

# The impact of assumptions about founder relationships on the effectiveness of captive breeding strategies

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**Abstract** Many breeding programs managed by zoos and aquariums employ strategies that minimize mean kinship as a way of retaining genetic diversity (MK strategies). MK strategies depend on accurate and complete pedigrees, but population founders are generally assumed to be unrelated and not inbred. This assumption was historically necessitated by the unavailability of data on founder relationships, but with DNA techniques it is sometimes now possible to estimate those relationships. We used computer simulations to investigate the impact of founder assumptions on the effectiveness of MK strategies. Individuals with known pedigrees were managed in groups of 10, 30, and 100 founders at two different rates of reproduction and two different degrees of founder relationship. The impact of assuming founders were unrelated was quantified by calculating the differences in gene diversity and inbreeding that were observed between simulations that used known relationships and simulations that assumed founders were unrelated. Results indicated that utilizing known relationships retained 0–2% more gene diversity over ten generations than assuming founders were unrelated, with specific results dependent on the conditions of a given scenario. Similar results were observed for inbreeding, with long-term levels of inbreeding being 0–2% lower when relationships were known. There were higher benefits to knowing founder relationships as reproductive rate increased, as well as when full-siblings were included in small groups of founders. Overall, however, long-term benefits gained from knowing founder relationships were generally small. Therefore, MK strategies probably often

produce near optimal results when standard founder assumptions are made.

**Keywords** Captive breeding · Gene diversity · Inbreeding · Kinship · Relatedness · Simulation

## Introduction

As ex-situ breeding efforts have become components of modern conservation initiatives, many zoos and aquariums have established scientifically managed, long-term captive breeding programs. While the specific conservation goals of these programs can vary, most aim to maintain a self-sustaining population that retains genetic variation and experiences minimal inbreeding (Foose and Ballou 1988; Hedrick and Miller 1992; Lacy 1994; Ballou and Lacy 1995). As captive population management has evolved, a number of different breeding strategies have been proposed to meet these genetic goals. A breeding strategy that minimizes the overall kinship in a population has been shown by both computer simulations (Ballou and Lacy 1995; Fernandez and Toro 1999; Toro et al. 1999) and empirical data (Montgomery et al. 1997) to be the best strategy for retaining genetic variation in the population while limiting inbreeding. As such, many captive breeding programs use this type of strategy to manage their populations.

The kinship ( $f$ ) of a pair of individuals is the probability that two alleles at a given locus, one randomly drawn from each individual, are identical by descent from a common ancestor (Falconer 1981). An individual's mean kinship ( $mk$ ) is then the average of pairwise  $f$ s between that individual and all living individuals in the population, including itself (Ballou and Lacy 1995). The majority of

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captive breeding programs use pedigree-based estimates of  $f$ , rather than molecular estimates, to recommend breeding pairs that minimize a population's kinship. Because an individual's  $mk$  is the expected inbreeding coefficient of its progeny under random mating, the average mean kinship of the population ( $\overline{mk}$ ) is the expected mean inbreeding coefficient of all progeny under random mating. Thus,  $1 - \overline{mk}$  is a population's proportional gene diversity (i.e., average expected heterozygosity).

Because the calculation of kinship values for captive breeding programs depends on a population's pedigree, breeding strategies intended to minimize average kinship (MK strategies) are only effective when the relationships among individuals in a population are accurately known. Unfortunately, all captive populations have some percentage of uncertainty present in their pedigrees. Incomplete or unknown pedigrees can arise for a variety of reasons. Many of the captive populations managed today were founded during a time when pedigrees were, at best, inconsistently recorded. While accurate records may now be maintained, missing information deeper in a pedigree still affects how a manager perceives relationships between animals currently in a population. In some cases incomplete or inaccurate pedigrees can continue to be perpetuated today. This primarily occurs when species are housed in groups; a situation where parentage (generally paternity) is often inherently uncertain. Even if a complete pedigree has been kept from the time a captive population was initiated, relationships between founding individuals are still unknown. Thus, at the very least, every captive population has incomplete pedigree records associated with its founders.

The founders of a captive population are those ancestors for whom no past relationship information (i.e., pedigree) is known. Thus, founders are usually the individuals that were captured in the wild and subsequently contributed descendants to the captive population. For the purposes of captive population management, founders usually are considered to be unrelated and not inbred (Ballou 1983). One of the central questions surrounding founders is how assumptions about founder relatedness affect the amount of genetic variation retained and the level of inbreeding experienced by a population managed under a MK breeding strategy. Research to date has addressed only a few aspects of this broad question. For example, Willis and Wiese (1993) characterized the number of new founders that must be imported into small captive populations on a per generation basis to maintain specified levels of gene diversity. In another line of research, Willis (1993) used statistical theory to provide general recommendations for when individuals of unknown ancestry should be included in captive breeding programs as founders versus when these individuals should be removed from managed

populations altogether. Haig et al. (1994) addressed founder relationships by attempting to improve upon the assumption that the founders of a captive Guam rail population were unrelated. The authors used five hypothetical patterns of founder relationships to calculate pedigree-based estimates of relatedness. The pedigree-based estimates of relatedness were then compared to estimates of genetic similarity calculated from DNA profiles. The pattern of founder relationships that produced pedigree-based estimates of relatedness that most closely correlated with the DNA-based estimates of similarity represented the best founder relationship assumptions. A number of studies have also used genetic methods to directly estimate relatedness among founders (e.g., Brock and White 1992; Geyer et al. 1993; Haig et al. 1995; Jones et al. 2002; Gautschi et al. 2003; Russello and Amato 2004), but these studies represent only a small percentage of captive populations, and most population managers must still assume founders were unrelated. While all of these studies have contributed to a better understanding of how captive populations should be managed, none quantified the effects of assuming founders were unrelated relative to the optimal, if hypothetical, case of knowing true founder relationships.

In this paper we describe computer simulations used to investigate how the retention of genetic variation and the accumulation of inbreeding in captive populations managed by MK breeding strategies are affected by assuming founders are unrelated and not inbred. Computer simulations are particularly powerful methods for studying these questions because they allow for the generation of complex pedigrees for population founders. Knowing the true relationships that existed between founders is critical for quantifying the detriment to assuming founders were unrelated. For a given group of founders, we compared the genetic variation and inbreeding present through time for simulations that either used the known founder relationships or assumed founders were unrelated. Our results are pertinent to the founding and early management of future captive populations, but they are also applicable to populations that already have been established.

## Materials and methods

### Overview of simulations

Two computer simulations were written in the C programming language, for which code is available upon request. Both simulations modeled dioecious populations with overlapping generations (tests on populations with discrete generations showed similar, but weaker, trends for knowing founder relationships). The first simulation randomly mated individuals to create a population with a

complex pedigree. The second simulation used individuals sampled from the first simulation to found populations that were subsequently managed by the MK breeding strategy. For both simulations pairwise  $f_s$  were calculated as  $f_{xy} = 0.5(f_{xs} + f_{xd})$ , where the subscripts  $s$  and  $d$  refer to the parents of individual  $y$  (Falconer 1981). Figure 1 is a general schematic of both simulations, and the specific details of each simulation are described below.

*Simulation 1*

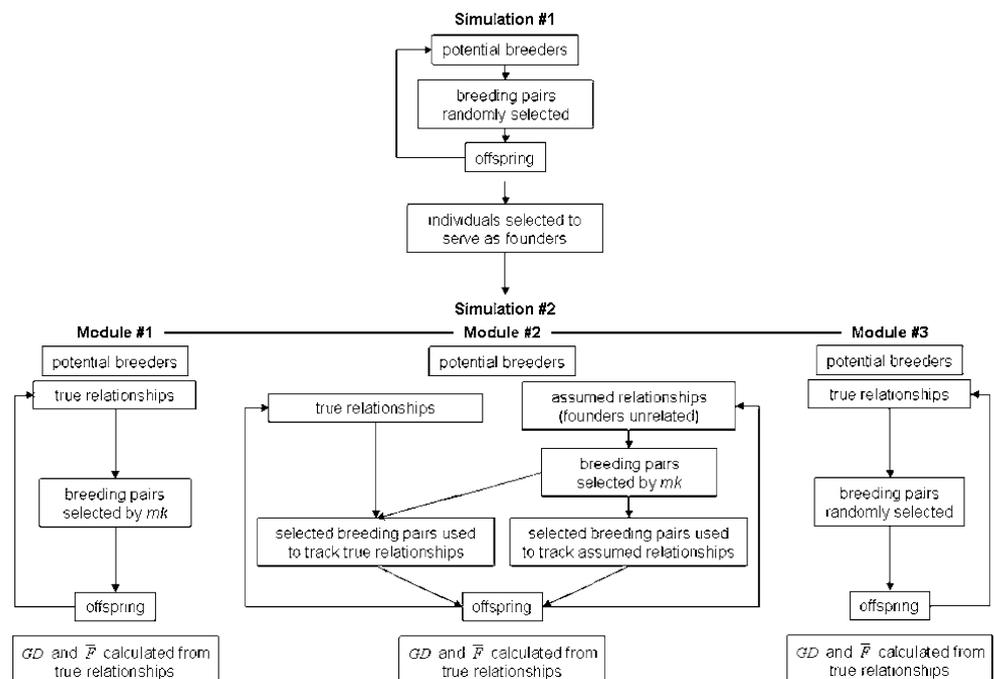
1. An initial population of 200 unrelated individuals was created with a 50:50 sex ratio. Individuals could breed starting in the first timestep after their birth and remained in the population for three timesteps, resulting in a mean generation time of approximately two timesteps.
2. Breeding pairs were randomly selected without replacement from the available individuals. The number of pairs selected was designed to maintain the population at size 200 and was equal to  $n/ptm$ , in which  $n$  was the target population size,  $p$  was the probability that a breeding pair produced offspring,  $t$  was the number of timesteps that individuals remained in the population, and  $m$  was the mean number of offspring produced by successful breeding pairs.
3. Each pair selected for breeding had a 0.8 probability of producing offspring ( $p$ ). If a pair was successful, the number of offspring produced was drawn from a Poisson distribution with a specified mean. To avoid

drawing an infinitely large number of offspring, the Poisson distribution was truncated to a specified maximum. Each offspring was assigned one sex or the other with a 0.5 probability.

4. After all offspring were produced, the relationships 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 parents' kinship; Falconer 1981) were calculated each timestep.
5. Individuals were aged one timestep and removed from the population if they were older than three timesteps.
6. Steps 2–5 were repeated for a specified number of timesteps. If there were not enough individuals available to make the number of breeding pairs specified in Step 2, the maximum number of pairs possible was made instead.
7. At the end of Simulation 1 a specified number of individuals was randomly sampled from the final population to create founders for Simulation 2, which quantified the effects of assuming founders are unrelated and not inbred when implementing a MK breeding strategy. A matrix of all possible pairwise  $f_s$  and each individual's  $F$  were exported for the sampled individuals.

Simulation 2 was used to study the effects of founder assumptions on MK breeding strategies. The simulation had three modules that all utilized a single group of

**Fig. 1** A diagram describing Simulations 1 and 2. Simulation 1 randomly mated individuals to create a population with a complex pedigree. Simulation 2 used individuals sampled from Simulation 1 to found populations for three modules. Module 1 used the true founder relationships to manage the population by an MK strategy, Module 2 assumed founders were unrelated and not inbred to manage the population by an MK strategy, and Module 3 employed random mating. Gene diversity (GD) and the average inbreeding coefficient ( $\bar{F}$ ) were calculated as summary statistics each timestep



founders imported from Simulation 1. To implement an MK breeding strategy for a given group of founders, Module 1 utilized known founder relationships and Module 2 assumed founders were unrelated and not inbred. For comparative purposes, Module 3 randomly mated individuals rather than implementing an MK breeding strategy. Due to its stochastic nature, Simulation 2 was run 1,000 times for each scenario that was tested and results were averaged over all iterations.

#### Module 1—True founder relationships

1. A specified number of founders were imported from Simulation 1 with their accompanying  $f$  matrix and individual  $F$ s (i.e., the true relationships present among the founders were known).
2. Breeding pairs were chosen from the available individuals in such a way as to select a group of individuals that exhibited the lowest overall mean kinship. The number of pairs selected was designed to maintain a population size of 100, utilizing the demographic method described for Simulation 1. It is important to note that different individuals can have the same mean kinship by virtue of different sets of relationships to others in the population, and overall mean kinships of many different groups of individuals could be nearly identical. Thus, we tested the effect of making alternate selections from among those individuals with the same or nearly the same mean kinships. We found that the protocol described below would yield a set of breeders with an overall mean kinship that was usually the lowest or otherwise within 0.1% of the best set we could find, and would lead to similarly nearly identical projections of loss of gene diversity and accumulation of inbreeding. Selection of breeding pairs was as follows:
  - a. The  $mk$  of each individual was initially calculated.
  - b. If there was an excess of one of the sexes, that sex was reduced in number until there was an equal number of males and females. This was accomplished by removing the individual of the over-represented sex that exhibited the highest  $mk$ . After that individual was removed from the pool of potential breeders, the  $mks$  of all individuals still in the pool were recalculated accordingly. Individuals continued to be removed from the population by this process until a 50:50 sex ratio was attained.
  - c. Males and females were ranked in two separate breeding lists by a process that utilized  $mks$  iteratively to determine the set of pairs with the lowest overall mean kinship. First, the individual with the highest  $mk$  was placed in its sex-specific list. That individual was removed from the pool of potential breeders and the  $mks$  of all remaining individuals were recalculated accordingly. Next, the individual of the opposite sex with the highest  $mk$  was placed in its sex-specific list. That individual was also removed from the pool of potential breeders and  $mks$  were again recalculated. Individuals of alternating sex continued to be ranked and removed from the pool of potential breeders in this manner until all available individuals were placed in the lists. As individuals were selected and ranked, they were added to the top of their respective sex-specific lists.
    - d. The specified number of breeding pairs was created from the sex-specific breeding lists. In general the top male was paired with the top female, these two individuals were removed from the breeding lists, then the next male was paired with the next female until the target number of breeding pairs was reached. However, to avoid close inbreeding, any breeding pair that exhibited an  $f$  of 0.25 or greater (i.e., first-order relatives in a non-inbred population) was rejected. If a pair was rejected, all females remaining to be paired were evaluated from the top of the list down 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 breeding list and an attempt was made to pair the new top male. In the event that the specified number of breeding pairs could not be made, the maximum number of pairs possible was made instead.
3. Each pair selected for breeding had a 0.8 probability of producing offspring. If a pair was successful, the number of offspring produced was drawn from a Poisson distribution with a specified mean. To avoid drawing an infinitely large number of offspring, the Poisson distribution was truncated to a specified maximum. Each offspring was assigned one sex or the other with a 0.5 probability.
4. After all offspring were produced, a matrix of all possible pairwise  $f$ s and each individual's  $F$  were calculated and recorded.
5. Individuals were aged one timestep and removed from the population if they were older than three timesteps.
6. Steps 2–5 were repeated for a specified number of timesteps. Genetic variation and inbreeding were evaluated on a per timestep basis, immediately following Step 5. Inbreeding was measured as the average inbreeding coefficient ( $\bar{F}$ ) and genetic variation was measured as proportional gene diversity (GD). GD was calculated as  $1 - \overline{mk}$ , where  $\overline{mk}$  was the average mean kinship in the population (Ballou and Lacy 1995). While genetic variation can be quantified

in a number of ways, proportional gene diversity (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.

#### Module 2—Assumed founder relationships

1. A specified number of founders were imported from Simulation 1 with two accompanying datasets: (1) the true  $f$  matrix and individual  $F$ s, which were used to track the true relationships among individuals and (2) the assumed  $f$  matrix and individual  $F$ s, which were used to calculate the  $mks$  that were used to choose breeding pairs. The assumed data designated all founders as unrelated and not inbred. Thus all pairwise  $f$ s were 0, an individual's  $f$  with itself was 0.5, and all individual  $F$ s were 0.
2. Steps 2–6 of Module 1 were executed with the following modifications. Two sets of relationships were maintained; one set was calculated from the true founder relationships and the other set was calculated from the assumed founder relationships. The set of assumed relationships was always used to calculate the  $mks$  that were used to select breeding pairs. After breeding pairs were selected and offspring were produced, a matrix of all possible pairwise  $f$ s and each individual's  $F$  were calculated separately for each set of relationships. Thus, while the MK breeding strategy was being driven by the assumed relationships, the true relationships among individuals were still being tracked and quantified. Each generation,  $\bar{F}$  and GD were calculated from the set of true relationships.

#### Module 3—Random mating

1. A specified number of founders were imported from Simulation 1 with their accompanying  $f$  matrix and individual  $F$ s (i.e., the true relationships present among the founders were known).
2. Steps 2–6 of Module 1 were executed with one exception; breeding pairs were randomly assigned as described in Simulation 1. Each generation  $\bar{F}$  and GD were calculated from the set of true relationships.

#### Scenarios

The founding and initial management of captive populations can vary in a number of ways. To summarize the

effects of the stated founder assumptions across a variety of situations, we investigated scenarios under three basic conditions: groups of 10, 30, and 100 founders. Simulation 1 was used to create appropriately sized groups of founders, which were subsequently utilized by Simulation 2 to run the following scenarios.

#### Groups of 10 and 30 founders

Rapid population growth is often given priority over genetic management in the early stages of many captive breeding programs. We modeled this situation by starting populations with either 10 or 30 founders, allowing the maximum number of breeding pairs possible to be made each timestep until the populations reached 100 individuals, then maintaining that number of individuals in the population per the methods outlined in the simulation descriptions. Note that because all individuals were used for breeding during early timesteps, genetic management was limited to avoiding close inbreeding. To better characterize the effects of the stated founder assumptions, scenarios were also run with two different types of founder groups. One group consisted of mostly unrelated individuals, while the second group included collections of full-siblings. We used four groups of 2–4 full-siblings for scenarios with 10 founders, and seven groups of 2–4 full-siblings plus 10 unrelated individuals for scenarios with 30 founders.

#### 100 Founders

The condition of 100 founders was designed to be applicable to established captive populations that have been maintained previously without accurate pedigrees. If it became desirable for such a population to be managed under an MK breeding strategy, much of the living population would likely be designated as founders. We modeled this situation by starting populations with 100 founders, then maintaining the populations at that number of individuals per the methods outlined in the simulation descriptions.

To further characterize the effects of the stated founder assumptions, scenarios for groups of 10, 30, and 100 founders were also run under two different reproductive conditions. The number of offspring created by each successful breeding pair was drawn from a truncated Poisson distribution. Low reproduction was modeled by a Poisson distribution with a mean of three and a maximum of five, while high reproduction was modeled by a Poisson distribution with a mean of six and a maximum of ten.

Founder groups with differing degrees of relationship and inbreeding were created by running Simulation 1 for

varying numbers of timesteps. Ultimately, Simulation 2 was used to run eight scenarios for both groups of 10 and groups of 30 founders; they included all possible combinations of two rates of reproduction, two levels of initial relationship, and groups of unrelated individuals versus groups of full-siblings. Four scenarios were run for groups of 100 founders, which included all combinations of two rates of reproduction and two levels of initial relationship.

#### Data analysis

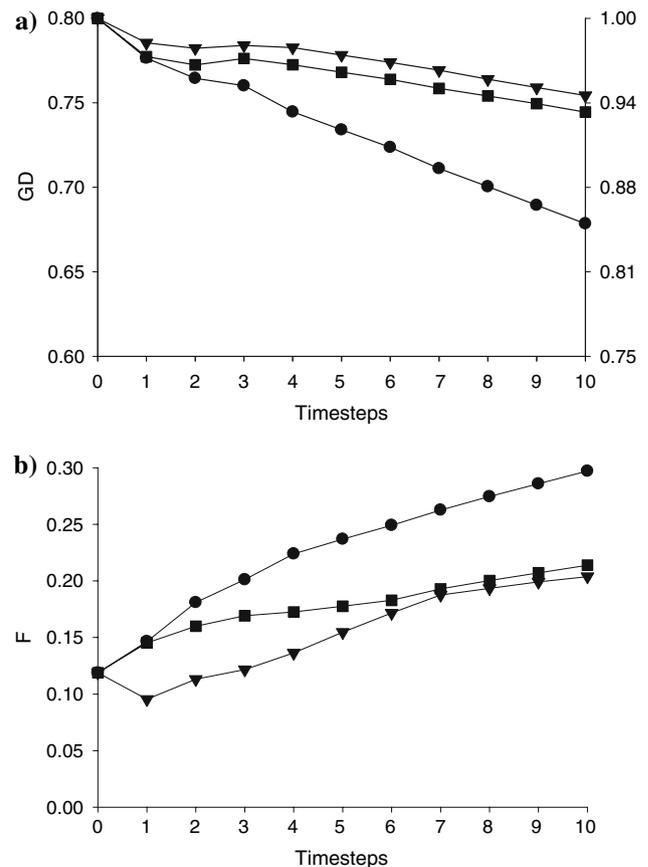
After Simulation 2 was used to test a scenario, the impact of assuming founders were unrelated and not inbred was quantified by calculating the differences in GD and  $\bar{F}$  observed between Modules 1 and 2. Module 1, which used true founder relationships, was hypothesized to retain higher values of GD and lower values of  $\bar{F}$  than Module 2, which assumed founders were unrelated. Thus, differences in GD were always calculated by subtracting the results of Module 2 from Module 1. Conversely, differences in  $\bar{F}$  were always calculated by subtracting the results of Module 1 from Module 2. Negative differences in both GD and  $\bar{F}$  were observed when Module 2 out-performed Module 1 (i.e., assuming founders were unrelated retained higher values of GD and lower values of  $\bar{F}$  than using the true founder relationships, which was contrary to expectation). Values of both GD and  $\bar{F}$  can range from 0 to 1 thus, differences in the values could be directly converted to percentages of the maximum value (the case of a totally inbred population). For example, a difference of 0.02 was equivalent to 2%. Finally, for each scenario, 95% confidence intervals based on 1,000 iterations were generated for all GD and  $\bar{F}$  averages.

#### Results

Simulation 1 was used to create four sets of unrelated founders for each of the three basic conditions tested (10, 30, or 100 founders). These four sets of founders represented all possible combinations of two levels of relationship and two rates of reproduction. All sets of low relationship had  $\bar{F}$ s that ranged from 0.02 to 0.04 and  $\overline{mks}$  that ranged from 0.04 to 0.08. All sets of high relationship had  $\bar{F}$ s that ranged from 0.12 to 0.15 and  $\overline{mks}$  that ranged from 0.13 to 0.19. Four additional sets of founders were created for scenarios that required groups of 10 or 30 founders to include full-siblings. These sets of founders had  $\bar{F}$ s equivalent to those described for groups of unrelated founders and  $\overline{mks}$  that ranged from 0.06 to 0.21.

#### 10 Founders

Populations founded by ten individuals were evaluated for ten timesteps (five generations). Iterations that were allowed to run for longer numbers of timesteps often crashed (i.e., population sizes declined to zero) because  $\overline{mks}$  exceeded the inbreeding restrictions placed on pairs allowed to breed. A small number of iterations also crashed before the tenth timestep, however, these failures were primarily due to demographic instability. Because only a maximum of 5 out of 1,000 iterations failed before the tenth timestep, iterations that failed were discarded and re-run until 1,000 successful iterations were collected for each scenario. When groups of ten founders were considered, Simulation 2 was used to evaluate eight scenarios that represented all possible combinations of two rates of reproduction, two levels of initial relationship, and unrelated individuals versus groups of full-siblings. Similar trends in GD and  $\bar{F}$  were observed across all scenarios, thus only the graphs from the scenario that



**Fig. 2** Trends in (a) GD and (b)  $\bar{F}$  observed for groups of ten founders. Data from the high reproduction, high initial relationship scenario are shown for ten founders that included groups of full-siblings. Values for timestep 0 were calculated from the founders. For GD, the left y-axis was scaled relative to the initial founders of Simulation 1 and the right y-axis was scaled relative to the founders of Simulation 2. Module 1 (▼); Module 2 (■); Module 3 (●)

exhibited the largest difference in both variables are presented (Fig. 2; high reproduction, high initial relationship, groups of full-siblings). GD experienced a general decline over timesteps and, within a scenario, the difference in GD between Module 1 (true founder relationships) and Module 2 (assumed founder relationships) varied for several timesteps before becoming approximately constant (Fig. 3). Across all scenarios and over all timesteps, the difference in GD between Modules 1 and 2 ranged from  $-0.0001$  to  $0.0108$ .  $\bar{F}$  experienced a general increase over timesteps and, within a scenario, the difference in  $\bar{F}$  between Modules 1 and 2 either noticeably declined or remained approximately constant (Fig. 3). The difference in  $\bar{F}$  across all timesteps and scenarios ranged from  $0.0003$  to  $0.0499$ . Across Modules 1 and 2, the 95% confidence intervals for both GD and  $\bar{F}$  ranged from  $0.0002$  to  $0.0023$ .

### 30 Founders

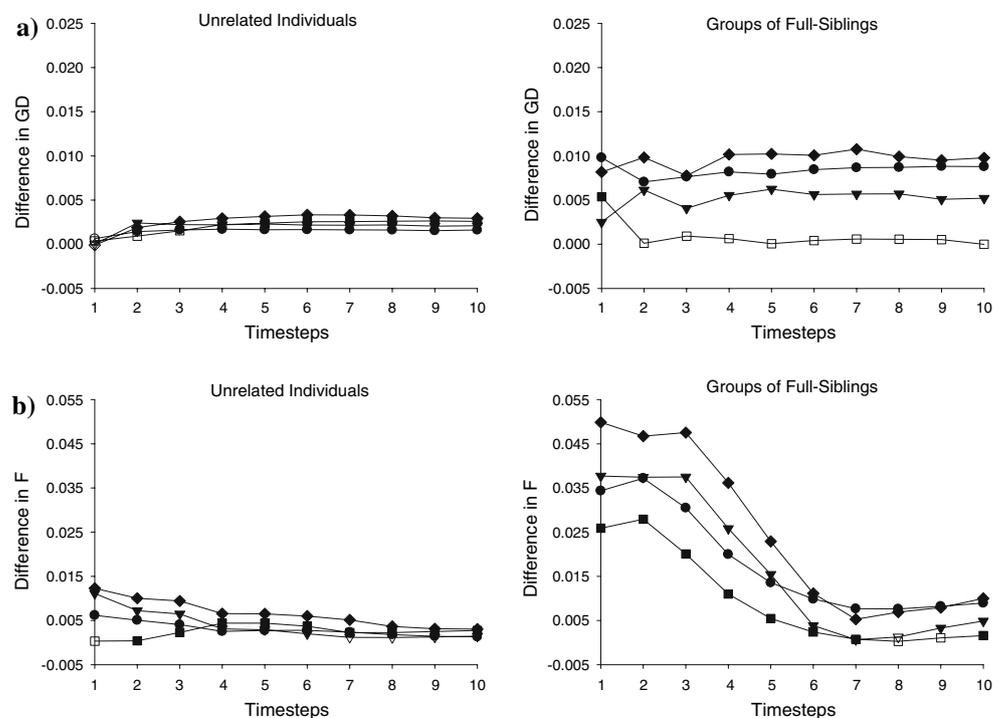
Populations founded by 30 individuals were evaluated for 20 timesteps (ten generations). Simulation 2 was used to evaluate eight scenarios that represented all possible combinations of two rates of reproduction, two levels of initial relationship, and unrelated individuals versus groups of full-siblings. Similar trends in GD and  $\bar{F}$  were observed across all scenarios, thus only the graphs from the scenario that exhibited the largest difference in both variables are presented (Fig. 4; high reproduction, low initial relationship, groups of full-siblings). GD experienced a general

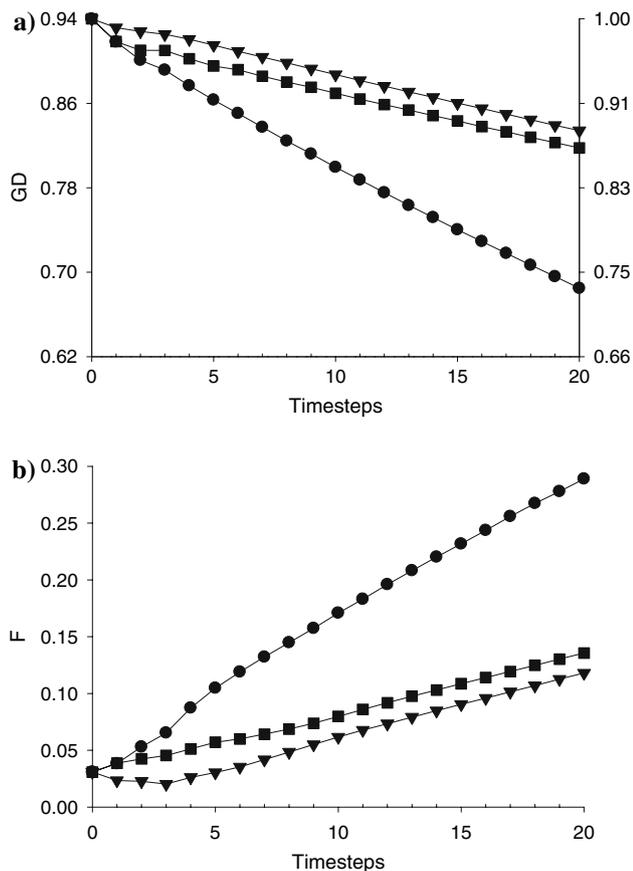
decline over timesteps and, as in the scenarios presented for ten founders, the difference in GD between Module 1 (true founder relationships) and Module 2 (assumed founder relationships) varied for several timesteps before becoming approximately constant. Across all scenarios and over all timesteps, the difference in GD between Modules 1 and 2 ranged from  $0.0017$  to  $0.0198$  (Fig. 5).  $\bar{F}$  experienced a general increase over timesteps, with the difference in  $\bar{F}$  between Modules 1 and 2 ranging from  $0.0065$  to  $0.0273$  across all timesteps and scenarios (Fig. 5). The difference in  $\bar{F}$  initially increased across all scenarios, then decreased before becoming stable around the tenth timestep. Across Modules 1 and 2, the 95% confidence intervals for both GD and  $\bar{F}$  ranged from  $0.0001$  to  $0.0010$ .

### 100 Founders

Populations founded by 100 individuals were evaluated for 20 timesteps (ten generations). We evaluated four scenarios that represented all possible combinations of two rates of reproduction and two levels of initial relationship. Similar trends in GD and  $\bar{F}$  were observed across all scenarios, thus only the graphs from the scenario that exhibited the largest difference in both variables are presented (Fig. 6; high reproduction, low initial relationship). GD experienced a general decline over timesteps and, in contrast to the scenarios presented for 10 and 30 founders, the difference in GD between Module 1 (true founder relationships) and Module 2 (assumed founder relationships) noticeably increased before stabilizing around the sixth timestep.

**Fig. 3** Differences in (a) GD and (b)  $\bar{F}$  observed between Modules 1 and 2 for groups of ten founders. Open shapes represent timesteps in which the 95% confidence intervals for the averages of Modules 1 and 2 overlapped. Low reproduction, low initial relationship (●); high reproduction, low initial relationship (▼); low reproduction, high initial relationship (■); high reproduction, high initial relationship (◆)





**Fig. 4** Trends in (a) GD and (b)  $\bar{F}$  observed for groups of 30 founders. Data from the high reproduction, low initial relationship scenario are shown for 30 founders that included groups of full-siblings. Values for timestep 0 were calculated for the founders. For GD, the left y-axis was scaled relative to the initial founders of Simulation 1 and the right y-axis was scaled relative to the founders of Simulation 2. Module 1 ( $\blacktriangledown$ ); Module 2 ( $\blacksquare$ ); Module 3 ( $\bullet$ )

Across all scenarios and over all timesteps, the difference in GD between Modules 1 and 2 ranged from 0.0029 to 0.0179 (Fig. 7).  $\bar{F}$  experienced a general increase over timesteps and, as in the scenarios presented for 30 founders, the difference in  $\bar{F}$  between Module 1 (true founder relationships) and Module 2 (assumed founder relationships) initially increased, then decreased before becoming stable around the tenth timestep. The difference in  $\bar{F}$  ranged from 0.0058 to 0.0335 across all timesteps and scenarios (Fig. 7). Across Modules 1 and 2, the 95% confidence intervals for both GD and  $\bar{F}$  ranged from 0.0001 to 0.0010.

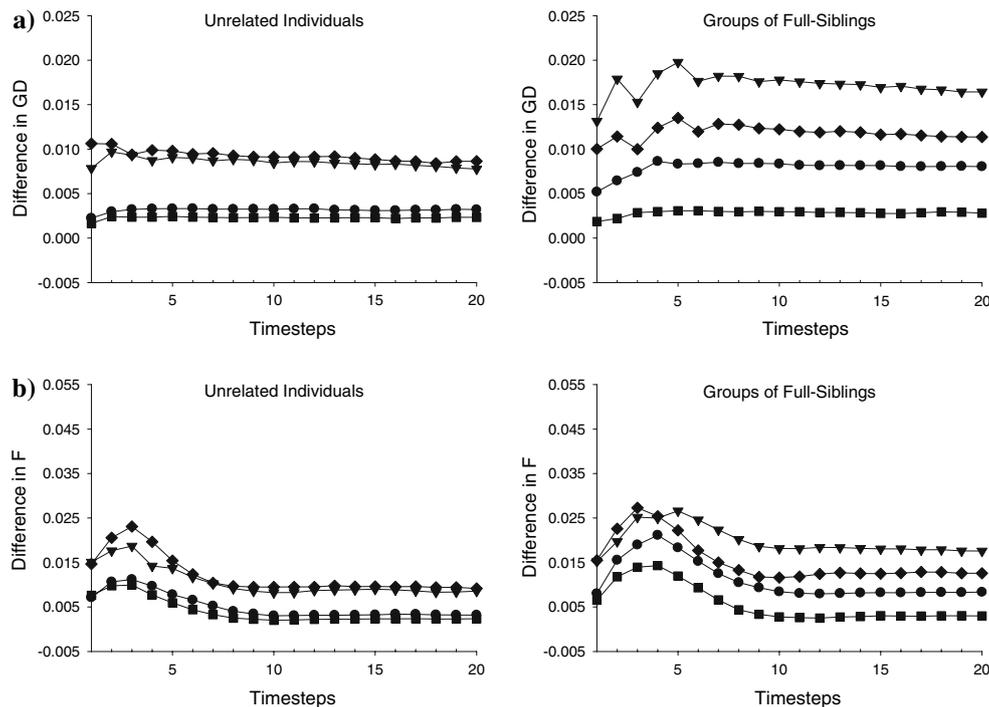
## Discussion

We investigated the effects of assuming that the founders of captive populations are unrelated and not inbred on breeding strategies that minimize the overall kinship in a

population. While several studies have examined issues surrounding founders (e.g., Willis 1993; Willis and Wiese 1993; Haig et al. 1994), none has quantified how assuming that founders were unrelated affects the ability of MK breeding strategies to retain genetic variation and limit inbreeding in future generations. Because MK strategies require accurate pedigrees, the effectiveness of these strategies should be improved when founder relationships are known. MK strategies preferentially breed individuals that are genetically underrepresented (Ballou and Lacy 1995), which continually corrects for the stochastic and probabilistic factors that impact the genetic composition of actual captive populations (e.g., uncertainty of mortality, reproductive success, variable litter size). To summarize the effects of the founder assumptions under a wide variety of conditions, we tested numerous scenarios that incorporated these types of factors.

For all scenarios tested, the difference in GD between when founder relationships were known and when founders were assumed to be unrelated was relatively constant over all timesteps. Differences in  $\bar{F}$ , however, initially increased for several timesteps before subsequently declining to an approximately steady value. This indicated that, for inbreeding, the benefit for knowing founder relationships changed over time. The most likely explanation for the decrease in benefit is that for MK breeding strategies, recent ancestry has a greater influence on the accumulation of inbreeding than deeper ancestry. Because complete pedigrees were kept for simulated populations after they were founded, recent ancestries became more accurate over time and the benefit to  $\bar{F}$  for knowing deeper ancestries (e.g., among the founders) declined.

When populations were founded by and subsequently maintained at 100 individuals, utilizing known founder relationships retained between 0.5% and 2% more GD for 5–10 generations than assuming founders were unrelated and not inbred (Fig. 7). When small groups of unrelated founders were allowed to grow into populations that were subsequently maintained at about 100 individuals, there was less of a benefit to knowing founder relationships; differences in GD were generally less than 0.5% for populations founded by 10 individuals and less than 1% for populations founded by 30 individuals (Figs. 3, 5). Data on  $\bar{F}$  also exhibited similar trends. Again, the most likely explanation for those trends is that the effectiveness of MK strategies depends more on the accuracy of recent, rather than historical, ancestries. When populations founded by small numbers of individuals were allowed to grow, the maximum number of breeding pairs possible was made each timestep until the population reached carrying capacity (100 individuals). Thus, even though breeding pairs were selected by *mk*, true genetic management was not occurring immediately. After carrying capacity was



**Fig. 5** Differences in (a) GD and (b)  $\bar{F}$  observed between Modules 1 and 2 for groups of 30 founders. The 95% confidence intervals for the averages of Modules 1 and 2 did not overlap for any timesteps. Low

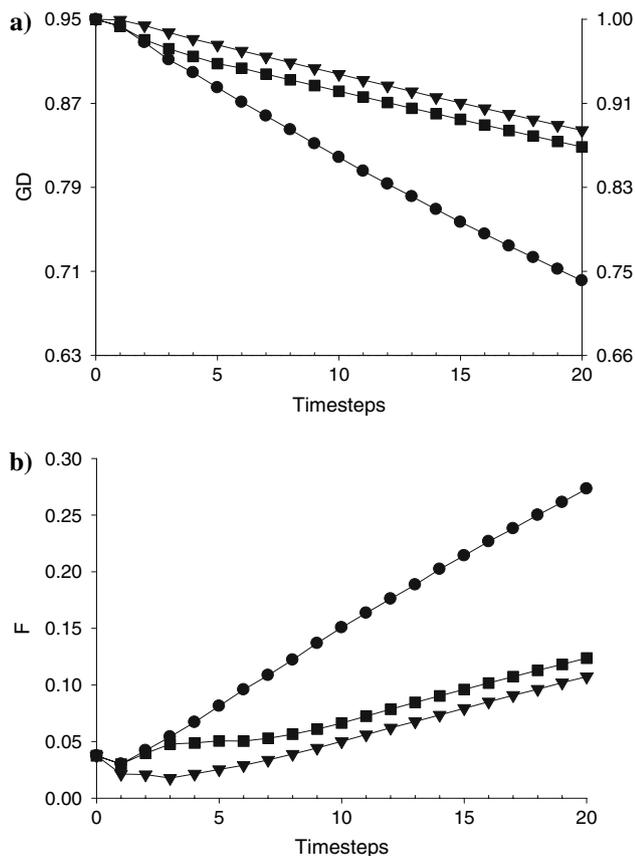
reproduction, low initial relationship (●); high reproduction, low initial relationship (▼); low reproduction, high initial relationship (◆); high reproduction, high initial relationship (■)

reached and genetic management became effective, there was less of a benefit to knowing historical founder relationships because recent ancestries were driving the effectiveness of the MK strategy.

Another trend was observed when the scenarios that were run for small groups of unrelated founders were compared to those run for founder groups comprised of full-siblings. When small groups of founders included full-siblings, the range of differences in GD and  $\bar{F}$  observed across all scenarios run for a given number of founders widened. As previously stated, when small groups of founders were unrelated the difference in GD between using founder relationships and assuming founders were unrelated was generally less than 0.5% for populations founded by 10 individuals and less than 1% for populations founded by 30 individuals. When founder groups included full-siblings, some scenarios produced equivalent results. However, for other scenarios, differences in GD rose as high as 1% for scenarios using 10 founders (Fig. 3) and as high as 1.5–2% for scenarios using 30 founders (Fig. 5). Increased benefit for knowing founder relationships when full-siblings were present was also demonstrated for  $\bar{F}$ . This trend was most striking for scenarios run with 10 founders, where differences in  $\bar{F}$  between knowing founder relationships and assuming

founders were unrelated reached 3–5% during initial timesteps (Fig. 3). While data on both GD and  $\bar{F}$  indicated that the potential benefit to knowing founder relationships was higher when full-siblings were present, it was unclear under what circumstances an increase in benefit was actually realized. Additional simulations were run for groups of 10 and 30 founders that included full-siblings, and it was discovered that the benefit for knowing founder relationships was highly dependent on the exact composition of the founding group (e.g., number of full-sibling groups, kinship between groups of full-siblings). Still, it is clear that, relative to scenarios run for unrelated founders, higher benefits for knowing founder relationships occurred when founders included full-siblings. However, even in these relatively extreme cases of founder relatedness, the overall long-term impact to knowing founder relationships was only modest.

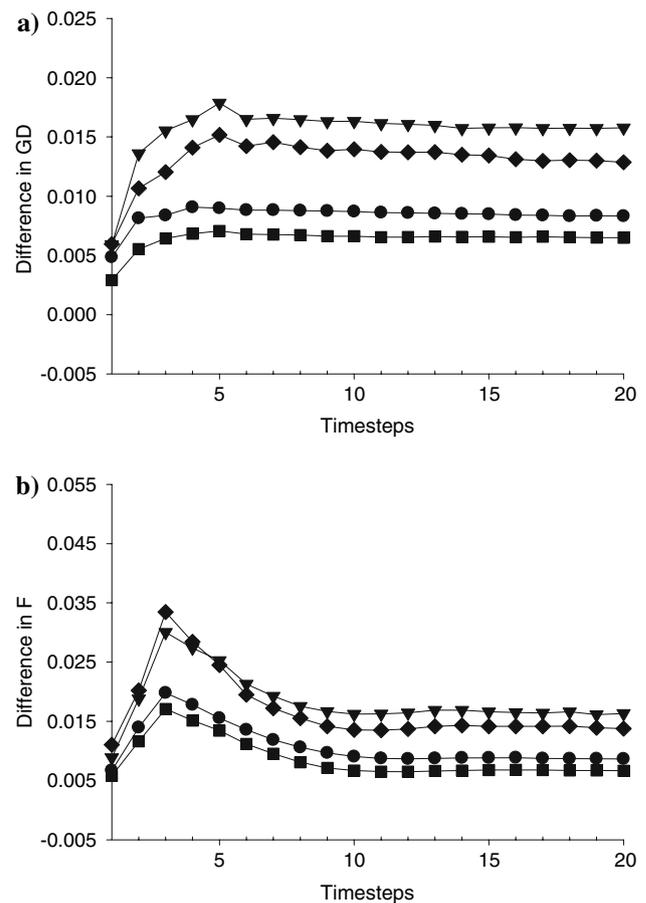
Scenarios for a given number of founders were run under two different rates of reproduction and two different levels of initial founder relationship. For scenarios run with 30 and 100 founders, greater benefits for knowing founder relationships were observed with higher rates of reproduction (Figs. 5, 7). At a low rate of reproduction long-term differences in both GD and  $\bar{F}$  for scenarios run with 30 unrelated founders fell below 0.5%, while at a



**Fig. 6** Trends in (a) GD and (b)  $\bar{F}$  observed for groups of 100 founders. Data from the high reproduction, low initial relationship scenario are shown. Values for timestep 0 were calculated for the founders of Simulation 1 and the right y-axis was scaled relative to the founders of Simulation 2. Module 1 ( $\blacktriangledown$ ); Module 2 ( $\blacksquare$ ); Module 3 ( $\bullet$ )

higher rate of reproduction differences in GD and  $\bar{F}$  were near 1%. A similar trend was observed for scenarios run with 100 founders; at a low reproductive rate differences in GD and  $\bar{F}$  were below 1%, while at a high reproductive rate differences in both values were 1–2%. When scenarios varying initial founder relationship were considered, no clear trend was observed. For populations founded by 30 and 100 individuals, there was greater benefit to knowing founder relationships when both initial founder relationship and rate of reproduction were low (Figs. 5, 7). However, for scenarios run at higher rates of reproduction, the level of initial founder relationship providing the most benefit was dependent on the number of founders (Figs. 5, 7).

Overall, results suggest that there is some benefit gained from knowing founder relationships when implementing an MK breeding strategy. Because molecular markers can provide information on unpedigreed individuals, genetic data on founders may be valuable for captive breeding



**Fig. 7** Differences in (a) GD and (b)  $\bar{F}$  observed between Modules 1 and 2 for groups of 100 founders. The 95% confidence intervals for the averages of Modules 1 and 2 did not overlap for any timesteps. Low reproduction, low initial relationship ( $\bullet$ ); high reproduction, low initial relationship ( $\blacktriangledown$ ); low reproduction, high initial relationship ( $\blacksquare$ ); high reproduction, high initial relationship ( $\blacklozenge$ )

programs. For example, DNA fingerprints identified three population subgroups within the captive California condor population for which the between-group relatedness was significantly less than the within-group relatedness (Geyer et al. 1993). Ely et al. (2005) used mitochondrial sequence data to provide a different type of information about captive population founders; the authors identified the subspecies of chimpanzee founders, which subsequently allowed them to describe the representation of those subspecies in a current captive population. As a final example, Ramirez et al. (2006) used microsatellites to retrospectively estimate the effective number of founders for a captive Iberian wolf population. Because the estimated effective number of founders closely matched the recorded number of founders, the authors concluded the founders were generally unrelated.

While the previous examples demonstrate that molecular data can provide a wealth of information about population founders, few studies to date have attempted to

directly integrate this information into MK breeding strategies. Breeding recommendations for MK strategies are based on an individual's  $mk$ , which can only be calculated if a complete pairwise  $f$  matrix is available. This means that, to most effectively improve MK breeding strategies, molecular markers must provide accurate estimates of pairwise founder relatedness. A limited number of studies have used molecular estimates of founder kinship to calculate  $mk$  and subsequently demonstrate how the genetic importance of individuals changed relative to assuming founders were unrelated (e.g., Jones et al. 2002; Russello and Amato 2004). However, we have not been able to find a study that explicitly advocated breeding recommendations should be based on  $mks$  estimated from molecular data the authors collected. While a number of genetic methods for estimating relatedness exist, all estimators suffer from the large variances that surround single, pairwise estimates (Lynch 1988; Lynch and Ritland 1999; Glaubitz et al. 2003). Given the marginal gains we observed for knowing founder relationships precisely, it seems unlikely that molecular estimates of founder relatedness would produce significantly better results than simply assuming founders were unrelated. Additional investigations are needed however, to conclusively determine the impacts and potential benefits of integrating molecular estimates of  $f$  into the kinship matrices used for MK breeding strategies.

While large variances hinder the usefulness of molecular estimates of pairwise  $f$  for calculating  $mk$ , the accuracies of many genetic relatedness estimators have been shown to be sufficient for distinguishing first-order relatives from unrelated individuals (Piper and Rabenold 1992; Blouin et al. 1996; Glaubitz et al. 2003). As the greatest benefit for knowing founder relationships was realized in the average inbreeding initially accumulated in populations that were founded by small groups of full-siblings, the incorporation of genetic estimates of relatedness into MK breeding strategies could prove the most valuable for limiting a population's initial inbreeding. However, while the benefits that could be gained from knowing founder relationships should be closely considered on a per population basis, our results suggest that captive breeding strategies aimed at minimizing a population's overall kinship probably often produce near optimal results when standard assumptions are made about founder relationships.

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