

A Comparison of Strategies for Selecting Breeding Pairs to Maximize Genetic Diversity Retention in Managed Populations

JAMIE A. IVY AND ROBERT C. LACY

From the Department of Life Sciences, San Diego Zoo Global, San Diego, CA 92112-0551 (Ivy);
Department of Conservation Science, Chicago Zoological Society, Brookfield, IL (Lacy)

Address correspondence to Jamie A. Ivy at the address above, or e-mail: jivy@sandiegozoo.org.

Abstract

Captive breeding programs aim to maintain populations that are demographically self-sustaining and genetically healthy. It has been well documented that the best way for managed breeding programs to retain gene diversity (GD) and limit inbreeding is to select breeding pairs that minimize a population's average kinship. We used a series of computer simulations to test 4 methods of minimizing average kinship across a variety of scenarios with varying generation lengths, mortality rates, reproductive rates, and rates of breeding pair success. "Static MK Selection" and "Dynamic MK Selection" are 2 methods for iteratively selecting genetically underrepresented individuals for breeding, whereas "Ranked MK Selection" and "Simultaneous MK Selection" are 2 methods for concurrently selecting the group of breeding individuals that produce offspring with the lowest average kinship. For populations with discrete generations (24 tested scenarios), we found that the Simultaneous and Ranked MK Selection methods were generally the best, nearly equivalent methods for selecting breeding pairs that retained GD and limited inbreeding. For populations with overlapping generations (198 tested scenarios), we found that Dynamic MK Selection was the most robust method for selecting breeding pairs. We used these results to provide guidelines for identifying which method of minimizing average kinship was most appropriate for various breeding program scenarios.

Key words: captive breeding, conservation, gene diversity, inbreeding, kinship, simulation

Captive breeding programs aim to maintain populations that are both demographically self-sustaining and genetically healthy (Foose and Ballou 1988; Hedrick and Miller 1992; Lacy 1994; Ballou and Lacy 1995). From a genetic perspective, the specific goals of captive population management commonly are to retain gene diversity (GD), which is indicative of a population's future adaptive potential (Falconer 1981), and limit the accumulation of inbreeding, which can reduce fitness (Lacy et al. 1993; Crnokrak and Roff 1999). It has been well demonstrated that the best way to meet these genetic goals is to minimize the average kinship (i.e., coancestry) in a population (Ballou and Lacy 1995; Fernández and Toro 1999; Sonesson and Meuwissen 2001). The kinship (f) between 2 individuals is the probability that 2 alleles at a given locus, 1 randomly drawn from each individual, are identical by descent from a common ancestor (Falconer 1981). An individual's mean kinship (\overline{mk}) is the average of pairwise kinships between that individual and all living individuals in the population

including itself (Ballou and Lacy 1995). Mean kinships provide a measure of current genetic value to the population; animals with lower \overline{mks} have fewer relatives, thus, their genes are more valuable for retaining GD. In general, breeding animals with low \overline{mks} minimizes the average kinship within a population.

A population's average kinship can be managed through breeding recommendations that consider both the individuals that should produce offspring (parental contributions) and how those individuals should be arranged in breeding pairs (mating scheme). A variety of methods for minimizing a population's average kinship have been proposed for both discrete and overlapping generations; some determine parental contributions and mating scheme simultaneously (Ballou and Lacy 1995; Fernández et al. 2001), whereas others use a two-step method that determines parental contributions first and mating scheme second (Fernández and Caballero 2001; Sonesson and Meuwissen 2001). Many of the methods designed to minimize a population's average

kinship share 1 characteristic that complicates their application to a majority of captive breeding programs—when parental contributions are determined, it is assumed that the exact contributions desired are successfully obtained (i.e., all pairings are successful and produce the designated number of offspring). Although some methods have been tested under models that incorporated limited allowances for reproductive failure or the production of nonoptimal parental contributions (Fernández et al. 2003), none of the cited methods for minimizing a population's average kinship have been rigorously tested under stochastic simulations that closely model a variety of scenarios that realistically represent a majority of wildlife breeding programs.

The Association of Zoos and Aquariums (the regional zoo and aquarium association in the United States) currently has more than 400 captive breeding programs that regularly receive management and breeding recommendations (Long et al. 2011). Similar collaboratively managed breeding programs are maintained by the European Association of Zoos and Aquariums, the Zoo and Aquarium Association in Australasia, and other regional zoo associations. Thus, given the number of breeding programs managed by these institutions, the commonalities shared by these programs should be considered when modeling population management. Most zoo-based breeding programs aim to maintain populations that are demographically self-sustaining in addition to being genetically healthy. To maintain demographic stability, the number of offspring that need to be produced in a given management cycle (generally a year) is usually determined by target population size and the expected proportion of a population that will need to be replaced due to loss (mortality, reintroduction, transfer outside the managed population, etc.). Then, the number of breeding pairs needed to produce that number of offspring is based on expected reproductive success. Thus, population managers first focus on the number of breeding pairs needed to meet demographic needs, then decide which individuals should be paired to meet genetic goals. The number of breeding pairs needed in each management cycle is sensitive to a number of factors, but mortality rates, reproductive rates, generation lengths, and rates of breeding pair success are 4 key life history characteristics that drive this variable for many managed breeding programs.

We used a series of computer simulations to test 4 methods designed to minimize average kinship in captive populations: “Static MK Selection,” “Dynamic MK Selection,” “Ranked MK Selection,” and “Simultaneous MK Selection.” Two of the methods tested, Static MK Selection and Dynamic MK Selection, are variants of the *mk* approach described by Ballou and Lacy (1995) to iteratively select genetically underrepresented individuals for breeding. These 2 methods have historically formed the basis for breeding pair selection in the PM2000 software package (Pollak et al. 2002) used to guide many captive breeding programs around the world. The remaining methods, Ranked MK Selection and Simultaneous MK Selection, represented 2 approaches for selecting the group of breeding individuals that produce a set of offspring with the lowest average kinship.

Simultaneous MK Selection is a modification of the “mate selection procedure” described by Fernández et al. (2001), whereas Ranked MK Selection is a method for minimizing average kinship that has not before been widely applied in management but was used in the simulations of Rudnick and Lacy (2008; their Simulation 2). We modeled both discrete and overlapping generations, as well as numerous combinations of varying mortality rates, reproductive rates, generation lengths, and rates of breeding pair success. Proportional GD and the average inbreeding coefficient were used to compare the performance of all methods tested.

Materials and Methods

Computer simulations were used to test 4 methods of minimizing kinship in captive populations. For comparative purposes, we also tested a random selection of breeding pairs. Simulations were designed to model standard wildlife breeding programs, although the following simplifications were incorporated: simulations ran on a yearly timestep, simulated populations were dioecious and maintained at approximately 100 individuals (a common size for zoo-based breeding programs; Long et al. 2011), and monogamous breeding pairs were selected once a year (with pairs reassigned between years). Numerous factors may impact which breeding pair selection method performs best at attaining the genetic goals of a breeding programs. Thus, the following focal parameters were varied across simulations: mortality rate, reproductive rate, generation length, pairing success rate, and reproductive success rate. A discussion of the 4 breeding pair selection methods is followed by a detailed description of the general simulation and its varying parameters.

Methods for Minimizing Kinship

Static MK Selection

The first method for selecting breeding pairs to minimize kinship used a static list of *mks* to rank animals by their genetic value. At each timestep, all individual *mks* were calculated and 2 sex-specific lists were created that ranked individuals from lowest to highest *mk*. In general, the male and female with the lowest *mks* were paired, followed by the male and female with the next lowest *mks*. This process continued until the specified number of breeding pairs was created. Because close relatives exhibit similar *mks*, any breeding pair that exhibited an *f_g* greater than the average *f* in the population was rejected to avoid close inbreeding. If a pair was rejected, all remaining unpaired females were evaluated in order of *mk* (from lowest to highest) to determine if one was a suitable match for the male of the rejected pair. A pair was made if a suitable female was found. If a suitable female was not found that male was removed from the pool of potential breeders and an attempt was made to pair the next male in the *mk* list (i.e., the male with the next lowest *mk*).

Dynamic MK Selection

The second method for selecting breeding pairs to minimize kinship used a dynamic list of *mks* to rank animals by their genetic value. At the beginning of the pairing process, each

individual's mk was calculated and the male and female with the lowest mk s were paired. After a breeding pair was selected, the pair produced hypothetical offspring (litter size determined as described below) that were temporarily added to the kinship matrix to allow adjustment of the resulting genetic value of possible breeders. The prospective offspring remained in the kinship matrix throughout the pairing process and were included in all subsequent mk calculations, however, they could not be selected to breed. After offspring from the selected breeding pair were added to the population, all mk s were recalculated. The male and female with the new lowest mk s were paired, and the process continued until the desired number of breeding pairs was created. To avoid close inbreeding, the inbreeding restriction described in the Static MK Selection method was imposed on breeding pairs. At the end of pair selection, all hypothetical offspring that had been created for the dynamic mk calculations were removed from the kinship matrix.

Ranked MK Selection

The third method for selecting breeding pairs to minimize kinship used ranked lists of individuals to identify the group of breeding pairs that would produce offspring with the lowest average kinship. The approach ranked males and females into separate sex-specific lists, and the lists were used to select breeding pairs after all possible parents were ranked. Ranked lists were created from the pool of all possible parents through the following process. 1) The individual with the highest mk was removed from the pool of possible parents and placed in its sex-specific list. 2) The mk s of all unranked individuals remaining in the pool of possible parents were recalculated. 3) The individual of the opposite sex with the highest mk was removed from the pool of possible parents and placed in its sex-specific list and the mk s of all unranked individuals remaining in the pool of possible parents were recalculated. 4) Steps 1–3 were repeated until all possible parents were moved to the sex-specific lists. As individuals were added to a list, they were placed just above the previous individual that had been added to that list. Thus, lists were populated from the bottom to the top. If a population exhibited an unequal sex ratio, individuals of the overrepresented sex were added to the appropriate list by the same process as described above, until an equal number of males and females remained in the pool of possible parents. Then, Steps 1–3 continued as described.

Breeding pairs were selected after all possible parents were added to their sex-specific lists. In general, the top-ranked male (the one added last in the above iterative procedure) was paired with the top-ranked female. The second-ranked male was then paired with the second-ranked female, and pairing continued in this fashion until a specified number of breeding pairs was reached. To avoid close inbreeding, the inbreeding restriction described in the Static MK Selection method was imposed on breeding pairs.

Simultaneous MK Selection

The fourth method for selecting breeding pairs to minimize kinship was a modification of the “mate selection procedure”

described by Fernández et al. (2001). The mate selection procedure was designed to optimize the production of a specific number of offspring, rather than the selection of a specific number of breeding pairs. In the original model, the breeding pairs selected by the optimization algorithm were assumed to produce exactly the number of offspring desired, as might be approximately attained in breeding programs for domestic livestock. To make the mate selection procedure compatible with our approach to modeling wildlife breeding programs, we modified the procedure so that the selection of breeding pairs and the production of offspring were decoupled. The modified procedure produced a list of breeding pairs, which were subsequently used by the general simulation to produce offspring through an approach that incorporated realistic stochasticity.

The modified mate selection procedure used simulated annealing (Kirkpatrick et al. 1983; Press et al. 1992) to minimize the function $\overline{mk} + \gamma\overline{F}$, where \overline{mk} was the average mean kinship of the offspring produced by the selected breeding pairs, \overline{F} was the average inbreeding coefficient of the offspring, and γ was a weighting factor. For the purposes of this investigation, γ was always equal to 0.01, so retaining long-term genetic diversity was given higher priority than avoiding current inbreeding. The simulated annealing process incorporated a Metropolis Monte Carlo algorithm and minimized the function by the following process: 1) A specified number of breeding pairs was randomly selected and all pairs produced a number of offspring equal to the mean of the modeled reproductive rate. The current solution (i.e., set of breeding pairs) was used to evaluate the function, with the value of the function representing the “energy” of the solution. 2) An alternate solution was generated by randomly changing a small number of breeding pairs, new offspring were produced, and the function was reevaluated. 3) If the energy of the alternate solution was lower, the alternate solution was accepted. To avoid local minima, a less optimal solution was accepted with a probability of $\Omega = \exp(-\Delta/T)$, where Δ was the difference in energy between the alternate and current solutions and T was the value of the current “temperature” (a measure of the degree of nonoptimality considered acceptable to allow escape from local minima). If an alternate solution was accepted, it became the current solution. Otherwise, the current solution remained unchanged. 4) Steps 2–3 were repeated for 100 repetitions (i.e., Monte Carlo steps). 5) Steps 2–4 were repeated for 100 temperatures. The starting value of the temperature (T) was 0.7, and this value was reduced by a factor of 0.9 for each of the subsequent temperature iterations. As T decreased, it was harder to accept an alternate solution that was worse than the current solution. Furthermore, as fewer alternate solutions were accepted, fewer breeding pairs were changed to create the alternate solution; at a given temperature, the number of altered breeding pairs was $10x + 1$, where x was the proportion of alternate solutions accepted at the previous temperature. The solution of breeding pairs that exhibited the lowest energy (i.e., the best solution to the

function) during the simulated annealing process became the list of breeding pairs.

During simulation development, we investigated the convergence of the simulated annealing algorithm, or the ability of the algorithm to settle on a single solution, by varying the number of Monte Carlo steps and temperatures under different model parameter sets then graphing the energy of the updating solution as it changed over time. We found that 100 Monte Carlo steps and 100 temperatures were sufficient to ensure that the simulated annealing algorithm successfully converged across a series of randomly tested parameter sets (data not shown). Thus, rather than optimizing the algorithm for every parameter set tested, we used what we considered to be robust algorithm settings across all modeled scenarios.

Simulation Overview and Parameters

- 1) An initial population of 30 unrelated individuals was created with an equal sex ratio. Ages for the starting individuals were distributed evenly among all possible age classes, up to a maximum specified age. Individuals could breed starting in the first timestep after their birth.
- 2) A list of breeding pairs was generated according to one of the methods being tested. The number of offspring (N) needed to reach or maintain a target size of 100 (T) was calculated from $N = (a + b + c) \times (1 + Q_0)$, where a represented the deficit between the current number of individuals and the target size ($T - n$), b represented the number of individuals that had reached a specified maximum age and would be removed from the simulation at the end of the timestep, c represented the number of individuals >1 timestep old that were expected to be lost to mortality during the timestep (“adult” mortality; $Q_{1+} \times (n - b)$), and Q_0 incorporated expected offspring mortality prior to the end of the timestep (“infant” mortality). The number of breeding pairs needed was subsequently calculated from $N/rmN/rm$, where r was the probability that a breeding pair successfully reproduced and m was the mean number of offspring produced by successful breeding pairs. If the specified number of breeding pairs could not be made because the population size was too small or the sex ratio was too skewed, the maximum number of pairs possible was made instead.
- 3) A specified probability of “pairing success” was applied to each selected breeding pair to model the reality that a set of breeding recommendations cannot always be explicitly followed due to unforeseen factors (unexpected mortality, medical conditions, behavioral issues, etc). Three rates of pairing success were modeled: 100%, 80%, and 50%. Breeding pairs were scored as successes or failures, then all individuals of unsuccessful pairs were returned to the pool of potential breeders and a number of alternate breeding pairs equal to the number of failed pairs were randomly selected. The addition of these randomly selected pairs was necessary to retain the demographic robustness of the simulated populations but was also used to incorporate the occurrence of some nonrecommended breeding pairs. The final set of selected pairs included the “successful” pairings identified at the beginning of the step, plus the randomly chosen pairs selected as alternates for the “failed” pairings.
- 4) A specified probability of “reproductive success” was applied to each selected breeding pair. Three rates of reproductive success were modeled: 100%, 80%, and 50%. For each breeding pair that was reproductively successful, the number of offspring produced was drawn from a Poisson distribution with a specified mean. The Poisson distribution was zero truncated so that some number of offspring were always produced. To avoid drawing an arbitrarily large number of offspring, the Poisson distribution was also truncated to a specified maximum. The following 3 reproductive rates were modeled: 1) each reproductively successful pair produced exactly 1 offspring (low reproduction), 2) a mean of 3 and a maximum of 5 offspring produced per reproductively successful pair (intermediate reproduction), and 3) a mean of 6 and a maximum of 10 offspring produced per reproductively successful pair (high reproduction). Each offspring was assigned one sex or the other with equal probability.
- 5) After all offspring were produced, the kinships between all individuals currently in the population were quantified and recorded. For relationships to be tracked through time, a matrix of all possible pairwise f_s (including an individual's f with itself) and each individual's inbreeding coefficient (F , equal to the kinship between the individual's sire and dam; Falconer 1981) were calculated each timestep. Pairwise f_s were calculated as $f_{xy} = 0.5(f_{xs} + f_{xd})$, where the subscripts s and d refer to the sire and dam of individual y (Falconer 1981).
- 6) A probability of mortality was applied to each individual. Two mortality rates were specified for each simulation; an “infant” mortality rate (Q_0) was applied to newly created offspring of age class 0 and an “adult” mortality rate (Q_{1+}) was applied to individuals in and above age class 1. Two Q_0 rates were modeled, 0.15 and 0.30, and two Q_{1+} rates were modeled, 0.05 and 0.10. These rates are typical of those observed in large mammal species, as well as many wildlife breeding programs.
- 7) Individuals were aged 1 timestep, with individuals older than a specified maximum lifespan removed from the population. Three generation lengths were modeled; individuals could remain in a simulation for a maximum of 1, 5, or 20 timesteps, which resulted in mean generation lengths, calculated as the average age of mothers, of approximately 1.0, 3.6, and 13.4 years. For scenarios with a maximum lifespan of 5 years, approximately 20% of the population was replaced on a yearly basis. For maximum longevity of 20 years, approximately 5% of populations were replaced on a yearly basis.
- 8) Steps 2–7 were repeated for 100 timesteps. Genetic variation and inbreeding were evaluated on a per timestep basis, immediately following step 7. Inbreeding was measured as the average inbreeding coefficient (\bar{F}) and

genetic variation was measured as proportional GD. GD was calculated as $1 - \overline{mk}$, where \overline{mk} was the average mean kinship in the population (Ballou and Lacy 1995). Although genetic variation can be quantified in a number of ways, proportional GD (i.e., average expected heterozygosity) is a common measurement (Wright 1969; Nei 1973; Lacy 1995). Furthermore, it should be noted that, in general, breeding strategies that retain GD also retain allelic diversity (Allendorf 1986; Ballou and Lacy 1995). Thus, for these simulations, high GD also indicates high allelic diversity.

Summary of Scenarios

A total of 324 scenarios were evaluated, representing all possible combinations of the described parameters; 108 scenarios were tested for discrete generations and 216 scenarios were tested for overlapping generations. For each scenario, the performances of the 4 methods for selecting breeding pairs were quantified each timestep by GD and \overline{F} in the simulated populations. Scenarios were run 1000 times and results were averaged over all iterations. An exception was made for the Simultaneous MK Selection method because it proved to be quite computationally intensive; when that method was tested, scenario results were averaged over only 100 iterations. For each timestep, 95% confidence intervals for average GD and \overline{F} values were calculated across all iterations. Because wildlife breeding programs typically set goals for genetic management according to years rather than generations, we ran all of our simulations for 100 timesteps (i.e., years) to mimic a common time frame over which captive population management goals are often set (Foose et al. 1995; Ballou et al. 2010). Initial testing of the simulations (data not shown) confirmed our previous experiences with similar models (Ballou and Lacy 1995; Rudnick and Lacy 2008) that suggested the performances of the different pair selection strategies should remain consistent after initial generations.

Some scenarios proved to be demographically unstable and failed (i.e., population sizes declined to zero) prior to reaching 100 timesteps. Those that failed demographically represented combination of parameters (e.g., short generation length with low reproduction) that would not be viable life histories. Consequently, scenarios that exhibited iteration failure rates of greater than 1% were discarded from our analyses. For all remaining scenarios, the few iterations that failed were discarded and rerun until 1000 successful iterations were collected.

Results

Discrete Generations

The 4 methods of minimizing kinship in captive populations were initially evaluated for a total of 108 discrete generation scenarios. Of those 108 scenarios, only 24 proved to be sufficiently demographically robust for analyses. For the remaining 84 scenarios, simulations quickly crashed (i.e.,

population sizes declined to zero) due to a combination of demographic instability and increasing limitations on the growth rate as an inbreeding restriction was placed on pairs allowed to breed. Scenarios removed from further analyses were all those with low or intermediate reproduction (72 total), as well as those with high reproduction and only 50% reproductive success (12 total). The set of scenarios that was ultimately analyzed continued to encompass multiple rates of both infant and adult mortality, pairing success, and reproductive success.

For discrete generation scenarios, results indicated that the best breeding pair selection method for retaining the highest GD and accumulating the lowest \overline{F} varied according to pairing success. When pairing success was 100%, Ranked MK Selection and Simultaneous MK Selection consistently retained the highest GD (Figure 1) and accumulated the lowest \overline{F} (Supplementary Material) across all timesteps of all relevant scenarios. However, although the performances of both methods were nearly equivalent, Ranked MK Selection did consistently outperform Simultaneous MK Selection to a slight degree; at the end of 100 timesteps, across all discrete generation scenarios, Ranked MK Selection retained between 0.0026 and 0.0089 more GD and accumulated between 0.0054 and 0.0107 less \overline{F} than Simultaneous MK Selection. As pairing success declined to 80%, Dynamic MK Selection joined Ranked and Simultaneous MK Selection as the most robust methods for retaining the highest GD and accumulating the lowest \overline{F} (Figure 1, Supplementary Material). Although Ranked and Simultaneous MK Selection continued to be the best breeding pair selection method for some of the relevant scenarios, the performances of these 3 pair selection methods were generally comparable with 0.0175 being the greatest difference in either GD or \overline{F} observed between any of the 3 methods at any given timestep. As pairing success dropped to 50%, Dynamic MK Selection retained the highest GD and accumulated the lowest \overline{F} across all timesteps of all relevant scenarios (Figure 1, Supplementary Material). The 95% confidence intervals for both GD and \overline{F} ranged from 0.0000 to 0.0040 across all timesteps of all scenarios and methods tested.

Overlapping Generations

The 4 methods of minimizing kinship in captive populations were initially evaluated for a total of 216 overlapping generation scenarios. Of those 216 scenarios, a total of 198 proved to be sufficiently demographically robust for analyses. The 18 scenarios removed from further analyses were all from the shortest overlapping generation length tested (~ 3.6 years, maximum longevity of 5 years); of these, all scenarios with low reproduction and 50% reproductive success were removed from further analyses (12 total), as were those scenarios with low reproduction, 80% reproductive success, and 30% infant mortality (6 total). The set of scenarios that was ultimately analyzed continued to encompass multiple generation lengths and multiple rates of

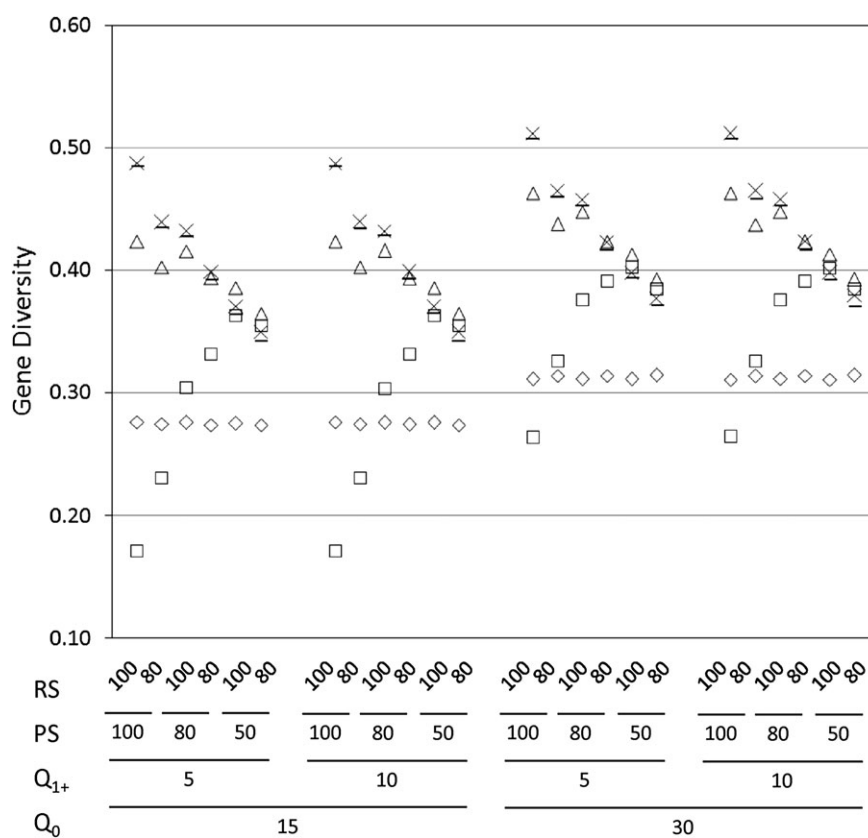


Figure 1. Values for GD at 100 timesteps for scenarios with discrete generations. Rates of reproductive and pairing success (RS and PS), as well as infant and adult mortality (Q_0 and Q_{1+}), are provided as percentages below each figure. Pair selection methods: Static MK Selection (\square), Dynamic MK Selection (\triangle), Ranked MK Selection (\times), Simultaneous Selection ($-$), and Random Selection (\diamond).

reproduction, both infant and adult mortality, pairing success, and reproductive success.

Across all overlapping generation scenarios, Dynamic MK Selection consistently performed the best of the 4 breeding pair selection methods and was ultimately identified as the most robust method for retaining GD and limiting the accumulation of \bar{F} . Across all timesteps of all relevant scenarios, the GD retained by Dynamic MK Selection was either greater than or equivalent to that retained by any of the other breeding pair selection methods (Figures 2 and 3; additional data presented in Supplementary Material). With respect to \bar{F} , the best breeding pair selection method varied between Dynamic and Ranked MK Selection (Supplementary Material). In general, when both pair and reproductive success were high, Ranked MK Selection accumulated slightly less \bar{F} than Dynamic MK Selection. As pair and/or reproductive success started to decline, Ranked MK Selection accumulated slightly less \bar{F} during initial timesteps and Dynamic MK Selection accumulated slightly less \bar{F} during later timesteps. Eventually, as pair and/or reproductive success continued to decline, Dynamic MK Selection accumulated notably less \bar{F} than Ranked MK Selection across all timesteps. This trend was observed across both overlapping generation lengths that were tested,

but it took a greater number of timesteps for the performance of Dynamic MK Selection to surpass Ranked MK Selection as generation length increased. For the timesteps of those scenarios where Ranked MK Selection outperformed Dynamic MK Selection, the largest difference in \bar{F} observed between the selection methods was 0.0088. Thus, given that 1) Dynamic MK Selection accumulated less \bar{F} in the majority of timesteps across all relevant scenarios, 2) Ranked MK Selection only slightly outperformed Dynamic MK Selection with regard to \bar{F} in the minority of timesteps across all relevant scenarios, and 3) Dynamic MK Selection consistently performed the best at retaining GD, Dynamic MK Selection was identified as the most robust breeding pair selection method for populations with overlapping generations. The 95% confidence intervals for both GD and \bar{F} ranged from 0.0000 to 0.0062 across all timesteps of all scenarios and methods tested.

Discussion

A number of studies have demonstrated that the best breeding pair selection strategies for meeting the genetic goals of wildlife breeding programs are those that minimize average kinship (Ballou and Lacy 1995; Fernández and Toro

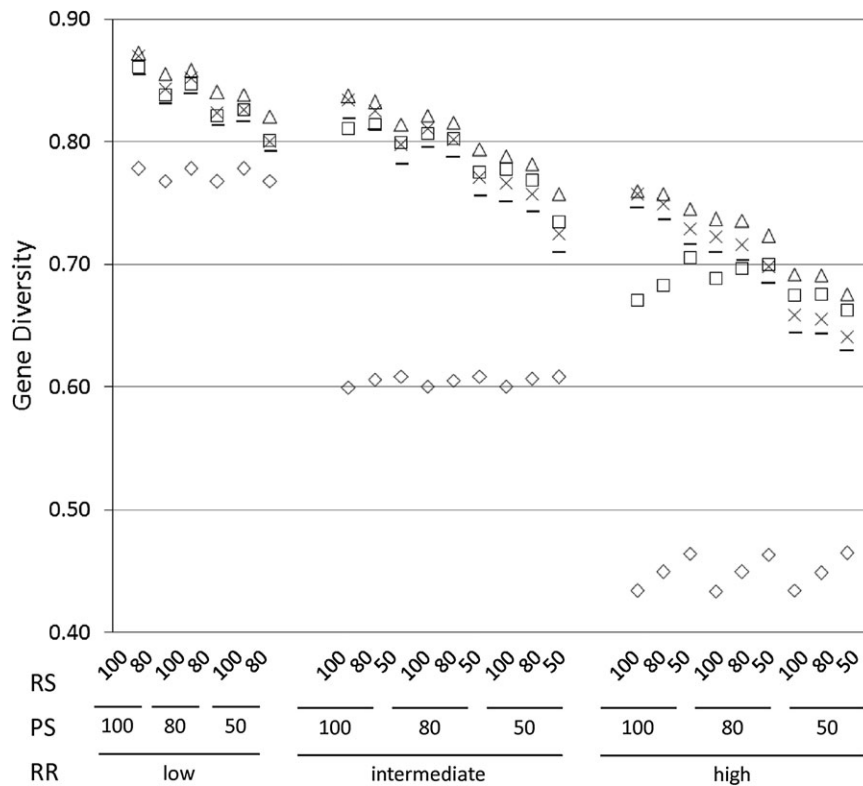


Figure 2. Values for GD at 100 timesteps for scenarios with an overlapping generation length of ~ 3.6 years. Rates of reproductive and pairing success (RS and PS) are provided as percentages below each figure. Reproductive rates, defined as the number of offspring produced per reproductively successful pair, were as follows: low = 1 offspring, intermediate = a mean of 3 and a maximum of 5 offspring, and high = a mean of 6 and a maximum of 10 offspring. All results are from scenarios with infant and adult mortality rates of 0.15 and 0.05, respectively. Comprehensive results from all scenarios are provided in the online Supplementary Material. Pair selection methods: Static MK Selection (\square), Dynamic MK Selection (\triangle), Ranked MK Selection (\times), Simultaneous Selection ($-$), and Random Selection (\diamond).

1999; Sonesson and Meuwissen 2001). However, to date, research in this area has primarily focused on testing pair selection methods that minimize kinship against alternate types of selection methods (e.g., those designed to minimize inbreeding, are based on other measures of genetic importance, or random mating; Ballou and Lacy 1995) or testing one-step versus two-step methods for minimizing kinship when determining parental contributions and mating scheme (Fernández and Caballero 2001; Fernández et al. 2001). We expand on this previous work by formally describing a novel method for minimizing average kinship (Ranked MK Selection) and rigorously testing the 2 historically predominant means by which many zoo-based breeding programs are managed (Static and Dynamic MK Selection).

We used a series of computer simulations to test 4 breeding pair selection methods designed to minimize average kinship in breeding programs. To model a range of possible species types, our simulations incorporated numerous combinations of varying generation lengths, mortality rates, reproductive rates, and rates of breeding pair success (measured as both pairing success and reproductive

success). With the exception of generation length and breeding pair success, we ultimately found that varying these parameters had very little impact on either 1) which breeding pair selection method was identified as the best for retaining GD and limiting the accumulation of \bar{F} or 2) the relative performances of the breeding pair selection methods when compared with each other. However, varying these parameters did have an impact on the values of GD and \bar{F} observed at the end of a simulation, as well as the degree to which the best breeding pair selection method outperformed the others.

The genetic management of captive populations should become less effective as stochastic factors, rather than careful breeding pair selection, more heavily influence the retention of GD and the accumulation of \bar{F} . Thus, we expected that as mortality rates increased and rates of breeding pair success decreased, a lower GD and a higher \bar{F} would be observed at the end of our simulations. Although this proved true for many scenarios, we actually observed the opposite trend in some instances. In some cases, the ultimate performance of a breeding pair selection method (measured as GD and \bar{F} at 100 timesteps) improved as

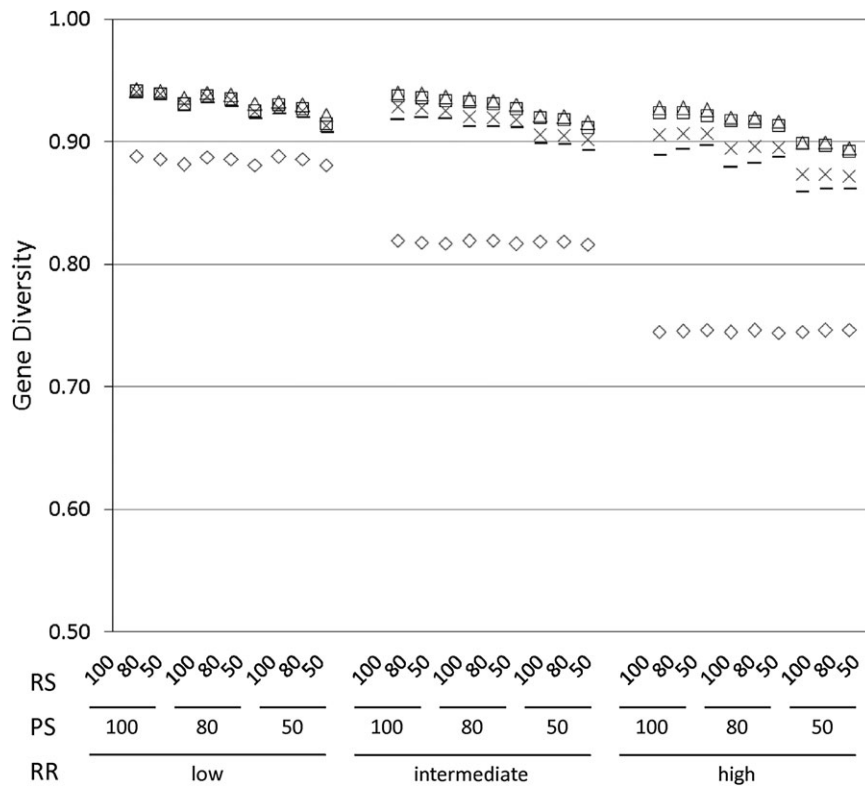


Figure 3. Values for GD at 100 timesteps for scenarios with an overlapping generation length of ~ 13.4 years. Rates of reproductive and pairing success (RS and PS) are provided as percentages below each figure. Reproductive rates, defined as the number of offspring produced per reproductively successful pair, were as follows: low = 1 offspring, intermediate = a mean of 3 and a maximum of 5 offspring, and high = a mean of 6 and a maximum of 10 offspring. All results are from scenarios with infant and adult mortality rates of 0.15 and 0.05, respectively. Comprehensive results from all scenarios are provided in the online Supplementary Material. Pair selection methods: Static MK Selection (\square), Dynamic MK Selection (\triangle), Ranked MK Selection (\times), Simultaneous Selection ($-$), and Random Selection (\diamond).

mortality rates increased and/or breeding pair success declined (e.g., Figure 2). We hypothesize that this contrary trend was a result of fewer close relatives being produced and/or surviving from among the offspring produced each timestep (in effect, the parameters themselves were minimizing kinship in the population). This hypothesis is supported by the evidence that this contrary trend was observed not only when a breeding pair selection method was utilized but also when breeding pairs were selected at random, and a stronger contrary trend was observed as reproductive rates increased (e.g., Figure 2).

From among the 4 breeding pair selection methods tested, the relative performance of Static MK Selection proved the most variable and was influenced by both of our measures of breeding pair success. The Static MK Selection method should exhibit optimum performance when individual mks remain largely unchanged (or static) from one timestep to the next. Thus, as reproductive and/or pairing success declined, the performance of Static MK Selection improved across some scenarios because the set of offspring produced each timestep was an increasingly random representation of the pool of potential breeders,

which meant that changes to the kinship matrix and thus to individual mks were less predictable. For scenarios with discrete generations, Static MK Selection actually performed worse than random mating when both reproductive success and pairing success were high (Figure 1). This occurred because only the individuals with the lowest mks successfully produced offspring for a given generation, which meant that no overrepresented individuals (those with high mks) from generation x were represented in generation $x + 1$. Thus, GD declined quicker and \bar{F} accumulated faster under Static MK Selection because the set of selected parents were actually a poorer genetic representation of the pool of available breeders than a set of randomly selected parents.

For discrete generations, the nearly equivalent Ranked and Simultaneous MK Selection were identified as the best breeding pair selection methods when pairing success was high. The Simultaneous MK Selection method was based on previously described approaches that performed well at retaining GD, limiting \bar{F} , and maintaining fitness in populations with discrete generations (Fernández and Caballero 2001; Fernández et al. 2001, 2003); thus, our results provide

additional support for the robustness of the general method under a range of newly tested captive breeding models. The Simultaneous and Ranked MK Selection methods used different approaches for selecting the group of breeding individuals that would produce a set of offspring with the lowest average kinship. Because both methods were different ways of achieving comparable sets of breeding pairs, we expected the performances of the methods to be similar. For scenarios with discrete generations, the performances of the Simultaneous and Ranked MK Selection methods were nearly equivalent, but Ranked MK Selection did consistently outperform Simultaneous MK Selection to a slight degree. Upon further post hoc investigation, we discovered that the simulated annealing algorithm used by the Simultaneous MK Selection occasionally failed to find the group of parents that would produce the set of offspring with the absolute lowest average kinship (or the algorithm converged on a local rather than the global minimum of the evaluated function). Thus, it seems likely that the occasional selection of a suboptimal set of breeding pairs explains why Simultaneous MK Selection slightly underperformed Ranked MK Selection. Because we used only a single set of simulated annealing algorithm settings when testing Simultaneous MK Selection, rather than optimizing the algorithm for each modeled scenario, it is possible that the performance of this pair selection method could be improved by modifying parameters intrinsic to the simulated annealing algorithm (e.g., the number of Monte Carlo steps).

Although Ranked and Simultaneous MK Selection performed well for discrete generations when pairing success was high, Dynamic MK Selection became the best breeding pair selection method as pairing success declined toward 50%. We propose that this shift in best breeding pair selection method occurred as a consequence of random breeding pairs replacing the failed pairs as pairing success declined. As pairing success declined, greater proportions of breeding pairs were randomly selected and greater proportions of the resultant offspring were a random genetic sampling of the pool of potential parents. In terms of the performance of the various pair selection methods, this created the genetic equivalent of overlapping generations, with some genes being transmitted to the next generation without having been subjected to the pair selection protocol. Thus, since Dynamic MK Selection was the most robust pair selection method for overlapping generations, that method also performed well for discrete generation scenarios with low pairing success.

For overlapping generations, Dynamic MK Selection was identified as the best breeding pair selection method for retaining GD. However, breeding pair success influenced whether Dynamic or Ranked MK Selection was the best breeding pair selection method for limiting the accumulation of \bar{F} . In general, when both pair and reproductive success were high, Ranked MK Selection accumulated slightly less \bar{F} than Dynamic MK Selection. As pair and/or reproductive success declined, Dynamic MK Selection clearly outperformed Ranked MK Selection by accumulating notably less

\bar{F} . Ranked MK Selection simultaneously selected the group of breeding individuals that would produce a set of offspring with the lowest average kinship, thereby minimizing future inbreeding, whereas Dynamic MK Selection iteratively selected breeding pairs that would minimize average kinship as offspring were added to an existing population, thereby not being quite as efficient at reducing inbreeding in the offspring generation. However, as breeding pair success declined and a larger number of breeding pairs were selected to compensate for decreased reproduction, the next generation more closely mirrored the parents and the inclusion of the parental generation in the Dynamic MK Selection calculations led to better performance by that method than the Ranked MK Selection method that was based on a projected optimal offspring generation. Given the results across all relevant scenarios, Dynamic MK Selection was identified as the most robust breeding pair selection method for overlapping generations because it accumulated less \bar{F} than Ranked MK Selection in the majority of timesteps across all relevant scenarios and consistently performed the best at retaining GD.

The Simultaneous and Ranked MK Selection methods were not expected to perform well for overlapping generations because those methods were designed to select the group of breeding individuals that would produce a set of offspring with the lowest average kinship. When generations overlap and both parents and offspring remain in a population, minimizing the average kinship within a single cohort of offspring does not minimize the average kinship in the larger population. There is no simple way to modify the Ranked MK Selection method to account for both parents and offspring remaining in a population, but the performance of the Simultaneous MK Selection method might be improved if the function $\overline{mk} + \gamma\bar{F}$ could be evaluated for the current population plus the expected future offspring. Other published methods for managing breeding in populations with overlapping generations account for both parents and offspring remaining in a population by incorporating individual reproductive values (Sonesson and Meuwissen 2001; Nomura 2005). Similarly, the selection methods based on mk included in this investigation can be modified to use weighted mean kinships that adjust for the expected reproductive value of each kin (Ballou and Lacy 1995).

For each of the breeding pair selection methods we tested, with Simultaneous MK Selection being a possible exception, selected breeding pairs consisted of males and females with similar mk s. For scenarios in which breeding pair success was low, this meant that 2 genetically valuable individuals failed to breed when a pairing of low mk individuals was unsuccessful. It has been proposed that an alternative pair selection strategy for captive breeding programs is compensatory pairing of individuals with disparate mk s (Caballero et al. 1996). Although this type of strategy might improve the reproductive success of low mk individuals when breeding pair success is low by distributing those individuals more evenly across selected

breeding pairs, it has been suggested that compensatory pairing is a poor strategy for wildlife breeding programs because it irreversibly links rare and common alleles in the offspring generation, thereby preventing future genetic management from being able to effectively equalize founder lineages in later generations (Lacy 1994; Ballou et al. 2010). However, Fernández and Caballero (2001) found that for two-step pair selection methods that determine parental contributions first and mating scheme second, compensatory pairing in the second step retained levels of GD equivalent to minimizing the average kinship between pairs. Thus, although this was beyond the scope of our research to investigate, compensatory pairing could have some merit when combined with parental contributions that are selected in such a way as to minimize kinship in a population.

Conclusions

For captive breeding programs to meet both demographic and genetic goals, managers must carefully consider breeding pair selection. For the past decade, PM2000 (Pollak et al. 2002) has been the most popular software package for managing captive populations of wildlife, and nearly all zoo-based breeding programs have used this software to produce regular breeding recommendations. The 2 options for selecting breeding pairs in PM2000 are what we have termed Static MK Selection and Dynamic MK Selection, and those 2 methods have historically been the predominant means by which many captive breeding programs are managed. PM2000 has recently been significantly revised and the new version of the software, named PMx (Lacy et al. 2011), also offers users the option to select a set of breeding pairs by Ranked MK Selection. We tested all 3 methods of breeding pair selection available in PMx, as well as the additional Simultaneous MK Selection method, across a range of scenarios that modeled realistic managed breeding programs for wildlife with varying life history characteristics. Results indicate that, in general, the best methods for retaining GD and limiting \bar{F} in species with high fecundity and short life spans (e.g., insects and many amphibians) are the nearly equivalent Ranked and Simultaneous MK Selection methods. However, if compliance with breeding recommendations is low ($\leq 50\%$) and a large number of nonrecommended pairings are made each generation, results indicate that Dynamic MK Selection is the best pair selection method for retaining GD and limiting \bar{F} . For populations of wildlife species with low fecundity and long life spans (e.g., many mammals, birds, and reptiles), results indicate that Dynamic MK Selection is the most robust pair selection method for captive breeding programs to meet genetic goals.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

Funding

Institute of Museum and Library Services (CP Grant #1C-03-05-0027-05); Zoological Society of San Diego; and Chicago Zoological Society.

Acknowledgments

We thank J. Fernández for his kind help and advice on implementing the simulated annealing algorithm.

References

- Allendorf F. 1986. Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biol.* 5:181–190.
- Ballou JD, Lacy RC. 1995. Identifying genetically important individuals for management of genetic diversity in pedigreed populations. In: Ballou JD, Gilpin M, Foose TJ, editors. *Population management for survival and recovery*. New York: Columbia Press. p. 76–111.
- Ballou JD, Lees C, Faust LJ, Long S, Lynch C, Bingaman Lackey L, Foose TJ. 2010. Demographic and genetic management of captive populations. In: Kleiman DG, Thompson KV, Baer CK, editors. *Wild mammals in captivity: principles and techniques for zoo management*. 2nd ed. Chicago (IL): University of Chicago Press. p. 219–252.
- Caballero A, Santiago E, Toro MA. 1996. Systems of mating to reduce inbreeding in selected populations. *Anim Sci.* 62:431–442.
- Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity.* 83:260–270.
- Falconer DS. 1981. *Introduction to quantitative genetics*. 2nd ed. New York: Longman Inc.
- Fernández J, Caballero A. 2001. A comparison of management strategies for conservation with regard to population fitness. *Conserv Genet.* 2:121–131.
- Fernández J, Toro MA. 1999. The use of mathematical programming to control inbreeding in selection schemes. *J Anim Breed Genet.* 116:447–466.
- Fernández J, Toro MA, Caballero A. 2001. Practical implementation of optimal management strategies in conservation programmes: a mate selection method. *Anim Biodivers Conserv.* 24:17–24.
- Fernández J, Toro MA, Caballero A. 2003. Fixed contributions designs vs. minimization of global coancestry to control inbreeding in small populations. *Genetics.* 165:885–894.
- Foose TJ, Ballou JD. 1988. *Population management: theory and practice*. *Int Zoo Yearb.* 27:26–41.
- Foose TJ, deBoer L, Seal US, Lande R. 1995. Conservation management strategies based on viable populations. In: Ballou JD, Gilpin M, Foose TJ, editors. *Population management for survival & recovery. Analytical methods and strategies in small population conservation*. New York: Columbia University Press. p. 273–294.
- Hedrick PW, Miller P. 1992. Conservation genetics: theory and management of captive populations. In: Sandlund OT, Hindar K, Brown AHD, editors. *Conservation of biodiversity for sustainable development*. Oslo (Norway): Scandinavian University Press. p. 70–87.
- Kirkpatrick S, Gelatt CD Jr, Vecchi MP. 1983. Optimization by simulated annealing. *Science.* 220:671–680.
- Lacy RC. 1994. Managing genetic diversity in captive populations of animals. In: Bowles ML, Whelan CJ, editors. *Restoration and recovery of endangered plants and animals*. Cambridge (UK): Cambridge University Press. p. 63–89.
- Lacy RC. 1995. Clarification of genetic terms and their use in the management of captive populations. *Zoo Biol.* 14:565–578.

- Lacy RC, Ballou JD, Pollak JP. 2011. PMx: software package for demographic and genetic analysis and management of pedigreed populations. *Methods Ecol Evol*. doi: 10.1111/j.2041-210X.2011.00148.x.
- Lacy RC, Petric A, Warneke M. 1993. Inbreeding and outbreeding depression in captive populations of wild animals. In: Thornhill MW, editor. *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*. Chicago (IL): University of Chicago Press. p. 352–374.
- Long S, Dorsey C, Boyle P. 2011. Status of association of zoos and aquariums cooperatively managed programs. *WAZA Mag*. 12:15–18.
- Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci U S A*. 70:3321–3323.
- Nomura T. 2005. Methods for minimizing the loss of genetic diversity in conserved populations with overlapping generations. *Conserv Genet*. 6:655–663.
- Pollak JP, Lacy RC, Ballou JD. 2002. *Population management 2000*, version 1.163. Brookfield (IL): Chicago Zoological Society.
- Press WH, Teukolsky SA, Vetterling WT, Flannery BP. 1992. *Numerical recipes in FORTRAN*. 2nd ed. Cambridge (UK): Cambridge University Press.
- Rudnick JA, Lacy RC. 2008. The impact of assumptions about founder relationships on the effectiveness of captive breeding strategies. *Conserv Genet*. 9:1439–1450.
- Sonesson AK, Meuwissen THE. 2001. Minimization of rate of inbreeding for small populations with overlapping generations. *Genet Res*. 77:285–292.
- Wright S. 1969. *Evolution and the genetics of populations*. Vol. II: the theory of gene frequencies. Chicago (IL): University of Chicago Press.

Received August 2, 2011; Revised October 17, 2011;
Accepted October 18, 2011

Corresponding Editor: Robin Waples