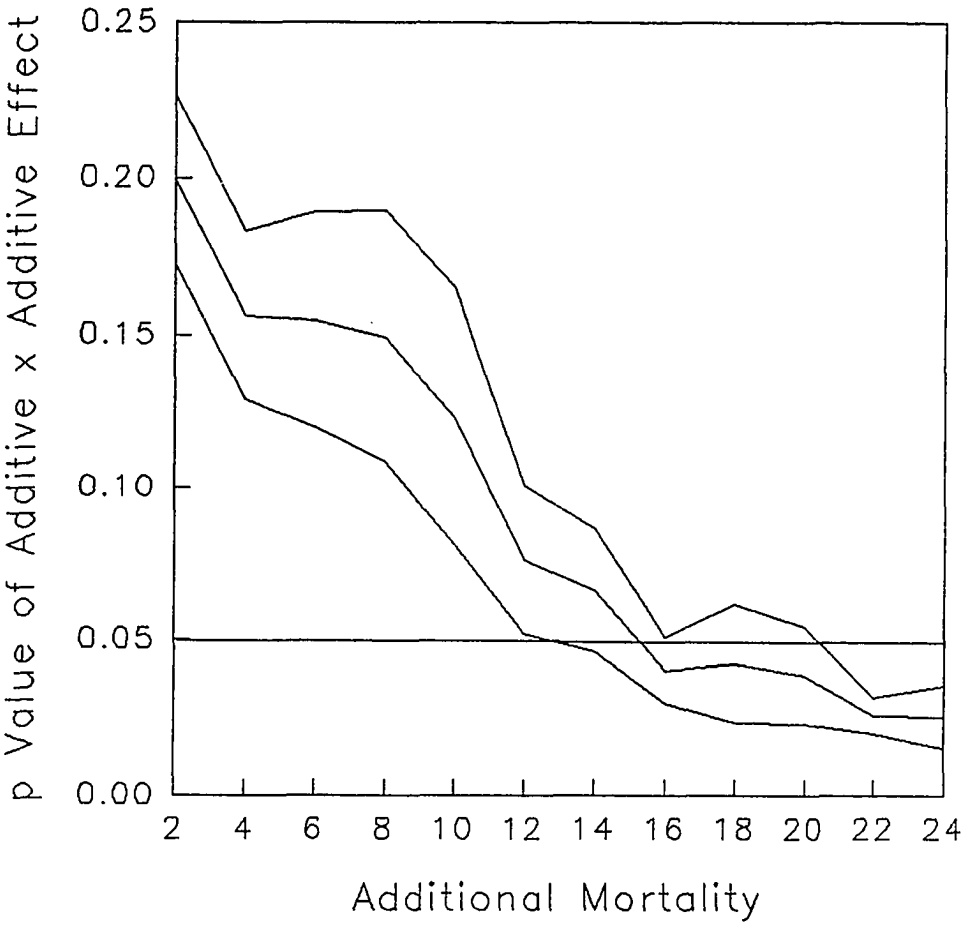

Figure 13. P values associated with the significance of the additive x additive outbreeding parameter as a function of additional mortality randomly imposed on individuals with $\theta_s^2 > 0$ in the orang utan data. The mean, upper and lower 95% confidence intervals, based on 10 simulations, are shown.

First, the hybridity coefficient model alone will not detect outbreeding depression in the F_1 generation, because the hybridity coefficients of the F_1 individuals are always zero (figure 8). The most serious outbreeding depressions are usually apparent in the F_1 generation and tend to involve chromosomal differences between parental forms. Secondly, additive and dominance effects may be confounded with epistatic effects when only certain generational types are present (figure 9, table 8). Failure to account for these confounding effects may result in concluding that outbreeding effects are present when effects are due to other factors. Third, the hybridity model may not detect all kinds of outbreeding depression effects present. Additional models (e.g., the Kinghorn or Lynch-based models) may detect effects not indicated by sole use of the Templeton model.

A more significant problem with any model is that often data on the origin of founders are absent or incorrect for most captive populations (e.g., Ariga et al. 1978). As we have done for the golden lion tamarin, Templeton and Read (1984) and Lacy et al. (1993) both assume, in the absence of any other information, that each founder (or founding event) in the populations analyzed originated from a different source population. However, errors in assumptions on the origin of founders reduce the probability of detecting outbreeding depressions that really exist. If the analysis assumes each founder came from a separate population, whereas in reality some founders came from the same population, some non-hybrids will be

incorrectly classified as hybrids, thus erroneously increasing hybrid survival rates. Conversely, if a group of founders are assumed to come from the same source population when they came from separate populations, some hybrids will be incorrectly classified as non-hybrids, thus erroneously decreasing non-hybrid survival rates. In either case, errors in assuming founder origin decrease the likelihood of detecting an outbreeding depression that really exists. The analysis would be more powerful if the origin of founders was known. Unfortunately, this is not possible for most existing captive populations due to lack of information on capture localities.

There are many reasons why a large proportion of wild-caught, F_1, or later generation individuals may fail to produce viable young. However, outbreeding depression is a testable hypothesis that should be considered for populations exhibiting these symptoms. Such populations may contain more than one "evolutionarily significant unit" or ESU. The term ESU has been adopted by the zoo community "out of a sense of frustration with the limitations of current mammalian taxonomy in determining which named subspecies actually represent significant adaptive variation." (Ryder 1986). Identification of the ESU's within a species may be difficult and requires the integration of data on distribution, morphology, and molecular genetic relationships (Ryder 1986; Avise 1989; Avise and Nelson 1989). If more than one ESU is found, one solution is to divide the population into these ESU's and manage each separately (Maguire and Lacy 1990). This may solve the outbreeding depression

problem but the resulting smaller population size can increase the risk of inbreeding depression. In this case, the relative risks of inbreeding and outbreeding depression need to be evaluated.

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CHAPTER III

PURGING AND INBREEDING DEPRESSION IN CAPTIVE POPULATIONS

INTRODUCTION

Inbreeding depression has been documented in numerous plant (Charlesworth and Charlesworth 1987) and animal species (Wright 1977; Ralls et al. 1988; Lacy et al. 1993; Thornhill 1993). Two genetic mechanisms have been proposed as the cause of inbreeding depression. Both relate to the decrease in heterozygosity during the inbreeding process. The *dominance* hypothesis proposes that fitness depression results from the increased expression of deleterious recessive alleles (mutational load) during inbreeding while the *overdominance* hypothesis proposes that depression is the result of declining heterozygosity among alleles exhibiting heterozygote superiority (Wright 1977; Charlesworth and Charlesworth 1987). In general, dominance effects (the presence of deleterious recessive alleles) are thought to account for a large proportion of the inbreeding depression observed (Morton et al. 1956; Simmons and Crow 1977; Wright 1977; Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Lande and Barrowclough 1987).

Regardless of the genetic mechanism, fitness is expected to decline as inbreeding increases. However, in the presence of selection, inbreeding effects due to deleterious alleles can be mitigated. Selection against deleterious recessive alleles is intensified under inbreeding because inbreeding increases the frequency of the recessive homozygous genotype (Crow and Kimura 1970), and, in the absence of mutation, the population can be "purged" of its mutational load (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Barrett and Charlesworth 1991; Hedrick 1994). Fitness can increase and return to or even exceed that of the randomly breeding (non-inbred) population (Hedrick 1994, Lande and Schemske 1985). This is supported by numerous experiments showing lower inbreeding depression in populations with a history of inbreeding than in populations with a history of outbreeding (Slatis 1960; Lorenc 1980; MacNeil et al. 1984; Abplanalp 1990; Bryant et al. 1990; Barrett and Charlesworth 1991; Ribble and Miller 1992; Dole and Ritland 1993).

Templeton and Read (1983, 1984) suggest that purging populations of deleterious alleles may be a useful approach in captive breeding programs of endangered species that suffer from severe inbreeding depression. They claimed to have significantly reduced inbreeding depression in the captive population of Speke's gazelle (*Gazella spekei*) over two- to three-generations by maximizing retention of genetic diversity and selecting healthy, inbred animals as breeders. Inbred animals surviving to reproductive age are less likely to carry deleterious alleles than non-inbred animals. The

inbreeding depression that was present in the population before selection was imposed was lower in offspring born to these selected and inbred parents (Templeton and Read 1983, 1984; but see Willis and Wiese, Submitted). Based on these results, this "purging" strategy has been recommended for use in other populations suffering from severe inbreeding depression (Ballou 1989, Templeton et al. 1986; Foose et al. 1986; Ralls and Ballou 1986; Hedrick and Miller 1992).

If the strategy to purge inbreeding depression by selectively breeding inbred animals is successful in captive populations, then we might expect to see evidence of purging in many captive populations that have inbred ancestry (Hedrick 1994). As in the Speke's gazelle, an inbred animal with inbred ancestry should be less susceptible to inbreeding depression than an inbred animal with non-inbred ancestors because surviving and reproducing inbred adults are less likely to be carriers of deleterious alleles (Templeton and Read 1984). In this paper, I examine pedigrees of 25 populations of captive mammals for evidence that inbreeding depression has been purged or reduced through selection upon ancestry of inbred animals. Inbreeding depression is measured on three components of fitness: neonatal survival (survival to 7 days of age), survival from 7 days to age of weaning, and, where appropriate, litter size. Two models of purging are presented and applied to each measure of inbreeding depression.

MODELS OF PURGING

The two models used here are based on analyses of the population's pedigree and measure the extent to which inbred ancestors of inbred individuals modify the inbred individuals' susceptibility to inbreeding.

Cumulative Ancestral Inbreeding Model

To evaluate the potential effect of ancestral inbreeding on inbreeding depression, a cumulative ancestral inbreeding coefficient (f_a) is calculated for each individual in the population. The value of f_a is defined as the cumulative proportion of an individual's genome that has been previously exposed to inbreeding in its ancestors:

$$f_a = [f_{a(s)} + (1-f_{a(s)})\, f_{(s)} + f_{a(d)} + (1-f_{a(d)})\, f_{(d)}]/2 \qquad \text{Eq. (1)}$$

where f_a is the ancestral inbreeding coefficient for an individual, f is Wright's inbreeding coefficient (Wright 1922) and the subscripts (s) and (d) represent these values for the sire and dam of that individual, respectively. The value f_a is then the proportion of a parent's genome that has been previously exposed to inbreeding (f_a of the parent) plus the effect of the parent's inbreeding coefficient on the proportion that has not been previously exposed (1-

f_a), averaged across both parents. Its range is 0 to 1. Calculation of f_a values for a simple pedigree are shown in figure 14.

Typically, inbreeding depression effects are modeled by regressing some component of fitness against inbreeding coefficient:

$$u = u_0 + \beta_f * f \qquad \text{Eq. (2)}$$

where u is a measure of fitness, u_0 is mean fitness for non-inbred animals, f is the inbreeding coefficient and β_f is the slope (regression coefficient) of f regressed against fitness. The severity of inbreeding depression is determined by the magnitude and sign of β_f. When u = -log(survival), $2\beta_f$ is a measure of the number of lethal equivalents per diploid genome in the population (Morton et al. 1956; Templeton and Read 1984; Ralls et al. 1988).

The model used here includes the cumulative ancestral inbreeding coefficient as a modifier of the inbreeding depression effect, as well as effects for time (year of birth, *YOB*, to control for changes in husbandry over time) and maternal inbreeding (inbreeding coefficient of dam, f_d). Although maternal inbreeding coefficient is a component of the cumulative ancestral inbreeding coefficient (eq. 1), it was included as a covariate because maternal inbreeding is often associated with poor offspring survival independent of the inbreeding coefficient of the offspring (Ralls et al. 1980). Furthermore, detrimental maternal effects can mask positive purging

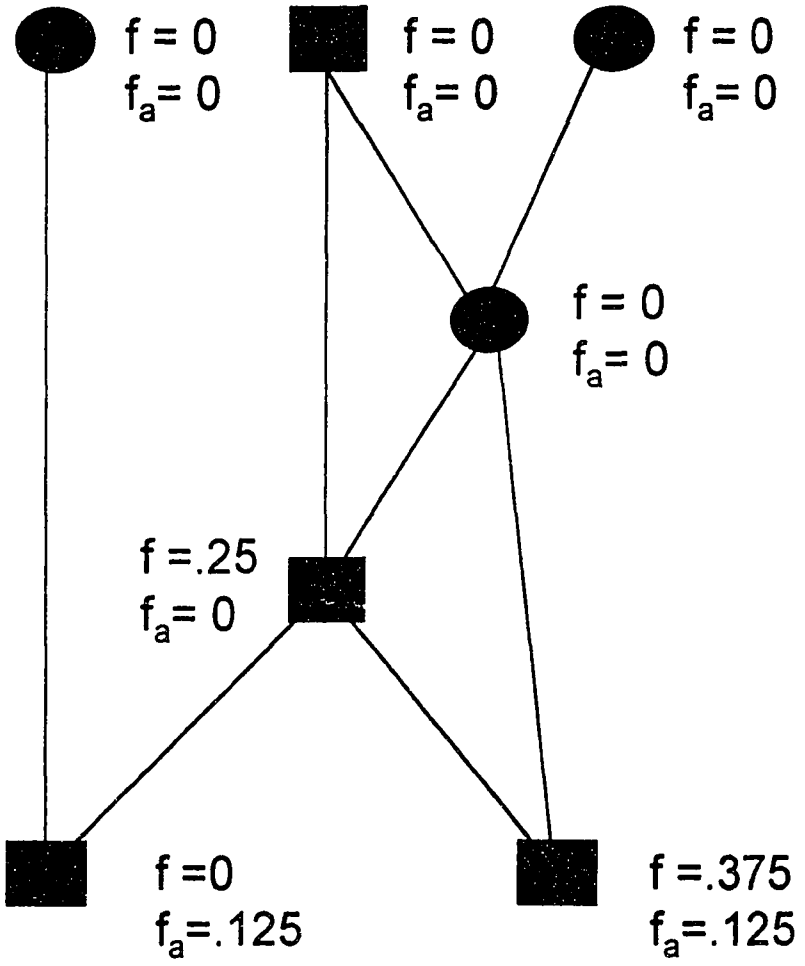

Figure 14. Simple pedigree illustrating the calculation of f and f_a (ancestral inbreeding coefficient).

effects if they are not considered separately. The model then becomes

$$u = u_0 + \beta_t YOB + \beta_f f + \beta_{f_a} f f_a + \beta_{f_d} f_d \quad \text{Eq. (3)}$$

where:

u, u_0, β_f, and f are as described above in equation (2);

β_t is the regression coefficient associated with year of birth (YOB);

β_d is the regression coefficient associated with maternal inbreeding (f_d);

ff_a is the interaction between inbreeding and cumulative ancestral inbreeding; and

β_{fa} is the regression coefficient associated with interactive term.

With this model, survival of non-inbred animals is independent of ancestral inbreeding but the inbreeding effect can be mitigated by the level of ancestral inbreeding. Note that f_a is entered in the equation only as a modifier of the inbreeding coefficient and thus is a measure of the modification of the inbreeding depression effect (β_f). This can be seen by re-expressing equation 3 as

$$u = u_0 + (\beta_f + \beta_{f_a} f_a) f. \qquad \text{Eq. (4)}$$

Inbreeding depression is characterized by $\beta_f < 0$. If there has been purging, then we predict that the coefficient β_{fa} will be positive, mitigating the inbreeding effect.

Lethal Recessive (*LL*) Model

Slatis (1960) first proposed a model of purging based on the assumption that each founder of a pedigreed population carried a single, lethal recessive allele at a different locus. Using path analysis, he estimated the probability of lethal homozygosity (*LL*) as the probability that an individual was homozygous for any lethal allele under the assumption that none of the individual's ancestors could have been homozygous for any lethal allele. Slatis (1960) proposed that if inbreeding depression was due to the presence of lethal recessives in the population, then the relationship between survival and homozygosity would be better predicted by the regression of *LL* on survival than by regressing inbreeding coefficient on survival.

Slatis applied this model to the captive population of European bison (*Bison bonasus*; Slatis 1960), and, not very convincingly, claimed that the *LL* model better fit the survival data than did the inbreeding coefficient model.

I used a similar approach to test the *LL* model against the inbreeding coefficient model as a means of examining pedigrees for the presence of a purging effect. As mentioned by Slatis (1960), calculating *LL* values is computationally complex in complicated pedigrees, and Slatis' original calculations and methods were only approximate. In addition, Slatis seems to have assumed that the probability of inheritance of alleles from the dam and sire were independent (which is not the case in inbred populations), leading to errors in his calculations of some *LL* values.

Because of these complications, *LL* values were estimated here using Monte Carlo simulations. Each founder was assumed to carry one lethal recessive at a different locus. For each individual in the pedigree, the probability of receiving homozygous lethal alleles at any locus was estimated by simulating gene transmission (dropping genes, MacCluer et al. 1986) from the founders to the individual under the assumptions of random assortment and Mendelian segregation. During a simulation, if any ancestor of the individual received a homozygous lethal genotype at any locus, its parents were re-sampled until a non-lethal genotype for that locus was obtained. Ten-thousand simulations were conducted for each individual and *LL* was defined as the proportion of simulations in which the individual received a homozygous lethal genotype at any locus.

The value *LL* was used in the inbreeding depression regression model with time effect (*YOB*):

$$u = u_0 + \beta_t YOB + \beta_{LL}(LL) \qquad \text{Eq. (5)}$$

where β_{LL} is the regression coefficient associated with *LL*.

To test for the presence of purging of lethal recessives, the fit of equation 5 was compared to the fit of equation 2, with the time effect ($\beta_t YOB$) being added to equation 2 as well. If inbreeding depression is caused by lethal recessives, than it is predicted that equation 5 will have the better fit (Slatis 1960).

DATA AND STATISTICAL ANALYSES

The twenty-five populations of captive mammals analyzed are listed in table 10. Values for *f*, f_a, f_m and *LL* were calculated for each individual. The fitness components analyzed were survival to 7 days-of-age (neonatal survival), survival from 7 days to age of weaning and litter size. For those species not producing litters, each individual was coded as either surviving to or dying before each of the survival ages. For those species producing litters, I controlled for non-independence of within-litter mortality by analyzing survival of litters rather than individuals. A litter was coded as surviving if average survivorship of litter mates to age of weaning was at or above the average weaning age survivorship in the population. Litter size at time of weaning was also recorded and coded either larger or smaller than average non-inbred litter size.

Table 10. Sources and sample sizes of data for taxa analysed.

Taxon	Weaning Age	N	Source
Elephant shrew * *Elephantulus rufescens*	30 D	189	National Zoological Park Records
Golden lion tamarin * *Leontopithecus rosalia*	12 W	1136	1993 Studbook (Ballou 1994)
Golden-headed lion tamarin * *Leontopithecus chrysomelas*	12 W	300	1993 Studbook (de Bois 1994)
Black lion tamarin * *Leontopithecus chrysopygus*	12 W	112	1993 Studbook (Padua 1994)
Goeldi's marmoset *Callimico goeldii*	12 W	1228	1994 Studbook (Warneke 1994)
Brown lemur *Lemur fulvus*	5 M	136	Oregon Regional Primate Center, 1983
Greater galago *Galago c. crassicaudatus*	3-4 M	255	Oregon Regional Primate Center, 1983
Melanotic galago *Galago c. argentatus*	3-4 M	40	Oregon Regional Primate Center, 1983
Orang utan *Pongo pygmaeus*	2 Y	1128	1993 Studbook (Perkins 1994)
Kerodon * *Kerodon rupestris*	35 D	165	National Zoological Park Records
Boris * *Octodontomys gliroides*	4 W	27	National Zoological Park Records
Punare * *Cercomys cunicularus*	3 W	78	National Zoological Park Records
Maned wolf * *Chrysocyon brachyurus*	4 M	434	M. Rodden (National Zoo) and 1992 Studbook
Red panda * *Ailurus fulgens*	6 M	641	M. Roberts (National Zoo) and 1993 Studbook (Glatson 1994)
Asiatic lion * *Panthera leo persica*	6 M	151	1993 Studbook (Fouraker 1994)
Sumatran tiger * *Panthera tigris sumatrae*	6 M	346	S. Christie (London Zoo) and 1993 Studbook (Seifert and Müller 1994)
Przewalski's horse *Equus przewalskii*	10 M	1940	O. Ryder (San Diego Zoo) and 1992 Studbook (Volf 1992)
Pygmy hippopotamus *Choeropsis liberiensis*	6 M	641	1993 Studbook (Tobler 1993)
Muntjac *Muntiacus reevesi*	3 M	136	National Zoological Park Records
Elds deer *Cervus eldi thamin*	35 W	314	1993 Studbook (Wemmer 1993)
Gaur *Bos gaurus*	9 M	518	D. Morris (Henry Doorly Zoo) and 1991 Studbook (Klös 1992)

Continued...

Table 10 (Continued). Sources and sample sizes of data for taxa analysed.

Taxon	Weaning Age	N	Source
European bison *Bison bonasus*	7 M	2878	1988 Studbook (Pilarski 1988)
Dorcas gazelle *Gazella dorcas*	75-90 D	184	National Zoological Park Records
Spekes gazelle *Gazella spekei*	12 W	162	1988 Studbook (Read 1988)
Nilgiri tahr *Hemitragus hylocrius*	6 M	168	1994 Studbook (Swengel 1994)

Animals with unknown death dates or ancestry were excluded, as were any animals born within weaning age of the cutoff date of the data.

Multiple logistic regression was used to estimate the regression coefficients for survival and litter size. Logistic regression is particularly suited for this purpose because it allows analysis of binary dependent data (e.g., binary coding of survival for each individual/litter as having lived or died) and provides flexibility in fitting independent data to probablistic dependent variables (Hosmer and Lemeshow 1989).

The multivariate logistic regression takes the form:

$$s = \frac{\exp(-x)}{1 + \exp(-x)} \qquad \text{Eq. (6)}$$

where s is the probability associated with the dependent variable (neonatal survival, weaning survival or probability of "large" litter);

$$x = \mu_0 + \beta_t YOB + \beta_{f_d} f_d + \beta_f f + \beta_{f_a} f_a \qquad \text{Eq. (7)}$$

for the cumulative ancestral inbreeding model;

$$x = \mu_0 + \beta_t YOB + \beta_{LL}(LL) \qquad \text{Eq. (8)}$$

for the lethal recessive model, and

$$x = \mu_0 + \beta_t YOB + \beta_f f \qquad \text{Eq. (9)}$$

for the inbreeding coefficient model.

The SAS LOGISTIC procedure was used to fit the data to the logistic regression (SAS 1991). Coefficients are estimated using maximum likelihood procedures and their statistical significance tested by a likelihood ratio test (Hosmer and Lemeshow 1989). Examination of collinearity among variables was conducted using the SAS REG procedure invoking the COLLINOINT option (SAS 1991). Comparison of model fits (eq. 8 to eq 9) was based on comparison of the Akaike Information Criterion (AIC) values for each model. Lower AIC values indicate better fit (SAS 1991). Trends across species were tested using the sign-test.

Composite Inbreeding Effects

From equation 4, the relationship between inbreeding and survival, taking into consideration purging effects, is ($\beta_f + \beta_{fa} f_a$), the slope of the inbreeding coefficient regressed on survival. Defining ($\beta_f + \beta_{fa} f_a$) as the composite inbreeding effect (β_c), the β_c can be compared with estimates of β_f to determine if inbreeding depression has been eliminated. The β_c and their variances were calculated for each species using the species' mean f_a and the estimates of β_f and β_{fa} obtained from the logistic regression analysis. Variance of β_c is calculated as

$$\sigma^2(\beta_c) = \sigma^2(\beta_f) + f_a^2\sigma^2(\beta_{f_a}) + 2f_a\sigma(\beta_f, \beta_{f_a}) \quad \text{Eq. (10)}$$

Change in Inbreeding Depression Due to Purging

Inbreeding depression can be expressed as $\delta = 1 - w_f/w_o$, where w_f is the fitness of inbred animals (at some defined level of inbreeding) and w_o is the fitness of non-inbred animals (Lande and Schemske 1985). Inbreeding depression in neonatal survival, weaning survival and litter size was calculated for each taxon from the regression estimates in two ways: 1) δ for inbreeding at the level of $f = 0.25$ using the estimated inbreeding effect β_f; and 2) δ' for inbreeding at the level of $f = .25$ using the estimated composite inbreeding effect β_c. The first method estimates the inbreeding depression without purging, while the second estimates the inbreeding effect under purging at the average level of purging experienced by each species (mean f_a). The difference ($\delta' - \delta$) is then the change in inbreeding depression at $f = .25$ due to purging.

RESULTS

Cumulative Ancestral Inbreeding Model

Analysis of the cumulative ancestral inbreeding effects could not be conducted in six populations because of limitations in the distribution of f_a values. These populations were the golden-headed lion tamarin, black-lion tamarin, brown lemur, melanotic galago, orang utan and punare (table 10). Furthermore, in four more populations (elephant shrew, golden lion tamarin, boris and red panda),

maternal inbreeding was sufficiently confounded with ancestral inbreeding (ancestral inbreeding was limited to maternal inbreeding) that inclusion of both f_d and f_a was not possible. In these cases, the model was run with only f_a, recognizing that interpretation of maternal and ancestral effects were confounded.

Estimates of inbreeding effects (β_f) for neonatal survival ranged from 1.104 to -5.914, and were significantly less than zero (indicating statistically significant inbreeding depression) in seven of the 19 populations (table 11). Inbreeding effects were less than zero in 17 of the 19 populations, indicating an overall trend consistent with inbreeding depression (p = 0.0004, sign-test). Estimates for the coefficient associated with purging effects (β_{fa}) for neonatal survival ranged from 709 to -7.76. The differences in magnitude between the estimates for β_{fa} and β_f are due to differences in magnitude between f and $f*f_a$ values. Purging effect was significantly greater than zero in only one species, the Sumatran tiger. However, overall purging effects were in the predicted direction (> 0) in 15 of the 19 populations (p = 0.0096, sign-test). Purging effects are only expected in those species that show inbreeding depression in the first place (Wright et al. In press). If the trend analysis is restricted to those species whose inbreeding effects are consistent with inbreeding depression ($\beta_f < 0$), the trend is even more apparent: 15 of the 17 β_{fa} effects are consistent with purging in the neonatal data (p = 0.0012, sign test, table 11).

Table 11. Logistic regression coefficients for the neonatal survival data.

Species	Time β_t Est	Time β_t SE	Inbreeding β_f Est	Inbreeding β_f SE	Ancestral Inbreeding β_{fa} Est	Ancestral Inbreeding β_{fa} SE	Maternal Inbreeding β_{fd} Est	Maternal Inbreeding β_{fd} SE	Composite Inbreeding β_c Est	Composite Inbreeding β_c SE	$\delta'-\delta$	Mean f_a
Klephant shrew	-0.278**	0.101	-4.973	5.431	709.500	535.300	----	----	-1.915	5.386	-0.233	0.004
Golden lion tamarin	0.030***	0.009	-4.741***	1.373	10.016	15.802	----	----	-4.503***	1.211	-0.019	0.024
Goeldi's monkey	-0.020	0.014	-5.659***	1.178	42.045	28.099	-1.000	2.117	-4.915***	1.092	-0.062	0.018
Greater galago	-0.030	0.034	-2.456	3.059	61.590	166.200	3.719	7.283	-1.821	3.232	-0.047	0.010
Kerodon	-0.094	0.079	-0.044	2.774	6.321	18.850	-6.519*	3.318	0.189	2.358	-0.004	0.037
Boris	-0.506	0.281	-1.665	6.361	2.641	22.165	----	----	-1.457	5.486	-0.013	0.079
Maned wolf	-0.066***	0.020	0.932	1.288	-7.760	7.422	-1.668	1.528	0.595	1.102	0.032	0.044
Red panda	0.003	0.022	-4.908	2.819	38.065	34.691	----	----	-3.633	2.374	-0.106	0.033
Asiatic lion	0.015	0.055	-0.630	2.532	-3.158	11.377	-4.131	2.304	-1.130	1.557	0.019	0.158
Sumatran tiger	0.000	0.013	-0.688	1.393	12.444*	5.545	-3.446*	1.482	0.521	1.111	-0.086	0.097
Przewalski's horse	-0.015*	0.008	-1.762	1.801	-0.238	2.793	-0.683	0.579	-1.892***	0.612	0.003	0.542
Pygmy hippopotamas	0.008	0.006	-4.082***	1.131	1.693	9.140	1.084	1.354	-4.011***	0.897	-0.006	0.042
Muntjac	0.015	0.061	1.104	4.867	-5.256	17.042	-0.283	2.993	0.517	3.586	0.013	0.112

Continued...

Table 11. (Continued) Logistic regression coefficients for the neonatal survival data.

Species	Time β_t Est	SE	Inbreeding β_f Est	SE	Ancestral Inbreeding β_{fa} Est	SE	Maternal Inbreeding β_{fd} Est	SE	Composite Inbreeding β_c Est	SE	$\delta'-\delta$	Mean f_a
Kids deer	-0.027	0.025	-4.798***	1.324	12.853	7.540	-1.490	1.420	-3.911***	1.075	-0.070	0.069
Gaur	-0.082*	0.032	-3.415	1.818	1.389	6.142	0.476	1.078	-3.164**	1.103	-0.010	0.184
European bison	0.001	0.005	-3.103**	1.069	1.807	1.594	1.779*	0.709	-2.180***	0.643	-0.027	0.508
Dorcas gazelle	0.005	0.033	-5.168*	2.443	16.692	11.839	-4.835*	1.976	-3.346*	1.570	-0.105	0.109
Spekes gazelle	0.094	0.058	-5.914***	2.290	6.686	10.332	-3.782	3.003	-5.188**	1.893	-0.048	0.109
Nilgiri tahr	0.013	0.056	-2.955	2.322	4.040	5.670	-4.407*	2.111	-2.113	1.846	-0.039	0.208

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Maternal inbreeding had a significantly negative effect on neonatal survival in four populations (kerodon, Sumatran tiger, Dorcas gazelle, and Nilgiri tahr), and a significantly positive effect in European bison. Estimates of year-of-birth effects (β_t) were significantly greater than 0 (indicating improved survival over time) in the golden lion tamarin, and significantly less than zero in four species (elephant shrew, maned wolf, Przewalski's horse and gaur).

The majority of the mortality in most of the species analyzed occurred during the neonatal period, providing very little data for the analysis of purging effects on survival from 7 days to age of weaning. In 12 of the 19 species, survival during this period exceeded 90%. In only three of the 19 species were inbreeding effects significant (Eld's deer, goeldi's monkey and kerodon), but 14 of the 19 inbreeding effects were negative ($p = .0318$, sign-test). In 11 of the 19 species, the purging effects were in the predicted direction ($\beta_{fa} > 0$; $p = .3238$), and in the 14 species showing inbreeding effects consistent with inbreeding depression, purging effects were positive in 10 ($p = .0898$, sign-test). Due to the paucity of mortality data between 7 days and weaning, the remaining analyses of survival will focus solely on neonatal survival.

For litter sizes, the inbreeding effect was significant only in the golden lion tamarin (table 12), and in the predicted direction in six of the eight taxa analyzed ($p = 0.1445$, sign-test).

Table 12. Logistic regression coefficients for the cumulative ancestral inbreeding model applied to litter size data.

Species	Time β_t Est	Time β_t SE	Inbreeding β_f Est	Inbreeding β_f SE	Ancestral Inbreeding β_{fa} Est	Ancestral Inbreeding β_{fa} SE	Maternal Inbreeding β_d Est	Maternal Inbreeding β_d SE	Composite Inbreeding β_c Est	Composite Inbreeding β_c SE	$\delta'-\delta$
Elephant shrew	-0.244*	0.107	-6.271	6.364	-75.756	527.300	-----	-----	-6.598	6.378	0.019
Golden lion tamarin	0.038***	0.009	-5.369***	1.558	13.657	17.558	-----	-----	-5.043***	1.366	-0.027
Kerodon	-0.099	0.066	0.215	1.913	-23.779	23.632	-0.934	3.871	-0.662	1.704	0.139
Boris	-0.775*	0.386	-8.790	6.350	36.578	24.734	-----	-----	-5.912	5.526	-0.207
Maned wolf	-0.059***	0.018	0.133	1.223	-5.276	7.541	-0.403	1.577	-0.097	1.052	0.032
Red panda	-0.003	0.021	-4.530	3.398	-83.561	58.982	-----	-----	-7.328*	2.795	0.200
Asiatic lion	-0.057	0.047	-0.645	2.116	4.449	9.854	-5.385**	2.006	0.059	1.423	-0.056
Sumatran tiger	-0.007	0.012	-2.358	1.295	10.627*	4.774	-1.780	1.438	-1.326	1.043	-0.119

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Estimates of purging effects were significant in the Sumatran tiger, however, there was no overall trend of the sort seen in the neonatal and weaning survival analysis (4 of the 8 purging effects in the predicted direction; p = 0.6367, sign-test). Maternal inbreeding had a significant negative impact on litter size in only the Asiatic lion. Litter size was significantly negatively associated with time in the elephant shrew, boris and maned wolf but positively associated with time in the golden lion tamarin.

Composite Inbreeding Effects

Estimates of composite inbreeding effects for neonatal survival are shown in table 11 and for litter size in table 12. For neonatal survival, composite inbreeding depression is statistically significant in nine species, and in the direction of inbreeding depression ($\beta_c < 0$) in 15 of the 19 species (p=0.0096, sign test; table 11). For litter size, the effect is significant for two species and less than zero in 7 out of 8 (p=0.0352, sign test, table 12).

Change in Inbreeding Depression due to Purging

Changes in inbreeding depression due to purging ($\delta'-\delta$) for each fitness measure are also shown in tables 11 and 12. For neonatal survival, differences ranged from 0.03 (3% increase in inbreeding depression) to -0.23 (23% decrease in inbreeding depression)

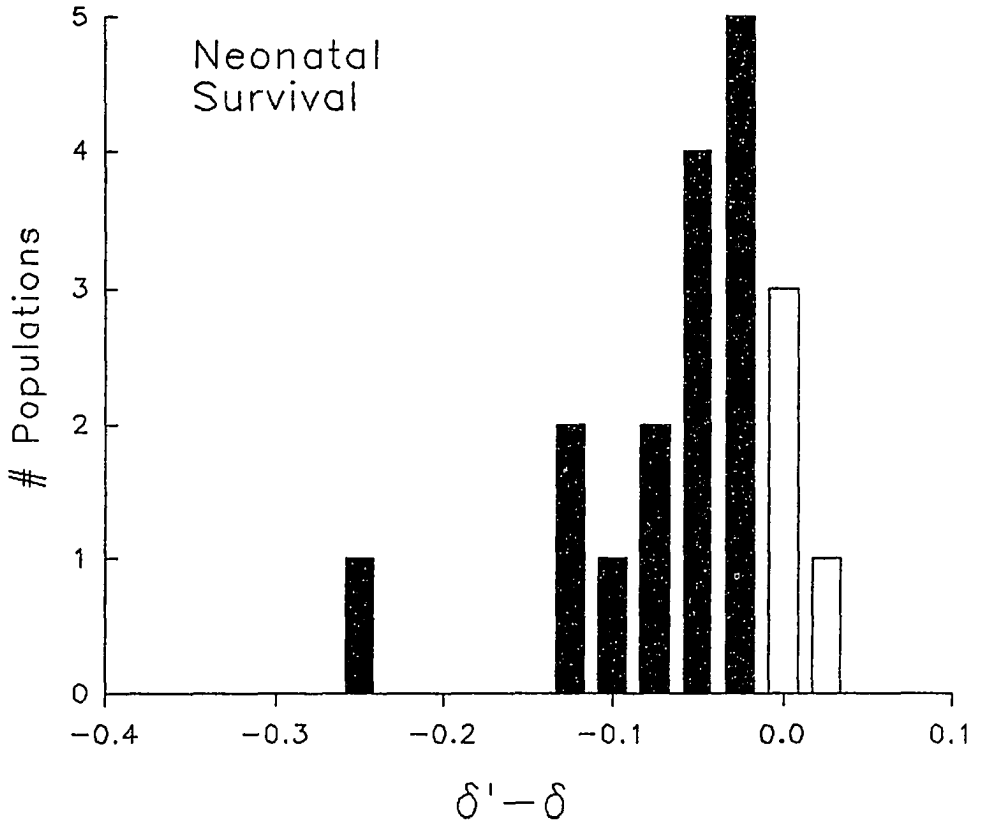

Figure 15. Distribution of change in inbreeding depression due to purging (δ'-δ) in neonatal survival. Shaded bars indicate populations in which inbreeding depression was reduced.

with a median value of -0.02 (table 11, figure 15). Inbreeding depression decreased in 15 of the 19 populations (p=0.0096, sign test). Median value of the change in inbreeding for litter size was -0.01 (table 12) with only 4 of 8 populations showing a decrease (p=.6367, sign test).

Comparison of Lethal Recessive and Inbreeding Models

The lethal recessive model fit the data better than the inbreeding model (lower AIC values) in 11 of 25 comparisons in the neonatal data ($p > 0.10$, sign test), and in 6 of 12 comparisons in the litter size data ($p = 0.6128$). Differences in fit were most apparent in the European bison (*LL* model better fit both the neonatal and weaning survival data), Asiatic lion (inbreeding model better fit for weaning survival) and red panda (inbreeding model better fit for litter size). Overall, there was no trend indicating one model fit better than the other.

DISCUSSION

Although purging effects, as measured by the cumulative ancestral inbreeding coefficient, were significant in only one species (Sumatran tiger), the overall trend in the sign of the purging effect was consistent with purging reducing inbreeding depression. This trend was highly significant for neonatal (table 11), but not for litter size (table 12).

Analysis of survival from day 7 to weaning failed to find any similar trend in purging effects across species, even though there was a trend in inbreeding depression effects across species. However, the inbreeding trend was not as strong as it was in neonatal survival. While the analysis was limited due to the small number of deaths that occurred during that period, the results do suggest that the genetic effects are expressed more strongly earlier in life than later.

Were the purging effects sufficiently large to have eliminated inbreeding depression? In no species did purging eliminate a statistically significant inbreeding effect for any of the three fitness components measured. In fact, inbreeding depression changed from statistical non-significance to significance at the $p = 0.01$ level in the Przewalski's horse for neonatal survival and in the red panda for litter size. In both cases, change in the significance of inbreeding effects was due to a reduction in the variance of β_c rather then by a change in sign of the inbreeding effect.

Purging was sufficient to change the sign of inbreeding effects from negative to positive in two species for neonatal survival (kerodon and Sumatran tiger) and one species for litter size (Asiatic lion). Despite these changes in sign, overall, purging had little effect on eliminating inbreeding depression. These results suggest that while purging may not be a statistically significant factor in reducing inbreeding within species in general, there are

certainly indications that it consistently has a minor effect across a wide variety of species.

Changes in the level of inbreeding depression ($\delta' - \delta$) varied widely among species (tables 11 and 12) and are not inconsistent with the diversity of inbreeding effects observed in other multi-taxa studies of inbreeding depression (Ralls et al. 1988, Lacy et al. 1993; Brewer et al. 1990). The variation in results among species could be due to a number of factors. An important consideration is the degree of inbreeding in the population prior to establishing the captive population (historical inbreeding). Purging of deleterious alleles may already have occurred in populations derived from previously inbred sources. A number of studies have shown that populations derived from inbred sources exhibit less inbreeding depression than populations from outbred sources (mice, Lorenc 1980; Japanese quail, MacNeil et al. 1984; chickens, Abplanalp 1990; house flies, Bryant et al. 1990; hyacinth, Barrett and Charlesworth 1991; *Peromyscus*, Ribble and Miller 1992; *Mimulus*, Dole and Ritland 1993).

Within the taxa analyzed here, the Asiatic lion is one species with a known history of inbreeding or small population size. While once distributed throughout Asia Minor, Iran and central India, the Asiatic lion has been restricted to a relatively small and closed population in the Gir forest in northwestern India for at least the last 100 years (O'Brien et al. 1987). Asiatic lions exhibit extreme-

ly low levels of genetic diversity and high percentages of abnormal sperm (Wildt et al. 1987) suggesting extreme inbreeding in the natural population. The results here are consistent with what is known about the taxon's recent history. Inbreeding depression was non-significant for all three fitness measures and for neonatal and weaning survival, the Asiatic lion was one of the only few species where ancestral inbreeding failed to decrease inbreeding depression (table 11 and 12). For litter size, however, the purging effect decreased inbreeding effects (table 12).

On the other hand, the estimates of β_{fa} for the European bison, also known to have been reduced to a small size in the late 19th century (Slatis 1960), were in the direction consistent with purging effects, but not statistically significant (table 11 and 12). Regardless of purging effects, and despite the historical bottleneck, this species still exhibits significant inbreeding depression for neonatal survival. Lacy et al. (1993) found an inbreeding effect of similar magnitude (but not statistically significant) in European bison at the Brookfield Zoo, and Slatis (1960), found significant inbreeding depression in survival to 2 years but not in survival to 30 days. Slatis used data only through 1958. The results here indicate that the historically small population size has not resulted in elimination of inbreeding depression.

Other studies confirm that purging or prior history of inbreeding (due to historically small population size or mating sys-

tem) is often not successful in completely eliminating inbreeding depression, although, as mentioned above, inbreeding depression might be reduced (Lorenc 1980; Charlesworth and Charlesworth 1987; Charlesworth et al. 1990; Wolf 1993; Ågren and Schemske 1993; Dole and Ritland 1993; Frankham et al. 1993; Wright et al. In press). Brewer et al. (1990) were not able to predict susceptibility to inbreeding depression in *Peromyscus* based on either levels of genetic diversity or estimated effective size of the source population. Likewise, the Pere David's deer, Elaphurus davidianus, which is known to have gone through a severe bottleneck (Jones 1983) and the cheetah, Acinonyx jubatus, which genetic data suggest has had a history of intense inbreeding (O'Brien et al. 1985), both show statistically significant levels of inbreeding depression in captivity (Foose and Foose 1983, Hedrick 1987).

One possible explanation for the persistence of inbreeding depression in small or inbred populations is that inbreeding depression is due primarily to overdominance, in which case fitness is not expected to recover over prolonged inbreeding (Charlesworth and Charlesworth 1987; Ziehe and Roberds 1989). However, studies on other species, summarized by Charlesworth and Charlesworth (1987), show that dominance effects, rather than overdominance, seems to account for a large part of the observed inbreeding depression and that inbreeding depression is maintained in inbreeding populations by the high rates of mutation for deleterious alleles (Charlesworth et al. 1990).

Hedrick (1994), using stochastic simulation models, also examined factors affecting purging success. Purging was most successful when levels of selection were high, as might be the case when inbreeding depression is due to lethal recessives. Ehoibu et al. (1989) found that in equally inbred lines of *Drosophila melanogaster* inbreeding depression was lower in lines with slow rates of inbreeding than in lines with more rapid rates of inbreeding, presumably because of the greater opportunity (i.e., more generations) for selection to act. The variation in results here might then be due to difference in degree of inbreeding or other factors within the population. Level of inbreeding (as measured by average f), the opportunity to detect purging (as measured by average f_a of inbred animals) and selection (as measured by the ratio of inbred to non-inbred survival rates) varied greatly among species. To determine which factors might most affect the estimates of purging effects, I used a stepwise multiple regression (PROC REG with STEPWISE option; SAS 1991) to determine if a number of population parameters could predict the estimated purging effect. To normalize the distribution of β_f and β_{fa} estimates across species, β_f and β_{fa} were standardized by multiplying them by the standard deviations of the f and f_a values, respectively, within each species. This resulted in standardized β_f and β_{fa} values equivalent to those that would have been obtained if the logistic regression had been conducted on standardized f and f_a values in the first place. The following variables were included as independent variables and predictors of purging effects in neonatal survival: total sample size, average f_a for

individuals with $f > 0$, average f, overall mortality rate (as an index on absolute selection), ratio of inbred to non-inbred survival rates (an index of selection operating on inbred animals relative to non-inbred), and the standardized estimate of the inbreeding effect (β_f). This analysis was not conducted on litter size data because of the limited number of species producing litters.

The only significant predictor of the purging effect in neonatal survival was the inbreeding effect ($p = 0.030$). Thus, the best predictor of purging appears to be whether or not the population exhibits inbreeding depression - the higher the inbreeding effect, the higher the purging effect. Since the inbreeding effect is a function of the number of lethal equivalents in a population (Morton et al. 1956), these results suggest that purging is most effective in populations with the highest number of lethal equivalents, which agrees with the simulation results of Hedrick (1994).

The lack of significant purging effects within species, but the overall trend across species, is consistent with the hypothesis that inbreeding depression is not due entirely to lethal alleles, but more likely due to less deleterious alleles or a combination of detrimental and lethal alleles, as is the case with *Drosophila* (Simmons and Crow 1977). If inbreeding depression was due entirely to lethal recessive alleles, purging is expected to be rapid (Hedrick 1994). In addition, the failure of the *LL* model to fit the data

better than the inbreeding model also suggests that depression is not due exclusively to lethals.

CONCLUSIONS

Templeton and Read (1983, 1984) use Speke's gazelle to illustrate the effectiveness of their recommendation to reduce inbreeding depression by purging populations of their lethal or deleterious genes. More recently, however, Willis and Wiese (submitted) in a re-analysis of Templeton and Read's data, found that the apparent reduction in inbreeding depression may have been due to the sample size correction factor applied to the data rather than to purging, *per se*. Willis and Wiese, however, failed to compensate for maternal inbreeding effects, which may affect the interpretation of purging effects. In the re-analysis of the Speke's gazelle data here, purging effects were shown to have only minimally reduced, but not eliminated inbreeding depression (table 11). In fact, inbreeding depression in this species was the highest of any of the species analyzed here. Nevertheless, regardless of the effect of purging in Speke's gazelle, the general issue of using purging to eliminate inbreeding depression is still one that needs to be addressed.

The results presented here suggest that although the purging that occurs naturally in small inbreeding populations may have a slight impact on reducing inbreeding depression, it is not sufficient to eliminate inbreeding depression. Eliminating inbreeding

depression is likely to require rapid rate of inbreeding and high levels of selection (Hedrick 1994). This will almost certainly incur some risk to the population during the purging period and will likely result in long term detrimental effects. If any inbreeding depression is due to deleterious recessive alleles, rather than lethals (which seems to be the case as suggested by the failure of the *LL* model to fit the data better than the inbreeding model), the chance of fixing deleterious alleles during the inbreeding process is high and the probability of population extinction increased (Barrett and Charlesworth 1987; Hedrick 1994, Mills and Smouse 1994). Furthermore, a program of intensive purging genetically alters the population, adapting it more rapidly to both its captive environment and its inbred genetic environment (Templeton and Read 1983), neither of which is desirable for species of conservation interest (Arnold, in press; Frankham et al. 1986).

Because of these concerns, strategies to purge inbreeding depression in species being bred for conservation purposes are ill-advised. Breeding schemes that maintain genetic variation in large populations, while avoiding inbreeding to the extent possible, will not only minimize selection to the captive environment, but also mitigate inbreeding effects (Ballou and Lacy, in press). As shown here, some level of purging will occur naturally as healthy inbred animals survive and reproduce. This probably will not eliminate inbreeding depression, but may reduce its effects.

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