GENETIC MANAGEMENT CONSIDERATIONS

FOR THREATENED SPECIES

WITH A

DETAILED ANALYSIS OF THE FLORIDA PANTHER

(*Felis concolor coryi*).

A report by the participants in a genetic augmentation workshop sponsored by the U. S. Fish and Wildlife Service in cooperation with the Captive Breeding Specialist Group SSC/IUCN.

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Introduction And General Position Statement

Biodiversity is maintained and enhanced by natural, geographic structure in the environment. To take a large scale example, different continents contain distinctive floras and faunas such that overall global species diversity is much higher than would otherwise be expected. Human-mediated introductions of exotic animals and plants have resulted in reduced global species diversity and are increasingly recognized as highly undesirable in terms of ecological effects on recipient biotas. Numerous examples exist in which extinction of native species was attributable to the introduction of exotic taxa. Recent experience in North America with exotics such as the zebra mussel and grass carp exemplify additional and sometimes disastrous ecological problems that can attend species introductions.

Perhaps less well appreciated is that geographic translocation of conspecifics within the range of a species can also have strong negative consequences. In the last two decades, the evidence from molecular genetics has confirmed and extended earlier suspicions based on morphological comparisons that geographic populations within many species are genetically differentiated to varying but often substantial degrees. Geographic differentiation within a species may have both historical and adaptive components. The following are some of the likely consequences of ill-conceived translocations of individuals and genetic material from one population to another:

1. Homogenization of the genetic composition of populations through decay of between-population differences;

2. Blurring or irretrievable loss of genetic information on the intraspecific evolutionary histories of populations;

3. Placement in jeopardy or outright destruction of local adaptations, through introduction of foreign genetic material, breakup of coadapted gene complexes, or genetic swamping;

4. Creation of reproductive difficulties when transplanted individuals differ from recipients in karyotype or other genetic characteristics that may decrease fitness of intercross progeny or their descendants;

5. Disruption, in some species, of the social structure and population stability of the recipient population;

6. Subsequent spread of introduced forms into unintended areas;

7. Unintentional introduction or spread of parasites or disease vectors;

8. Creation of a false sense of management accomplishment (and a masking of underlying environmental difficulties) in situations where repeated translocations from a demographically strong source population are absorbed lost in a recipient population that is not self-sustaining and represents a demographic sink.

Human-mediated translocation of plants and animals is fraught with dangers and should be strongly discouraged as stated in the IUCN Policy on Translocation (IUCN, Gland, 1990). However, in some special circumstances, translocation (managed gene flow and population augmentation) may be warranted and desirable to maintain small populations that are isolated because of human-induced fragmentation of the environment. The burden of proof in any proposed translocation program should rest squarely on the advocates rather than on the opponents of this management option. The purpose of this document is to outline the necessary procedure for considering or initiating a translocation program.

Exceptions To The General Position On Translocation Of Plants And Animals:

Identifying Candidate Species for Genetic Management and Population Augmentation.

Translocation (managed gene flow and population augmentation) may be necessary when a population is small and artificially isolated due to human-induced habitat fragmentation. The guidelines outlined here apply to the augmentation and genetic management of existing populations. They do not apply to introduction of exotic species for game, food or amusement, reintroductions of species into formerly occupied areas, introductions for biological control or environmental remediation (e.g. release of natural or genetically engineered organisms to metabolize or sequester pollutants).

Two types of threats to continued existence of a population could lead to categorizing it as a candidate for population augmentation:

1. Demographic threats. Current or past rates of population decline, current or anticipated achievement of a critical small size, and skewed sex ratios or age structure that would threaten the existence of a population.

2. Genetic threats. Current or anticipated loss of genetic variability that is currently or potentially adaptive, and inbreeding depression.

Demographic Threats

Current trends in population size should be assessed in the context of historical demographic

information. Data on the life history and age structure, the temporal and spatial structure of the population, and its behavioral/social system including territoriality and cultural transmission are especially important.

The possibility of a critical threshold size or density of a population necessary to its survival should be investigated. Such a threshold could result from the difficulty of finding a mate in a sparsely distributed population, cooperative hunting or group defense behavior, dispersal from limited areas of suitable habitat into unsuitable habitat, or the dynamics of local extinction and colonization in a fragmented habitat (Lande, R. 1988. Genetics and demography in biological conservation. Science 241:1455-1460.).

The first course of action in response to a perceived demographic threat should be to remove the cause of the threat, and to allow the population to increase by itself. If the demographic threat cannot be removed in time to allow natural recovery, then temporary augmentation of the population from the same or closely similar genetic stock should be considered. Use of a genetically differentiated stock for purposes of demographic augmentation should be avoided if possible. In the absence of genetic threats, local amplification of the population, e.g. by captive breeding, is preferred.

Genetic Threats

The main criteria for genetic threats are small population size and (geographic) isolation caused by human action, e.g habitat destruction. Often, if not usually, genetic threats will be manifested only after demographic threats are apparent; that is, genetic threats become important at smaller population sizes. Loss of genetic variability in all types of characters becomes a significant concern for populations below an effective size of a few hundred individuals. However, some characters such as disease resistance may be based on genetic variants that are usually rare and found only in very large populations. It is conceivable that a specific genetic threat, such as lack of resistance to a particular disease, could be met by introduction of a specific resistant allele or genotype into a population, rather than random gene flow.

Desirability of preserving a population in a given area can be based on a number of considerations, including the degree of genetic differentiation from other populations of the same species as indicated by morphological, molecular and reproductive traits. The risks from demographic and genetic factors have to be weighted against the risk of diluting or swamping local genetic differences or adaptations by artificial gene flow or introduction of genetic incompatibilities such as major chromosomal rearrangements. A level of gene flow much less than the local selective advantage of a character is unlikely to result in swamping of that character by gene flow, although other less adaptive characters may be significantly diluted or swamped. The adaptive value of a character can be inferred from behavioral or ecological observations. However, its adaptive value can be directly demonstrated in terms of fitness effects only by measurements of natural selection in the natural environment. This requires studying

individual variation within a population or transplantation experiments among populations in different environments, which may not be feasible in many species.

Different manifestations of inbreeding depression should be distinguished, along with the types of evidence for their occurrence. Within a population, decrease of the mean of a character such as body size upon inbreeding can be estimated from pedigree data or breeding experiments. Fitness components including reproductive rates and offspring viability are often subject to substantial inbreeding depression upon matings between close relatives in historically large outcrossing populations. Inbreeding depression affecting an entire population, e.g. due to the fixation of a deleterious recessive gene, can be documented by transplantation experiments among populations (which again may be impractical for many species), or implicated by extensive comparative data among populations.

Inbreeding depression usually is manifested only upon matings between close relatives, or continued random mating in small population of effective size at or below a few dozen individuals.

Before augmenting a population to reduce current or future inbreeding depression, ideally it should be verified by experimental intercrossing, e.g., in a captive stock initiated as part of an augmentation program, that inbreeding depression does indeed exist and can be ameliorated by artificial gene flow. Augmentation should be based on the most similar genetic stock available, even another, small, isolated population that may be suffering from genetic problems provided that these are not identical in detail to those of the target population (i.e. the source population could show inbreeding depression in morphological traits different from those in the target population). Augmentation through a captive population to a wild population allows control of the rate and amount of genetic material to be introduced.

Time scales for action should be evaluated by balancing the relative risks of extinction or genetic damage to the population versus the risks associated with artificial gene flow.

Levels of Gene Flow

The level of artificial augmentation should be commensurate with the demographic or genetic risks faced by the population. Demographic augmentation should counteract (artificial) causes of population decline (including interaction with exotic introduced species), until these can be ameliorated or removed.

Current genetic problems, especially inbreeding depression, require enough gene flow to solve the problem. This may initially be greater than the level of original gene flow to prevent anticipated genetic risks from small population size and geographic isolation caused by human action. However, the cumulative genetic augmentation necessary to mitigate current inbreeding depression generally should not require addition (or substitution) of more than several percent

(2-5%) of the total genetic material in the target population.

After currently existing genetic problems have been solved by genetic augmentation, if the only apparent genetic risk is in the future, the management goal should be to achieve a natural level (that which occurred before isolation of the population) of gene flow. This can be assessed from historical and current observations of dispersal and geographic distribution, and/or (with caution) from molecular genetic studies (e.g. estimates of number of migrants per generation among population subdivisions [Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 16:393-430; Slatkin, M. and N.H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349-1368.). The level of artificial gene flow should be lower than the estimated natural level if the only remaining source is more genetically differentiated than the historical source(s).

Procedural Overview For Population Augmentation Program

I. Overall Procedural Issues - Advanced Planning & External Review of Plan.

A. The decision trees and sequence of steps below should be documented in advance and sent out for external peer review.

B. Suitable reviewers, in addition to other agencies and academic reviewers, should include 3 Specialist Groups of the IUCN - World Conservation Union (who offer expertise based in part on assembly of experience and mistakes made by others worldwide):

1. Reintroduction Specialist Group

2. Captive Breeding Specialist Group

3. Relevant Taxon Specialist Group (e.g., Cat Specialist Group)

II. Source of Stock

A. Demographic supplementation of an existing local population should generally be accomplished with stocks known to be very similar genetically, and evaluated in advance for risk of disease transmission.

1. Stock from another historically nearby (i.e. contiguous) population which formerly exchanged significant numbers of dispersing individuals with the

threatened population and is genetically very similar, is suitable.

2. Stock withdrawn directly from the population at risk and amplified by captive breeding or other means, is suitable.

3. When a population has been completely extirpated, the preferred source of stock for re-establishment may be a more complex issue which warrants further analysis.

B. Genetic supplementation of an existing local population should generally be accomplished with stocks known to be very similar but not identical genetically, and evaluated in advance for risk of genetic incompatibilities and disease transmission. See the following section of this document, where this issue is developed in more detail.

III. Method of Introduction - the emphasis here is on practical means of lowest disease risk which can be monitored for success and effect - see Follow-up section.

A. Introduction of early life stage material offers the advantage of natural integration and cultural transmission. Possibilities include:

1. Artificial insemination where techniques exist.

2. Embryo transfer where techniques exist.

3. Egg-swapping

4. Youngster swapping

B. Introduction of adults with relevant wild experience

C. Introduction of captive-bred individuals trained for release.

IV. Follow-up

A. Sound follow-up study design (in advance) is critical:

1. Managers need to know outcomes.

2. Techniques can only be improved if their success and failure is measurable.

3. Success must be recognizable so the effort can stop when success has been assured or if it becomes apparent that the chosen strategy will not succeed.

4. Poor follow-up has made many release programs a wasted effort from which little is learned.

B. Follow-up is part of the necessary ongoing monitoring and evaluation needed for a population at risk. This should consist of at least:

1. Creation of studbook data sets for the wild population and any existing captive populations.

2. Analytical evaluation of age- and sex-specific fecundities, mortalities, age structure, and population growth or decline rates.

3. Before/after evaluation of genetic composition of population, changes in fitness traits.

4. Public reactions before, during, and after the translocation.

5. Evaluation (of a surviving population) should begin with a 3-5 year baseline study prior to treatment (concurrently with capture studies) and continue for 3-5 years following treatment. Reevaluation of the program should occur at least every 3-5 years.

Criteria For Assessing Appropriateness Of Population Augmentation Program

The following steps should be examined prior to any genetic augmentation of a natural population. The urgency of preventing imminent extinction might necessitate action based on an assessment of partial information before each step can be addressed fully, but adequate attention to the concerns below should not be needlessly postponed until a crisis demands sudden action on behalf of a population. Translocation of organisms involves considerable risks not only to that population, but to all components of the natural communities affected (see introductory section). Concern for the natural environment and biodiversity demands that artificial intercrossing (see definitions) be undertaken only after careful deliberation, after all reasonable precautions have been taken, and after alternatives have been examined. Possible benefits of augmentation must be weighed against costs and risks of artificial translocations, and lack of knowledge concerning any points below must be viewed as contributing substantially to the risks.

Verify that the problems facing the population include genetic loss.

Possible indicators of genetic loss include (temporal trends or traits relative to other populations): (1) Projection of a high rate of genetic loss in the past and/or in the future based on population size and/or structure; (2) Low genetic variation observed in the population; (3) High rate of observed close inbreeding; (4) High prevalence of morphological abnormalities; (5) Health problems; (6) Compromised reproductive status (e.g., poor sperm count or viability, lack of regular cycling of females); (7) Low reproductive output; (8) Poor survival. For the last four possible indicators of genetic problems, attempts should be made to assess whether non-genetic causes (e.g., poor nutrition, social stress, shrinking habitat, or disease) might be responsible for poor performance.

Confirm that genetic problems can be ameliorated by intercrossing.

Experimentally verify potential reversal of genetic problems by intercrossing. This would likely be in a captive setting, in which non-genetic factors could be controlled and data easily collected while not placing the wild population at risk. Such studies could be concurrent with the genetic and demographic studies of the wild population.

Evaluate habitat availability, occupancy, quality, and trends to demonstrate the existence of sufficient habitat to allow the population to benefit from the introduction of additional genes.

There is rarely value in augmenting a population in already saturated habitat, or if continued habitat deterioration is likely to preclude population recovery. Restocking should not be used to continually replenish areas that are functionally population "sinks", and it would commonly be difficult for translocated animals to become established in a resident population that fully occupies available habitat. However, there may be situations in which genetic problems could be remedied while steps are taken to recover habitat quality or to prepare alternative habitat. The purpose would not be to bolster numbers of animals (genetic augmentation might take the form of demographic exchange rather than addition), but rather to improve the genetic health of a population in order to increase resiliency to perturbations and to allow for population expansion when habitat becomes available or to increase viability within existing habitat with lessening of human related pressures.

Demonstrate lack of negative effects of intercrossing (before gene pools are irreversibly mixed).

Serious problems are much less likely to arise if the source and recipient populations had exchanged migrants prior to human-caused habitat or population fragmentation. Notwithstanding the perceived similarity of the populations, it would be prudent to test experimentally, or otherwise under controlled circumstances, the viability, fecundity, and morphological continuity of first and second generation intercrosses.

Confirm availability and appropriateness of potential source population(s).

To minimize negative impacts (foreseen and otherwise) while achieving desired goals of restoring genetic and demographic viability to a small, isolated population, the following ranked list of criteria is suggested for choosing source population(s):

1. Use source population(s) historically in closest geographic proximity, preferably one(s) formerly in contact with the remnant (recipient) population and not formerly separated by geographic barriers to natural dispersal. The goal of the translocation is to restore, to the extent possible, processes that augmented genetic variation prior to human disruption of natural gene flow.

2. Use source population(s) demonstrated to be genetically similar to the recipient population. Karyotypic differences between populations are often indicative of difficulties in intercrossing (often not apparent until the second generation), and information on karyotypic similarity can often be obtained relatively quickly. The diversity of molecular (allozyme, immunological, DNA) techniques available allow quantification of the degree of genetic divergence over a very wide range, from relationships among higher order taxa down to familial relationships within a local pedigree. Study of genetically based morphological variation can be important in revealing adaptive divergence among populations.

3. Use source population(s) from similar habitats. The goal is to allow restoration of potentially adaptive genetic variants into a population that is so small as to be subjected to considerable non-adaptive drift (loss of alleles adapted to components of the habitat and fixation of deleterious alleles).

Establish ability (and plan) to monitor impacts of translocations for intercrossing.

Expected outcomes should be specified. Potential dangers must be identified. Methods need to be designed for determining if anticipated benefits are achieved without serious negative impacts. Contingency plans should be made for changing, halting or, if possible, reversing a management plan that fails to meet pre-defined acceptable levels of performance. Data collection throughout is essential to evaluate success and to help guide future efforts at recovery of endangered populations.

The evidence from each of the above considerations must be evaluated relative to each other and to the perceived urgency of action. The required level of assurance of benefit and minimization of risk could be less if the population is unlikely to persist for long in the absence of action. If risks are judged to be low (e.g., source and recipient populations are known to have regularly exchanged migrants until recently), modest benefits (e.g., sustenance of historic levels

of variation) may lead to a decision to proceed with intercrossing. Higher risks should be accepted only if benefits are large and highly likely to be obtained and if the cost of not taking the action is judged to outweigh the risks.

Definitions

1. Problems With The Word "Hybrid": Mixing Species, Subspecies, And Populations

Words such as "hybrid", "intergrade", "mongrel", and "interbreed" tend to carry strong but varying connotations to most people. For example, many taxonomists confine the use of "hybrid" to the products of crosses between distinct species, whereas geneticists commonly use the word in reference to products of crosses between any organisms that carry different genetic markers. "Intergrade" usually refers to progeny from crosses between "subspecies", but definitions and criteria for identification of subspecies vary. Furthermore, many traditionally-recognized named subspecies (i.e., populations within a species assigned a Latin trinomial) are often highly suspect as guides to the major evolutionary or genetic partitions within species, having originally been described from very limited assessments of morphological or other attributes with unknown genetic basis (See next section on Subspecies).

To provide a general and neutral word to be used in initial discussion regarding progeny resulting from parents of different sources, we suggest "intercross". This word should be used in a generic sense to encompass what is normally meant by more conceptually loaded terms such as hybrid or intergrade. Additional refinement of what is meant by the term in particular instances must be achieved by reference to additional sources of information, such as the magnitude and pattern of genetic divergence and reproductive relationships of the populations or taxa involved. A schematic representation of the term is as follows:

INTERCROSS

"Hybrid"	"Intergrade"	"cross",interbreed"
(Species level)	(subspecies level)	(geographic populations)

<<<<<<<<<<<< Continuum of Possibilities >>>>>>>>>>>>>>>>>>>>

(requiring additional knowledge on such factors as magnitude of genetic differentiation, reproductive relationships, geographic partition, etc).

2. Problems of Using Named Subspecies As Units For Conservation, Management, And Recovery Decisions

The use of named subspecies as units in conservation, management, and recovery decisions is plagued with a series of problems. These problems are highlighted by the history of using subspecies nomenclature in systematics. For several decades the prevailing practice among systematists has been to avoid naming subspecies. The move away from naming subspecies was spurred by the observation that different traits often show different patterns of geographic variation within a species (discordant geographic variation (Wilson, E.O. and W.L. Brown. 1953. The subspecies concept and its taxonomic application. Syst. Zool. 2:97-111.). In this common situation, the naming of subspecies depends on which traits or characters are being considered. Because of this arbitrary aspect of subspecies designation, most systematists stopped naming subspecies in the 1960's or earlier. Nevertheless, subspecies names persist in the literature.

Increasing application of molecular techniques in the past 30 years has further weakened the case for using named subspecies as units for conservation. The molecular techniques now enable us to estimate the phylogenetic relationships of populations within a species (Avise, J.C. and R. Martin. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys in Evolutionary Biology). Sometimes the new molecular results show geographic or phylogenetic patterns that coincide with the old subspecies names. Frequently, however, the new results conflict with the old nomenclature. The conflicts may reflect: (a) discordance between molecular and phenotypic patterns of geographic variation, (b) molecular resolution of units not represented by named subspecies (e.g., units within subspecies), (c) failure of named subspecies to reflect phylogenetic relationships. Thus, valid units commonly exist within species, but often these are not reflected by the named subspecies.

Valid units within a species could be diagnosed by searching for geographic concordance between different sets of traits. The issue in making a conservation decision is whether new molecular information shows geographic concordance with older subspecies names based on analysis of phenotypic traits or geographic separation. Taking analysis a step further, one can ask whether the phylogenetic relationships of populations are concordant across traits (Ball, R.M. and J.E. Nigel, J.C. Avise. 1990. Gene genealogies within the organismal pedigrees of random mating populations. Evolution in press.). Using this more detailed analysis requires data on multiple, genetically-based traits. For example, when multiple genetic differences concordantly distinguish populations, those populations might be considered a candidate unit for conservation, regardless of whether they reside in the same or different named subspecies.

Application Of Decision Criteria To Florida Panther Genetic Management:

Augmenting The Florida Panther Population By Intercrossing With *FELIS CONCOLOR* From Other Populations

1. Does the Florida panther meet requirements to be a candidate as an exception to the general guideline that proscribes augmentation with genetically divergent stock?

The Florida panther was formerly widespread throughout the southeastern United States and was contiguous with other populations (subspecies) of *Felis concolor.* Due to human destruction of habitat and direct persecution of animals, the subspecies has been reduced over the past few centuries to a remnant population existing only in south Florida. The south Florida population is very small, numbering no more than 30-50 adult panthers. The number of breeding animals may be no more than 20-30. Extensive surveys of possible habitat and investigations of reported sightings has demonstrated that the only remaining viable, breeding population of *Felis concolor coryi* is the south Florida population under intensive study and management. Isolated animals elsewhere, if they exist, could not be part of the breeding population. The remnant population of Florida panther is well-separated from the next closest population of *F. concolor*, in western Texas, and the two cannot exchange migrants.

Thus, the Florida panther population meets the criteria of being very small and totally isolated from all conspecifics, due to human-induced fragmentation and destruction of habitat and animals.

2. Is the Florida panther population at substantial risk of extinction?

The Population Viability Analysis conducted on the Florida panther projects, under existing demographic and genetic conditions, the extinction of the population within 25-40 years. The population size is well below criteria that have been suggested for numbers needed to assure viability (see above; O.H. Frankel and M.E. Soule. 1981. Conservation and Evolution. Cambridge University press; Franklin, I.R. 1980. Evolutionary change in small populations. In: Soule, M.E. and R.A. Wilcox (eds.). Conservation Biology. Sunderland, MA, Sinauer. Pp. 135-150.). The habitat available to the south Florida population is not sufficient to allow for expansion of the population to a size that would assure self-sustaining capabilities. Recovery of the population, whether or not it includes genetic augmentation, will require habitat preservation and management, and the identification and/or development of additional suitable habitat within the historic range of the subspecies.

3. Do the problems facing the Florida panther include genetic loss with adverse effects?

The Florida panther PVA projected a loss of 3% to 7% of genetic diversity (heterozygosity) per generation under current conditions of population size and structure. This loss is expected to accelerate unless aggressive management reverses habitat contraction and population decline. During the past decade (1981-1991), mortality of founder animals (those containing genes not known to be contained elsewhere among the living panthers) has been 49% per 24 months (M. Roelke, pers. comm., FL GFWFC). Of the 5 populations of *Felis concolor* that have been investigated by molecular genetic methods, the Florida population has the least genetic variation (7.5% polymorphic loci, 0.028 mean heterozygosity). Much of the genetic variation that does exist in the Florida panther population is contained in those animals believed to be intergrades between *F. concolor coryi* and as yet unidentified subspecies from Central or South America. Assuming that the ancestral population of Florida panthers contained as much genetic variation as do other populations of the species, approximately 50% of the genetic variation that once characterized the subspecies has already been lost.

The pedigree available information demonstrate that close inbreeding (matings between parents and offspring) has been documented in at least 3 breeding events. Second generation inbreeding is probable but undocumented.

There are a number of indicators that inbreeding and losses of genetic diversity are having damaging effects on the population. Male Florida panthers average more than 93% abnormal sperm, more than any of 5 other felid species examined to date. Of male panthers examined since 1985, 44% are cryptorchid (having only one descended testicle), and the rate of cryptorchidism has been increasing markedly since then. As of 1991, 90% of living male Florida panthers are cryptorchid (M. Roelke, FL GFWFC). Vaginal fibropapillomas were observed in at least six female panthers. These papillomas are thought perhaps to impede penile penetration during copulation and or impede transport of sperm through the female tract. Two of the females did not breed during 6.5 years of observation even though they were in regular contact with breeding males (1990 FP Report, FL GFWFC).

Recently, heart murmurs have been detected in Florida panther young adults and kittens. It is not known whether this condition is genetic in cause or whether it will change with age. However, 2 panthers have died since 1988 due to complications associated with congenital atrial septal defects.

Several unusual morphological traits that have traditionally been used to help characterize the subspecies are likely non-adaptive genetic traits that have become common in the small population by chance. Prior to 1990, all panthers thought to be historic *F. c. coryi* have a kink in the end of the tail, while this abnormality is rare among those panthers with some South American ancestry. Likewise, the majority of the historic *F. c. coryi* have a cowlick on the back. The cowlick shows up in museum specimens, and may have been common in the Florida

panther population for at least 100 years. Differences in skull morphology distinguishing Florida panthers from other subspecies are probably indicative of genetic divergence among subspecies, perhaps representing adaptive differentiation. These differences would not be taken as indications of deleterious effects of inbreeding.

Inbreeding is known to cause increased juvenile mortality and decreased reproduction in many populations (Falconer, D.S. 1990. Introduction to Quantitative Genetics. 3rd Ed. Longman, New York; Ralls, K. and J. Ballou. 1983. Extinction: lessons from zoos. Pages 164-184 in C.H. Schonewald-Cox, S.M. Chambers, B. MacBryde, L. Thomas eds. Genetics and Conservation. Menlo Park, CA: Benjamin/Cummings; Ralls, K., J.D. Ballou, and A.R. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2:185-193; Wildt, D.E., M. Bush, K.L. Goodrowe, C. Packer, A.E. Pusey, J.L. Brown, P. Joslin, and S.J. O'Brien. 1987. Reproductive and genetic consequences of founding isolated lion populations. Nature 329:328-331.). Juvenile mortality has not been noted to be elevated in Florida panthers but it has not yet been well-quantified. Similarly, there is not yet evidence of poor reproductive performance by those panthers that have been breeding. Of those female panthers that have not been breeding, non-genetic causes (e.g., poor nutrition, lack of available males) have been implicated.

The above observations together strongly indicate that loss of genetic variation has been and continues to be substantial in the Florida panther population and that inbreeding and genetic loss has increasing impacts on the panthers. The lack of demonstrated loss of fitness (survival and reproduction) attributable to inbreeding may suggest that genetic losses have not so damaged the population as to preclude recovery of the population as it exists genetically at this time.

4. Are the perceived genetic problems correctable via intercrossing?

It is possible that managed translocation of animals already within the south Florida population could ameliorate immediate effects of close inbreeding. (Known pedigrees are not sufficiently deep to provide detailed knowledge of the genealogical relationships between animals in the ENP and in the Big Cypress subpopulations.) The PVA for the Florida panther and the consequent management decisions outline courses of action designed to manage the existing gene pool to recover the population. If the existing genetic variation can be captured and the founder base expanded rapidly, it is hoped that the population can be recovered without intercrossing to other subspecies. If deleterious genetical traits persist in spite of aggressive management of the existing gene pool, it may be possible and desirable, and perhaps necessary for population survival, to augment the genetic variability of the Florida panther with genetic material from other subspecies. The history of intercrossing of Florida panthers in captivity and in the Everglades National Park suggest that the reproduction and health concerns identified above may be reversible. Neither the ENP sub-population nor the Piper captive stock (both thought to be composed of mixtures between Florida panthers and South American panthers) show

cryptorchidism and kinked tails occur only rarely.

The histories of neither the Piper stock nor the ENP animals (thought to be partly derived from the Piper stock) are well documented. Controlled, experimental crosses among populations would be needed to confirm that deleterious traits could be prevented by genetic augmentation via intercrossing. Animals produced by experimental inter-populational crosses could be examined for sperm quality, presence or absence of health problems (e.g., heart murmur, vaginal fibropapillomas), and morphological traits (e.g., cryptorchidism, kinked tails).

5. Would there be negative effects of intercrossing?

The amount of genetic divergence between the Florida panther and other *F. concolor* subspecies appears to be slight (O'Brien data, 1990 FL GFWFC Report), indicating a recent and shallow evolutionary separation of populations that formerly would have been connected by gene flow. The weak inter-populational differentiation is consistent with observations that pumas are capable of long-distance dispersal. Based on the success of crossing between much more divergence populations of other carnivores, and the apparent success in crossing between Florida panthers and a South American stock (among the populations most genetically divergent from the Florida panther) likely during the creation of the Piper stock and the intergradation into the ENP population, it seems very unlikely that crosses between Florida panthers and similar subspecies from elsewhere in North America would display any negative effects in the first or later generations. The crossing experiments proposed above to verify the benefits of intercrossing also would provide an opportunity to confirm the lack of deleterious effects of intercrossing. Any evidence of "hybrid breakdown" in health, viability, or reproduction should be examined carefully in experimental crosses through at least 2 generations.

6. Are appropriate source populations available for intercrossing with Florida panthers?

The closest extant geographic population to the Florida panther is in south and west Texas. Animals from this source have already been used for experimental releases in northern Florida, and those animals appeared to adapt well to that habitat. Molecular evidence indicates that this population is genetically similar to the Florida panther, although not necessarily the most similar of the extant subspecies. Given the apparent close genetic relationships among all the North American populations, any other population could probably be used to augment the Florida panther population.

Further genetic research should be done to quantify more precisely the relationship of the Florida panther to other populations. Although the Texas population seems suitable for intercrossing experiments, other candidate populations may be found to show much closer genetic

affinities, more genetic variation, or more similar habitat use. In particular, nothing is yet known of the relationships of Central American populations of *F. concolor* to Florida, other North American, or South American populations. Central American populations inhabiting approximately comparable environments may be found to have close genetic affinities to the Florida panther. Another group of animals of interest and deserving of more extensive genetic analysis, is the Piper stock. Although it is inbred and exhibits hip problems, this captive stock has some Florida panther ancestry, and may contain Florida panther genes no longer present in the wild.

It should be noted that an option to utilize the most genetically divergent population of *F. concolor* available for intercrossing was considered. Such a strategy could maximize the input of new genetic material into the Florida panther population. If the desire were to replace the Florida panther with a healthy population of the species (but not necessarily most closely related to the animal that formerly inhabited the SE US and that still inhabits south Florida), then use of a source population or a mixture of multiple source populations to maximize genetic variation could be appropriate. At this time, however, it is still hoped that the Florida panther can be saved from extinction with as little genetic alteration as possible. The attempt should be to preserve and restore a population that resembles the ancestral populations of the subspecies as closely as possible, augmenting the gene pool of the population as much as is necessary to assure continued viability of the population.

7. Strategy for incorporating intercrossing into the recovery of the Florida panther

As stated in the Recovery Plan, in the Population Viability Analysis, and above, it is believed that the Florida panther can be recovered to viable populations with aggressive management of the existing animals. Recovery will require management and restoration of habitat combined with measures to increase productivity and survival in the wild and in a captive population. The survival and continued adaptive evolution of the Florida panther is far from assured, however, and many uncertainties in our data on the current demographic and genetic status of the population, concerning the future changes in the environment, and in our understanding of basic population processes are recognized in the various recovery documents. The extent of genetic deterioration of the Florida panther and the impact that past and ongoing genetic losses will have on the viability of individual animals and the population remains one of the areas of greatest uncertainty and concern.

The recovery of the Florida panther should proceed through three levels of increasingly interventive management. First, ongoing attempts to secure and enhance the wild population must continue. Second, the newly established captive population has been identified as an important demographic and genetic back-up for the existing wild population and as the probable only source of sufficient Florida panthers for translocation to re-establish populations in other parts of the former range. Third, having identified (above) the evidence that genetic problems

are likely contributing to the vulnerability of the Florida panther, and that opportunity probably exists to utilize other populations of the species to genetically augment the Florida panther population, it would be recommended that panther management and recovery should include intercrossing experiments. Given the urgency of action to protect the panther (PVA projects extinction in 25-40 years if current genetic and demographic trends continue), and the necessarily long development time to implement captive propagation and intercrossing wisely, it is important that the three components of panther management proceed simultaneously. Sequential implementation of the three phases, rather than overlapping implementation, would leave the population highly vulnerable to extinction between phases if one were found to be insufficient and then next phase became the primary focus of recovery efforts. Time exists now to cautiously and wisely investigate options while not jeopardizing complementary components of an overall program and while animals exist with which to undertake such actions.

The PVA projects the captive breeding program to be implemented over a 20 period. During that time, concurrent experiments on intercrossing Florida panthers with other populations can proceed. Given the potential for serious deleterious consequences of unwise and poorly planned intercrossing of populations (see opening section), and the necessity for producing two generations of intergrades to confirm the presumed benefits and the lack of dangers in intercrossing, it would be prudent to begin investigations of intercrossing as soon as is possible. It is fortunate that experimental verification of the assumptions of a program of intercrossing can be done before such a program becomes the only and last hope for preserving Florida panthers. By crossing male *F. c. coryi* to females from other populations (perhaps by artificial insemination), investigations of intercrossing could proceed without harm to the wild population in Florida nor to the captive breeding program designed to propagate Florida panthers. Panthers from Texas or elsewhere could be used immediately. Knowledge gained from such crosses would be valuable even if future genetic investigations reveal better source populations for augmentation of the Florida panther. If intercrossed panthers are never needed because efforts to protect the Florida panther with its existing gene pool are successful, any such animals produced would be good subjects for planned trial releases into candidate reintroduction sites. If it becomes desirable or necessary to augment the Florida panther population by intercrossing, the animals produced experimentally could be used as the initial stock for such augmentation.

For the purpose of restoring genetic health to the Florida panther population it should not be necessary to introduce many animals from (an)other population(s). Demographic recovery and stability of the Florida panther population (and considerable genetic stability) can be afforded by the captive breeding program with Florida panthers bred solely from the genetic stock already existing in south Florida. However, intercrossing would be necessary to accelerate improvement of the genetic health and variation of the existing population and with relatively few animals should be sufficient to restore genetic variation, should that course be determined to become necessary. In that case, further analyses will be needed to determine the optimal amount and rate of genetic augmentation.

Any efforts to genetically augment Florida panthers by intercrossing must be closely monitored. Releases of panthers produced in captivity, especially if by intercrossing, should be made in areas where the resident population (if any) is well monitored and, consequently, social interactions between resident and translocated animals can be documented. The social structure of the recipient population should be evaluated and prepared for the introduction of individuals from captivity to reduce the likelihood of social disruption and death of important individuals in the population. All released or translocated panthers should be monitored by radio-collars, in order to track dispersal, habitat use, and social interactions with other panthers, and to indicate quickly death or serious injury. If possible, unique genetic markers for each translocated panther should be identified, permitting later verification of which animals successfully enter the breeding population in the wild. The ongoing data collection that serves now to provide understanding of the population status and structure will become the baseline data for comparison to similar data taken following translocations or other manipulations of the population.

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Estimates of Lethal Equivalents and the Cost of Inbreeding in Mammals

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Abstract: *The costs of inbreeding in natural populations of mammals are unknown despite their theoretical importance in genetic and sociobiological models and practical applications in conservation biology. A major cost of inbreeding is the reduced survival of inbred young. We estimate this cost from the regression of juvenile survival on the inbreeding coefficient using pedigrees of 40 captive mammalian populations belonging to 38 species.*

The number of lethal equivalents ranged from −1.4 to 30.3, with a mean of 4.6 and a median of 3.1. There was no significant difference between populations founded with wild-caught individuals, a mixture of wild-caught and captive-born individuals, and individuals of unknown origin. The average cost of a parent-offspring or full sibling mating was 0.33, that is, mortality was 33% higher in offspring of such matings than in offspring of unrelated parents. This is likely to be an underestimate.

Resumen: *Los costos de procreación en consanguinidad en poblaciones naturales de mamíferos son desconocidos a pesar de su importancia teórica en los modelos genéticos y sociobiológicos y en sus aplicaciones prácticas para la biología de la conservación. Uno de los costos mayores de la procreación en consanguinidad es la disminución en la sobrevivencia de las crías consanguíneas. Estimamos este costo por medio de la regresión de la sobrevivencia juvenil en el coeficiente de procreación en consanguinidad utilizando pedigrís de 40 poblaciones de mamíferos en cautiverio pertenecientes a 38 especies.*

El número de equivalentes letales varió de −1.4 a 30.3, con una media de 4.6 y una mediana de 3.1. No hubo diferencia significativa entre poblaciones formadas a partir de individuos silvestres capturados, a partir de una mezcla de individuos silvestres capturados, y a partir de individuos de origen desconocido. El costo promedio del apareamiento de padre-cría o hermanos completamente consanguíneos fue de 0.33, es decir, la mortalidad fue 33% más alta en las crías de tales apareamientos que en las crías de especies no relacionadas. Es probable que este cálculo sea una subestimación.

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Introduction

Many studies of laboratory, domestic, and zoo animals have documented reduced survival and fecundity of inbred young (Wright 1977; Ralls & Ballou 1983; Sausman 1984; Templeton & Read 1984). Inbreeding depression is thus a major concern in the management of small populations, and estimates of the cost of inbreeding are of considerable importance to conservation biology.

However, inbreeding can increase an individual's inclusive fitness by producing young that share more of its genome. Thus, when inbreeding has little or no genetic cost, there should be strong selective advantage for inbreeding as well as recognition and cooperation among kin (Wilson 1976; May 1979). The cost of inbreeding is therefore of theoretical importance as well.

Calculations of the total cost of inbreeding in natural populations would involve considering the effects of inbreeding on several components of fitness. However, the "cost of inbreeding" that appears in a variety of theoretical models (Dawkins 1976; Bengtsson 1978; Parker 1979; Smith 1979; Feldman & Christiansen 1984) is defined solely in terms of the survival of inbred young relative to non-inbred young. There are almost no estimates of this quantity in natural populations of mammals (Packer 1979).

We estimate this cost from pedigrees of 40 captive mammalian populations belonging to 38 species.

Methods

Morton, Crow, & Muller (1955) developed a log model for estimating the cost of inbreeding from the rate at which juvenile survival decreases with increasing amounts of inbreeding. Specifically,

$$S = e^{-(A + BF)} \qquad (1)$$

where S is the proportion of individuals surviving to some age, F is the inbreeding coefficient, A is considered a measure of death due to environmental causes and the genetic damage expressed in a randomly mating population, and B is a measure of the rate at which survival decreases with increasing inbreeding.

Makov & Bittles (1986) evaluated the use of this and several other equations to estimate effects of inbreeding in humans. They found that many different models could adequately detect significant inbreeding effects; however, different models resulted in different values of A and B. Because of the limited range of inbreeding levels in available data from human populations (F = 0–0.125), they were unable to determine which equation most adequately modeled data on inbreeding effects in humans. They suggested that different equations could more effectively be evaluated in animal populations with wider ranges of inbreeding levels.

We evaluated the log transformed equation (1) and two other equations, using several of our largest data sets with relatively wide ranges of inbreeding levels (F = 0–0.5). The two additional equations were

$$S = A + B(F) \qquad (2)$$

$$\arcsin\sqrt{S} = A + B(F) \qquad (3)$$

where S, A, B, and F are the same values as in equation (1). Model 2 was used because it represents the simplest linear relationship between the variables. Model 3 (angular transformation) was used since it is often recommended for estimating proportions (Sokal & Rohlf 1969). Weighted least squares regression, with a small sample size correction (Templeton & Read 1984), was used to estimate the parameters for each of the models. The total percentage of variation explained by the equation (R^2) was used to evaluate which model best fitted the data.

When analyzing pedigrees of zoo animals, care must be taken to distinguish inbreeding depression from hybridity effects or "outbreeding depression" (Templeton & Read 1984; Templeton et al. 1986). We therefore carried out the analysis developed for this purpose by Templeton & Read (1984) on those pedigrees with adequate sample sizes but found no evidence of outbreeding depression (Templeton & Read 1984; unpublished data).

Inbreeding coefficients (F) were calculated for each animal in each pedigree, relative to the founders of the population. Methods for calculating F from pedigree data are given by Ballou (1983). F is the probability that the two alleles present at a given locus are "identical by descent"—that is, are derived by replication of a single allele from a common ancestor. F ranges from 0 in a non-inbred individual to 1.0 in a completely inbred (homozygous) individual (Crow & Kimura 1970). The effect of inbreeding is often less severe in individuals with inbred ancestors (Bowman & Falconer 1960; Lorenc 1980; Templeton & Read 1984), but we were unable to exclude them from the analysis because this eliminated all levels of inbreeding except F = 0.25 in many pedigrees.

Levels of inbreeding varied among pedigrees (Table 1). For each level of inbreeding represented in a particular pedigree, we calculated the proportion of animals that survived to a criterion age. This was 180 days for the larger species and one-half the age at sexual maturity for the smaller ones (Table 2). Ideally, studies of the relationship between inbreeding and juvenile mortality should be based upon the total mortality before reaching reproductive age (Cavalli-Sforza & Bodmer 1971), but we were unable to follow many individuals for this period because zoo animals are often transferred to other institutions before reaching reproductive age. Considering survival to a criterion age less than repro-

Table 1. Comparison of models used for estimating cost of inbreeding.

SPECIES[a]	Maximum Inbreeding level	Comparison of R^2 Values MODEL		
		Log (1)	Linear (2)	Arcsin (3)
Short bare-tailed opossum	.328	80	.79	.77
Elephant shrew	.125	05	.06	.07
Golden lion tamarin	.375	.35	.26	.26
Greater galago	.250	.17	.14	.13
Maned wolf	.312	.77	.83	.83
Bush dog	.500	.02	.00	.00
Pygmy hippopotamus	.375	.45	.55	.55
Dorcas gazelle	.375	.64	.66	.63

[a] *Scientific names listed in Table 2.*

ductive maturity tends to underestimate the cost of inbreeding, as inbred mortality increases more rapidly than non-inbred mortality with increasing age in some species (Ralls, Brugger, & Glick 1980; unpublished data).

Results

Table 1 shows the results of the three models applied to eight of the largest data sets. R^2 values were highest for the Linear model (2) in 2 populations, highest for the Arcsin model (3) in 2 populations, and highest for the log model (1) in 4 populations. As Makov and Bittles (1986) concluded, no one model was clearly better than the others; R^2 values ranged over only a few percentage points across the models.

The log transformed model (1) has been used extensively in the literature to estimate number of lethal equivalents and is the theoretically expected model, if it is assumed that genetic and environmental influences are independent of each other with respect to survival (Morton, Crow & Muller 1955). Use of this model also facilitates comparisons with A and B values already published in the literature. We therefore selected it for all subsequent analyses.

Estimates for A and B are shown in Table 2. Values of A ranged from 0.03 to 1.11 with a mean of 0.33 and a median of 0.32. Values for B ranged from −0.68 to +15.16, with a mean of +2.33 and a median of +1.57 (Fig. 1). Of the 40 populations, 36 had positive slopes, which clearly indicates an overall trend towards higher levels of juvenile mortality with increasing inbreeding coefficients (Sign test, $P < .001$). This relationship was statistically significant—that is, the slope of the line was significantly greater than zero—in only 9 (23%) of the populations. However, most of our sample sizes were small and distributed over only a few levels of inbreeding. The statistical power to detect slopes significantly greater than zero was therefore limited. Considering only those populations in which the relationship between inbreeding and survival is significant would be likely to greatly overestimate the average cost of inbreeding in mammals. Limiting the analysis to only those species with relatively large data sets increases the power of the statistical comparisons but reduces the number of species that can be analyzed. Only 10 species had more than five levels of inbreeding and total sample sizes over 100. Six of these 10 had slopes significantly different from zero; the average B value was 1.98, with a median of 1.64. These B values did not differ significantly from those in the overall data set (Mann-Whitney U test, $P > 0.05$).

The distributions of B by order are shown in Figure 2. Median values were between one and two except for the Carnivora. There were no statistically significant differences between average B values in populations founded with wild-caught individuals ($\bar{x} = 2.57$, $n = 18$), a mixture of wild-caught and captive individuals ($\bar{x} = 2.42$, $n = 11$), and individuals of unknown origin ($\bar{x} = 1.95$, $n = 10$) (Kruskal-Wallis Test, $P = 0.88$).

The number of lethal equivalents per gamete lies between B and A but is usually very close to B (Cavalli-Sforza & Bodmer 1971; Crow & Kimura 1970). The number per zygote or individual is twice the number per gamete, thus our estimates of the average number of lethal equivalents per individual are twice the values of B in Table 2, with a mean of 4.6 and a median of 3.1. We estimated the cost of inbreeding for matings between first-degree relatives (parents and their offspring or full siblings) by solving equation (1) for each species using $F = 0$ and $F = 0.25$ to obtain the predicted survivorship at these levels of inbreeding. The cost of inbreeding (i) at $F = 0.25$ is then equal to

$$i = 1 - \left[\frac{\text{Survivorship at } F = 0.25: e^{-(A+.25B)}}{\text{Survivorship at } F = 0: e^{-(A)}}\right]$$

$$= 1 - e^{-.25B}. \qquad (4)$$

The average cost of inbreeding between first degree relatives, calculated by averaging the costs across all populations, was 0.33 (Table 2). Solving equation (4)

Table 2. The cost of inbreeding in 40 mammalian populations.

TAXON	Survival to Age (Days)	N	Founder[a] Type	No. of Inbred Levels	Model Estimates A	Model Estimates B	Model R^2	Cost of Inbreeding[c] at F = 0.25	Data Source
MARSUPIALIA									
Short bare-tailed opossum (*Monodelphis domestica*)	75	251	W	6	0.03	0.43[b]	0.80	.10	National Zoo
Parma wallaby (*Macropus parma*)	180	17	W	5	0.32	1.69	0.47	.34	National Zoo
INSECTIVORA									
Elephant shrew (*Elephantulus rufescens*)	21	218	W	7	0.28	2.12	0.05	.41	National Zoo
PRIMATES									
Black spider monkey (*Ateles fusciceps robustrus*)	180	23	W	3	0.23	2.22	0.88	.43	National Zoo
Saddle-backed tamarin (*Saguinus fuscicollis*)	180	233	U	2	1.11	1.86	—	.37	Monell Chemical Senses Center
Illiger's saddle-backed tamarin (*Saguinus f. illigeri*)	180	406	U	4	0.40	1.92	0.40	.82	Rush-Presbyterian St. Luke's Medical Center
Golden lion tamarin (*Leontopithecus r. rosalia*)	180	974	W	18	0.54	2.15[b]	0.35	.42	1984 Studbook
Ring-tail lemur (*Lemur catta*)	180	53	M	4	0.34	0.13	0.01	.03	Oregon Primate Research Center
Black lemur (*Lemur macaco*)	180	43	W	3	0.52	2.78	0.87	.50	Oregon Primate Research Center
Brown lemur (*Lemur fulvus*)	180	136	M	6	0.32	9.17[b]	0.94	.90	Oregon Primate Research Center
Greater galago (*Galago c. crassicaudatus*)	180	251	M	29	0.45	1.69[b]	0.17	.34	Oregon Primate Research Center
Melanotic galago (*Galago c. argentatus*)	180	54	M	4	0.36	0.48	0.19	.11	Oregon Primate Research Center
Crab-eating macaque (*Macaca fascicularis*)	180	237	U	3	0.37	0.29	0.56	.07	New England Primate Research Center
Celebes black ape (*Macaca nigra*)	180	86	U	3	0.38	2.84	0.70	.51	Oregon Primate Research Center
Chimpanzee (*Pan troglodytes*)	180	247	U	4	0.35	1.05	0.67	.23	Yerkes Primate Center
RODENTIA									
Climbing rat (*Tylomys nudicaudus*)	45	49	U	5	0.23	−0.14	0.02	−.04	National Zoo
Wied's red-nosed rat (*Wiedomys pyrrhorhinos*)	30	23	W	2	0.05	15.16	—	.98	National Zoo
Rock cavy (*Kerodon rupestris*)	90	132	U	3	0.12	0.77	0.87	.18	National Zoo
Salt-desert cavy (*Dolichotis salinicola*)	90	17	W	2	0.08	7.21	—	.34	National Zoo
Acouchi (*Myoprocta pratti*)	135	36	U	5	0.30	2.20	0.17	.42	National Zoo
Boris (*Octodontomys gliroides*)	75	53	U	6	0.26	1.15	0.33	.25	National Zoo
Punare (*Cercomys cunicularus*)	60	161	W	4	0.10	0.94[b]	0.91	.21	National Zoo
CARNIVORA									
Maned wolf (*Chrysocyon brachyurus*)	180	338	M	4	0.52	−0.68	0.77	−.19	1983 Studbook
Bush dog (*Speothos venaticus*)	180	176	W	9	0.54	0.24	0.02	.06	1983 Studbook
Sumatran tiger (*Panthera tigris sumatrae*)	180	427	M	12	0.49	0.01	0.00	.003	1983 Studbook
PERISSODACTYLA									
Zebra (*Equus burchelli*)	180	50	U	2	0.30	1.56	—	.32	National Zoo
ARTIODACTYLA									
Pygmy hippopotamas (*Choeropsis liberiensis*)	180	419	W	12	0.33	1.59[b]	0.45	.33	1982 Studbook

Table 2. Continued

TAXON	Survival to Age (Days)	N	Founder[a] Type	No. of Inbred Levels	Model Estimates A	Model Estimates B	Model R^2	Cost of Inbreeding[c] at F = 0.25	Data Source
Reeves muntjac (*Muntiacus reevesi*)	180	75	M	9	0.19	1.20	0.37	.26	National Zoo
Eld's Deer (*Cervus eldi thamin*)	180	24	M	2	0.31	7.57	—	.85	National Zoo
Pere David's Deer (*Elaphurus davidianus*)	180	39	C	7	0.17	0.63[b]	0.74	.15	National Zoo
Reindeer (*Rangifer tarandus*)	180	50	W	4	0.32	4.20	0.71	.65	National Zoo
Giraffe (*Giraffa camelopardalis*)	180	19	W	2	0.29	2.24	—	.43	National Zoo
Kudu (*Tragelaphus strepsiceros*)	180	25	W	2	0.37	−0.03	—	−.01	National Zoo
Bongo (*Tragelaphus eurycerus*)	180	74	W	3	0.23	−0.55	0.74	−.15	1984 Studbook
Gaur (*Bos gaurus*)	180	182	W	6	0.18	0.51	0.36	.12	Hinz & Foose, 1982
Scimitar-horned oryx (*Oryx dammah*)	180	81	M	2	0.09	4.63	—	.69	National Zoo
Wildebeest (*Connochaetes taurinus*)	180	42	W	11	0.33	0.28	0.02	.07	National Zoo
Dik-dik (*Madoqua kirki*)	180	20	M	3	0.80	0.59	0.12	.14	National Zoo
Dorcas gazelle (*Gazella dorcas*)	180	143	M	15	0.34	1.85[b]	0.64	.37	National Zoo
Spekes gazelle (*Gazella spekei*)	30	64	W	5	0.22	3.08[b]	0.92	.54	Templeton & Read, 1983
		Mean:			0.33	2.33		0.33	
		Median:			0.32	1.57		0.33	
		Lower Quartile:			0.23	0.45		0.09	
		Upper Quartile:			0.39	2.81		0.47	

[a] Founder Type: W = All founders wild-caught.
C = Founders captive-born.
M = Founders were a mix of wild-caught and captive-born.
U = Source of founders unknown.

[b] B (slope) significantly different than zero at the 0.05 level.

[c] Cost of inbreeding for F = 0.25: $= 1 - \left[\frac{\text{Predicted inbred survival: } e^{-(A + .25B)}}{\text{(Predicted non = inbred survival: } e^{-(A)}}\right] = 1 - e^{-.25B}$

using the average B value (2.3) results in a cost of inbreeding of 0.44. However, the statistic of interest here is the estimate of the expected value of the cost of inbreeding rather than the cost of inbreeding calculated from the expected value of B. We therefore base our discussion on an average cost of inbreeding of 0.33. The distribution of the cost of inbreeding between first degree relatives is shown in Figure 3.

Discussion

The costs of inbreeding varied widely among captive populations. This is not surprising since one would expect populations to differ in their level of susceptibility to inbreeding. However, in many cases, the models fit the data very poorly and only a small proportion of the variance was explained. These variable results probably reflect the heterogeneous data used for the analysis. The available data for the populations surveyed differed in sample size and the range and number of levels of inbreeding. Nevertheless, these results do provide data on the costs of inbreeding and number of lethal equivalents in a wide variety of captive populations and allow analyses of general trends and patterns.

The median number of estimated lethal equivalents for the captive mammalian populations we examined was 3.1. This figure is similar to estimates for other animal populations. Humans (May 1979), *Drosophila* (Dobzhansky 1970), and the great tit, *Parus major* (Bulmer 1973), are thought to have about two lethal equivalents per individual, and the Japanese quail, *Coturnix coturnix japonica*, is thought to have about 3.4 (Sittmann, Abplanalp & Fraser 1986). Our estimates for captive carnivores, although based on only three populations, were quite low. More carnivore populations should be studied to determine if this is characteristic of the order or unique to the data sets we examined.

May (1979), assuming the number of lethal equivalents in humans was 2.2, estimated the cost of breedin' in humans at F = 0.25 to be .42. However, his equation for calculating the inbreeding cost contained an error. The correct cost, based on formula (4), is .24. This es-

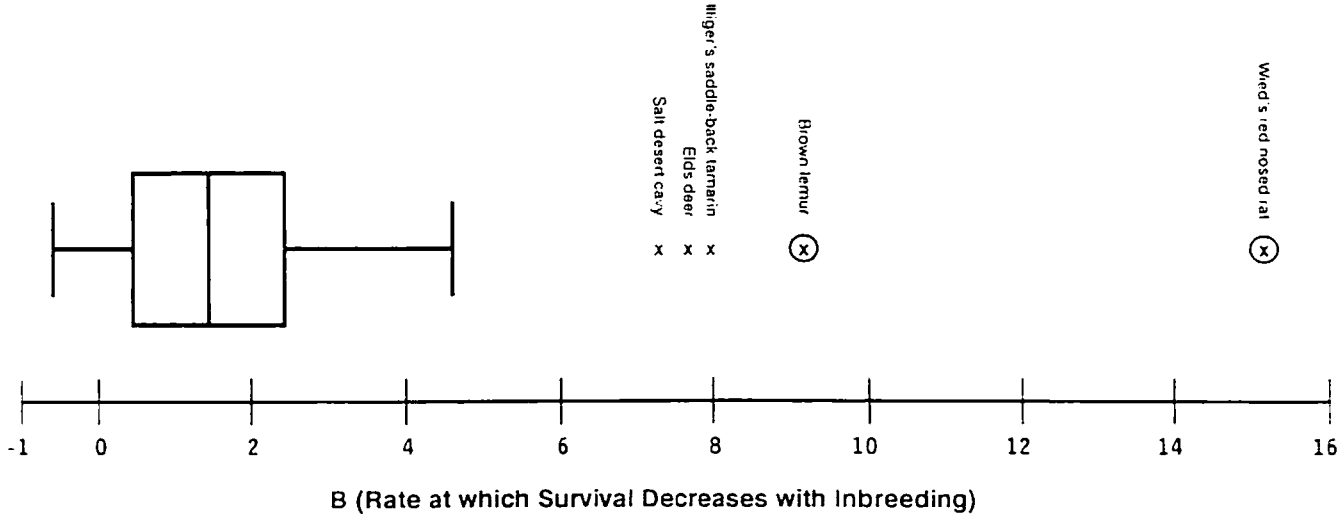

Figure 1. Box plots of B, a measure of the rate at which survival decreases with increasing inbreeding, for 40 mammalian populations. The median (middle vertical line in box), upper and lower quartiles (left and right ends of box), upper and lower inner fences (vertical lines), outlying values (x), and values beyond the outer fences (⊗) are shown (Hoaglin, Mosteller, & Tukey 1983).

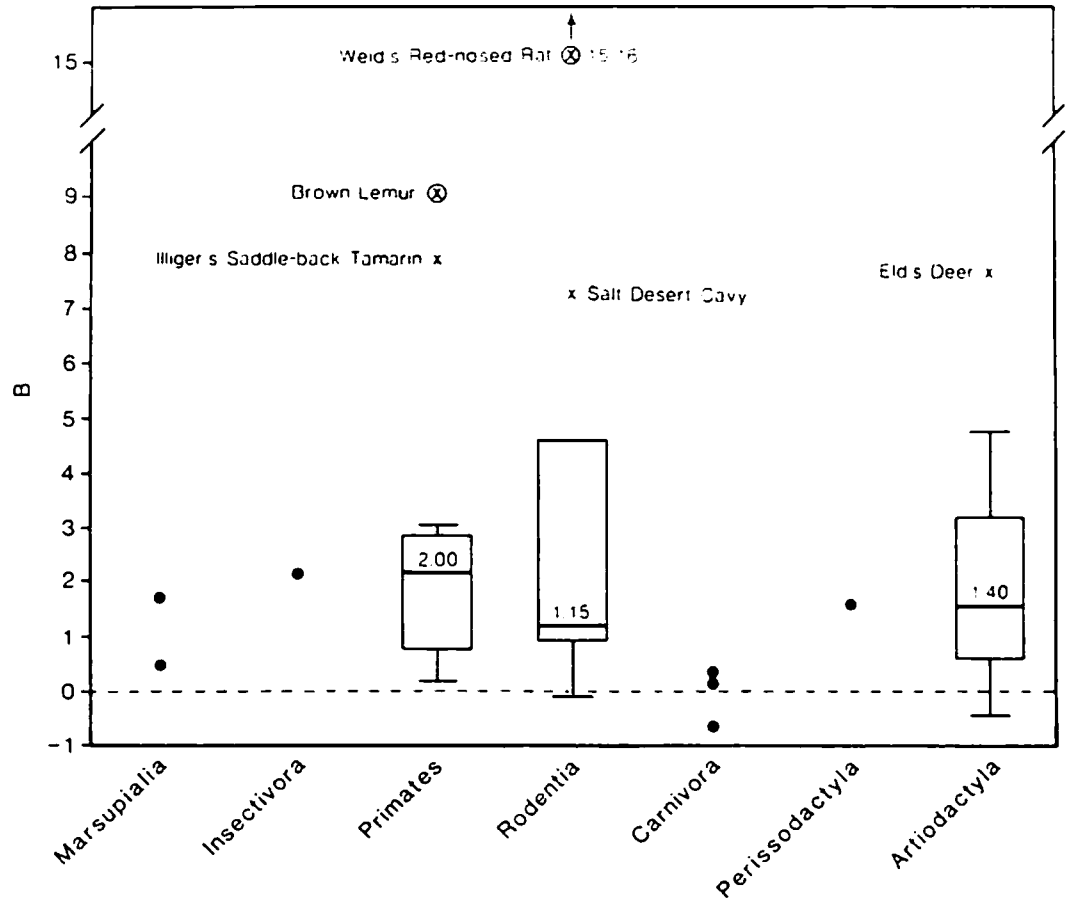

Figure 2. Box plots of B across 40 mammalian populations by order. Median effects (middle horizontal line in box), upper and lower quartiles (upper and lower ends of boxes), upper and lower inner fences (horizontal lines), outlying values (x), and values beyond the outer fences (⊗) are shown for the distribution of B in primates, rodents, and artiodactyls. Results for individual populations in other orders are shown by solid dots.

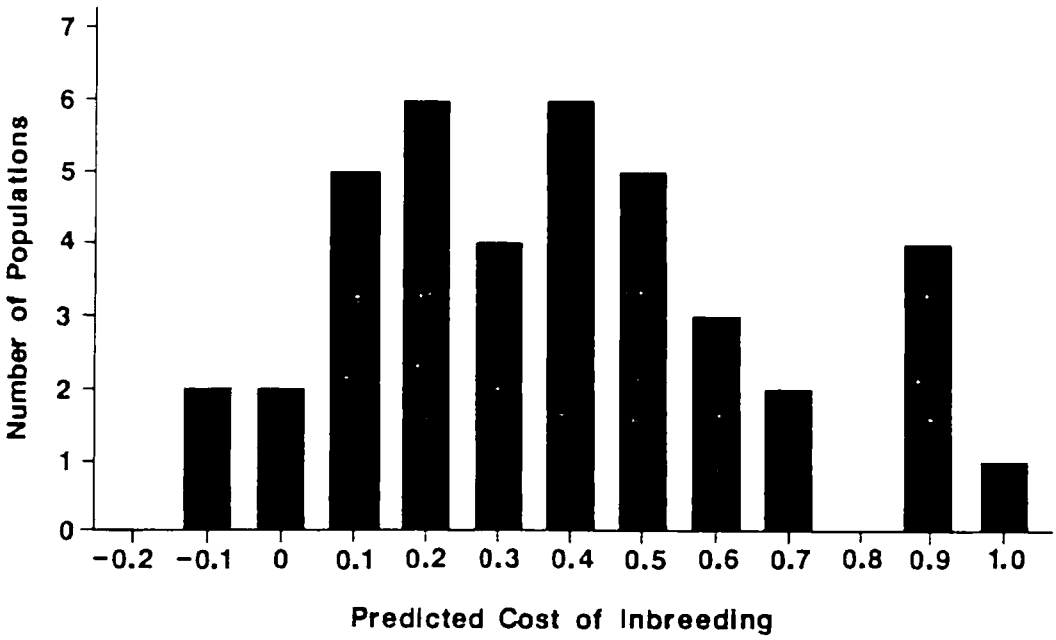

Figure 3. Distribution of the predicted cost of inbreeding in matings resulting in young with an inbreeding coefficient of 0.25 (i.e., matings between parents and offspring or full siblings) for 40 mammal populations.

timate is slightly lower than the average .33 cost of inbreeding found in our mammal populations.

The total costs of inbreeding in natural populations are probably considerably higher than our estimates. First, our estimate of the cost based on only one component of fitness (survival of young) is probably low. We were unable to count early embryonic deaths, exclude individuals with inbred ancestors, and follow individuals until the age of reproductive maturity. Furthermore, mortality rates of inbred young may be higher in natural populations, because many weak young that might die in the wild survive in captivity with the assistance of veterinary care. Second, there are likely to be additional costs of inbreeding in other components of fitness, such as litter size in species that normally bear multiple young and a reduction in fecundity of the inbred young that do survive to reproductive age (Wright 1977). (The reported higher recruitment rate of inbred young in the great tit (van Noordwijk & Scharloo 1981) is not supported by the data (Greenwood & Harvey 1982).) Third, inbred individuals with low levels of heterozygosity may be highly susceptible to viral epidemics (O'Brien et al. 1985).

Considering only the cost of inbreeding relative to the gain in inclusive fitness due to inbreeding, theory suggests that females should not mate with their fathers or sons unless the cost of inbreeding is less than .33 (Smith 1979). Although this is a highly oversimplified model, our data suggest that the cost of inbreeding in mammals is usually high enough (mean = .33) that females should not mate with their closest relatives. The limited data on the frequency of such matings in natural populations of mammals agree with this prediction. Estimates based on observations of identifiable individuals during long-term field studies range from zero to 2% in 9 of 14 well-studied mammalian populations, and the highest documented frequency is 5.5% (Ralls, Harvey & Lyles 1986).

Estimates of the cost of inbreeding also have important applications to conservation biology. The effects of the accelerated rate of inbreeding in small populations, in both captivity and the wild, can potentially drive a population towards extinction (Gilpin & Soulé 1986). The susceptibility of most small populations of conservation interest to elevated levels of inbreeding is unknown, and predicting the degree to which mortality may be increased as a result of inbreeding is impossible. The results presented here provide estimates of the general relationship between the rates of inbreeding and juvenile mortality in a large variety of captive mammal populations and will be useful in developing conservation management programs for small populations (Ballou, in press). Unfortunately, however, our estimates of the cost of inbreeding for individual populations varied greatly and were not clustered near the mean value. Thus, the severity of inbreeding effects in any unstudied mammalian population is quite likely to differ from that predicted by models based on average values.

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DRAFT

Cryopreservation and Banking of Animal Germ Plasm for Species Conservation: An Imperative for Action by the Captive Breeding Specialist Group

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SUMMARY

Conservation efforts for rare animal species currently focus on programs to protect populations in natural habitat (*in situ*) and in captivity (*ex situ*). The ultimate aim of both approaches is to maximize both global biodiversity and genetic diversity. The systematic cryopreservation and banking of germ plasm from free-living and captive populations provide new opportunities to control and manage bio- and genetic diversity. Despite the widely acknowledged benefits of this approach, the development of genetic resource banking programs is hampered by the lack of a mechanism to integrate this activity with other conservation activities.

We propose that the CBSG act immediately to provide leadership for international coordination. The CBSG should assume responsibility for developing programs that encourage germ plasm banking as an integral component of *in situ* and *ex situ* conservation efforts. Specifically, the CBSG should: 1) draft and seek adoption of an IUCN Position Statement on the role of germ plasm banking in management and research programs to conserve endangered species; 2) establish a Genetic Resource Banking Oversight Committee to formulate global guidelines for the establishment, operation and review of animal genetic resource banking programs; and 3) develop a formal process that would assist the development of Genetic Resource Banking Action Plans. It is likely that extensive regional and international planning is required to establish and operate such banking programs and ensure the ultimate utility of the banked materials.

INTRODUCTION

Increasing numbers of species face extinction in their native habitat usually as a result of the direct or indirect actions of man. The survival of a species in the wild is thought to depend on a secure native habitat that is sufficiently large to support a population meeting certain genetic and demographic requirements (Soule 1987). Most of the important requirements are related to the properties and characteristics of the population as a whole, such as its size, life-history characteristics and the nature of the gene pool contained therein. The latter, especially genetic variations (i.e. polymorphism) within populations or communities of individuals, plays an important role in many of the critical biological processes related to species conservation, including

extinction (Ehrlich and Ehrlich 1981), inbreeding depression (Ralls et al. 1988), speciation (Templeton 1989) and natural selection (Frankel and Soule 1981). The loss of biological resources as embodied in species resulting from aeons of evolutionary adaptations is recognized as a major international concern. For this reason, it is generally recognized that every possible avenue should be taken to conserve bio- and genetic-diversity (Wilson 1988).

Conservation efforts consist of both: 1) '*in situ*' conservation programs that protect and manage animal populations within their natural, native habitat; and 2) '*ex situ*' conservation programs that remove individuals, gametes or embryos from wild populations for controlled breeding and management in captivity. Although habitat protection is acknowledged to be the most efficient approach for conserving bio-and genetic-diversity, for some species, *in situ* conservation alone can not be relied upon to ensure the long-term viability of species at risk (Conway 1988, Soule 1991). Continued human population growth and the biopolitical, environmental and social consequences of that growth require *ex situ* approaches as critical components of integrated conservation (McNeely et al. 1990).

Currently, *ex situ* efforts for animal species at risk of extinction focus on captive propagation (Soule et al. 1986, Foose et al. 1986). The immediate goal of such programs is to manage populations of a species so as to retain maximum genetic diversity. Ultimately, such captive populations would serve as a source of individuals for release into restored habitat or to infuse genetic diversity into inbred, free-living populations. This can be accomplished only if a significant fraction of the overall genetic diversity existent in the wild population is incorporated into and retained by the captive population. Most captive breeding programs seek to maintain 90% of the captive population's initial genetic diversity for 200 years (Ballou 1991), as recommended by Soule et al (1986). Unfortunately, the world's zoos and bioparks do not have sufficient capacity to house the numbers of animals needed to meet the habitat crisis facing wild animals. For example, estimates suggest that space currently is available in North America for only about 100 mammalian species in populations large enough to meet the required genetic and demographic goals (Conway, 1987). This compares to the 815 mammalian species estimated by Soule et al. (1986) that would require captive propagation programs during the next 200 years.

UTILITY OF GENETIC RESOURCE BANKS FOR IN SITU AND EX SITU CONSERVATION PROGRAMS

The efficiency and efficacy of captive breeding can be increased many-fold by applying recent advances in reproductive biotechniques (Wildt 1989, 1991). Perhaps the most important advance is germ

plasm cryopreservation or the low-temperature storage and banking of spermatozoa, embryos and oocytes. Germ plasm cryopreservation currently plays an important role in domestic livestock agriculture, especially in the international movement of disease-free, genetically-superior individuals. The development of banks of cryopreserved germ plasm for nondomesticated species offers many important advantages for conserving and managing the genetic diversity within existing populations. Specifically, an animal genetic resource bank:

1. Reduces the number of animals that must be maintained in captivity by extending the generation interval of a species indefinitely. Thus, the genetic diversity of a founder does not die with the animal, but remains viable and available for use in future generations.

2. Provides a high degree of security against the loss of diversity or entire species from epidemics, natural disasters and social/political upheavals.

3. Serves a vital, interactive role between in situ and ex situ conservation programs. Such interactions prevent unintended selection pressures in captivity, preserve new diversity resulting from natural evolutionary processes in free-living populations, and permit 'infusions' of genetic diversity into fragmented populations suffering from genetic drift or inbreeding depression. This strategy also eliminates the need to remove additional animals from the wild or introduce captive animals into wild free-living populations.

4. Provides a method for improving food production and the economy of local communities by inter-species hybridization with domesticated species (e.g. hybridization of rare species of cattle with domesticated breeds).

5. Allows ready access to systematic collections of rare biological specimens for research in conservation biology or other 'life' sciences.

The importance of germ plasm resource banks for conserving the genetic diversity of wild fauna has been recognized since the first reports of successful cryopreservation of spermatozoa (Polge et al. 1949) and mammalian embryos (Whittingham et al. 1972). Over the past two decades, reports of various public- and privately-sponsored task forces have stressed the need for germ plasm repository programs to be established for conservation purposes. These include:

1. Conservation of Germplasm Resources: An Imperative. National Research Council, National Academy of Sciences, Washington DC, USA, 1978.
2. Animal Genetic Resources: Conservation and Management.

Proceedings of the FAO/UNEP Technical Consultation, FAO Animal Production and Health Paper No. 24, Rome Italy, 1981.
3. Animal Germplasm Preservation and Utilization in Agriculture. Council for Agricultural Science and Technology, Report No. 101, September 1984, Ames, Iowa, USA.
4. U.S. Strategy on the Conservation of Biological Diversity. Interagency Task Force Report, U.S. Agency for International Development, Washington DC, USA, 1985.
5. Technologies to Maintain Biological Diversity. U.S. Congress, Office of Technology Assessment, Report OTA-F-330, U.S. Government Printing Office, Washington DC, USA, 1987.
6. Research Priorities for Single Species Conservation Biology. A workshop sponsored by the U.S. National Science Foundation, Washington, DC, 1989.

STATEMENT OF THE PROBLEM

Despite all the publicity directed at the issues of declining habitat, species extinction, loss of genetic diversity and the potential contributions of germ plasm banking, it is remarkable that no organized programs exist to sample, evaluate, cryopreserve, maintain and use germ plasm from wild animal species. Furthermore, there are no guidelines for establishing such germ plasm banking programs or integrating them with other conservation programs. As yet, no single organization with a role in the international coordination of conservation efforts has provided guidance or oversight.

There are several organizational and procedural matters that must be addressed before the full potential of genetic resource banks can be realized for international conservation purposes. We propose that the CBSG immediately provide a leadership role to remedy the lack of international oversight and coordination. The CBSG should assume responsibility for developing programs that encourage germ plasm banking as an integral component of in situ and ex situ conservation efforts. Specifically, the CBSG should: 1) draft and seek adoption of an IUCN Position Statement on the role of germ plasm banking in management and research programs to conserve endangered species; 2) establish a Genetic Resource Banking Oversight Committee to formulate global guidelines for the establishment, operation and review of animal genetic resource banking programs; and 3) develop a formal process that would assist the development of Genetic Resource Banking Action Plans. Other important elements of these overall activities include the coordination of activities within the Species Survival Commission to identify species conservation programs that would benefit from germ plasm banking, and assisting efforts to secure sources of funding for international germ plasm banking activities. Discussion of each of these critical needs follows.

ENCOURAGE INTERNATIONAL GERM PLASM BANKING ACTIVITIES

Germ plasm banking activities can best be encouraged by education programs to inform the public, conservation managers and conservation researchers of the benefits resulting from the systematic banking of genetic resources. Examples of current applications and the conservation and research benefits of germ plasm banking can be drawn from type-culture collections of microorganisms and cell cultures (Colwell 1976, Edwards 1988), the commercial cattle breeding industry (Seidel, G.E. 1990) and banks of embryos from genetically-defined strains of laboratory rodents (Mobraaten 1981).

Ongoing international programs for the ex situ conservation of plant genetic resources provide a useful model (Cohen et al. 1991). Efforts for developing collections of crop germ plasm are well advanced. International coordination of crop germ plasm conservation is provided by the International Board for Plant Genetic Resources (IBPGR) and the Consultative Group on International Agricultural Research (CGIAR). At present, 14 major agricultural research centers have been established in developing regions, each developing base collections of germ plasm for the major food crops. Funding for these activities is approximately US$300 million per year. Comparable efforts for domestic animal species are modest. Currently there is no 'International Board of Animal Genetic Resources' to coordinate international efforts to conserve agriculturally-important sources of animal germ plasm. However, the Food and Agriculture Organization (FAO) of the United Nations has established an initiative to establish germ plasm banks in developing regions. Coordination of FAO and wild animal conservation and germ plasm banking activities would be best provided through the CBSG.

IUCN POSITION STATEMENT ON ANIMAL GENETIC RESOURCE BANKING

One method of highlighting the potential benefits of active genetic resource banking programs is to seek an official position statement by the IUCN. The statement should be drafted jointly by the CBSG and the Chairman of the Species Survival Commission (SSC) of the IUCN. Information and review of the statement should be solicited from other SSC Specialist Groups prior to submission to the IUCN for approval. We suggest that the statement emphasize the importance of coordinated in situ and ex situ conservation programs for endangered species. The role of germ plasm banking in preserving important sources of genetic diversity and in providing a means for moving genetic diversity between captive and free-living populations should be stated. The CBSG should be designated to be responsible for oversight of germ plasm banking activities within the Species Survival Commission. Finally, the CBSG should be directed to coordinate and review international aspects of banking programs for nondomesticated animal species.

FORMULATE GLOBAL GUIDELINES FOR THE ESTABLISHMENT, OPERATION AND REVIEW OF ANIMAL GENETIC RESOURCE BANKS

A key factor to ensuring the success of animal genetic resource banks (GRBs) is to ensure that they are established using rigorous scientific criteria and state-of-the-art technology. Because limited resources are available, difficult choices will need to be made on which species can derive the maximum benefit from this approach. At present no guidelines exist to assist in formulating action plans for establishing and operating a genetic resource bank.

To assist the CBSG in developing such guidelines, we suggest the following sequence as a first attempt to address many of the important issues. This working plan was modified from one suggested recently by one of us (Rall 1992).

GENETIC RESOURCE BANKING OVERSIGHT COMMITTEE

STEP 1. The first step in establishing integrated GRBs is to establish a GRB Oversight Committee under the auspices of the CBSG. This committee should be composed of 8 to 15 members. The composition must include one or more experts from each of the following areas: 1) cryobiologist; 2) reproductive physiologist; 3) population biologist; 4) geneticist; 5) veterinarian; 6) *in situ* conservation biologist; 7) *ex situ* conservation manager; and 8) the chairmen of regional cryopreservation task force committees. Furthermore, the chairmen (or their representative) of all SSC specialist groups should serve as *ad hoc* members.

STEP 2. The second step is to define the responsibilities of the committee and formulate a formal process for establishing GRBs. We propose five basic missions for the GRB Oversight Committee:

1. Coordinate GRB activities within the SSC and regional propagation groups. The GRB Oversight Committee would assist SSC taxon Specialist Groups, regional taxon advisory and captive propagation groups achieve their goals of conserving rare species. This can be accomplished by integrating the consideration of GRBs directly into the framework of strategic planning processes of population viability assessment and conservation action plan (PVA/CAP) workshops. These activities require that an expert resource network be established to provide advise on all technical matters related to GRBs and their utility.

2. Establish guidelines for identifying candidate taxa, species or populations that would benefit from a GRB program. These guidelines should be detailed and assist in the development of strategic GRB Action Plans for conserving specific animal populations. The single most important consideration is to ensure that there is a defined conservation goal that requires the collection and storage

of biological materials. This requires that a integrated plan for a goal-oriented conservation program be established prior to initiating banking activities. We list three scenarios below to illustrate our proposed process.

3. Provide expert technical assistance to the appropriate taxon groups to assist in the development of GRB Action Plans. This would include identifying institutions with an interest in providing long-term repository storage space or local/regional assistance in collecting and preserving material. Furthermore, the GRB Oversight Committee would work with the CBSG, the SSC Financial Development Officer and other interested organization to identify sources for supporting international GRB activities. Proposals for funding might be submitted individually or jointly with these and other organizations to private foundations, national research granting agencies and multinational organizations.

4. Provide a mechanism for the review of proposed GRB Action Plans. Plans that meet recommended requirements should be approved formally by the CBSG. (Formal 'sanction' may assist in the securing of external funding.)

5. Develop a periodic review process for individual GRB programs. This would be best accomplished by shared responsibility with the appropriate regional GRB Task Force Committee. For example, the annual reports of individual GRBs could be presented by the chair of the appropriate regional GRB Task force for review of recent progress, problems and future directions of banking activities.

THREE SCENARIOS OF APPROPRIATE GENETIC RESOURCE BANKING PROGRAMS

Scenario 1. An ongoing captive propagation program seeks to increase safety and management options for maintaining genetic diversity in a population, and achieve the same goals with fewer animals. We propose that such a population would be a candidate for a GRB program if the following minimum requirements are met:

a. Populations in captivity and/or the wild must be potentially viable by demographic and genetic criteria. This information is best obtained from a recent population viability assessment (PVA).

b. Ongoing captive propagation (e.g. SSP, EEP), studbook and conservation research programs have been established for the candidate animal population(s).

c. The current level of success of captive breeding must be sufficient to provide reasonable assurance that GRB-associated reproductive biotechniques will be successful.

d. Animals with known genetic backgrounds should be available to serve as founders of a GRB.

e. Sufficient numbers of 'surplus' females and males must be available to act as recipients to demonstrate the viability of cryopreserved germ plasm and serve as a source of material for

research and protocol development.

f. The effects of potential restrictions on the importation and exportation of animals and animal products must be evaluated.

g. And other factors as appropriate for the specific candidate species or population.

Scenario 2. An animal population has declined to low numbers (<100) and is expected to recover slowly. The population is expected to lose heterozygosity rapidly (>0.5% per generation and be subjected to genetic drift. A propagation/management plan has been initiated with the goals of protecting current levels of genetic diversity, preventing the loss of diversity in specific elderly founders and increasing the size of the population. We propose that such a population would be a candidate for an emergency GRB program if the following minimum requirements are met:

a. The populations must be potentially viable by demographic and genetic criteria. This information is best obtained from a recent population viability assessment (PVA).

b. There is a reasonable expectation that captive propagation will be successful. For example, a taxonomically-related species or subspecies has been successfully bred in captivity.

c. There is a reasonable expectation that GRB-associated reproductive techniques (e.g. germ plasm collection and cryopreservation, artificial insemination, embryo transfer) will be successful. For example, these procedures have been successfully applied in a taxonomically-related species or subspecies.

d. And other factors as appropriate for the specific candidate species or population.

Scenario 3. A free-living population has declined rapidly and satisfies the 'critical' or 'endangered' categories of the Mace-Lande criteria for threatened taxa (Mace and Lande 1991). Factors leading to the decline have been identified and a management plan has been initiated to maintain the population at low numbers (<2000) for many generations (5 to 20) before an increase in population size is expected. The population remains at risk to a further rapid decline that may reduce genetic diversity to unacceptable levels. One management goal is to develop a secure ex situ program to provide a reinfusion of genetic diversity in the event of a future decline. We propose that such a population would be a candidate for a GRB program if the following minimum requirements are met:

a. There is a reasonable expectation that GRB-associated reproductive techniques (e.g. germ plasm collection and cryopreservation, artificial insemination, embryo transfer) will be successful. For example, these procedures have been successfully applied in a taxonomically-related species or subspecies.

b. Animals with known or identifiable genetic backgrounds should be available to serve as founders of a GRB.

c. Sufficient numbers of 'surplus' females and males must be available to act as recipients to demonstrate the viability of cryopreserved germ plasm and serve as a source of material for

research and protocol development.

d. And other factors as appropriate for the specific candidate species or population.

DEVELOPMENT OF ACTION PLANS FOR GENETIC RESOURCE BANKS

STEP 3. The primary responsibility for developing GRB Action Plans properly resides with those groups with specific responsibilities for *in situ* and *ex situ* conservation of specific taxa, species and populations (e.g. taxon Specialist Groups, Taxon Advisory Groups and regional captive propagation groups). These groups should be encouraged to include the development of GRBs as an integral component in their strategic conservation planning (e.g. Captive Action Plans, Taxon Action Plans). The first step in the process occurs when a group identifies a specific conservation goal for a taxon, species or population that requires the collection and storage of biological materials. The needs and characteristics of the candidate animal population(s) would be evaluated in terms of the requirements listed in the appropriate scenario listed above. If analysis of these factors suggest that conservation efforts would be enhanced or ensured by a GRB program, the group would petition the CBSG of their intent to develop such an action plan.

The GRB Oversight Committee would review the petition and, if approved, would assist the conservation group in organizing a working session meeting to further evaluate the conservation needs and develop a detailed action plan. The role of the Oversight Committee would be to identify technical experts who can assist in this effort. The specific goals of the meeting would be to:

1. Assemble and evaluate available information on the life-, reproductive- and genetic histories of *ex situ* and *in situ* populations of interest. Much of this information would be available for recent propagation/management (e.g. SSP, TAG) and PVA materials.

2. Evaluate the efficiency and efficacy of reproductive technologies for the candidate species, such as artificial insemination, embryo transfer, *in vitro* fertilization, gamete and embryo cryopreservation and collection of spermatozoa, oocytes and embryos. Areas requiring further research or development would be identified.

3. Identify the types of biological material requiring storage. It should be noted that a wide variety of different biological materials might be cryopreserved and stored depending on the goals and needs of the conservation program (see Table 1).

4. Specify the appropriate protocols for banking activities. These include:

a. The criteria used to select material(s) for accession, determine the quantity of material from each donor and identify

appropriate uses of the material.

b. Procedures for collection, processing, cryopreservation, shipping, thawing and other treatments. The minimum quality control standards for each process and overall viability would be identified.

c. The appropriate repository equipment, facilities, security and management systems that ensure the ultimate utility of the banked materials would be identified.

d. If any of the above items are unknown, specific areas requiring further research should be identified.

5. Determine the location of the primary repository for storage of cryopreserved materials and secondary backup sites.

6. Develop strategies for the use of banked materials in breeding and conservation research programs.

7. Identify sources of funding for the GRB Action Plan.

If analysis of the these factors indicates that a GRB program would benefit conservation, the petitioning organization would prepare a written Action Plan for developing a GRB program.

REVIEW AND APPROVAL OF PROPOSED ACTION PLAN FOR A GENETIC RESOURCE BANK

STEP 4. Identifying the appropriate authority for reviewing GRB Action Plans is complicated by the overlapping purviews of national, regional and international organizations and their animal propagation/management programs. We suggest that the proposed GRB Oversight Committee is the most appropriate organization because of the very nature and responsibilities of the CBSG. First, by definition, genetic resource banking programs represent a form of *ex situ* captive propagation. Second, GRB activities are international in that technical experts and populations of most rare species are located on several continents. Third, GRB programs require integration with other *in situ* and *ex situ* conservation programs. However, in many cases, regional cryopreservation task force committees will play an important role in regional coordination and development of these programs. In those cases, we propose that the GRB Action Plan be reviewed by both the regional banking authority *and* CBSG GRB Oversight Committee. After approval, the plan would be implemented and collection, storage and use of biological materials can begin.

CONCLUSION

The development of animal Genetic Resource Banks offers unique opportunities to control and manipulate the effects of time in the management and conservation of rare species. The ideas proposed

here are intended to help stimulate discussion about the process on a formal basis. Many important questions remain to be resolved, including the translation of banking germ plasm into live offspring. However, many recent reports of successes using artificial breeding techniques indicate the potential of reproductive biotechnology. The further development of strategies proposed here will ensure that GRBs are not merely an interesting idea or static warehouses of biological materials but facilitators for conservation.

Table 1. Biological Materials for Germ Plasm Banking and Conservation Research.

Material Type	Long-term Storage Conditions	Examples of Potential Uses
Sperm, oocytes	below -130°C[a]	Controlled breeding; international shipment; gene banking
Embryos	below -130°C	Control of generation interval and gene flow; population amplification; international shipment
Cell lines	below -130°C	Genetic and physiological research
DNA		Molecular biology:
-Isolated	dried, 4°C[b]	Sequence detection and identification (e.g. by PCR)
-Isolated and frozen tissues	below -60°C[c]	Pedigree determination; genomic and mitochondrial libraries
Serum, plasma	below -60°C	Disease status (detection of microbial antibodies and disease organisms); endocrine status (measure hormones or hormonal metabolites)
Urine, milk	below -60°C	Endocrine and health status (measure hormonal and other metabolites)

[a]Liquid nitrogen refrigerator.
[b]Refrigerator or cold room.
[c]Low-temperature mechanical refrigerator.
[from Rall 1992, with modifications]

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PROPOSED
IUCN RESOLUTION STATEMENT ON ANIMAL GENETIC RESOURCE BANKING FOR SPECIES CONSERVATION

Captive Breeding Specialist Group Annual Meeting
Singapore, September 29, 1991

PROBLEM STATEMENT

The IUCN holds that the successful conservation of species requires integrated management efforts to sustain available genetic diversity. These efforts include programs to protect and manage animal populations within their natural, native habitat (in situ conservation) and supporting programs that manage individuals, gametes and/or embryos outside of natural environments (ex situ conservation).

The IUCN recognizes that, although habitat protection is the most desirable approach for conserving biological diversity, supportive ex situ programs are essential in many cases. For example, such programs can deal effectively with short-term crises and with maintaining long-term potential for continuing evolution.

The IUCN further recognizes that the efficiency and efficacy of ex situ conservation can be increased many fold by applying recent advances in reproductive technology. These include assisted or 'artificial' breeding and the low temperature storage (banking) of viable animal germ plasm, namely spermatozoa, embryos and oocytes. Germ plasm banks: 1) offer a high degree of security against the loss of diversity and, therefore, entire species from unforeseen catastrophes; 2) minimize depression effects of genetic drift and inbreeding; and 3) provide a powerful method for managing the exchange of genetic diversity among populations. Other conservation benefits include banks of serum, DNA and cultured cell lines from germ plasm donors which permit studies on disease status, detection of microbial antibodies, pedigree determination, taxonomic status, geographical substructure and cellular physiology.

The IUCN also recognizes that the establishment of a genetic resource bank must, through basic research, be matched by the development of technologies for its use as a genuine and practical conservation asset.

The development of genetic resource banking programs is hampered by the lack of guidelines for establishing such banks and for integrating them with overall conservation programs. As yet, no single organization with a role in the international coordination of conservation efforts has provided guidance.

RECOMMENDATION

The IUCN regards development of genetic resource banks as an essential component of integrated conservation programs. Therefore, the Captive Breeding Specialist Group recommends that a formal process be developed to formulate global guidelines to establish, operate, use and review animal genetic resource banking programs for species at risk. The framework for international coordination of this type of program must be based upon agreements to cooperatively manage such species for demographic security and genetic diversity.

To achieve this recommendation, a Coordination Committee under the auspices of the Captive Breeding Specialist Group and others to be identified will:

a) Coordinate animal genetic resource banking activities within the Species Survival Commission and among regional captive propagation groups. This will be accomplished by integrating the genetic resource banks directly into the framework of population viability assessments and conservation Action Plans. These activities require an expert resource network to provide advice on all technical matters.

b) Establish guidelines for identifying taxa, species or populations that would benefit from genetic resource banks. These guidelines should be detailed and assist in the development of strategic Action Plans for conserving targeted animal populations. The single most important consideration is to ensure that there is a defined conservation goal that requires the collection and storage of biological materials. This requires that an integrated plan for a goal-orientated conservation program be established prior to initiating banking activities.

c) Establish a globally-standardized, record-keeping database for cataloging, managing and pooling data on banked materials. It will be essential that these biological materials are linked to individually identifiable source animals.

d) Provide expert technical advice to the appropriate taxon groups to assist in developing animal genetic resource Action Plans. The primary responsibility for developing Action Plans resides with those groups with specific responsibilities for in situ and ex situ conservation of specific taxa, species and populations. These groups should be encouraged by the Coordination Committee to include genetic resource banks as an integral component in their strategic conservation planning. The Coordination Committee will support the appropriate taxon groups to integrate information on: reproductive and genetic histories of ex situ and in situ populations; efficiency of reproductive technologies; areas requiring further research; types of biological materials requiring storage; appropriate protocols for banking biological materials; primary and secondary repository sites; strategies for using banked materials; and sources of funding.

e) Provide a mechanism for approval and periodic review of animal genetic resource banking Action Plans.

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SCIENCE

Translocation as a Species Conservation Tool: Status and Strategy

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Translocation as a Species Conservation Tool: Status and Strategy

BRAD GRIFFITH, J. MICHAEL SCOTT, JAMES W. CARPENTER, CHRISTINE REED

Surveys of recent (1973 to 1986) intentional releases of native birds and mammals to the wild in Australia, Canada, Hawaii, New Zealand, and the United States were conducted to document current activities, identify factors associated with success, and suggest guidelines for enhancing future work. Nearly 700 translocations were conducted each year. Native game species constituted 90 percent of translocations and were more successful (86 percent) than were translocations of threatened, endangered, or sensitive species (46 percent). Knowledge of habitat quality, location of release area within the species range, number of animals released, program length, and reproductive traits allowed correct classification of 81 percent of observed translocations as successful or not.

A TRANSLOCATION IS THE INTENTIONAL RELEASE OF animals to the wild in an attempt to establish, reestablish, or augment a population (*1*) and may consist of more than one release. To date, translocations have been used to establish populations of nonnative species and restore native species extirpated by hunting. An increasing perception of the value of biological diversity has focused attention on translocations of rare native species. These latter translocations are expensive (*2, 3*) and are subject to intense public scrutiny (*4*). They have varied goals (*3*) that include bolstering genetic heterogeneity of small populations (*5–7*), establishing satellite populations to reduce the risk of species loss due to catastrophes (*8, 9*), and speeding recovery of species after their habitats have been restored or recovered from the negative effects of environmental toxicants (*2*) or other limiting factors.

In the face of increasing species extinction rates (*10–12*) and impending reduction in overall biological diversity (*12*), translocation of rare species may become an increasingly important conservation technique. If current patterns of habitat loss continue, natural communities may become restricted to disjunct habitat fragments and intervening development may disrupt dispersal and interchange mechanisms (*2*). Increased rates of extinction may be expected in small fragmented habitats (*13*) and translocation may be required to maintain community composition, especially for species with limited dispersal abilities.

The immediacy of reduction in biodiversity (*14*) demands a rigorous analysis of translocation methodology, results, and strategy. We need to know how well it works, what factors are associated with success, and what strategies suggest greatest potential success.

We conducted three surveys of contemporary (1973 to 1986) translocations of native birds and mammals in Australia, Canada, Hawaii, New Zealand, and the United States (*15*). In the first survey, we obtained general information on the number of programs completed by various organizations. In the later surveys, we sought detailed information on translocations of (i) threatened, endangered, or sensitive species and (ii) native game birds and mammals.

Current Status

At least 93 species of native birds and mammals were translocated between 1973, the year the Endangered Species Act became law, and 1986. Most (90%) translocations were of game species; threatened, endangered, or sensitive species accounted for 7%. Ungulates (39%), gallinaceous birds (43%), and waterfowl (12%) dominated translocations of game species; raptors (28%) and marsupials (22%) dominated threatened, endangered, or sensitive species translocations.

A typical translocation consisted of six releases over the course of 3 years. Many (46%) released 30 or fewer animals and most (72%) released 75 or fewer animals.

The average number of translocations per reporting organization doubled from 1974 (5.5) to 1981 (10.6) suggesting contemporary totals of 700 translocations per year. Most (98%) of these were conducted in the United States and Canada. Effort was not uniformly distributed; 21% of North American agencies conducted 71% of North American translocations. Only 27% of reporting organizations had protocols that specified the types of information to be recorded during translocation programs.

Theoretical Considerations

A translocation is a success if it results in a self-sustaining population; conversely, the founder group may become extinct. Theoretical considerations predict that population persistence is more likely when the number of founders is large, the rate of population increase is high, and the effect of competition is low (*13*). Low variance in rate of increase (*16*), presence of refugia (*9*), reduced environmental variation (*16*), herbivorous food habits (*17*), and high genetic diversity among founders (*18*) may also enhance persistence. Suitable, protected, and maintained habitat, control of limiting factors, and proper care and training of captive reared

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animals (*3*, *19*) are also considered prerequisites of a successful translocation.

We found that several factors were associated with success of translocations (Table 1). Native game species were more likely to be successfully translocated than were threatened, endangered, or sensitive species. Increased habitat quality was associated with greater success. Translocations into the core of species historical ranges were more successful than were those on the periphery of or outside historical ranges. Herbivores were more likely to be successfully translocated than either carnivores or omnivores. Translocations into areas with potential competitors of similar life form were less successful than translocations into areas without competitors or areas with a congeneric potential competitor. Early breeders with large clutches were slightly more likely to be successfully translocated than were species that bred late and had small clutches.

Translocations of exclusively wild-caught animals were more likely to succeed than were those of exclusively captive-reared animals (Table 1). Among translocations of exclusively wild-caught animals, success depended ($P \leq 0.10$) on whether the source population density was high (77% success, $n = 109$), medium (78%, $n = 37$), or low (37%, $n = 8$). Success of translocations of wild-caught animals was also associated ($P \leq 0.10$) with whether the source population was increasing (83% success, $n = 93$), stable (63%, $n = 49$), or declining (44%, $n = 9$). Successful translocations released more animals than unsuccessful translocations (160 compared to 54, respectively; $P = 0.024$).

Our results are consistent with analyses of naturally invading or colonizing species that show (i) larger founder populations are more successful (*20*, *21*), (ii) that habitat suitability is important (*21*), and (iii) increased number and size of clutches enhances successful invasion (*22*). Our data also support the hypothesis that herbivores are more successful invaders than carnivores (*17*) and the conclusion that, for birds, morphologically similar species have a greater depressing effect on successful invasion than do congeneric species (*23*).

We found no consistent association of translocation success with number of releases, habitat improvement, whether the release was hard (no food and shelter provided on site) or soft, immediate or delayed release on site, or average physical condition of animals at release. We were unable to directly evaluate genetic heterogeneity, sex and age composition, or specific rearing and handling procedures for released animals because of inadequate response to survey questions.

Table 1. Percentage success of intentional introductions or reintroductions (translocations) of native birds and mammals to the wild in Australia, Canada, Hawaii, New Zealand, and the United States between 1973 and 1986. Data were obtained from a survey conducted in 1987 (*15*). The data include 134 translocations of birds and 64 translocations of mammals. For all variables listed, χ^2 was statistically significant ($P \leq 0.10$), implying true differences in the percentages of successful translocations among the categories. Animals that first give birth at age 2 or less with average clutch size of three or more are considered early breeders with large clutches; all others are late breeders with small clutches.

Variable	Translocations (n)	Success (%)
Threatened, endangered, or sensitive species	80	44
Native game	118	86
Release area habitat		
Excellent	63	84
Good	98	69
Fair or poor	32	38
Location of release		
Core of historic range	133	76
Periphery or outside	54	48
Wild-caught	163	75
Captive-reared	34	38
Adult food habit		
Carnivore	40	48
Herbivore	145	77
Omnivore	13	38
Early breeder, large clutch	102	75
Late breeder, small clutch	96	62
Potential competitors		
Congeneric	39	72
Similar	48	52
Neither	105	75

Evaluating Alternative Strategies

Analyses of individual factors associated with translocation success do not adequately reflect the multivariate nature of actual translocations. To overcome this problem, we used stepwise logistic regression (*24*, *25*) to develop preliminary predictive equations for estimating the success of translocations (Table 2). An expanded data set or independent sample would probably yield different regression coefficients and estimates of success than we report. As a result, extrapolation to conditions much different than those represented by our data and applications to individual species are discouraged.

The coefficients from Table 2 can be used to plot predicted success of different kinds of translocations as a function of continuous variables such as the number released. We present an example for a threatened, endangered, or sensitive bird (Fig. 1).

This exercise (Fig. 1) illustrates that the increase in success associated with releasing larger numbers of organisms quickly becomes asymptotic. Releases larger than 80 to 120 birds do little to increase the chances that a translocation will be successful for this particular set of conditions. The asymptotic property is consistent across other classifications of the data but the inflection point varies. For large native game mammals the asymptote is reached at releases of 20 to 40 animals with a concurrently higher predicted success.

The asymptotic property of the association of translocation success and number released (Fig. 1) is consistent with theoretical predictions (*13*) and analytical treatments (*26*) that suggest a threshold population size below which extinction is likely, primarily due to chance events affecting birth and death of individuals. The existence of the inflection (Fig. 1) is also consistent with the prediction of a threshold density below which population social interactions and mating success are disrupted (*27*), again leading to diminished population viability.

The coefficients from Table 2 and relationships presented in Fig. 1 can be used to assess alternative strategies. Suppose 300 threatened and endangered birds are available for a translocation program and they must be released during a 3-year time frame. Further suppose that two potential translocation areas are available within the core of the species historical range. If the goal of the translocation is to establish at least one geographically disjunct population to reduce the risk of catastrophic loss of the species, how should the birds be distributed between the two potential translocation areas to minimize the probability that both translocations will fail?

If both release areas have excellent habitat quality, and the areas are independent, the answer is obvious. The birds should be divided between the areas. The coefficients from Table 2 allow us to estimate the probability that a single release of 300 birds will fail (1.0 minus probability of success) is 0.257. Two releases of 150 birds each have individual probabilities of failure of 0.312. The probability that both will fail is $0.312 \times 0.312 = 0.097$; substantial gain is achieved by splitting the birds between areas.

If we complicate the picture and say that one potential area has excellent habitat quality and the other has only good habitat quality; we see that it remains slightly advantageous to split the birds between areas. Predicted probabilities of failure are 0.312 for excellent and 0.698 for good habitat, respectively. The probability that both translocations will fail is $0.312 \times 0.698 = 0.218$ compared to 0.257 for putting all birds in a single excellent habitat quality area. In this example, slight advantage to splitting the translocated birds between areas is maintained down to a total release of 40 birds. However, with so few birds released the probability that both translocations will fail is increased to about 0.42.

The model coefficients in Table 2 may be used to evaluate other scenarios. For example, given two alternatives, should a given number of birds be released in good habitat quality in the core of the historical species range or in excellent habitat quality on the periphery or outside the historical range? Good habitat quality in the core of the range is the better choice regardless of the number of birds released. This suggests that the physiological amplitude of a species may influence local population viability.

Enhancing the Chances of Success

Without high habitat quality, translocations have low chances of success regardless of how many organisms are released or how well they are prepared for the release. Active management is required. Limiting factors must be identified and controlled and assurances of maintenance of habitat quality obtained prior to translocation.

Identification and retention of adequate habitat will require a combined species and ecosystem approach. Ecological information will be necessary to identify critical life history traits, factors determining habitat quality, species interactions, and minimum habitat fragment size (*28*). Regional approaches to maintaining diversity (*29*) will be essential to ensure that existing species and habitat assemblages are identified, their interactions are understood, and remnant habitats are protected. The latter approach may ultimately reduce the number of species that require translocation if it enhances understanding of the effects of habitat fragmentation on persistence of multiple disjunct populations.

Table 2. Stepwise logistic regression (*24*) model coefficients for predicting probability $[P = 1/(1 + e^{-x})]$ of success of intentional introductions or reintroductions (translocations) of native birds and mammals in Australia, Canada, Hawaii, New Zealand, and the United States between 1973 and 1986; x is the sum of applicable coefficients for categorical variables plus the applicable coefficient times the value of continuous variables. The model is based on 155 translocations; 100 were of birds and 55 were of mammals. Data were obtained from a survey conducted in 1987 (*15*). The stepwise procedure was run at the $\alpha = 0.10$ level for entry of terms and the $\alpha = 0.15$ level for removal of terms. Probability of larger test statistics for the model were χ^2, $P = 0.90$ (*24*); Hosmer-Lemeshow χ^2, $P = 0.121$ (*24*); Brown's χ^2, $P = 0.537$ (*24*). The model correctly classified 81.3% of observed translocations based on a cutpoint of 0.50 in predicted probability of success.

Variable	Coefficient (SE)
	−1.418 (0.738)
Threatened, endangered, or sensitive species	−0.972 (0.253)[1]*
Native game	0.972 (0.253)[1]
Birds	−0.919 (0.374)[6]
Mammals	0.919 (0.374)[6]
Release area habitat	
Excellent	1.681 (0.438)[2]
Good	0.053 (0.314)[2]
Fair or poor	−1.734 (0.450)[2]
Release area	
Core of historic range	1.028 (0.267)[3]
Periphery or outside	−1.028 (0.267)[3]
Early breeder, large clutch	1.080 (0.355)[5]
Late breeder, large clutch	−1.080 (0.355)[5]
Log(number released)	0.887 (0.405)[7]
Program length (years)	0.181 (0.074)[4]

*Numbers in brackets represent order of entry.

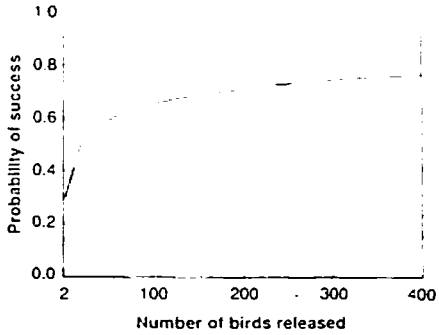

Fig. 1. Predicted probability of successful translocation as a function of the number of animals released during a 3-year period in the core of the historic species range in either excellent (solid line) or good (dashed line) habitat quality for a threatened, endangered, or sensitive bird species that first breeds at 2 years of age or more with average clutch size of three or less. Probabilities are based on stepwise logistic regression model coefficients (Table 2).

We may reduce the need for and increase the success of translocations if we can improve our ability to identify potentially tenuous situations and act before we are faced with a rescue. Simulation modeling (*28, 32*) of the behavior of small populations of species or of groups of species with similar reproductive strategies can provide guidance for establishing minimum population and vital rate goals. Simulations will be most productive if set in a regional context that addresses the interaction among metapopulations and the spatial relation among reserves or potential release sites (*28*).

The asymptotic nature of the relation between translocation success and number of animals released emphasizes the point that releasing large numbers of animals does little to increase the success of translocations. Lack of demonstrated success after translocating large numbers of animals is cause for reevaluating other variables associated with success.

The asymptotic levels do suggest that there is a minimum number of animals that should be released. Because longer translocation programs are more successful (Table 2), the minimum number may be released over several years if insufficient animals are available for a single release. Captive rearing programs that are focused on translocation should have the goal of establishing multiple self-sustaining populations so they can provide sufficient animals over a number of years and increase the success of these expensive (*2, 3*) programs.

Those planning translocations should adopt rigorous data recording procedures (*19, 30*). Details of translocation attempts should be assembled in a database. It is critical that both failures and successes be adequately documented. Permit-granting agencies may need to assume the role of ensuring that adequate records are kept so the database can be increased and predictability of success enhanced.

Because of the low success of translocations of small numbers of endangered, threatened, or sensitive species, even in excellent habitat quality, it is clear that translocation must be considered long before it becomes a last resort for these species—before density has become low and populations are in decline. Both these traits are associated with low chances of successful translocation. In addition, obtaining sufficient numbers of animals to achieve reasonable chances of success may be impossible. The greatest potential for establishing satellite populations may occur when a candidate population is expanding and numbers are moderate to high. These conditions are the ones that tend to make endangered species biologists relax; our analysis suggests that these conditions may point out the time for action.

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33. This work would not have been possible without the voluntary efforts of cooperators who responded to our survey. We thank S. I. Batten, S. Hill, and L. J. Miller for assistance in the project and T. J. Cade, S. H. Fritts, J. Hatfield, H. R. Perry, Jr., K. Ralls, D. P. Scott, and T. G. Shoemaker for comments on the manuscript. Conducted under the auspices of the Idaho Cooperative Fish and Wildlife Research Unit, which is funded and supported by Idaho Department of Fish and Game, University of Idaho, U.S. Fish and Wildlife Service, and the Wildlife Management Institute; contribution number 465 from the University of Idaho Forest, Wildlife, and Range Experiment Station.

Captive Breeding Specialist Group

Species Survival Commission
IUCN – The World Conservation Union
U. S. Seal, CBSG Chairman

TRANSPONDERS

C.B.S.G. Working Group on
Permanent Animal Identification

Report on Transponder System Testing and Product Recommendation:
A Global Standard for Zoo and Aquarium Specimens.

BACKGROUND

Transponders offer a technology for unobtrusive permanent individual animal identification applicable across nearly all vertebrates and some invertebrates. Registration of this number with ISIS provides the world's zoos with an important technique for following individual animals throughout their life span which is essential for scientific management of animal populations in captivity. These and other needs prompted the formation of an international working group, at the CBSG meeting 25-26 August 1989 in San Antonio, to assemble and evaluate information on available technologies.

This group reported back to the CBSG meeting in Copenhagen and indicated that there were several products available with apparently different capabilities, availability, and costs. A copy of the report and recommendations from the meeting was distributed in the report from the meeting and summaries were provided in the CBSG Newsletter (Vol. 1, Numbers 2 and 3). These products appeared to have different capabilities and to be incompatible requiring their own readers. The assembled participants (140 people from zoos in 24 countries) agreed that it was desirable to choose one for recommended use by the entire international zoo and aquarium community. The following recommendations were adopted and transmitted to the international zoo and aquarium community (1) that all zoos and aquariums agree to use the same type of transponder, (2) that the final choice be delayed until the working group made its final report, and (3) that the international working group compare and test the different devices and recommend a preferred choice by the end of January 1991. This recommendation and the report, presented here, would then be printed in the regional zoo Newsletters and the first 1991 issue of the CBSG Newsletter.

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REPORT

Introduction

At the CBSG meeting in Copenhagen, Denmark (15-16 September 1990), a report was presented on the applications and standardization of transponders for permanent identification of non-domestic vertebrates including mammals, birds, reptiles, amphibians and aquatic species. Previous reports had addressed issues such as central registration, medical concerns, costs, and limited read-ranges. However, indications of a changing technology, uncertainty concerning international availability, differences in standards, and a lack of consistent information available to the Working Group at the time did not allow a recommendation as to which system should be selected for use by the international zoo and aquarium community.

As a result of this report the CBSG, at the Copenhagen meeting, then recommended and urged all concerned parties to postpone their selection of a specific transponder system until the competing systems could be independently evaluated. This evaluation was to be completed and a report provided by the end of January 1991. This side-by-side evaluation, conducted by members of the Working Group, has been completed and a recommendation can now be made.

Criteria and Methods

The criteria used for evaluating the systems were:

1) Product performance (read range) under a standard set of conditions.
2) International commercial availability by January 1, 1991.
3) International distribution and availability for the tests.
4) Costs including acquisition and preparation of the transponders for use and cost of the readers.

In addition to the findings listed above, several other factors were considered by the Working Group:

5) The vast majority of specimens to receive transponder implants will be of a size in which medium (3 x 18mm) and large (3.5 x 29mm) implants would not be acceptable. Therefore, product choice should be based on the performance and price of the small size (approximately 2 x 11mm) transponders.

6) Most previous experience with transponders has been based on bulk-packed implants which were sterilized by the user, and required re-use of the implanter needle. The availability of pre-packaged, sterile transponders packaged in needles will result in easier and less traumatic use of these systems.

Product performance was evaluated by reading implants against a measured grid background. All systems were tested ten (10) times each and an average reading distance was calculated. In each instance, the transponders were placed flat on a table top oriented parallel to the reader. Although this orientation produced the shortest read-range for all systems, it most closely approximates the actual orientation of the transponder in most implanted specimens. System testing was recorded on videotape and copies are available upon request. The "original" transponder systems, manufactured by Destron/I.D.I. and distributed previously by Euro I.D., Biosonics, Biomedics, A.V.I.D., etc. have been included in the testing to demonstrate and document the improvements made in the development of the current systems.

Results

Table 1. **Results of Transponder System Tests**

Manufacturer	Manufacturer's Suggested Read-Range	Actual Read-Range
		Mean±S.E. #
	cm	cm
"Original"		
Destron/I.D.I.	5	2.6 ± .13
A.V.I.D.	5.8	5.2 ± .14
Destron/I.D.I.		
small	11.4	5.6 ± .61**
medium	29.2	12.9 ± .24
large	38.1	16.4 ± .38**
Trovan/A.E.G.	15	10.7 ± .38

** Actual Read-Range calculated from only 5 readings due to battery problems in the reader.

Statistical analysis (Repeated measures ANOVA) of the read distances for the 4 products of similar size yielded an "F"=91.3 with P<.0001. The Trovan product had a significantly greater (p<.01) read distance than the small Destron/I.D.I and the A.V.I.D. products.

Table 2. Costs of the systems.

Manufacturer	Reader	Transponder Plain	Transponder Sterile	Availability Commercial	Availability International
A.V.I.D.	$1,250.00	8.50	N/A*	Yes	No
Destron	$ 815.00++			No++	Yes
S		5.50	11.25	Yes	Yes
M		7.75	N/A	Yes	Yes
L		8.25	N/A	Yes	Yes
Trovan/ A.E.G.	$ 837.00	N/A	5.85	Yes	Yes

++ New Dual Coil Reader, which was used for this test. Other, shorter distance reading readers are available. * N/A = Not available.

Recommendations

Based upon the listed criteria and the results of the testing, the CBSG Transponder Working Group has chosen the **Trovan/A.E.G. transponder system** as the recommended system for use by the world's zoos and aquariums.

Distribution of Report

These findings and recommendations are being provided through the CBSG Newsletter to national and regional zoo associations and to more than 1200 zoos and aquariums in 147 countries. It is also being provided to other regional and international agencies and organizations (S.S.C, I.U.C.N., C.I.T.E.S., E.E.C., Trade International, U.S.F.W.S., etc.) along with the recommendation that they consider adoption of similar standards.

Additional Tentative Recommendations

1. Registration of ID numbers of zoo and aquarium specimens needs to be done in regional central databases and in ISIS for zoos and aquariums. ARKS III allows entry of these numbers and numbers for implants from other systems.
2. Standardized locations of placement of the implants in different groups of animals have been suggested but need more discussion.

3. Choice of species and priorities have focused on endangered, studbook, regional management plan, and CITES listed species. Each institution will need to develop a systematic program for their installation as animals are handled for any purpose. The use of an injectable device should simplify this process. All animals shipped from one institution to another should have them installed and the ID number registered.

Availability:

The Trovan/A.E.G. system can be purchased from:

North America

International Infopet Systems
31264 La Baya Drive, Suite A
Westlake Village, CA 91362, U.S.A.
Telephone: (818) 707-9942; 800-463-6738
Telefax: (818) 707-9947
Contact: Lindy Harton

Europe

Euro I.D.
Grossbuellesheimer Str. 56
5350 Euskirchen 16
West Germany
Telephone: (02251) 7 11 25
Telefax: (02251) 7 34 88
Contact: Joe Masin

Additional Regional distributors are being developed.

Additional Information

Copies of the previous reports and the Newsletter are available from the CBSG office.

Questions or requests for additional information should be sent to:

Dr. Evan S Blumer
Fossil Rim Wildlife Center
P. O. Drawer 329
Glen Rose, Texas 76043, U.S.A.
Telephone: (817) 897-3147
Fax: (817) 897-3785

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Fax: 03-231-00-18

KENYA BLACK RHINOCEROS

METAPOPULATION WORKSHOP

BRIEFING BOOK

SECTION 10
SOFTWARE DOCUMENTATION

VORTEX
Simulation model of stochastic population change

Written by Robert Lacy
Chicago Zoological Park
Brookfield, IL 60513
21 August 1991

STOCHASTIC SIMULATION OF POPULATION EXTINCTION

Life table analyses yield average long-term projections of population growth (or decline), but do not reveal the fluctuations in population size that would result from variability in demographic processes. When a population is small and isolated from other populations of conspecifics, these random fluctuations can lead to extinction even of populations that have, on average, positive population growth. The VORTEX program (earlier versions called SIMPOP and VORTICES) is a Monte Carlo simulation of demographic events in the history of a population. Some of the algorithms in VORTEX were taken from a simulation program, SPGPC, written in BASIC by James Grier of North Dakota State University (Grier 1980a, 1980b, Grier and Barclay 1988).

Fluctuations in population size can result from any or all of several levels of stochastic (random) effects. Demographic variation results from the probabilistic nature of birth and death processes. Thus, even if the probability of an animal reproducing or dying is always constant, we expect that the actual proportion reproducing or dying within any time interval to vary according to a binomial distribution with mean equal to the probability of the event (p) and variance given by $Vp = p * (1 - p) / N$. Demographic variation is thus intrinsic to the population and occurs in the simulation because birth and death events are determined by a random process (with appropriate probabilities).

Environmental variation (EV) is the variation in the probabilities of reproduction and mortality that occur because of changes in the environment on an annual basis (or other timescales). Thus, EV impacts all individuals in the population simultaneously -- changing the probabilities (means of the above binomial distributions) of birth and death. The sources of EV are thus extrinsic to the population itself, due to weather, predator and prey populations, parasite loads, etc.

VORTEX models population processes as discrete, sequential events, with probabilistic outcomes determined by a pseudo-random number generator. VORTEX simulates birth and death processes and the transmission of genes through the generations by generating random numbers to determine whether each animal lives or dies, whether each adult female produces broods of size 0, or 1, or 2, or 3, or 4, or 5 during each year, and which of the two alleles at a genetic locus are transmitted from each parent to each offspring. Mortality and reproduction probabilities are sex-specific. Fecundity is assumed to be independent of age (after an animal reaches reproductive age). Mortality rates are specified for each pre-reproductive age class and for reproductive-age animals. The mating system can be specified to be either monogamous or polygynous. In either case, the user can specify that only a subset of the adult male population is in the breeding pool (the remainder being excluded perhaps by social factors). Those males in the breeding pool all have equal probability of siring offspring.

Each simulation is started with a specified number of males and females of each pre-reproductive age class, and a specified number of male and females of breeding age. Each animal in the initial population is assigned two unique alleles at some hypothetical genetic locus, and the user specifies the severity of inbreeding depression (expressed in the model as a loss of viability in inbred animals). The computer program simulates and tracks the fate of each population, and outputs summary statistics on the probability of population extinction over specified time intervals, the mean time to extinction of those simulated populations that went extinct, the mean size of populations not yet extinct, and the levels of genetic variation remaining in any extant populations.

Extinction of a population (or meta-population) is defined in VORTEX as the absence of either sex. (In some earlier versions of VORTEX, extinction was defined as the absence of both sexes.) Recolonization occurs when a formerly extinct population once again has both sexes. Thus, a population would go "extinct" if all females died, and would be recolonized if a female subsequently migrated into that population of males. Populations lacking both sexes are not considered to be recolonized until at least one male and at least one female have moved in.

A population carrying capacity is imposed by a probabilistic truncation of each age class if the population size after breeding exceeds the specified carrying capacity. The program allows the user to model trends in the carrying capacity, as linear increases or decreases across a specified numbers of years.

The user also has the option of modelling density dependence in reproductive rates, i.e., one can simulate a population that responds to low density with increased (or decreased) breeding, or that decreases breeding as the population approaches the carrying capacity of the habitat. To model density-dependent reproduction, the user must enter the parameters (A, B, C, D, and E) of the following polynomial equation describing the proportion of adult females breeding as a function of population size:

$$\text{Proportion breeding} = A + BN + CN^2 + DN^3 + EN^4$$

in which N is total population size. Note that the parameter A is the proportion of adult females breeding at minimal population sizes. A positive value for B will cause increasing reproduction with increasing population sizes at the low end of the range. Parameters C, D, and E dominate the shape of the density dependence function at increasingly higher population sizes. Any of the values can be set to zero (e.g., to model density dependence as a quadratic equation, set D = E = 0). To determine the appropriate values for A through E, a user would estimate the parameters that provide the best fit of the polynomial function to an observed (or hypothetical) data set. Most good statistical packages have the capability of doing this. Although the polynomial equation above may not match a desired density dependence function (e.g., Logistic, Beverton-Holt, or Ricker functions), almost any density dependence function can be closely approximated by a 4th-order polynomial.

After specifying the proportion of adult females breeding, in the form of the polynomial, the user is prompted to input the percent of successfully breeding females that produce litter sizes of 1, 2, etc. It is important to note that with density dependence, percents of females producing each size litter are expressed as percents of those females breeding, and the user does not explicitly enter a percent of females producing no offspring in an average year. (That value is given by the polynomial.) In the absence of density dependence, the user must specify the percent of females failing to breed, and the percents producing each litter size are percents of all breeding age females (as in earlier versions of VORTEX). Read the prompts on the screen carefully as you enter data, and the distinction should become clear.

VORTEX models environmental variation simplistically (that is both the advantage and disadvantage of simulation modelling), by selecting at the beginning of each year the population age-specific birth rates, age-specific death rates, and carrying capacity from distributions with means and standard deviations specified by the user. EV in birth and death rates is simulated by sampling binomial distributions, with the standard deviations specifying the annual fluctuations in probabilities of reproduction and mortality. EV in carrying capacity is modelled by sampling a normal distribution. EV in

reproduction and EV in mortality can be specified to be acting independently or jointly (correlated in so far as is possible for discrete binomial distributions).

Unfortunately, rarely do we have sufficient field data to estimate the fluctuations in birth and death rates, and in carrying capacity, for a wild population. (The population would have to be monitored for long enough to separate, statistically, sampling error, demographic variation in the number of breeders and deaths, and annual variation in the probabilities of these events.) Lacking any data on annual variation, a user can try various values, or simply set EV = 0 to model the fate of the population in the absence of any environmental variation.

VORTEX can model catastrophes, the extreme of environmental variation, as events that occur with some specified probability and reduce survival and reproduction for one year. A catastrophe is determined to occur if a randomly generated number between 0 and 1 is less than the probability of occurrence (i.e., a binomial process is simulated). If a catastrophe occurs, the probability of breeding is multiplied by a severity factor specified by the user. Similarly, the probability of surviving each age class is multiplied by a severity factor specified by the user.

VORTEX also allows the user to supplement or harvest the population for any number of years in each simulation. The numbers of immigrants and removals are specified by age and sex. VORTEX outputs the observed rate of population growth (mean of N[t]/N[t-1]) separately for the years of supplementation/harvest and for the years without such management, and allows for reporting of extinction probabilities and population sizes at whatever time interval is desired (e.g., summary statistics can be output at 5-year intervals in a 100-year simulation).

VORTEX can track multiple sub-populations, with user-specified migration among the units. (This version of the program has previously been called VORTICES.) The migration rates are entered for each pair of sub-populations as the proportion of animals in a sub-population that migrate to another sub-population (equivalently, the probability that an animal in one migrates to the other) each year. VORTEX outputs summary statistics on each subpopulation, and also on the meta-population. Because of migration (and, possibly, supplementation), there is the potential for population recolonization after local extinction. VORTEX tracks the time to first extinction, the time to recolonization, and the time to re-extinction.

Overall, the computer program simulates many of the complex levels of stochasticity that can affect a population. Because it is a detailed model of population dynamics, it is not practical to examine all possible factors and all interactions that may affect a population. It is therefore incumbent upon the user to specify those parameters that can be estimated reasonably, to leave out of the model those that are believed not to have a substantial impact on the population of interest, and to explore a range of possible values for parameters that are potentially important but very imprecisely known.

VORTEX is, however, a simplified model of the dynamics of real populations. One of its artificialities is the lack of density dependence of death rates except when the population exceeds the carrying capacity. Another is that inbreeding depression is modelled as an effect on juvenile mortality only; inbreeding is optimistically assumed not to effect adult survival or reproduction.

VORTEX accepts input either from the keyboard or from a data file. Whenever VORTEX is run with keyboard entry of data, it creates a file called VORTEX.BAT that contains the input data, ready for resubmission as a batch file. Thus, the simulation can be instantly rerun by using VORTEX.BAT as the input file. By editing VORTEX.BAT, a few changes could easily be made to the input parameters before rerunning VORTEX. Note that the file VORTEX.BAT is over-written each time that VORTEX is run. Therefore, you should rename the batch file if you wish to save it for later use. By using data file input, multiple simulations can be run while the computer is unattended. (Depending on the computer used, the simulations can be relatively quick -- a few minutes for 100 runs -- or very slow.) Output can be directed to the screen or to a file for later printing. I would recommend that VORTEX only be used on a 80386 (or faster) computer with a math co-processor. It should run on slower machines, but it might be hopelessly slow.

The program can make use of any extended memory available on the computer (note: only extended, not expanded, memory above 1MB will be used), and the extra memory will be necessary to run analyses with the Heterosis inbreeding depression option on populations of greater than about 450 animals. To use VORTEX with expanded memory, first run the program TUNE, which will customize the program EX286 (a Dos Extender) for your computer. If TUNE hangs up DOS, simply re-boot and run it again (as often as is necessary). This behavior of TUNE is normal and will not affect your computer. After TUNEing the Dos Extender, run EX286, and then finally run VORTEX. TUNE needs to be run only once on your computer, EX286 needs to be run (if VORTEX is to be used with extended memory) after each re-booting of the computer. Note that EX286 might take extended memory away from other programs (in fact it is better to

disable any resident programs that use extended memory before running EX286); and it will release that memory only after a re-boot. If you have another extended memory manager on your system (e.g., HIMEM.SYS), you will have to disable it before using EX286.

VORTEX uses lots of files and lots of buffers. Therefore, you may need to modify the CONFIG.SYS file to include the lines

FILES=25
BUFFERS=50

in order to get the program to run.

VORTEX is not copyrighted nor copy protected. Use it, distribute it, revise it, expand upon it. I would appreciate hearing of uses to which it is put, and of course I don't mind acknowledgement for my efforts. James Grier should also be acknowledged (for developing the program that was the base for VORTEX) any time that VORTEX is cited.

A final caution: VORTEX is continually under revision. I cannot guarantee that it has no bugs that could lead to erroneous results. It certainly does not model all aspects of population stochasticity, and some of its components are simply and crudely represented. It can be a very useful tool for exploring the effects of random variability on population persistence, but it should be used with due caution and an understanding of its limitations.

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VORTEX -- simulation of genetic and demographic stochasticity

k5m2p1i3.h4
Fri Aug 30 16:35:20 1991

1 population(s) simulated for 200 years, 1000 runs

HETEROSIS model of inbreeding depression
with 3.50 lethal equivalents per diploid genome
First age of reproduction for females: 6 for males: 6
Age of senescence (death): 35
Sex ratio at birth (proportion males): 0.5000

Population 1:

Reproduction is assumed to be density dependent, according to:
Percent breeding = 24.79020980
+ 0.03799530 N
+ 0.00244760 NN
+ -0.00016320 NNN
+ 0.00000000 NNNN
EV in reproduction (SD around the first term in the above Eq.) = 6.25

Of those females producing litters, in an average year ...
100.00 percent of adult females produce litters of size 1

11.00 (EV = 5.50 SD) percent mortality of females between ages 0 and 1
0.50 (EV = 0.25 SD) percent mortality of females between ages 1 and 2
0.50 (EV = 0.25 SD) percent mortality of females between ages 2 and 3
0.50 (EV = 0.25 SD) percent mortality of females between ages 3 and 4
0.50 (EV = 0.25 SD) percent mortality of females between ages 4 and 5
0.50 (EV = 0.25 SD) percent mortality of females between ages 5 and 6
2.50 (EV = 1.25 SD) percent annual mortality of adult females (6 <= age <= 35)
27.00 (EV = 13.39 SD) percent mortality of males between ages 0 and 1
1.00 (EV = 0.50 SD) percent mortality of males between ages 1 and 2
1.00 (EV = 0.50 SD) percent mortality of males between ages 2 and 3
1.00 (EV = 0.50 SD) percent mortality of males between ages 3 and 4
1.00 (EV = 0.50 SD) percent mortality of males between ages 4 and 5
1.00 (EV = 0.50 SD) percent mortality of males between ages 5 and 6
2.50 (EV = 1.25 SD) percent annual mortality of adult males (6 <= age <= 35)
EVs may have been adjusted to closest values possible for binomial distribution.
EV in mortality will be correlated among age-sex classes
but independent from EV in reproduction.

Frequency of type 1 catastrophes: 10.000 percent
with 1.000 multiplicative effect on reproduction
and 0.800 multiplicative effect on survival

Frequency of type 2 catastrophes: 100.000 percent
with 1.000 multiplicative effect on reproduction
and 0.980 multiplicative effect on survival

Polygynous mating; 60.00 percent of adult males in the breeding pool.

Initial size of Population 1:
(set to reflect stable age distribution)

Age 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		Total	
1	2	2	1	2	1	1	1	1	1	1	1	1	1	1	0	1	0	1
0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0		24	Males
3	2	2	2	1	2	2	1	2	1	1	1	1	1	1	1	0	1	1
0	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1		32	Females

Carrying capacity = 50 (EV = 2.50 SD)

Animals harvested from population 1, year 1 to year 4 at 1 year intervals:
2 female adults (6 <= age <= 35)
1 male adults (6 <= age <= 35)

Deterministic population growth rate (based on females, with assumptions of
no limitation of mates and no inbreeding depression):

r = 0.009 lambda = 1.009 R0 = 1.142
Generation time for: females = 15.61 males = 15.61

Stable age distribution:

Age class	females	males
0	0.043	0.043
1	0.036	0.030
2	0.034	0.028
3	0.032	0.026
4	0.031	0.025
5	0.029	0.023
6	0.028	0.022
7	0.026	0.021
8	0.024	0.019
9	0.022	0.018
10	0.021	0.016
11	0.019	0.015
12	0.018	0.014
13	0.016	0.013
14	0.015	0.012
15	0.014	0.011
16	0.013	0.011
17	0.012	0.010
18	0.011	0.009
19	0.011	0.008
20	0.010	0.008
21	0.009	0.007
22	0.008	0.007
23	0.008	0.006
24	0.007	0.006
25	0.007	0.005
26	0.006	0.005
27	0.006	0.005
28	0.005	0.004
29	0.005	0.004
30	0.005	0.004
31	0.004	0.003
32	0.004	0.003
33	0.004	0.003
34	0.003	0.003
35	0.003	0.003

Ratio of adult (>= 6) males to adult (>= 6) females: 0.800

Population1

Year 25
N[Extinct] = 0, P[E] = 0.000
N[Surviving] = 1000, P[S] = 1.000
Population size = 32.25 (0.31 SE, 9.67 SD)
Expected heterozygosity = 0.945 (0.001 SE, 0.020 SD)
Observed heterozygosity = 0.991 (0.001 SE, 0.018 SD)
Number of extant alleles = 27.60 (0.23 SE, 7.22 SD)

Year 50
N[Extinct] = 27, P[E] = 0.027
N[Surviving] = 973, P[S] = 0.973
Population size = 27.33 (0.37 SE, 11.51 SD)
Expected heterozygosity = 0.890 (0.002 SE, 0.049 SD)
Observed heterozygosity = 0.954 (0.002 SE, 0.052 SD)
Number of extant alleles = 15.24 (0.15 SE, 4.79 SD)

Year 75
N[Extinct] = 86, P[E] = 0.086
N[Surviving] = 914, P[S] = 0.914
Population size = 22.62 (0.37 SE, 11.21 SD)
Expected heterozygosity = 0.829 (0.003 SE, 0.084 SD)
Observed heterozygosity = 0.901 (0.003 SE, 0.096 SD)
Number of extant alleles = 10.06 (0.11 SE, 3.45 SD)

Year 100
N[Extinct] = 204, P[E] = 0.204
N[Surviving] = 796, P[S] = 0.796
Population size = 18.98 (0.39 SE, 10.90 SD)
Expected heterozygosity = 0.773 (0.004 SE, 0.107 SD)
Observed heterozygosity = 0.856 (0.005 SE, 0.129 SD)
Number of extant alleles = 7.46 (0.10 SE, 2.71 SD)

Year 125
N[Extinct] = 357, P[E] = 0.357
N[Surviving] = 643, P[S] = 0.643
Population size = 15.44 (0.40 SE, 10.25 SD)
Expected heterozygosity = 0.711 (0.005 SE, 0.130 SD)
Observed heterozygosity = 0.811 (0.007 SE, 0.166 SD)
Number of extant alleles = 5.78 (0.09 SE, 2.27 SD)

Year 150
N[Extinct] = 553, P[E] = 0.553
N[Surviving] = 447, P[S] = 0.447
Population size = 14.42 (0.44 SE, 9.36 SD)
Expected heterozygosity = 0.661 (0.007 SE, 0.153 SD)
Observed heterozygosity = 0.745 (0.009 SE, 0.192 SD)
Number of extant alleles = 4.90 (0.09 SE, 1.88 SD)

Year 175
N[Extinct] = 709, P[E] = 0.709
N[Surviving] = 291, P[S] = 0.291
Population size = 11.43 (0.46 SE, 7.86 SD)
Expected heterozygosity = 0.617 (0.010 SE, 0.168 SD)
Observed heterozygosity = 0.719 (0.013 SE, 0.214 SD)
Number of extant alleles = 4.17 (0.10 SE, 1.62 SD)

Year 200
N[Extinct] = 845, P[E] = 0.845
N[Surviving] = 155, P[S] = 0.155
Population size = 9.81 (0.59 SE, 7.37 SD)
Expected heterozygosity = 0.586 (0.014 SE, 0.174 SD)
Observed heterozygosity = 0.710 (0.019 SE, 0.233 SD)
Number of extant alleles = 3.75 (0.12 SE, 1.45 SD)

In 1000 simulations of 200 years of Population1:
845 went extinct and 155 survived.

This gives a probability of extinction of 0.8450 (0.0114 SE),
or a probability of success of 0.1550 (0.0114 SE).

845 simulations went extinct at least once.
Median time to first extinction was 143 years.
Of those going extinct,
mean time to first extinction was 130.73 years (1.39 SE, 40.28 SD).

No recolonizations.

Mean final population for successful cases was 9.81 (0.59 SE, 7.37 SD)

Age 1	2	3	4	5	Adults	Total	
0.17	0.18	0.17	0.23	0.23	3.52	4.50	Males
0.22	0.25	0.28	0.23	0.21	4.12	5.30	Females

During years of harvest and/or supplementation
mean lambda was 0.9152 (0.0012 SE, 0.0753 SD)

Without harvest/supplementation, prior to carrying capacity truncation,
mean lambda was 0.9879 (0.0003 SE, 0.1107 SD)

Note: 0 of 4000 harvests of males and 0 of 8000 harvests of females
could not be completed because of insufficient animals.

Final expected heterozygosity was	0.5864 (0.0140 SE, 0.1740 SD)
Final observed heterozygosity was	0.7098 (0.0187 SE, 0.2331 SD)
Final number of alleles was	3.75 (0.12 SE. 1.45 SD)

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1991 VORTEX SIMULATIONS OF JAVAN RHINO POPULATIONS IN UJUNG KULON

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INTRODUCTION

PVA analyses use computer models which incorporate demographic and genetic characteristics of the population(s) and conditions in the environment to simulate probable fates (especially extinction) of the population(s) under these circumstances.

Since the 1989 Workshop and Report on population viability assessment of the Javan Rhino in Indonesia, the computer simulation models have evolved and improved. A density dependence model, as described in the VORTEX documentation, is now incorporated into the VORTEX software. This permits the model to decrease reproduction as the population approaches carrying capacity or to increase reproduction as the population is reduced below carrying capacity. Hence, the model now permits the population to "recover" more realistically from declines below carrying capacity. The state of the art is described in the VORTEX section of this Briefing Book.

Using the improved models, a number of the population viability analyses are repeated here as a basis for further analysis at this 1991 Workshop. The results are presented in the next 6 tables (**Tables 1-6**) which attempt to develop the scenario of small population problems and risks in what hopefully is a logical sequence.

Each case investigated is represented by a row in the tables. A case is defined by the condition represented by the columns of the table. Blocks of rows defined by the double lines above and below represent cases subjected to similar sets of conditions.

The simulations for each case are repeated through 1000 runs, i.e. 1000 populations are subjected to the conditions of this case.

All populations are simulated for 200 years with results reported at the end of both 100 and 200 years.

The sequence of cases are:

(1) Basic scenarios are established by assigning demographic parameters for each case. "**POPULATION PARAMETERS** column". Important demographic variables include: the carrying capacity **K**; the pattern of survivorship L_x. (**Table 7**); the pattern of fertility or reproduction M_x.

After basic scenarios are constructed, a number of the problems that can afflict small populations are added.

(2) First, the effects of catastrophes are explored (**CATASTROPHE** columns).

(3) Then, the effects of inbreeding are investigated. "**INBRD**" column

(4) Lastly, the effects of removing rhino from the population are examined (**REMOVALS**" column).

All simulations are investigated at 3 levels of carrying capacity (K): 100, 70, 50.

The results of the population simulations are reported in terms of:

P(E):	Probability of extinction, i.e. the number of populations out of 1000 that became extinct in the simulations.
T_E:	The mean time to extinction for those populations that did not survive. The result is reported as the mean $\pm$the standard deviation to provide a view of the range of extinction times.
POP.:	The mean final size for those populations that survive, again presented as a mean $\pm$ the standard deviation.
H_E:	The expected fraction remaining in the surviving populations of the original heterozygosity (genetic diversity).

BASIC SCENARIOS - (Table 1)

Basic population parameters are derived from 3 sources:

(1) Demographic data on *Rhinoceros unicornis* in the wild in Nepal (Dinerstein & Price 1991. included in this Briefing Book)

(2) Demographic analysis of the captive population of *Rhinoceros unicornis* in captivity in North America. (SSP 1988, included in this Briefing Book)

(3) Limited data demography of *Rhinoceros sondaicus* in Ujung Kulon (Amman 1982, included in this Briefing Book)

Survivorship and mortality schedules are selected to produce an age structure approximating these three reference populations.

In formulating the basic parameters, there is an attempt to replicate the population structure and dynamics reported in these populations, e.g. the 7% annual growth rate (λ) observed in both the Nepal and Ujung Kulon populations during periods of maximal increase or the 4-5% growth rate more recently prevailing in the Nepal population. These two rates of growth are achieved by varying the average level of reproduction.

Level 1 (7% growth rate):	On the average, 33% of the females in the population produce a calf in a given year. This pattern is equivalent in the demographic models to each female producing a calf every 3 years.
Level 2 (5% growth rate):	On the average, 25% of the females in the population produce a calf in a given year. This pattern is equivalent in the demographic models to each female in the population producing a calf every 4 years.

Incorporating density dependence permits the model population to emulate these rates of growth when density is lower and still achieve zero population growth near carrying capacity. The pattern of density dependent change in reproduction used are presented in **Table 8.** These patterns also cause the interbirth intervals to increase near carrying capacity consistent with what has been suggested for the Javan rhino in Ujung Kulon.

The newer models also produce more reasonable estimates of generation time (G) than was the case in 1989, i.e. the G's are similar to what is calculated for *Rhinoceros unicornis* populations in Nepal and in North American zoos.

Results:

At both levels of reproduction, the populations maintain their sizes near carrying capacity and their heterozygosity at high levels over the 200 year period.

EFFECTS OF CATASTROPHES (Table 2)

Catastrophes can increase mortality and fertility below the level that occurs because of normal events in the population. Two types and severities of catastrophes suggested by the recent history of the Ujung Kulon population are investigated:

Type I: A "disease" catastrophe (suggested by the 1982 death event) occurring on the average once every 10 years (.1 frequency (**FRQ**) of occurrence). It is assumed here that the effect of the catastrophe will be to increase mortality (although VORTEX also permits decrease in fertility). Two levels of severity (**SRVT**) in mortality are imposed.

- Severity 1: .1 (10%) increase in mortality which is equivalent to a survivorship of .9 (90%) of what it is without the catastrophe. This level of mortality is suggested by the 5 carcasses actually discovered in the 1982 death event when the total population was estimated at about 50.
- Severity 2: .2 (20%) increase in mortality which is equivalent to a survivorship of .8 (80%) of what it is without the catastrophe. This level is suggested by the speculations that not all carcasses were discovered in 1982 (Van Strien report).

Type II: A "poaching" catastrophe. Poaching can be modelled as either a stochastic or a deterministic event. It is here modelled as a stochastic event, as a continuin catastrophe. The frequency is .5 (50%) which is equivalent to an event occurring every other year. The severity is .02 (2%) removal of the existing population which in a population of about 50-60 animals represents a loss of 1 individual. This level is consistent with estimates at the last Workshop.

The catastrophes are investigated with respect to both levels of reproduction (.33 and .25).

Results: **Four sets of cases:**

At the higher level of reproduction (.33) and the lesser severity of the "disease" Catastrophe I (.9), all populations maintain their sizes near carrying capacity. By year 200, genetic diversity is at high levels for populations with K = 100; lower for K = 70; and for K = 50, almost 25% of the original genetic diversity is lost. (As is true in all "50 K" cases in this Table.)

At the higher level of reproduction (.33) and the greater severity of Catastrophe I (.8), mean final population sizes are slightly lower and standard deviations around mean (instability) are higher. Moreover, the cases with carrying capacity of 50 are already manifesting some extinctions.

At the lower level of reproduction (.25) and the lesser severity of Catastrophe I (.9), mean final populations are again lower than in the basic scenarios and the populations with carrying capacity of 50 exhibit problems.

At the lower level of reproduction (.25) and the greater severity of Catastrophe I (.8), populations at all 3 carrying capacity levels have lower final population sizes and are experiencing extinctions. The smaller the carrying capacity, the greater the extinctions. Expected heterozygosity is appreciably reduced by year 200 in the populations with carrying capacities 70 and 50.

EFFECTS OF INBREEDING (Tables 3 & 4)

Inbreeding can reduce ("depress") the survival and fertility (fitness) of a small population. Inbreeding is incorporated using a heterosis model where level is measured by the number of lethal equivalents per diploid genome. The lethal equivalents are assumed to reduce fitness by increasing juvenile mortality. There is a simplistic and approximate way of appreciating what lethal equivalents are. A 10% loss of heterozygosity is equivalent to a 10% decline in fitness (as measured by increased juvenile mortality) which represents 1 lethal equivalent; 20% loss of heterozygosity = 20% decline in fitness = 2 lethal equivalents. For a fuller explanation the reader is referred to the VORTEX program as well as Ralls et. al (1988), both of which are provided in this Briefing Book.

Two levels of inbreeding are investigated:

Level 1: 3.5 lethal equivalents per diploid genome which is a value between the mean and the median for a wide range of mammals investigated by Ralls et al. (copy of paper provided in Section of this Briefing Book)

Level 2: 7 recessive lethals which represents a high value of the range reported by Ralls et al., e.g. approximates the value discovered for Eld's deer.

Inbreeding is investigated at two levels of severity of the "disease" Catastrophe I.

Severity 1: The 10% increase in mortality (i.e. the .9 survivorship value). **Table 3 - INBREEDING I.**

Severity 2: The 20% increase in mortality (i.e. the .8 survivorship value). **Table 4 - INBREEDING II.**

The "poaching" Catastrophe II is applied in all cases.

Results: **Eight sets of cases.**

INBREEDING I: (Lesser severity of Catastrophe I) 4 sets of cases.

At higher levels of reproduction (.33) and lower levels of inbreeding (3.5), there is some further reduction in final population sizes and genetic diversity over the "Effects of Catastrophe" cases.

At lower levels of reproduction (.25) and lower levels of inbreeding (3.5), the final populations and genetic diversity are reduced even more and for populations with carrying capacity 50, extinctions are occurring and appreciable decline in mean size occurs from Year 100 to Year 200. This latter trend is evident even for populations with carrying capacity 70.

At higher levels of reproduction (.33) but higher levels of inbreeding (7), declines of final population and expected heterozygosity are greater than at lower levels of inbreeding. Populations at all levels of carrying capacity have population sizes appreciably lower at Year 200 than at Year 100.

At lower levels of reproduction (.25) and higher levels of inbreeding (7), problems are evident for populations at all 3 levels of carrying capacity, but for K = 70 and especially K = 50, the populations clearly seem to be in an "extinction vortex".

INBREEDING II: (Greater severity of Catastrophe I) 4 sets of cases.

Populations at all levels of reproduction and degree of inbreeding are exhibiting extinction problems. Problems are least in the first set of cases (reproduction .33 and inbreeding 3.5) in Table 4. The problems increase for the 3rd set of cases (reproduction .33 and inbreeding 7) in Table 4. The problems are greatest and very severe in the two sets of cases with lower reproductive potential (.25) at either level of inbreeding but with the worse with inbreeding at 7. Populations at all levels of carrying capacity are clearly in "extinction vortices".

In general there seems to be a synergism between catastrophes and inbreeding that produce such "extinction vortices". This synergism is plausible. When catastrophes reduce the populations to low size, they experience genetic bottlenecks which increases inbreeding and can further reduce fitness and decrease the size of the population even more.

EFFECTS OF REMOVALS (Tables 5 & 6)

For purposes of this preliminary analyses, 12 adult rhino (4 males and 8 females) are removed from Ujung Kulon to establish a second population.

Animals are removed using the previous worst case scenario for catastrophes, i.e. EFFECTS OF INBREEDING II. A worst case scenario is initially investigated on the premise that the most secure approach for conservation is a strategy that will minimize regrets.

Two removal schedules are explored:

(1) removing all the animals at once in a single year (**Removal I**);
(2) removing 3 animals per year (1 male and 2 females) over 4 years (**Removal II**)

Results:

Results indicate that there is no significant effect on the population of removing this number of adult animals. Moreover, there is no significant difference between removing all the animals in one year or over 4 years. These results are consistent with the analyses conducted to produce the 1989 Javan Rhino PVA report. Obviously, other scenarios in terms of both numbers of animals removed and period over the removals occur can be explored.

CONCLUDING COMMENTS

One conclusion that emerges from these analyses appears to be the particular vulnerability of rhino populations with carrying capacity of 50 (and lower). Risks of extinction are appreciable to significant in many of the "50 K" cases. Moreover, loss of genetic diversity (heterozygosity) is significant (< 85%) by 200 years in all "50 K" cases investigated.

Many other analyses could and should be conducted. For example, it is possible also to simulate competition, e.g. from Banteng, in the models. Very importantly, it is possible to simulate metapopulation situation, i.e. what are the expected outcomes if there are 2 populations (Ujung Kulon and a second population, wild or captive). These simulations can be performed at the Workshop.

TABLE 1 - JAVAN RHINO PVA SIMULATIONS - BASICS SCENARIOS

POPULATION PARAMETERS					CATASTROPHIES					REMOVALS		PROJECTIONS			
					I		II								
YRS	K	λ	M_x	G	FRQ	SVRT	FRQ	SVRT	INBRD	TOT #	YRS.	P(E)	T_E	POP.	H_E
100	100	1.07	.33	16	0		0		0	0	0	0		97±5	.94
200												0		97±5	.89
100	70											0		68±4	.91
200												0		68±4	.84
100	50											0		48±3	.89
200												0		48±3	.79
100	100	1.05	.25	17	0		0		0	0	0	0		95±5	.94
200												0		95±8	.89
100	70											0		66±4	.92
200												0		66±4	.85
100	50											0		47±3	.89
200												0		47±3	.80

TABLE 2 - JAVAN RHINO PVA SIMULATIONS - EFFECTS OF CATASTROPHES

	POPULATION PARAMETERS				CATASTROPHES I		CATASTROPHES II			REMOVALS		PROJECTIONS			
YRS	K	λ	M_λ	G	FRQ	SVRT	FRQ	SVRT	INBRD	TOT #	YRS	P(E)	T_E	POP.	H_E
100	100	1.05	.33	16	.10	.9	.50	.98	0	0	0	0		93±7	.93
200												0		92±7	.92
100	70											0		65±5	.91
200												0		65±5	.83
100	50											0		46±4	.87
200												0		46±5	.77
100	100	1.04	.33	15	.10	.8	.50	.98	0	0	0	0		85±14	.92
200												0		85±13	.86
100	70											0		60±10	.89
200												0		60±10	.82
100	50											.001		41±8	.85
200												.007	129±47	42±8	.73
100	100	1.03	.25	16	.10	.9	.50	.98	0	0	0	0		86±10	.92
200												0		86±9	.86
100	70											0		60±8	.91
200												0		60±8	.82
100	50											.001		42±6	.87
200												.001	66	42±7	.76
100	100	1.02	.25	16	.10	.8	.50	.98	0	0	0	0		71±20	.90
200												.007	141±31	71±20	.82
100	70											.001		50±14	.88
200												.019	153±33	50±15	.77
100	50											.013		35±11	.83
200												.072	141±41	34±11	.68

TABLE 3 - JAVAN RHINO PVA SIMULATIONS - EFFECTS OF INBREEDING I

POPULATION PARAMETERS					CATASTROPHES I		CATASTROPHES II		INBRD	REMOVALS		PROJECTIONS			
YRS	K	λ	M_a	G	FRQ	SVRT	FRQ	SVRT		TOT #	YRS	P(E)	T_E	POP.	H_E
100	100	1.05	.33	.16	.10	.9	.5	.98	3.5	0	0	0		91±8	.93
200												0		89±9	.88
100	70											0		63±6	.91
200												0		61±7	.83
100	50											0		44±5	.88
200												0		40±7	.77
100	100	1.03	.25	.16	.10	.9	.5	.98	3.5	0	0	0		82±11	.93
200												0		77±13	.87
100	70											0		57±9	.91
200												0		50±12	.81
100	50											.003		38±8	.87
200												.034		30±11	.73
100	100	1.05	.33	.16	.10	.9	.5	.98	7	0	0	0		89±8	.93
200												0		82±11	.88
100	70											0		61±7	.91
200												0		53±11	.83
100	50											0		41±7	.88
200												.02		28±11	.75
100	100	1.03	.25	.16	.10	.9	.5	.98	7	0	0	0		78±13	.93
200												.002		60±18	.86
100	70											0		53±10	.91
200												.046		31±15	.79
100	50											.002		34±9	.86
200												.323		14±9	.68

TABLE 4 - JAVAN RHINO PVA SIMULATIONS - EFFECTS OF INBREEDING II

POPULATION PARAMETERS					CATASTROPHES I		CATASTROPHES II		INBRD	REMOVALS		PROJECTIONS			
YRS	K	λ	M_s	G	FRQ	SVRT	FRQ	SVRT		TOT #	YRS	P(E)	T_E	POP.	H_E
100	100	1.04	.33	15	.10	.8	.5	.98	3.5	0	0	0		81±15	.92
200												.003	177±29	76±18	.85
100	70											.002		56±12	.89
200												.016	157±39	49±15	.79
100	50											.012		38±10	.85
200												.080	156±41	29±12	.70
100	100	1.02	.25	16	.10	.8	.5	.98	3.5	0	0	.006		62±23	.90
200												.094	156±33	46±24	.80
100	70											.012		43±16	.87
200												.211	159±30	28±16	.73
100	50											.051		27±12	.82
200												.472	150±36	16±11	.64
100	100	1.04	.33	15	.10	.8	.5	.98	7	0	0	0		77±18	.92
200												.018	174±24	60±22	.84
100	70											0		53±13	.89
200												.085	171±26	32±18	.77
100	50											.011		32±11	.85
200												.403	166±26	15±9	.66
100	100	1.02	.25	16	.10	.8	.5	.98	7	0	0	.012		54±23	.90
200												.328	162±30	26±18	.77
100	70											.022		36±16	.87
200												.550	160±29	13±10	.69
100	50											.096		22±11	.81
200												.882	145±34	8±5	.63

TABLE 5 - JAVAN RHINO PVA SIMULATIONS - EFFECTS OF REMOVALS I

POPULATION PARAMETERS					CATASTROPHES I		CATASTROPHES II		INBRD	REMOVALS		PROJECTIONS			
YRS	K	λ	M_1	G	FRQ	SVRT	FRQ	SVRT		TOT #	YRS	P(E)	T_E	POP.	H_E
100	100	1.04	.33	15	.10	.8	.5	.98	3.5	12	1	.001		81±16	.91
200												.005	129±51	74±18	.84
100	70											.001		56±12	.89
200												.016	163±34	49±16	.79
100	50											.006		38±10	.85
200												.078	161±33	28±12	.70
100	100	1.02	.25	16	.10	.8	.5	.98	3.5	12	1	.016		60±22	.89
200												.113	151±37	44±24	.79
100	70											.037		42±16	.87
200												.228	153±40	26±16	.73
100	50											.071		27±12	.82
200												.488	145±38	16±11	.62
100	100	1.04	.33	15	.10	.8	.5	.98	7	12	1	.002		76±18	.91
200												.020	162±39	55±23	.83
100	70											.003		51±14	.89
200												.131	172±25	32±17	.76
100	50											.015		33±11	.84
200												.417	164±28	15±10	.67
100	100	1.02	.25	16	.10	.8	.5	.98	7	12	1	.035		49±23	.88
200												.403	157±35	22±17	.74
100	70											.042		34±16	.86
200												.384	155±33	14±11	.70
100	50											.097		21±11	.80
200												.895	143±34	8±6	.60