POPULATION BIOLOGY ASPECTS

OF

GENOME RESOURCE BANKING

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1 September 1994

POPULATION BIOLOGY ASPECTS

OF A

GENOME RESOURCE BANK PLAN

Prologue

The concept and potential of genome resource banks (GRBs) (the organized collection, storage and use of biomaterials) for wildlife species has been debated for more than two decades. The challenge has been in converting the concept into reality having an actual impact on conservation.

Beginning in 1991, the Conservation (Captive) Breeding Specialist Group (CBSG) under the umbrella of the World Conservation Union's Species Survival Commission began seriously considering how to systematically develop the GRB concept. The issue was first debated in both a plenary session and in a working group at the Annual CBSG Meeting in Singapore. It was determined that GRBs could have tremendous potential for both in situ and ex situ conservation programs, especially as a method for ensuring maintenance of genetic variation as well as moving genetic variation among isolated populations. One primary recommendation of the 1991 meeting was that GRBs should be considered as an ancillary conservation tool to be developed in the form of individual action plans, written documents that would ensure that biomaterials would be collected, stored and used in a systematic and scientific fashion and only for the purpose of real conservation. The topic was re-visited at the annual CBSG meeting in 1992 (Vancouver) where the working group developed a written set of guidelines precisely identifying all the factors that must be addressed in writing the Action Plan (these guidelines are appended to this document).

The next high priority was actually testing the guidelines by preparing a 'prototype' Action Plan. Because of (1) its precarious status in the wild, (2) a worldwide masterplan for the captive population and (3) a substantial database on reproduction, the tiger was chosen as the model species for developing a GRB Action Plan. During the formulation of the Plan, a number of population biology-type questions arose that (to our knowledge) had never been addressed in a systematic fashion by the scientific community. These unknowns were sufficiently important to dictate not only how the GRB should be developed, but also its potential and ultimate impact on the conservation of the species. It was for this reason that the CBSG in cooperation with the American Zoo and Aquariums Association held a 2-day workshop to discuss the population biology aspects of genome resource banking. The following are the resulting recommendations from these discussion.

Introduction

Effective conservation of endangered taxa requires making every attempt to retain all extant genetic variation to ensure continued evolution and adaptation through natural selection. Establishing a Genome Resource Bank (GRB) as part of an integrated conservation strategy can aid in the retention and protection of genetic variation. The formulation of genetic objectives for living populations (wild and/or captive) and the composition of germplasm and other biological materials in the GRB are essential prerequisites for applying biotechnologies (including assisted reproduction) to achieve conservation and management goals. The following goals, objectives and strategies are proposed to address population biology assumptions and guidelines needed for developing and using a Sumatran and Siberian tiger GRB.

The GRB will be divided into two major components. First a GRB is described that is designated for storage of the total genetic variation found in the species today (see Goal 1). Secondly, a GRB is described that is to be used interactively with living animals both in captivity and in the wild (Goal 2).

This draft document is intended to be reviewed and revised on a continuing basis as new information is gathered and as a better understanding of small population biology develops.

GOAL 1. The goal of this GRB is to cryopreserve gametic material in sufficient quantity to represent (at high probability) the genetic variation contained in the living taxon for the purpose of preserving indefinitely the evolutionary potential of the taxon.

Objective 1. Sampled gametes should retain at 95% probability of representation any allele with a frequency greater than 0.05.

Strategy 1. Gamete samples from each of 25-50 individuals, sufficient to yield 2 surviving offspring from each individual, (see Marshall & Brown, pp. 65-66) across the range of a homogeneous population should achieve this representation under most circumstances.

Strategy 2. Genetically subdivided populations would require additional sampled individuals divided among the subdivisions.

Strategy 3. The continued availability and safety of cryopreserved materials and related information on their source and characteristics must be ensured by storage at two or more locations.

Strategy 4. Both captive and wild populations should be sampled for placement in this GRB.

Strategy 5. For taxa in which gametes are derived from free-ranging individuals of possible close kinship, molecular genetic techniques (such as minisatellites and microsatellites) should be used to identify close relationships among sampled individuals. This information will be used to avoid inadvertent inbreeding within any future population derived from the GRB.

Objective 2. This GRB also is meant to serve as a representative population 'backup' to be accessed in the case of near extinction of the living taxon.

Strategy 6. This GRB should be used as needed to replenish genetic variability when the taxon is critically endangered because of low fitness resulting from lack of genetic variation or inbreeding depression.

GOAL 2. The goal of this GRB is to establish an active, additional repository, containing maximal gene diversity, to continually sustain maximal original genetic variation of the taxon by gametic exchange among the wild, captive and this specific GRB population.

Objective 1. Sampled gametes should retain at 95% probability of representation any allele with a frequency greater than 0.05 in the wild and captive population.

Strategy 1. Animals should be selected from the captive (based upon pedigree information) and wild (based upon randomized sampling or molecular genetic studies) populations to fully represent the genetic variation of the taxon in the GRB.

Strategy 2. Animals should be prioritized for inclusion in the GRB based upon minimizing mean kinships in the GRB.

Strategy 3. For taxa in which gametes are derived from free-ranging individuals of possible close kinship, molecular genetic techniques (such as minisatellites and microsatellites) should be used to identify close relationships among sampled individuals. This information will be used to avoid inadvertent inbreeding within any future population derived from the GRB.

Objective 2. At least 90% of gene diversity (heterozygosity) should be maintained continuously for 100 years as a correlate of maintaining high fitness (to retain genetic variation and avoid inbreeding depression) of the wild and captive populations. The GRB will be established by sampling the captive and, when possible, the wild populations.

Strategy 4. Avoiding or minimizing the loss of genetic variation per generation in the population should be the guide to achieving this objective.

Strategy 5. The living population should be managed on the basis of mean kinships to minimize the removal of gamete samples from the GRB.

Strategy 6. Gametes to be utilized from the GRB should be selected to further reduce mean kinship of the living population.

Strategy 7. Gametes from the GRB should be used to transfer genetic material between sites and populations rather than moving animals.

Strategy 8. The continued availability and safety of cryopreserved materials and related information on their source and characteristics must be ensured by storage at two or more locations.

Objective 3. When available and appropriate, additional characters potentially related to fitness or genetic variation should be considered in the decision for use of GRB gametes.

Strategy 9. When possible, survival rates (survivorships) should be monitored as a component of fitness.

Strategy 10. When possible, fertility rates should be monitored as a component of fitness.

Strategy 11. When possible, molecular genetic variation should be monitored as an indicator of heterozygosity.

Strategy 12. When possible, specific genes should be monitored that are known to affect fitness or as correlates of fitness.

Objective 4. Any artificial selection for genetically-affected traits should be avoided.

Strategy 13. Mean kinship relationships should be used for breeding, cryobanking, and utilizing gametic material from the GRB to counter selection of genetic traits in the captive population.

Objective 5. The captive population size necessary for maintaining genetic variation should be decreased to reduce the resources needed for captive management of the taxon.

Strategy 14. The population should not be reduced to levels that compromise the persistence of the population.

Strategy 15. Guidelines on minimum population size should be determined on a case-by-case basis when planning breeding programs that incorporate use of the GRB.

GOAL 3. Biological samples suitable for research that will contribute to the conservation of the taxon should be collected and maintained.

Objective 1. Research should be conducted to improve and enhance assisted reproduction.

Strategy 1. Sperm, embryos, and oocytes should be collected, maintained, and used for basic and applied research purposes.

Objective 2. Specimens should be maintained for identification and verification purposes.

Strategy 2. Tissue samples and fibroblast cell culture lines should be collected, maintained, and used as living genetic material to serve as reference voucher specimens for systematic verification on the individual and the taxon.

Objective 3. The health and physiology of animals providing GRB specimens should be monitored.

Strategy 3. Blood samples should be maintained as reference material for animal health care.

Goal 4. Guidelines should be formulated for sampling, cryobanking, and using biomaterials from the living population needed to stock and maintain the two GRBs (described in Goals 1 and 2).

Objective 1. The actual and potential efficiency should be estimated for using sperm samples for artificial insemination (AI).

Strategy 1. The efficiency of sperm collection and cryostorage should be evaluated for the species of interest.

Estimates for tigers:

This required estimating (mean and SE) of the following reproductive traits obtained from 17 Siberian and 27 Sumatran tiger ejaculates: ejaculate (EEJ) volume (6.9 $\pm$ 0.6 ml, Siberian; 5.6 $\pm$ 0.7 ml, Sumatran); sperm concentration (19.7 $\pm$ 4.9 x 10^6 sperm/ml, Siberian; 47.4 $\pm$ 6.3 x 10^6 sperm/ml, Sumatran); percentage of motile sperm (70%; both subspecies), 140.7 $\pm$ 35.5 x 10^6 (Siberian) and 240.3 $\pm$ 33.1 x 10^6 (Sumatran) total motile sperm per ejaculate (Siberian and Sumatran, respectively), percentage of post-thaw motile sperm (60%; both subspecies) and total yields of motile post-thaw sperm (59.1 x 10^6 sperm/ejaculate, Siberian; 100.9 x 10^6 sperm/ejaculate, Sumatran).

Strategy 2. The number of sperm required per AI attempt and the number of AI attempts required to achieve a pregnancy should be estimated.

Estimates for tigers:

Estimation of the number of motile sperm needed per AI attempt is based upon the single successful attempt in the tiger = 16 x 10^6 motile sperm (the amount used in other felid species averages 10 x 10^6 motile sperm). The mean expected yield for Siberian tigers: 59.1/16 = 3.7 AI doses/EEJ and the mean expected yield for Sumatran tigers: 100.9/16 = 6.3 AI doses/EEJ.

The number of AI attempts needed to achieve a pregnancy with viable offspring in tigers is based upon a controlled study in which one pregnancy resulted from 10 AI attempts (or 10% efficiency). Thus, the number of ejaculates required to produce a pregnancy or litter in tigers may be conservatively estimated at 10% efficiency as: *Siberian tigers = ~3 ejaculates per pregnancy (10 attempts/3.7 per EEJ) and Sumatran*

tigers = ~2 ejaculates per pregnancy (10 attempts/6.3 per EEJ). AI success rates in other felid species include cheetah = 40% and domestic cat = 60%.

Projections were made of the probable efficiency of AI procedures over the next 3-5 years based upon experience with other felid species. The major projected change is in enhanced efficiency in pregnancy rates per AI attempt (from 10% to 30-40%). This results in *estimates for Siberian tigers = 0.7 ejaculate per pregnancy and Sumatran tigers = 0.4 ejaculate per pregnancy.*

Objective 2. The number of AI contributions needed per species generation should be estimated to meet the program goal of maintaining 90% of the heterozygosity of the taxon.

Strategy 3. Appropriate estimation of the rate at which gamete samples from the GRB will need to be used to meet the genetic goal of maintaining 90% gene diversity in the captive population require simulation modeling and/or analytical calculations.

Estimates for tigers:

These models need to incorporate the gene diversity of the present captive population (e.g., about 96% for Siberian tigers), the gene diversity obtained within GRB described in Goal 2 (about 96% for Siberian tigers if all gamete collection is from the present captive population), the estimated effective population size of the maintained living population (perhaps 100 breeders), the generation time (about 7 years), and the extent to which optimal genetic management based on mean kinships can be achieved (most of the animals are in management programs). Such modeling will be completed during the next 6 months.

Strategy 4. A crude (until more work modelling is done) estimate of the rate of the use of the GRB to produce progeny by AI should be obtained as follows. The models of Johnston and Lacy (1991) for using sperm banks to preserve gene diversity in the Siberian tiger suggest that for a captive population with Ne of 120 animals (sex ratio 0.5), production of four offspring per generation by AI from a sperm bank created from males in the captive population, identified by the Mean Kinship selection strategy, leads to an expected retention of 91.6% gene diversity for 100 years. For the Sumatran tiger, modelling

results indicate that for a captive population with Ne of 120 animals (sex ratio 0.5), production of four offspring per generation by AI from a sperm bank created from sampling the wild population leads to an expected retention of 93.6% gene diversity for 100 years.

Comments:

Because all of the above estimates are at this point crude, and will be refined, we would provisionally recommend aiming for a target of 28 ejaculates in the sperm bank.

Other theoretical models, such as that of Lacy (1987) suggest that incorporation of two immigrants from a stable and genetically diverse source population (e.g., a GRB) can allow preservation of more than 90% gene diversity in a small randomly breeding population indefinitely. Progeny produced by AI represent half a genome from the GRB and half from the living dam. Thus, these theoretical models also suggest that about four offspring would need to be produced per generation by AI to keep a captive population above 90% gene diversity.

The above models all assume that immigrants into the captive population (e.g., from the GRB via AI) are randomly selected from the source population (GRB). Management will select the most genetically valuable semen samples for AI, but because tigers produce progeny in litters of mean size 2.5, the genetic material incorporated from the GRB into the captive population will be sampled over the short term from few sperm donors. Thus, over the first few generations, the retention of gene diversity in the captive population will not be as effective as would be the case if progeny produced by AI could have independent sires. (For example, the gene diversity represented in 25 individuals produced in five litters of two offspring and five litters of three offspring is 96.4%; the gene diversity represented in 25 unrelated individuals is 98%; the gene diversity represented in 10 unrelated individuals is 95%.). Over the course of many generations, semen samples from more valuable GRB donors would be planned to be used multiple times in a program that uses mean kinship to prioritize breeders. The disadvantage of producing multiple progeny per litter sired by a male will, therefore, diminish over generations.

Estimates for tigers:

Given all the above considerations and uncertainties, and until more detailed models are analyzed, it can be estimated provisionally that approximately *four progeny (in two litters) would need to be produced per generation by AI from the GRB* to keep that living captive population above 90% gene diversity.

The requirement of four offspring per generation in the captive population translates into 1.6 litters per generation (i.e. two litters / generation for purposes of these recommendations), and a total of 28 litters over the 100 year period (generation length, 7 years).

Siberian tiger: Under the estimates that 0.7 EEJ are needed per pregnancy, these 28 litters will require 19 EEJ to ensure retention of at least 90% gene diversity during the next 100 years in the captive population.

Sumatran tiger: Under the estimates that 0.4 EEJ are needed per pregnancy, these 28 litters will require 11 EEJ to ensure retention of at least 90% gene diversity during the next 100 years in the captive population.

Further comments:

As stated above, the number of different donor males required for these 14 EEJ depends on the genetic value of the potential donor pool relative to the current and future living population. Ideally, the 28 litters would be sired by sperm from 28 males that are unrelated to the population (i.e., ideally the GRB should be a bank comprised only of wild-caught males). However, it may be unnecessary to use gametes from this many donors to achieve genetic goals. Fewer males, but more samples per male, may be adequate.

Strategy 5. Identification of specific males, and number of samples/male should be accomplished through pedigree analysis of potential donors. A *strategy that minimizes mean kinship among samples* in the GRB is recommended as an efficient and effective method for donor selection.

Comments:

These estimates should be considered the minimum number of males/samples based on the currently known average values of the various needed parameters. Establishing a secondary (i.e., back-up) bank requires doubling these numbers (to a minimum of 56 samples

split among two or more geographically separated banks). Considerations of additional risk factors, such as failure of a specific male's ejaculate to survive Cryopreservation and thawing, will add to the number of samples required.

Objective 3 The safety factor needed for the quality control procedures and for potential bank losses should be estimated.

Comments:

Sample storage duplication is required because the viability of cryopreserved germplasm and other living cells requires continuous storage in vacuum insulated refrigerators containing liquid nitrogen. Although reliable storage technology has evolved over the past 40 years, repository management requires continuous electronic monitoring and human intervention. There have been numerous failures with sometimes catastrophic results. Prudent stewardship of banked materials dictates the use of state-of-the-art repository facilities with restricted access, sophisticated electronic monitoring and redundant refrigerators, liquid nitrogen manufacturing (or storage) capabilities, and a backup electrical generator.

Strategy 6. Risks due to the need to store cryopreserved germplasm and other living cells at cryogenic temperatures (-196° C) for at least one century requires that at least one duplicate copy of all materials in the GRB be stored at a secondary site.

Strategy 7. Repositories should be located in geologically and politically stable locations. Storage location, repository monitoring, security, and other procedures must be reviewed at least annually to ensure continued compliance with minimum requirements.

Strategy 8. Quality control testing for cryopreserved spermatozoa will be conducted on each new deposit. Upon receipt of each new deposit of cryopreserved sperm, a sample will be selected at random from each donor male for thawing and *in vitro* viability assessment.

Criteria for tigers:

Tests will include assessments of post-thaw sperm motility, percent normal acrosomes, and ability to bind and penetrate heterologous (cat) zonae pellucidae *in vitro*. Cryopreserved semen will be considered available for AI only when the following criteria are met:

1. Post-thaw motility is >30%.
2. Progressive forward motion status is 3.0 (on a 0-5 scale; 0=least).
3. Percent normal acrosomes is >10%.
4. Positive zonae binding and penetration are found.

Samples not meeting these criteria will be stored and used for basic and applied research purposes.

Objective 4. A sampling strategy should be developed to guide selection of donor individuals to provide an optimal representation of the genetic variation from the living population to establish the GRB.

Comments:

This selection strategy involves removal of all females from the "living" population in the database (since only sperm-banking is being considered). In calculating the mean kinship of each potential donor to the GRB, the kinships to all living males (wild-caught and captive-born) are averaged. Animals with high mean kinship values are those with many relatives and lowest priority, whereas animals with low mean kinship values have few living relatives and highest priority. The iterative removal of males with the highest mean kinship is an important part of the strategy in that the mean kinship of the males remaining in the pool will change as each male is removed. This iterative selection scheme which removes animals from the pool available for banking until gene diversity can no longer be improved should provide the optimal or nearly optimal GRB for long-term preservation of gene diversity.

Strategy 9. The optimal strategy for selecting males for sperm banking from the captive population is through the use of mean kinship. The calculations of mean kinship during the iterative removal of males, as well as the various metrics summarizing the genetic variation preserved within the bank are calculated from a modified version of the GENES pedigree analysis software which is distributed with SPARKS.

Strategy 10. Additions to the GRB should be made whenever a re-analysis of the male living population and the presently banked males indicates that inclusion of a male not yet banked would result in a higher gene diversity of the bank. This selection strategy provides the flexibility to manage an effective GRB through the uncertain future pedigree of a population.

Objective 5. The fragmented wild populations should be managed as a panmictic population and to maintain >90% of estimated current gene diversity (for 100 years) in the overall population.

Strategy 11. For each subpopulation, the expected rate of loss of gene diversity and the number of AIs per generation and their source (from other subpopulations) that will be required for each subpopulation to maintain at least 90% of heterozygosity should be estimated.

Strategy 12. For the subpopulations, the numbers of AIs per generation required and the pattern of exchange from other subpopulations to maintain a population in which there is not significant differentiation among the populations should be estimated.

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Glossary

Allele: Single copy of a gene. Each diploid individual contains two copies of a gene or alleles - one at the same gene on each of the pair of chromosomes. For many genetic loci (typically 50% to 90%), all individuals of a population are homozygous for a single allele (i.e., the gene is monomorphic).

Cryopreservation: The process by which living gametes, embryos, cells and tissues are cooled and stored at low temperatures without deterioration of future viability or function.

Effective Population Size: The size of a randomly mating population of constant size with equal sex ratio and a Poisson distribution of family sizes that would (a) result in the same mean rate of inbreeding as that observed in the population, or (b) would result in the same rate of random change in allele frequencies (genetic drift) as observed in the population. These two definitions are identical only if the population is demographically stable (because the rate of inbreeding depends on the distribution of alleles in the parental generation, whereas the rate of gene frequency drift is measured in the current generation).

Founder: A wild-caught animal that contributed to the living, captive-born descendant population by breeding. An animal with unknown ancestry may or may not be a founder.

Gamete: A mature haploid reproductive cell that fuses with a gamete of the opposite sex to form a diploid zygote (or embryo). The male gamete is the spermatozoan and the female gamete is the oocyte.

Gene: The basic unit if inheritance by which hereditary characteristics are transmitted from parents to offspring. Each gene consists of a sequence of DNA nucleotides on a chromosome that typically codes for one protein or RNA molecule.

Genome: The complete set of an organism's genes.

Gene Diversity: The average heterozygosity expected over all genes in a population if reproduction were to occur by random mating.

Heterozygosity: The observed proportion of genes (across a genome or for one gene across a population) that are heterozygous (i.e. having two different alleles).

Mean Kinship: The kinship coefficient between two individuals is the probability that two alleles of a gene, one sampled from each individual, are identical because of descent from an ancestor common to both individuals. The mean kinship coefficient between all pairs of individuals in the population is equal to the proportional loss of gene diversity of the population since the beginning of the pedigree and is also the mean inbreeding coefficient expected among progeny produced by random mating.

Taxon: Any group of organisms to which a category in a taxonomic system (e.g., kingdom, phylum, class, order, family, or species) can be applied.