



ACADEMIC
PRESS

Cryobiology 44 (2002) 122–131

CRYOBIOLOGY

www.academicpress.com

Computer simulations to determine the efficacy of different genome resource banking strategies for maintaining genetic diversity[☆]

V.K. Harnal,^{a,b,*} D.E. Wildt,^a D.M. Bird,^{b,c} S.L. Monfort,^a
and J.D. Ballou^a

^a *Conservation and Research Center, Smithsonian National Zoological Park, Smithsonian Institution, Front Royal, Virginia, USA*

^b *McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Que., Canada H9W 3W3*

^c *Avian Science and Conservation Centre, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Que., Canada*

Received 3 August 2001; accepted 21 March 2002

Abstract

Genome resource banks (GRBs) and assisted reproductive techniques are increasingly recognized as useful tools for the management and conservation of biodiversity, including endangered species. Cryotechnology permits long-term storage of valuable genetic material. Although the actual application to endangered species management requires technical knowledge about sperm freezing and thawing, a systematic understanding of the quantitative impacts of various germ plasm storage and use scenarios is also mandatory. In this study, various GRB strategies were analyzed using the historical data from three managed populations of endangered species with varied pedigrees (Eld's deer, Przewalski's horse, and Sumatran tiger). The following types of sperm banks were assessed: (1) a "Wild Bank" consisting of sperm (i.e., genes) from 5 to 10 males unrelated to the managed population and to each other; and (2) a "Best Male" bank containing sperm from only the most genetically valuable males alive in the ex situ population at the time the bank was established. These different bank types were then used to evaluate the effectiveness of different bank usage frequencies. The efficiency of each scenario was assessed by examining the level of inbreeding and gene diversity in the population. Overall, a sperm usage frequency of five times per year was determined to be the most efficient and "wild banks" were highly successful at enhancing genetic diversity. The value of a GRB established from the ex situ population depends on how closely related the banked males are to future generations. A GRB will have significantly less benefit when banked males also produce many successful offspring, or when donors are already genetically over-represented in the population at the time of establishing the GRB. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Genome resource banks (GRBs); Endangered species; Genetic diversity; Inbreeding; Przewalski's horse; Eld's deer; Sumatran tiger

[☆] V.K. Harnal was supported by FCAR (Fonds pour la Formation de Chercheurs et l'Aide à la Recherche). The project was funded by Smithsonian Institution Scholarly Studies Program.

*Corresponding author. Fax: +514-398-7990.

E-mail address: vharnal@hotmail.com (V.K. Harnal).

A genome resource bank (GRB) is the organized collection, storage, and use of biomaterials, including germ plasm (e.g., sperm) [24]. Establishing GRBs in concert with assisted breeding techniques has potential for preserving genetic diversity of many endangered species managed ex situ. Soulé et al. [17] estimated that nearly 815 mammalian species will require the assistance of captive propagation programs in the next 200 years. Unfortunately, zoos only have space for accommodating approximately 100 mammalian species in populations of sufficient size to ensure long-term genetic and demographic viability [5]. For any given species, the organized cryopreservation of gametes, combined with the ability to reinfuse these genes into subsequent generations, would reduce the number of captive individuals required to maintain genetic diversity [24]. This would also increase the amount of resources (cage spaces, keeper time, etc.) available for other species requiring conservation attention [8]. The systematic cryopreservation and use of sperm would also permit extending the generation length of a population or individual indefinitely thereby reducing the loss of genetic diversity over a fixed time period [1]. Combined with assisted reproductive techniques (such as artificial insemination), GRBs could also eliminate the need to translocate animals between breeding institutions, while surmounting the difficulties associated with behaviorally incompatible mating pairs [21,22,24]. The availability of germ plasm from wild, free-living animals and its importation into zoos would also obviate the need to collect animals from nature to augment the genetics of ex situ populations [24].

The use of GRBs for species conservation and methods for selecting sperm donors have been examined previously [7,8]. Johnston and Lacy [8] assessed four strategies for selecting genetically valuable males whose genes should be banked: (1) all males in the population; (2) only living founder males and early generation male progeny; (3) males with minimum mean kinship values (MK; i.e., maximum theoretical gene diversity [3,7]); and (4) a group of males equal in number to those identified using strategy 2, but with minimum MK values. Each of these strategies was tested using demographic data collected from four North American captive populations (okapi, *Okapia johnstoni*; golden-headed lion tamarin, *Leontopithecus chrysomelas*; Siberian tiger, *Panthera tigris altaica*; and gaur, *Bos gaurus*) with distinctly different pedigree structures.

In terms of preserving genetic diversity, the four strategies were found to be ranked identically across all four of the species populations as follows: 1 > 3 > 4 > 2. The “All Male Bank” provided the best results, but nearly optimal retention of allelic and gene diversity was obtained by strategy 3. Since an “all male” bank may not always be feasible or practical, a more cost-effective GRB incorporating only a portion of the most valuable males (identified through minimizing MK) may achieve a level of genetic variation that is quite comparable.

Johnston and Lacy [7] also investigated the effectiveness of employing sperm banks to maintain the genetic diversity of the North American ex situ gaur herd. Gene diversity in the population was significantly enhanced through the frequent use of a GRB established from the captive herd and even more so by a GRB established from wild-born unrelated individuals. The more frequently the GRB was used (e.g., once every generation vs. once every other generation), the more diversity was retained. Their simulations, however, assumed random breeding in the population (which is not the case in captive breeding programs) and did not consider the specific structure of the gaur pedigree. Johnston and Lacy [7] suggested that GRB use would be more effective if decisions on when and how to use sperm were based on pedigree analysis (i.e., minimizing MK). Thus, optimal GRB strategies might depend on each specific case study and require examination of the population’s pedigree structure and demographics.

This study builds upon the work of Lacy and Johnston by using pedigree analyses and computer simulations to determine the efficacy of different GRB-semen banking strategies in the context of case studies and a population’s history, structure or pedigree. First, using historical data on the captive populations of three species (Eld’s deer, *Cervus eldi thamin*; Przewalski’s horse, *Equus przewalskii*; and Sumatran tiger, *Panthera tigris sumatrae*), retrospective analyses were conducted to determine if GRBs, established years in the past, would have increased genetic diversity in the current populations. Second, the effectiveness of different semen banking strategies was tested in these three species to determine which approaches would be most effective for maintaining genetically viable, managed populations. Third, the frequency with which cryobanked gametes were infused into these populations was varied to determine if frequent use of a GRB was always

superior for maintaining genetic diversity compared to less frequent use.

Methods

The captive populations

Adequate historical records exist for the North American Eld's deer population from 1976 to the present (M. Rodden, pers. comm.). There are fewer than 2000 free-ranging Eld's deer in nature with most in Myanmar being limited to the central plains, predominantly on the Irrawaddy Plain and including the Pegu or Sittang Plain located in the east [13,15]. The North American Eld's deer population originates from 15 potential founders, and approximately 120 individuals are maintained in North American zoos and breeding facilities [20].

The Przewalski's horse originates from Asia and has been bred in captivity since the early 1900s. Because the contemporary population is derived from only 13 founders, it is highly inbred [14]. The species is extinct in the wild, and the ex situ population consists of more than 900 individuals [19]. Although the Przewalski's horse is genetically distinct from the domestic horse (*E. caballus*), the two species are capable of interbreeding and producing fertile offspring [16]. Management of the Przewalski's horse is complicated, since domestic horse genes were incorporated into the population by a domestic mare in 1906 [2]. The managed population consists of two major lineages: (1) the Prague line that is comprised of descendants originating from the domestic mare and (2) the Munich line that does not contain any domestic horse genes [2]. The population is managed to maintain the purity of the Munich line (i.e., gene flow is permitted from the Munich line to the Prague line, but not in the opposite direction). However, for the purpose of the present study, the two lineages were combined.

The Sumatran tiger exists solely on the island of Sumatra, Indonesia, and predominantly in five major protected areas [23]. Evidence indicates that it may survive in all eight provinces and a total of 26 protected areas [23]. There are probably fewer than 400 tigers roaming within the five major protected areas. The most current compilation of the demographic distribution of the world's ex situ population indicates that approximately 240 Sumatran tigers exist in captivity [12,18].

The three captive populations used for the analyses had very different histories and pedigrees and therefore provide the opportunity to compare the effect of different GRB strategies on maintaining (or improving) genetic diversity. The captive Eld's deer population is much smaller than the Przewalski's horse population (by approximately 10-fold), but contains more genetic diversity and a much lower level of inbreeding. The Sumatran tiger population is highly inbred and intermediate in size compared to Eld's deer and Przewalski's horse populations.

The historical data on the captive populations were contained in studbook data files using SPARKS (Single Population Analysis and Record Keeping System) [6] and provided by the studbook keepers for these species [12,19,20].

Establishing GRBs

Our objective was to determine levels of inbreeding and genetic diversity in these three populations if GRBs had been established and used effectively in the past. Four types of hypothetical GRB scenarios were compared, two using captive males and two using wild-caught males.

For the ex situ male scenarios, a GRB was established for each species using males that represented the maximum genetic diversity possible at a particular time early in the history of the population (but when the populations were beginning to expand and grow). Dates chosen for establishing the banks were 1976 for Eld's deer (when sperm from six males represented the maximum gene diversity), 1960 for the Przewalski's horse (eight males represented), and 1970–1971 for Sumatran tigers (seven males represented). Donor males were identified from among those living at that time by selecting those males with the lowest mean kinship rankings until the maximum level of genetic diversity was “captured” in the GRB [8]. This resulted in different numbers of males being used to establish the GRBs in the different species. However, in each case the set of males identified represented the minimum set needed to maximize genetic diversity in the GRB. GENES software [9] was used for these calculations. In the scenario “Best Male 1” (BM1), banked semen was used once per year, whereas in the other scenario “Best Male 5” (BM5), semen was used five times per year.

For the scenarios using wild-caught males, two hypothetical banks were created using five and 10 “wild-caught” males, respectively. These males

were assumed to be unrelated to each other or to their ex situ contemporaries (i.e., they were considered to be new founders). In the scenario “Wild Bank 1” (WB1), semen from the five male bank was used to produce one offspring per year, whereas in the “Wild Bank 5” (WB5) scenario, semen from the 10-male bank was used to produce five offspring per year. These banks were “established” at the same time as the captive-male banks.

Use of the GRBs

Each of these hypothetical sperm banking scenarios was used for each species in the following way. Each year after the bank was formed, females that gave birth (as indicated in the historical studbook data) were sorted by mean kinship to identify top-ranked breeding females in the population. The top females (or top five females depending on the scenario examined) were then re-paired (i.e., had their mates changed in the historical pedigree) with genetically valuable males (i.e., low mean kinship) chosen either from the existing population or from the sperm bank being tested. Pairing of close relatives was avoided. Living males were considered along with banked males because there were situations in which living males were genetically more valuable and therefore more suitable as mates than semen from banked males (or vice versa). Because some offspring from re-paired matings died before reproducing (i.e., actual mortality data from studbooks were used), re-pairing did not always influence future population genetics. The pattern of sire replacement was repeated each year (at the frequency prescribed by the scenario chosen) through the most current year of a studbook. Pedigrees of the populations were updated annually. By only substituting sires of actual offspring in the pedigree, it was possible to test genetic impacts of using a GRB without modifying the demographic history of the population.

Monitoring the impact of the GRB

The GENES and SPARKS software were used in tandem to incorporate changes in the pedigrees and to monitor changes in genetic diversity over time. Two measures of genetic diversity were recorded: gene diversity and mean inbreeding. Gene diversity (GD) was defined as the proportional retention of expected heterozygosity in the captive population [11]. Inbreeding was measured as the

average inbreeding coefficient of animals alive at the end of a particular year. Gene diversity and average inbreeding were calculated from the pedigrees using the GENES software for each year after the GRB was established. These measures were also calculated for the actual populations over time (i.e., without the use of the GRB).

Value of an unused GRB: 100 MK scenario

An additional scenario (“100 MK”) was used to examine a slightly different question: If semen were collected and stored early in the history of these populations but not used, how valuable would it be today for increasing gene diversity and reducing inbreeding in the current population? This scenario was used to assess the relevant genetic importance of banked unused semen versus that of semen from extant individuals. The GENES pedigree analysis program automatically determines the best 100 successive pairings needed to maximize gene diversity in a population by iteratively selecting pairs with the lowest mean kinships [10]. One-hundred automatic pairings were performed for each species using the contemporary population of females and both the contemporary population of males plus the males represented in the sperm bank created for scenarios BM1 and BM5 above. The frequency of banked versus living males used in these pairings was indicative of the relative value of the GRB. Levels of gene diversity with and without the use of the GRB were also compared.

Results

The levels of gene diversity and inbreeding over time for each strategy were compared to those of the actual populations in Figs. 1–3. Some of the scenarios were terminated before 1996 because studbooks were not current through this time. Additionally, in some instances, scenarios with a usage frequency of five times per year were terminated prematurely because the genes of the banked males eventually became over-expressed (i.e., their genes no longer served to increase the genetic diversity of the managed populations).

Compared to the “actual” genetic parameters, all the GRB scenarios improved gene diversity and decreased inbreeding in Eld’s deer and Przewalski’s horse populations. The BM5 scenario did not enhance gene diversity or reduce inbreeding in the Sumatran tiger population beyond 4 years

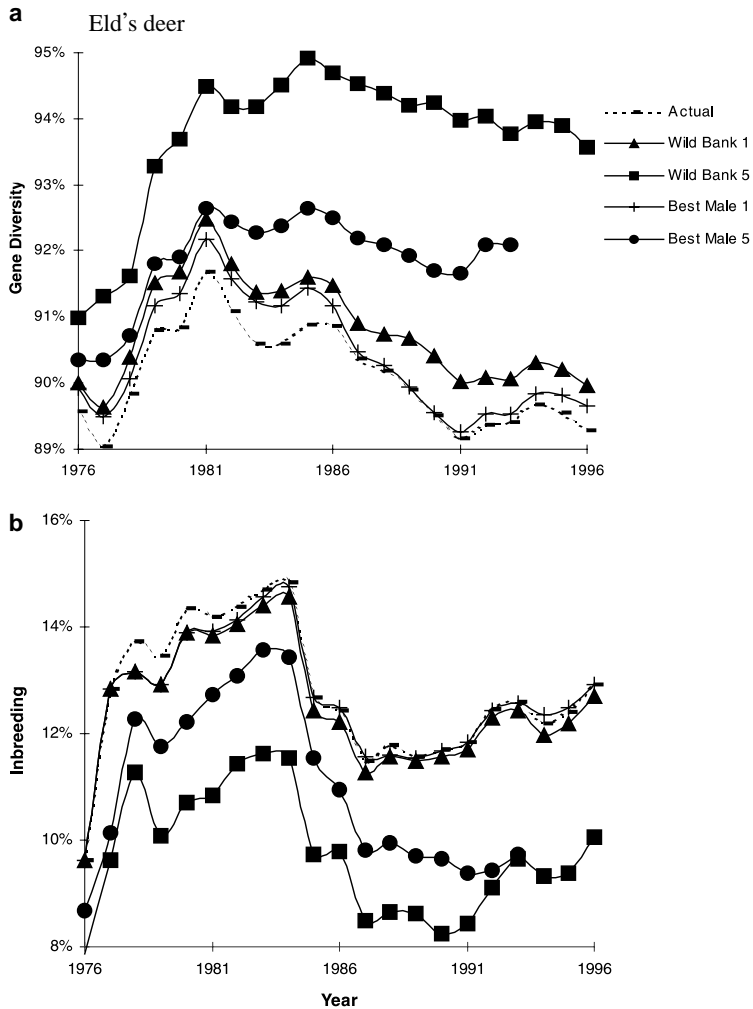


Fig. 1. Comparison of changes in gene diversity (a) and levels of inbreeding (b) through time for the managed Eld's deer population as a result of the application of different GRB strategies.

(Fig. 3), but was effective for more than 20 years in Eld's deer (Fig. 1) and Przewalski's horse (Fig. 2) populations. Separate examinations of the "Best Male" and "Wild Bank" scenarios across species revealed that five times per year was a more effective sperm usage frequency than once per year at strengthening genetic diversity (with the exception of the BM1 and BM5 strategies in the Sumatran tiger, see below). This trend was expected because higher yearly "dosages" of banked sperm provided the managed populations with a greater degree of new or rare genes.

Although the WB5 strategy was the most effective for enhancing gene diversity and reducing

inbreeding in all three managed populations (Figs. 1–3), there were species-specific differences related to which GRB strategy was the next most efficacious. For example, WB1 > BM5 in Przewalski's horse, BM5 > WB1 in the Eld's deer, and WB1 \cong BM1 in the Sumatran tiger.

Contrasting the genetic importance of banked but unused sperm with that of extant genes in living animals (100 MK scenario) revealed that the value of banked sperm increased with time for both Eld's deer and Przewalski's horse populations (Table 1). When banked males were incorporated into the managed populations and GENES was instructed to perform 100 MK-based

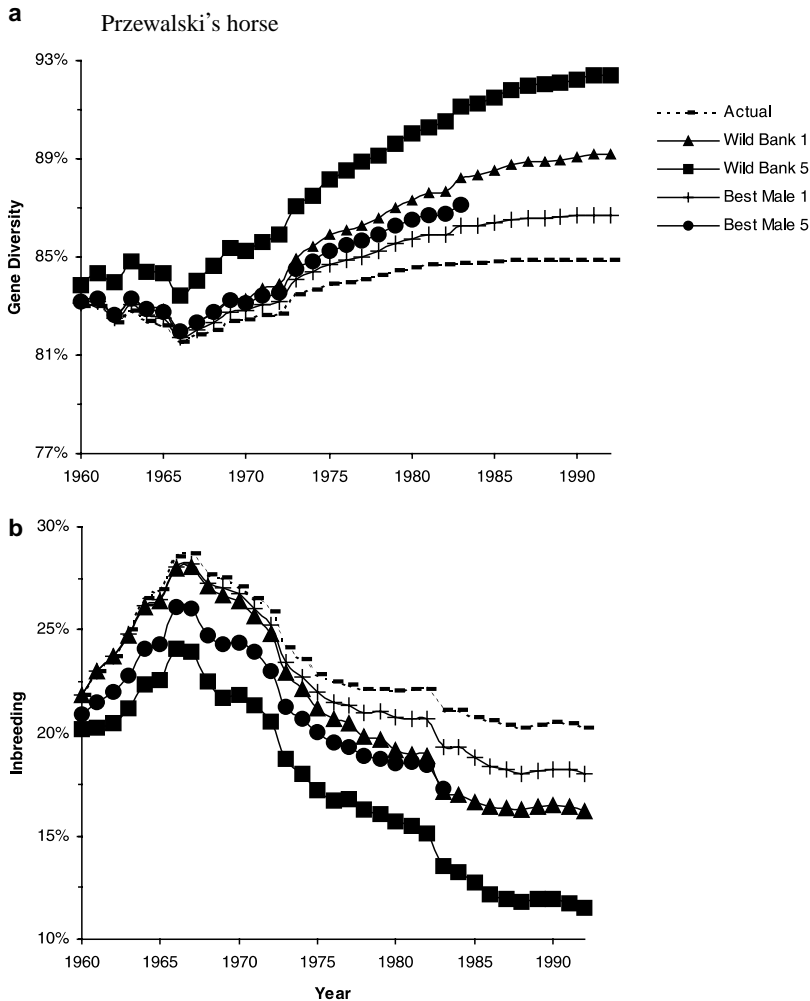


Fig. 2. Comparison of changes in gene diversity (a) and levels of inbreeding (b) through time for the managed Przewalski's horse population as a result of the application of different GRB strategies.

pairings, the pedigree analysis software selected the banked males for suggested pairings much more frequently than extant males in these two populations. With respect to Przewalski's horse, two of the banked males were selected for 100% of the proposed 100 pairings. Banked males were selected for 87% of the 100 suggested pairings in Eld's deer. The incorporation of the banked sperm into the managed populations also served to enhance gene diversity in the two populations (Eld's deer, 0.7%; Przewalski's horse, 0.5%). Conversely, banked semen was selected for only 26% of the 100 suggested pairings in the Sumatran tiger, but its incorporation into the managed population did increase gene diversity (0.1%).

Discussion

Johnston and Lacy [8] proposed that an ideal GRB would contain the following: (1) biomaterials from as few individual animals as possible; (2) the majority or all of the allelic diversity existing within the living population; (3) a considerable level of gene diversity; (4) genetic material available for the purpose of future genetic management; and (5) easily identified donors. Although the establishment of ideal GRBs may not always be feasible, it is critical that efficient GRBs are developed to counteract genetic changes in captivity (loss of genetic diversity through genetic drift, inbreeding, and genetic adaptation to the

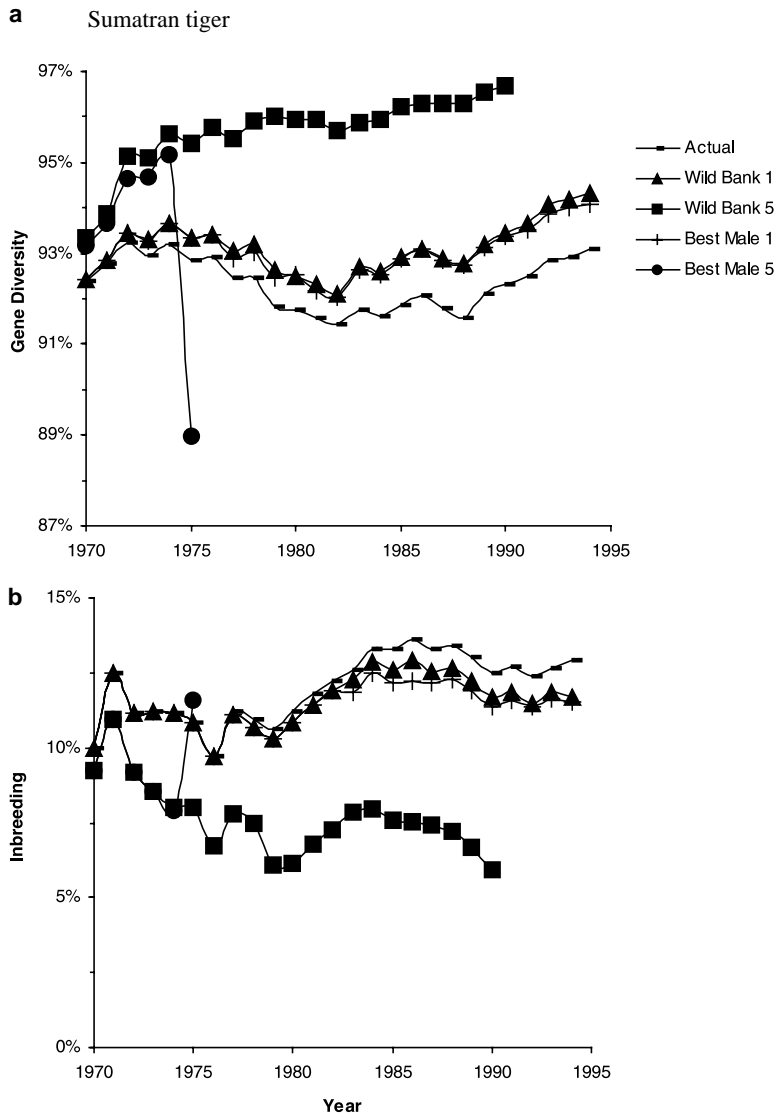


Fig. 3. Comparison of changes in gene diversity (a) and levels of inbreeding (b) through time for the managed Sumatran tiger population as a result of the application of different GRB strategies.

ex situ environment). Normally, the maintenance of large breeding populations is required to minimize genetic drift [7,9], but GRBs provide institutions with the option of housing smaller populations capable of retaining adequate levels of genetic diversity [1]. This is significant, especially since the combined enclosure space of the world's zoos could easily fit into an area similar in size to Brooklyn, New York [4].

Through the use of computer simulations, it is possible to investigate the effects of different

management strategies on the genetic composition of a population [9]. This study examined the effects of different GRB strategies on the genetic parameters of a given population. We believe that these simulations provide insight into which GRB approaches should be adopted for a particular species in future generations.

As expected, the WB5 (Wild Bank 5) scenario, founded with 10 males, greatly enhanced genetic diversity in all the populations assessed and performed significantly better than a wild bank based

Table 1

Results of the 100 MK scenario comparing the levels of gene diversity obtained with and without the incorporation of banked males into the respective populations

Bank	% Gene diversity	
	Without banked males	With banked males
Eld's deer	91.5	92.2
Przewalski's horse	85.3	85.8
Sumatran tiger	97.7	97.8

on only five animals used only once yearly (WB1). This is due both to the more frequent use and larger number of males contributing to the WB5 bank than to the WB1 bank. These results confirm the findings of Johnston and Lacy [7] that frequency of use and source of semen (wild vs. captive) are important considerations in GRB development. However, the value of the wild bank over time is inversely related to its use. With frequent use, the alleles from the wild bank become well represented in the population. Further inseminations using wild sperm then have decreasing impact on gene diversity over time. In the species modeled here, initially gene diversity increased rapidly relative to the actual population because sperm from the wild banks was unrelated to the ex situ gene pool (Figs. 1–3). Over time, however, the rate of divergence of the WB5 scenario from the Actual gene diversity decreased. Most of the differences between the levels of gene diversity in the WB5 and Actual scenarios were due to differences that accrued soon after initiation of the GRB strategy, and this was particularly apparent for the Eld's deer and Sumatran tiger. The WB5 scenario continued to accrue benefits for Przewalski's horse genetics through the 60-year test interval. Because of the relatively large size of Przewalski's horse population, even the addition of wild genes five times per year represented a relatively small percentage contribution compared to the smaller Eld's deer and tiger populations.

The effect of banking genetically valuable males originating from a managed population (BM1, BM5) was also examined, and the effectiveness of this strategy varied among species. For example, the BM5 strategy worked well for Eld's deer, only moderately well for Przewalski's horse and very poorly for the tiger. The success of this strategy in Eld's deer relates to: (1) donor males not being closely related to the future population

and (2) not having produced many offspring at the time the GRB was established. Thus, gene diversity increased and inbreeding decreased when these sperm were used. Importation of more founders after GRB establishment also added to the divergence between the ex situ and banked gene pools over time. In contrast, no new founders were added after forming the Przewalski's horse GRB, and all animals born subsequently were descendants of animals alive at that time. Our analyses suggested that Przewalski's horse GRB donors had descendants who were successful breeders, thereby attenuating the relative genetic contribution of donor sperm in subsequent generations. In Sumatran tigers, the BM5 bank substantially improved conditions over the first 4 years, but gene diversity and inbreeding deteriorated thereafter. The ineffectiveness of the BM5 strategy in tigers relates to the production of multiple offspring litters in this species, which more rapidly incorporated the GRB genes into the population and lead to the over-representation of GRB males in the ex situ gene pool. Furthermore, new founders (i.e., non-GRB animals) were regularly introduced into the ex situ population. Infusion of new founder genes would, in most instances, increase gene diversity and decrease inbreeding to a greater extent than using banked genes. It is possible that an interactive bank, updated every few years with wild genes, would prove to be highly beneficial for strengthening the genetic variation of a litter producing species such as the tiger.

It should also be noted that overall the BM5 scenario performed better than WB1, thus, revealing that a "wild bank" will not always be superior to a captive-generated bank. A wild bank will typically have more genetic diversity than the captive bank unless the wild males become over-represented in the captive population. However, as shown by these scenarios, more frequent use of the less variable captive bank may outweigh the advantages of limited use of the more variable wild bank.

This study examined the effect of continuous GRB usage throughout the captive management history of three species. An alternative strategy would be to delay GRB use until it is deemed vital for improving population genetic structure or recovering fitness in an inbred population. For example, a GRB held in reserve might be used when significant inbreeding depression was observed or to enhance the genetic diversity of animals for reintroduction to the wild. The "100 MK"

scenario illustrated that genes can become more valuable with time. Hence, it may be advantageous to store sperm from the most genetically valuable males of an extant population and to use it for offspring production at a later time (i.e., in 20–30 years). This strategy was slightly more effective for Eld's deer compared to the highly inbred Przewalski's horse. It is possible that storage periods >30 years would prove even more effective for populations like that of Przewalski's horse that have a vast pedigree and high levels of inbreeding.

The scenarios examined here show the relatively short-term effects of GRB usage. Longer-term effects will depend on the amount of stored sperm. Under ideal conditions, and an unlimited supply of stored sperm, levels of inbreeding and genetic diversity will equilibrate at the level of relatedness among the males whose sperm have been stored. Genetic diversity from all others will be lost to genetic drift over time but will be continuously reinvigorated by infusion of sperm from the GRB for those males represented in the GRB. However, if sperm supplies were limited, even with the GRB, the long-term effects will be the eventual loss of genetic diversity and an increase in inbreeding. The rate at which the population becomes inbred and loses diversity will depend on the frequency of use of the GRB and its level of genetic diversity, as has been shown in the simulations here.

GRBs have the potential to greatly enhance endangered species management and conservation, but much research is still required to develop the technology and protocols necessary to ensure the long-term storage of viable sperm. The simulations conducted in this study revealed that different species and/or populations will require different management strategies. One cannot simply assume that a GRB strategy that is efficient for one species will also be optimal for the genetic management of another species. The pedigree of a population must be examined carefully before delineating a specific GRB plan. The ultimate value of a GRB will depend on the genetic relationships between the GRB semen donors and the females alive in the extant population at the time when the semen is used. Clearly, the effectiveness of the GRB will be significantly diminished if all living females are descended from the banked males. This argues strongly for establishing banks using males that are genetically underrepresented in the population or males from unrelated (e.g., wild) populations. Likewise, breeding institutions must work together

to develop global GRB plans that allow adequate transfer of genetic material among breeding facilities to ensure that the correct sperm and females are identified [22–24]. Population geneticists must also work in concert with reproductive physiologists to investigate the numerous details involved with the cryopreservation and storage of gametes. If executed precisely, GRBs have the potential for ensuring the long-term survival of a substantial number of rare species.

Acknowledgments

The manuscript benefited significantly from helpful comments from two anonymous reviewers.

References

- [1] J.D. Ballou, Potential contribution of cryopreserved germ plasm to the preservation of genetic diversity and conservation of endangered species in captivity, *Cryobiology* 29 (1992) 19–25.
- [2] J.D. Ballou, Genetic studies of Przewalski's horses and their impact on conservation, in: L. Boyd, K.A. Houpt (Eds.), *Przewalski's Horse: The History and Biology of an Endangered Species*, State University of New York Press, Albany, 1994, pp. 75–113.
- [3] J.D. Ballou, R.C. Lacy, Identifying genetically important individuals for management of genetic diversity in captive populations, in: J.D. Ballou, M. Gilpin, T.J. Foose (Eds.), *Population Management for Survival and Recovery*, Columbia University Press, New York, 1995, pp. 76–111.
- [4] W.G. Conway, The practical difficulties and financial implications of endangered species breeding programmes, *Intl. Zoo Yb.* 24/25 (1986) 210–219.
- [5] W. Conway, Species carrying capacity in the zoo alone, in: *Proceedings, 1987 Am. Asso. Zoo Prk. Aquar. Annual Conference*, 1987, pp. 20–32.
- [6] ISIS. SPARKS (Single Population Analysis and Record Keeping System). International Species Information System, Apple Valley, MN, 1994.
- [7] L.A. Johnston, R.C. Lacy, Utilization of sperm banks to maintain genetic diversity in captive populations of wild cattle, in: D.L. Armstrong, T.S. Gross (Eds.), *Wild Cattle Symposium Proceedings*, Omaha, Henry Doorly Zoo, NE, 1991, pp. 107–118.
- [8] L.A. Johnston, R.C. Lacy, Genome resource banking for species conservation: selection of sperm donors, *Cryobiology* 32 (1995) 68–77.
- [9] R.C. Lacy, Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision, *Conserv. Biol.* 1 (1987) 143–158.

- [10] R.C. Lacy, GENES: a computer program for the analysis of pedigrees and genetic management of populations, Chicago Zoological Society, Brookfield, IL, 1992–1999.
- [11] R.C. Lacy, Clarification of genetic terms and their use in the management of captive populations, *Zoo Biol.* 14 (1995) 565–577.
- [12] P. Müller, International Sumatran Tiger Studbook, Leipzig Zoo, Germany, 1995.
- [13] W.J. McShea, P. Leimgruber, M. Aung, S.L. Monfort, C. Wemmer, Range collapse of a tropical cervid (*Cervus eldi*) and the extent of remaining habitat in central Myanmar, *Anim. Cons.* 2 (1999) 173–183.
- [14] O.A. Ryder, Przewalski's horse: putting the wild horse back in the wild, *Oryx* 22 (1988) 154–157.
- [15] R.E. Salter, J.A. Sayer, The brow-antlered deer in Burma—its distribution and status, *Oryx* 20 (1986) 241–245.
- [16] R.V. Short, The evolution of the horse, *J. Reprod. Fertil. Suppl.* 23 (1975) 1–6.
- [17] M. Soulé, M. Gilpin, W. Conway, T. Foose, The millennium ark: how long a voyage, how many state-rooms, how many passengers, *Zoo Biol.* 5 (1986) 101–113.
- [18] R.L. Tilson, T.J. Foose, F. Princee, Tiger Global Animal Survival Plan (GASP), IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, 1992.
- [19] J. Volf, Pedigree Book of the Przewalski's Horse, Zoological Garden Prague, Prague, Czechoslovakia, 1996.
- [20] C. Wemmer, North American Regional Studbook for Burmese Brow-Antlered Deer, National Zoological Park, Front Royal, VA, 1996.
- [21] D.E. Wildt, Genetic resource banks for conserving wildlife species: justification examples and becoming organized on a global basis, *Anim. Reprod. Sci.* 28 (1992) 247–257.
- [22] D.E. Wildt, Genome resource banking: impact on biotic conservation and society, in: A.M. Karow, J. Critser (Eds.), *Tissue Banking in Reproductive Biology*, Academic Press, New York, 1997, pp. 399–439.
- [23] D.E. Wildt, A.P. Byers, J.G. Howard, R. Wiese, K. Willis, S.J. O'Brien, J. Block, R.L. Tilson, W.F. Rall, Tiger Genome Resource Banking (GRB) Action Plan: Global Need and a Plan for the North American Region. IUCN-World Conservation Union Species Survival Commission's Conservation Breeding Specialist Group, Apple Valley, MN, 1995.
- [24] D.E. Wildt, W.F. Rall, J.K. Critser, S.L. Monfort, U.S. Seal, Genome resource banks: living collections for biodiversity conservation, *BioScience* 47 (1997) 689–698.