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Genome Resource Banking for Species Conservation: Selection of Sperm Donors

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The systematic banking of genome resources using cryopreserved germ plasm offers the opportunity to further conservation strategies of endangered species by assisting in the effective genetic management of captive populations. Cryopreserved germ plasm will allow indefinite preservation of the presently available gene diversity represented in either captive or wild populations. If properly utilized, genome resource banks have the potential to decelerate the loss of gene and allelic diversity in captive populations through reintroducing "original" genetic material through time to counter genetic drift. However, in order for any genome resource bank to be effective, strategies need to be developed to identify genetically valuable individuals to bank which will represent optimal gene diversity of the specific population. Four selection strategies were evaluated to identify individual donors from four North American captive populations representing differently structured pedigrees. The strategies consisted of selecting: (1) all males in the population ("All Male Bank"); (2) only living founders and early generation descendents ("Founder Method Bank," FMB); (3) males remaining after culling to minimize mean kinship ("Culled Male Bank 1"); and (4) males remaining after culling to minimize mean kinship, with the males reduced to the number in the FMB ("Culled Male Bank 2"). The effectiveness of each strategy was based on the comparison of genetic variation metrics in each bank with the genetic variation in the present living managed population. Although maximal retention of allelic diversity was achieved by banking genes from all living animals, nearly optimal retention of allelic and gene diversity was obtained by utilizing the selection strategy based on minimizing mean kinships. As a consequence, properly designed and utilized, genome resource banks can become effective tools for preserving gene diversity in future generations of living populations.

A fundamental problem facing captive and many wild populations is the loss of genetic diversity through random genetic drift (1, 8, 9, 17, 18). Theoretically, genetic drift can be controlled by the maintenance of large breeding populations in which all animals contribute equally to future generations. Also, in many cases, immigration of individuals into subpopulations can reduce genetic drift (11). In captive and, increasingly, in wild populations the number of breeding individuals is reduced and populations are isolated such that even short-term maintenance of genetic variation is placed in jeopardy. As recently as 5 years ago, the genetic goals of the American Zoos and Aquariums Species Survival Plans (SSP, designed to manage genetically and demographically self-sustaining captive populations) were defined to maintain at least 90% gene diversity (also termed the "expected heterozygosity", 7) for 200 years. During recent years, we have seen this genetic goal erode to 90% gene diversity for 100 years and, in several species, it is now possible to manage at 90% gene diversity for only 20–50 years. As more SSP programs develop, this downward trend will probably increase due to the lack of space and resources required to maintain any one species. Soulé *et al.* (19) have suggested that approximately 800 mammalian species will likely disappear unless they are maintained in captivity. Currently, there is enough space in zoos worldwide to house approximately

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100 mammal species in populations large enough to be genetically and demographically self-sustaining (6). Therefore, strategies need to be designed which will decelerate the loss of gene and allelic diversity in the captive populations while attempting to decrease space requirements for any given species. Genome resource banking (GRB) has the potential to accomplish this and complement the conservation strategies of both captive and, ultimately, wild populations by preserving original germ plasm indefinitely and reintroducing this material (as immigrants) through time to counter genetic drift (21, 22).

Ballou (4) has recently discussed the benefits of germ plasm cryopreservation (sperm, ova, embryos) for captive management programs. Cryopreserved germ plasm enables the captive program to: (1) maintain a population's original genetic variation indefinitely, (2) extend the generation length of either individuals or populations, and (3) decrease the number of individuals required to achieve the defined genetic goals. Long-term preservation of gene and allelic diversity will enable the population to retain the capacity for adaptive evolution. In addition, increasing generation length will provide fewer opportunities for losing genetic variation. The combined effect of preserving gene diversity and extending generation length is the ability to reduce population numbers while achieving genetic goals. Consequently, cryopreservation programs will allow additional spaces to become available for other critically endangered species.

While there has been much speculation on the effectiveness of gamete/embryo cryopreservation as a conservation tool (ranging from discussions of completely frozen zoos to dismissal of the concept), little effort has been made to evaluate quantitatively the role that reproductive technologies can play in wildlife conservation. Development of sampling and utilization strategies to guide selection of an optimal representation of the genetic and allelic diversity from any living population are required for a genome banking program. The usefulness of any GRB plan will ultimately depend on the selection of individuals used to establish the cryobank. In this paper, we present several strategies to select males whose genetic composition will comprise the foundation of a genome resource bank composed of sperm samples.

METHODS

Currently, there are more than 60 SSPs, with each being distinct with respect to the number of founders, founder representation, depth of pedigree, and population size. The populations selected to test the effectiveness of the different strategies for choosing individuals for a GRB were the cooperatively managed North American zoo populations of okapi (*Okapia johnstoni*, African ungulate), golden-headed lion tamarin (*Leontopithecus rosalia chrysomelas*, South American primate), Siberian tiger (*Panthera tigris altaica*), and gaur (*Bos gaurus*, Asian wild bovid). The studbooks, current through about mid-1993, were obtained from the respective studbook keepers as SPARKS (Single Population Animal Record Keeping System: ISIS) computerized databases. The pedigree of okapi in North America is relatively small (43 living animals), moderately shallow (ranging from wild-caught founders to a few fifth generation captive-born animals), and descends from a moderate number of founders (n = 25). The remaining three populations have similar living population sizes, ranging from 96 (golden-headed lion tamarins) to 125 (gaur). The golden-headed lion tamarin pedigree is shallow, representing founders through a few third generation captive-born animals, and descends from a moderate number of founders (n = 22). In contrast to the okapi and golden-headed lion tamarin pedigrees, both the Siberian tiger and gaur pedigrees are more complex and deep. The Siberian tiger pedigree is ex-

tensive, ranging from living founders to seventh generation captive-born, and descends from many founders (n = 40). The gaur pedigree is deep with approximately two-thirds of the population representing third to fifth generation to several seventh generation animals. Unlike the previously described populations, the gaur population descends from a very narrow founder base (n = 8).

Several of the pedigrees examined contain a few captive-born animals of unknown paternity and/or maternity. Although we did not undertake an exhaustive investigation of the circumstances surrounding each of these gaps in the pedigree, in each case it appeared as though the unknown parents were likely other animals within the pedigree, rather than animals unrelated to the known pedigree. For example, it is not known which of two possible males sired an okapi born in Europe, descendants of which subsequently were sent to North America and became incorporated into the SSP. To avoid overestimating genetic variation and, in particular, to avoid assigning higher genetic value to animals with partly unknown ancestries, the contributions of any genes that were not traceable to founders were omitted from the calculations. The algorithms for omitting unknown genes from kinship and inbreeding calculations are described by Ballou and Lacy (5) and are handled by the GENES software package (13).

GRB Selection Strategies Tested

Four strategies for selecting male donor animals for a GRB were evaluated through comparisons of various metrics of the genetic variation in each bank with the genetic variation in the present cooperatively managed populations. The first and most complete GRB tested, the "All Male Bank" (AMB), included sperm collected from all living males in the population. The second strategy tested a method proposed in the draft Tiger Genome Resource Banking Strategy document (23). This method, which we will term the "Founder Method Bank" (FMB), calls for the banking of sperm from (1) all living male founders, (2) up to two first generation progeny from dead founders or living female founders, (3) up to two second generation descendants from founders that are otherwise unrepresented in the GRB. This strategy endeavors to preserve, if possible, the genetic contributions of founders through early generation descendants (23).

The third and fourth strategies tested utilized the mean kinship (MK) of living animals to select males for sperm banking. The mean kinship of each animal is the mean of its coefficients of kinship to all living animals (3, 5). As normally used, MK is calculated by averaging only the kinships to captive-born (i.e., nonfounder) animals in the population, thereby providing a metric that summarizes and can help guide the preservation of the gene diversity within a captive propagation program. If founders were included in the calculation, then a population with many wild-caught individuals, but very poor and uneven breeding success, would appear to be in good genetic health. The purpose of selecting animals for a GRB, however, is to preserve as much of the genetic variation of the original wild-caught animals as possible, regardless of whether they have left descendants (and especially if they have not). Therefore, in calculating the mean kinship of each potential donor to the GRB, we averaged the kinships to all living males, wild-caught and captive-born. Animals with high values of MK are those with many relatives in the population and therefore lowest priority, whereas, animals with low values of MK have few living relatives and highest priority for genome preservation.

Both strategies utilizing MK involved the removal of all females from the "living" population in the database (since only sperm banking was considered) and then the iterative "culling" of males with the

highest MK. The objective of the culling process, in the absence of females, is to select males which represent maximum gene diversity to incorporate into the GRB. In the third strategy, "Culling Method Bank 1" (CMB1), males were removed from the pool of potential donors until the removal of another male would cause the gene diversity of the pool to decrease. The iterative culling of males with the highest MK is an important part of this strategy, because the MK of the males remaining in the pool will change as each male is removed. Males remaining are designated for semen cryopreservation. It may be possible to select a subset of males that will have a lower MK (i.e., will have more gene diversity), for example by using mathematical optimization techniques (20), but the iterative culling procedure can be employed easily with available computer programs for pedigree analysis and is likely to produce the optimal or nearly optimal set of males for maximizing gene diversity.

The last strategy for selecting males, termed "Culling Method Bank 2" (CMB2), continued the culling procedure of the CMB1 until the number of males remaining in the population matched the number selected for the FMB. In this way, the effectiveness of selecting males based on MK could be compared to the selection based on founders and early generation descendants (FMB), while holding constant the number of males and, therefore, the likely effort required to collect the sperm samples.

The calculations of MK during the iterative removal of males, as well as the founder contributions needed for the FMB and the various metrics summarizing the genetic variation preserved within each bank (see below), were all calculated from the GENES pedigree analysis software (13) which is distributed with SPARKS. In our use of the program, we included wild-caught animals in the MK calculations (as described above) and in calculations of the gene diversity retained within the captive population.

Measures of GRB Effectiveness

We compared the four GRB selection strategies described above, and the current living managed populations, for the following metrics commonly used for summarizing genetic variation within captive populations: (1) the number of founders represented; (2) the number of living founders represented within the bank; (3) the expected number of founder alleles surviving within the bank; and (4) the percent of the gene diversity of the source wild population retained within the bank. The number of founder alleles retained was determined with a "gene drop" (15) simulation of transmission of alleles through the pedigree, with the assumption that each founder carried two unique alleles at a hypothetical genetic locus. Gene diversity retained was determined by calculation of kinship coefficients between all pairs of animals (2). The proportion of gene diversity lost is equal to the overall mean kinship to the pedigree of the selected population (12, 14).

In addition to examining the genetic variation present in each population or GRB, we examined the variation that would be present if the population were propagated with a genetically optimal scheme for selecting mates. For each population or GRB, we iteratively selected 100 pairs to be mated and then assumed that each pairing would add a single living offspring to the population. For the living populations, pairs were chosen by selecting the male and the female with the lowest MKs, with the MKs recalculated each time a hypothetical offspring was produced. These calculations are carried out by the GENES software and are identical to the technique used by most SSPs to identify the genetically optimal pairings. For the four GRBs, the same iterative technique was used, except that the two males with the lowest MKs were chosen to be paired and produce an offspring.

This procedure was done to indicate both the approximate gene diversity that can be achieved by optimal genetic management of a population, or utilization of a gene bank, and the relative frequency with which each male or its stored semen would be used in a breeding program. Gene diversity will increase as hypothetical offspring are produced by this method, as the representation of founder alleles (those that have not been irretrievably lost from the population) will be made progressively more equal. By the time 100 pairs were selected, representation of founder alleles was quite even, as indicated by minimal further increases in gene diversity with subsequent pairings.

RESULTS

Table 1 shows the number of animals in each population and GRB and compares the metrics summarizing the genetic variation preserved within each bank. Also given in the table is the gene diversity that would be contained within each population, if it were "propagated" by the addition of 100 progeny produced by mating the optimal pairs of animals. (The number of founders and the retention of founder alleles are unaffected by propagation, as progeny can only contain alleles already present in their parents. Founder alleles can decrease when animals die.)

The CMB1 required the banking of sperm from many (52 to 72%) of the living males, while the FMB used fewer (15 to 42%) living males. The largest discrepancy with respect to the number of donors was with gaur, a very deep pedigree with no living founders, for which the FMB selected the smallest subset of males, while the CMB1 selected the largest proportion of the living males.

Three of the four populations examined (all but gaur), contained a wild-caught female with no living male descendants. These founders were subsequently lost from the GRB due to the inevitable exclusion of female gametes. In addition, the two GRB methods relying on mean kinships (CMB1 and CMB2) preserved some representation from each founder that contributed to the living male population, while the FMB lost representation from one or several founders in each case.

As the number of animals decreases, from the entire living population, to the GRBs, the founder alleles surviving declines as animals containing the only copies of some founder alleles are removed from the selected population. Across all four populations, the CMB1 retained almost all of the allelic diversity that was present in

TABLE 1
Comparison of the Current Living Populations and the Four Strategies Used to Select Donor Males

Bank	N	Nf	Lf	FAS	%GD	After 100 matings %GD
Okapi						
SSP	43	25	2	11.436	92.27	95.06
AMB	19	24	0	8.984	91.53	93.41
CMB1	12	24	0	8.508	93.00	93.28
CMB2	8	24	0	7.178	92.71	92.60
FMB	8	20	0	6.672	91.34	92.05
Golden-headed lion tamarin						
SSP	96	22	10	17.600	95.81	96.81
AMB	53	20	5	15.517	95.32	96.22
CMB1	28	20	5	15.009	95.81	95.99
CMB2	20	20	5	13.752	95.69	95.72
FMB	20	18	5	13.592	95.50	95.69
Siberian tiger						
SSP	122	40	4	19.259	95.60	97.01
AMB	61	39	2	17.771	95.64	96.41
CMB1	36	39	2	16.304	96.14	96.41
CMB2	18	39	2	12.431	95.88	95.88
FMB	18	36	2	10.788	95.13	95.36
Gaur						
SSP	125	8	0	6.534	89.11	89.64
AMB	39	8	0	6.112	88.08	88.55
CMB1	28	8	0	6.043	88.47	88.55
CMB2	6	8	0	4.121	85.12	85.03
FMB	6	7	0	4.021	84.49	84.52

Note. N, number of living animals; Nf, number of founders; Lf, number of living founders; FAS, founder alleles surviving; %GD, % gene diversity; SSP, population of managed males and females; AMB, all male bank; CMB1, culling method bank 1; CMB2, culling method bank 2; FMB, founder method bank.

the living males, while the FMB lost a moderate proportion of the founder alleles. The largest differential in allelic retention occurred with the deepest pedigrees (gaur and Siberian tiger), in which many founder alleles are present only in third and later generation descendants. In each population, the CMB2 retained slightly more founder alleles than did the FMB.

Gene diversity can decrease or increase as animals are removed from a population. Gene diversity is dependent upon both the allelic diversity retained within a population, which can only go down as animals are removed, and the evenness of the frequencies of the retained alleles, which can increase if animals with many kin (i.e., some of those from over-represented founders) are removed (12). The patterns of gene diversity in Table 1 illustrate complex determination by the structure of the pedigree. Gene diversity was lower in the living males than in the entire living population in the shallower pedigrees of the tamarins and okapis and, in the gaur, which keeps a lower proportion of males in the managed population. For the Siberian tiger SSP population, there is slightly more gene diversity in just the males than when the females are included (in part because more females than males happen to be descended from over-represented founders).

In each of the four managed populations, CMB1 had an increase in gene diversity relative to the entire population of males. (The method of producing this bank will ensure that this happens.) Except in the case of gaur, CMB1 also had equal or greater gene diversity than did the entire population. Gene diversity within the FMB varied from being slightly greater than the male population (AMB) in the tamarins, with many founders and first generation captive-born animals, to much less than the living male population (AMB) in the gaur. CMB2 always had a level of gene diversity between CMB1 and FMB.

Adding 100 hypothetical offspring to the living populations or GRBs revealed the varied potentials of the populations to achieve better gene diversity through optimal genetic management. The present living populations have the greatest capacity for improved gene diversity, as all animals are available for pairing. The scope for further improvement was limited in the case of the gaur, however, as it is already many generations removed from its few founders. Excluding females substantially reduced the opportunity for effective genetic management (even assuming, in the abstract, that males can mate with other males to produce offspring). CMB1 retained much or even all (in the case of the gaur and, almost, tigers) of the capacity to increase gene diversity that was present in the entire population of males. The FMB and the CMB2 were less able to produce through propagation most of the gene diversity present in the living males, presumably because the smaller number of males included in those banks provided lesser scope for further genetic management.

DISCUSSION

The ideal genome resource bank would include the following attributes: (1) gametes from as few animals as possible (to simplify logistics and reduce costs); (2) most or all of the allelic diversity present in the living population; (3) high gene diversity (perhaps even greater than the entire living population); (4) genetic material needed for future genetic management; and (5) donors easily and unambiguously identified. The sperm bank that provides maximal flexibility and long-term effectiveness will always be one that includes plentiful (and infinitely available) samples from every living male at the time the bank is created. In some cases, such an intensive strategy might be logistically feasible and desirable (for example, from a small captive population of a species which is extinct or nearly extinct in the wild). More often, however, the quality of a GRB will have to be compromised in order

to reduce costs and facilitate logistics. While one of the important advantages of GRBs is the opportunity they provide for efficient and low-cost preservation of genetic variation, the difficult issues of resource allocation that pertain to managing living animals (16) will affect GRBs as well.

Of the four strategies compared, their ranking with respect to retention of allelic diversity and with respect to the gene diversity achievable through propagation of the population was consistent across the four populations (AMB > CMB1 > CMB2 > FMB). The poor performance of the FMB in these metrics was especially notable considering that the FMB strategy was developed explicitly in an attempt to preserve founder allele diversity and the opportunity for future genetic management. Among the three banks that sampled only a subset of the male population, the ranking was consistent among the populations with respect to the gene diversity of the bank (CMB1 > CMB2 > FMB). The bank of all males (AMB) ranked second, third, or last in gene diversity relative to the selective banks.

It is instructive to examine the performance of each of the GRBs when applied to the four rather differently structured pedigrees. All do well when the pedigree is shallow, as in the case of the golden-headed lion tamarins. In deep pedigrees, when few early generation captive-born animals remain, however, FMB omits much of the allelic variation, gene diversity, and even all representation from some founders that are preserved within the other banks. In part, this is due to the few males selected for the FMB in such pedigrees, but CMB2, with the same number of males selected, always outperforms FMB.

While testing the effectiveness of these GRBs, we noted several differences between the banks in the case with which males are selected. First, FMB often does not lead to an unambiguous selection of males to be banked. If, for example, there are four living second generation descendants from an otherwise unrepresented founder, any two would be selected for FMB. Which descendants are included in the bank can affect the quality of the bank, however, as the other ancestors of these animals may be founders of varying degrees of representation in the bank. When we encountered such ambiguities, we generally selected the potential donors based on which we encountered first in the studbook. The exception was that for Siberian tigers, in which we selected the males for the FMB that were identified in the draft Tiger Genome Resource Banking document (23). Because animals with lower studbook number will usually be animals that were born earlier, they will often be the descendants that are fewer generations removed from the array of founders that contributed to them. Selecting these males for the FMB would seem to be in keeping with the intent of the FMB strategy and, we suspect, would often lead to better retention of genetic variation than would selection of alternative donors that were born later. Another potential difficulty in selecting animals for the FMB arose in that cross-generational matings are common in each of the pedigrees, making assignment of captive-born animals to first, second, and later generations confusing and not well defined. The F1, F2, etc. notation so commonly used in the captive breeding community should probably be reserved for its original use, which is identifying the descendant generations produced by crossing genetic strains, and not awkwardly applied to ill-defined generations of captive breeding. With respect to each founder that contributed to a living animal, the number of generations in captivity can usually be specified, allowing determination of the FMB, but such is not always the case. In the gaur pedigree there are inbred animals that are second, third, and fourth generation descendants from a single founder. Although these descendants contain a high propor-

tion of alleles from that founder (some in multiple copies), it is not clear whether they should be selected to represent that founder's genes in the sperm bank. In trying to apply the method, we treated animals with 50% or more of their genomes derived from a founder as if they were offspring (although they may be both offspring and grandchildren or other descendant relatives), and we treated animals with 25 up to 50% of their genes from a founder as if they were second generation descendants. We are aware of no computer program presently available that would facilitate the selection of males for a FMB, but one could be readily developed if the ambiguities identified above were resolved.

The selection of the males from which to sample gametes is only the first step in using a GRB to assist with the conservation of rare and endangered gene pools. At or near the outset of collecting, the amount of semen to collect from each male must also be determined. Whether to continue to add samples to the GRB needs to be decided as soon as future generations become available for collection. Finally, the optimal strategy for utilizing the stored gametes in a breeding program must be developed. Some of these issues were addressed in a preliminary way by Johnston and Lacy (10). Without trying to address each of these issues fully here, we can indicate some considerations that arose from our examination of strategies for establishing GRBs.

The relative amount of gametes to collect from each donor to a GRB can be determined, as was done in the draft Tiger Genome Resource Banking document (23), by observing how often each male would be used if a large number of pairings were to be selected based on minimizing mean kinships (with iterative recalculation of mean kinships each time a hypothetical offspring is produced). In the pedigrees we examined, the relative frequency with which each male was selected for breeding largely stabilized during the selection of 100 pairs of males to produce hypothetical offspring. The total number of sperm samples collected would depend on the strategy for using the samples in future breedings.

As long as gametes from only one sex are collected, there can be substantial benefits from managing a GRB as an open bank that continues to be augmented by collection from future generations. Clearly, any alleles present only in living females can be preserved in a sperm bank only if gametes are collected from their male descendants. This can be especially important in cases such as the golden-headed lion tamarin, okapi, and Siberian tiger, each of which contain wild-caught females with no male descendants. Even when there are no valuable females lacking male relatives from which sperm can be collected, there will likely be advantages to monitoring the genetic value (via mean kinship) of later generations and collecting semen from any living male that becomes more valuable genetically than are some of the males already in the bank. In the absence of such active management of the sperm bank, over time the captive population will necessarily become, at best, a mirror of the original bank, while the genetic contributions of the females present when the bank was created will all be lost. An actively managed bank open to the addition of samples in future generations can theoretically (and, in fecund organisms, practically) capture over time almost all the genetic variation available in the original males and females (through their male descendants). In practice, additions to a GRB could be made whenever a reanalysis of the optimal subset of males from among the living males and the presently banked males indicated that inclusion of a male not yet banked would result in a higher gene diversity of the bank. Such a strategy provides the flexibility to manage an effective GRB through the uncertain future pedigree of a population. For example, if the descendants of some

founders breed and survive poorly, the changing founder representations in the pedigree could be managed responsively to ensure the best possible preservation of gene and allelic diversity.

The optimal use of a GRB will be difficult to resolve fully, as it involves consideration of the management of multiple generations in a pedigree yet to occur (in contrast to the one-time analysis of a fully known pedigree needed to initiate a GRB). Johnston and Lacy (10) used simulation modeling to examine the effectiveness of several possible strategies for using a sperm bank, but their analysis assumed random (and therefore less than fully effective) selection of males to be banked and future utilization sperm within a breeding program. We have begun investigation of the effectiveness of strategies that use mean kinship for optimally developing and then utilizing GRBs to preserve genetic variation in a captive population.

CONCLUSION

Increasingly, "assisted" reproductive technologies are becoming available to allow the use of genome resource banking to improve the efficiency with which captive propagation programs can preserve genetic variation of rare and endangered fauna. Techniques very similar to those presently used by SSPs for selecting mates, based on mean kinship, can be easily employed to produce an effective gene bank. Maximal retention of allelic diversity is achieved by banking genes from all living animals, but nearly optimal retention of founder alleles is obtained by a selection based on minimizing mean kinships with the GRB. An iterative selection scheme that culls animals from the pool available for banking until gene diversity can no longer be improved should identify the remaining animals as optimal or nearly optimal for inclusion into the GRB for long-term preservation of gene diversity. If logistics, costs, or other factors require that gametes from fewer animals be banked, the same procedure for using mean kinships can be employed to determine a subset of animals for banking that is as effective as possible given the number of animals whose gametes are to be incorporated into the bank. Whatever strategy is chosen for establishing a GRB, it is important to assess the effectiveness of the bank to be created by various measures of the genetic variation contained within it.

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