
Improvement of the Maximum Avoidance of Inbreeding by the Use of Cell Manipulation Technique in Gametogenesis

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Abstract: Reduction of genetic drift for preservation of genetic variability is one of the primary concerns for maintenance of endangered species in captivity. For this purpose, a number of selection schemes to equalize parental contributions to the next generation have been widely accepted as a simple guideline, but genetic drift due to random segregation of heterozygote parents, so-called Mendelian sampling, has remained unavoidable. In the past, the use of cell manipulation techniques developed in a field of mammal reproductive technology has been suggested to restrict this genetic drift. However, its potential benefit has been examined only for a randomly mating population of equal sex ratio. In this study, we assumed the situation where the cell manipulation technique is applied to the population under the mating system of maximum avoidance of inbreeding (MAI), and examined its effect on the progress of inbreeding by developing a recurrence equation of panmictic indices of the population. Inbreeding coefficient was substantially suppressed at the locus site where the mean number of crossovers between the site and centromere (m) was small. Although inbreeding coefficient inflated as m increased, its effect diminished as m increased. These tendencies were observed irrespective of the size of the population.

Keywords: Maximum Avoidance of Inbreeding, Mendelian Sampling, Recurrence Equation, Cell Manipulation Technique, Gametogenesis

1. Introduction

When preservation of endangered wild species in captivity is planned, relaxation of rapid decrease of expected heterozygosity due to genetic drift, i.e., random change in allele frequency, is one of the key issues to be addressed for a successive reintroduction to the wild condition [1]. The amount of genetic drift is determined by the effective size of the population [2], for which many predictive equations taking into account of demographic variables have been previously developed by many researchers [3, 4].

Genetic drift is measured by variance of a change in allele frequency from one generation to the next, and generally ascribed to two main sources. The first one is the different contributions of parents to the next generation, which is expressed as variance and covariance of their progeny numbers. The other one is a Mendelian sampling, which arises when allele is transmitted to the progeny from a heterozygote parent.

For preservation of genetic variability in the artificially controlled population, equalization of family sizes to relax the former source of genetic drift has been suggested based on the implications from the effective size theory [5, 6]. Especially, it is well known that in a population with equal sex ratio, equalization of family sizes completely restricts the former source of genetic drift, and inflates the effective size twice as large as a randomly mating population of the same size. For a population with unequal sex ratio, selection scheme proposed by Gowe et al. [7] had been considered to be of most efficient in equalizing family sizes of parents. However, Wang [8] have found more efficient selection scheme to reduce genetic drift due to variance in the family sizes.

On the other hand, selection method to reduce the latter source of genetic drift, i.e., Mendelian sampling, have also been studied. Wang and Hill [9] proposed the selection method of offspring using information given by genetic markers, and revealed its efficiency theoretically and also by simulation. At the same time, Santiago and Caballero [10]

proposed the potential use of cell manipulation technique in the processes of gametogenesis and fertilization. They supposed a situation where four gametes from the same meiosis were identifiable and available for reproduction, and showed that appropriate selection of multiple gametes from the four gametes effectively restrict genetic drift due to Mendelian sampling.

Their idea was based on a number of existing cell manipulation techniques in mammal species. For example, because production of normal offspring from the first and second polar bodies transferred to enucleated oocytes had already been achieved [11, 12], it is theoretically possible to produce more than one offspring from the same meiosis of female parents. In addition, in vitro culture of premeiotic germ cells [13] and microinjection of primary spermatocytes into oocytes [14] will allow the identification of four spermatogenic products. Evaluation of potential benefits of such reproductive technologies prior to the application to the genetic conservation of endangered species in captivity should be of importance.

In this paper, we will show that the cell manipulation technique can be applied to the population under the mating system of maximum avoidance of inbreeding (MAI) and improve the mating system.

2. Materials and Methods

2.1. Recurrence Equation of Panmictic Indices of the Population Under MAI

The mating pattern of MAI with $N=8$ individuals are illustrated in Figure 1. Let F_t be the inbreeding coefficient of an individual in generation t , and $\theta_t(k)$ be the probability that two randomly chosen homologous genes in two individuals k ($=1 \sim N/2$) steps removed are identical by descent, i.e., coancestry between two individuals k steps removed to each other.

Putting $P_t(0)=1-F_t$ and $P_t(k)=1-\theta_t(k)$,

$$h_t = Gh_{t-1}. \quad (1)$$

Here, for example, when $N=8$,
 $h'_t = [P_t(0) P_t(1) P_t(2) P_t(4)]$ and

$$G = \begin{bmatrix} 0 & 1 & 0 & 0 \\ 0 & 1/2 & 1/2 & 0 \\ 0 & 1/2 & 0 & 1/2 \\ 1/4 & 1/2 & 0 & 0 \end{bmatrix}$$

In general, h_t includes $P_t(0)$ and $P_t(2^x)$ ($x=0, 1, 2, \dots, \log_2 \frac{N}{2}$), and the elements of $G=[g_{ij}]$ are shown in Table 1. The last row of h_t describes the relationships between full-sibs born in generation t .

Table 1. Elements of matrix $G=[g_{ij}]$ in MAI.

i (rows)	j (columns)	g_{ij}
1	2	1
	$\neq 2$	0
k ($=2, \dots, \log_2 N$)	2	1/2

i (rows)	j (columns)	g_{ij}
	$k+1$	1/2
	$\neq 2$ and $k+1$	0
	1	1/4
$\log_2 N + 1$	2	1/2
	$\neq 1$ and 2	0

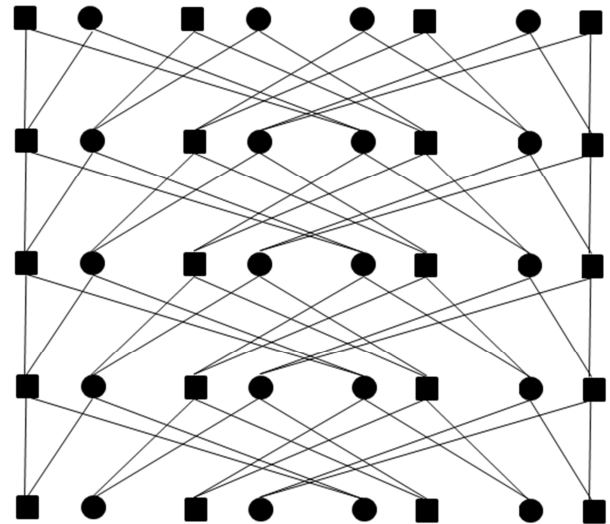


Figure 1. Maximum avoidance of inbreeding with the population size of $N=8$. Squares are males and circles are females. Generation progresses from top to down.

2.2. Selection of Two Gametes by the Cell Manipulation Technique in Gametogenesis

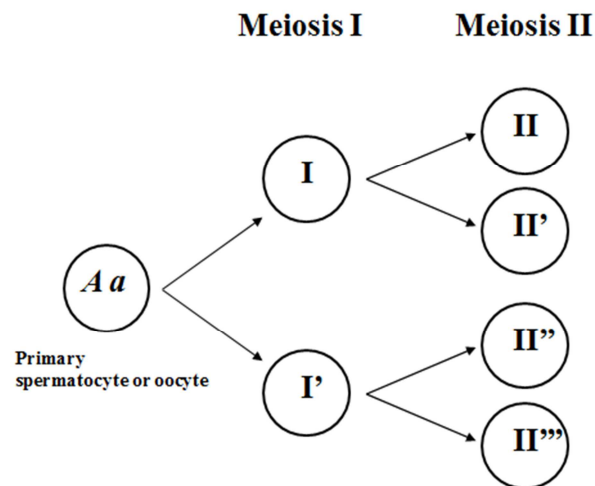


Figure 2. Process of gametogenesis from primary spermatocyte or oocyte. I and I' represent the two products of meiosis I and II, II', II'', and II''' represent the four products of meiosis II. Aa represents heterozygote.

Figure 2 depicts the process of gametogenesis from one primary spermatocyte or primary oocyte. In this study, we assume that, throughout the cell manipulation technique, four gametes from the same meiosis are identifiable in any male and female parents, and all of these gametes are available for reproduction. Furthermore, from which cell of I and I' each gamete originates is assumed to be distinguishable.

Male parents produce one male progeny and one female

progeny under MAI. So, two gametes derived from different cell after meiosis I (II or II' and II'' or II''') are assumed to be used for reproduction, since X chromosome and Y chromosome in a male parent should segregate at meiosis I. According to Santiago and Caballero [10], such sampling method of gametes suppresses genetic drift most efficiently. Therefore, the same sampling method of gametes is assumed to be applied to the female parents.

2.3. Recurrence Equation of Panmictic Indices When the Cell Manipulation Technique Is Applied to MAI

When the cell manipulation technique is applied to the population under MAI, modification is needed for the computation of coancestry between full-sibs. Suppose that full-sibs of G and H are produced by the mating between parents of E and F. Letting the frequency of an arbitrary neutral allele, say A , in the individual i be x_i ($=0, 1/2$ or 1), and Mendelian sampling term which arises when individual i produces individual j be δ_{ij} ($=-1/4, 0$ or $1/4$), frequencies of an arbitrary neutral allele in the individuals G and H are

$$x_G = \frac{1}{2}(x_E + x_F) + \delta_{EG} + \delta_{FG}$$

and

$$x_H = \frac{1}{2}(x_E + x_F) + \delta_{EH} + \delta_{FH}$$

respectively. Ignoring expected covariance between haploid gametes in the base population,

$$\begin{aligned} \theta_{GH}q(1-q) &= \text{cov}[x_G, x_H] \\ &= E \left[\left\{ \frac{1}{2}(x_E + x_F) + \delta_{EG} + \delta_{FG} \right\} \left\{ \frac{1}{2}(x_E + x_F) + \delta_{EH} + \delta_{FH} \right\} \right] - \frac{1}{2}(E[x_E] + E[x_F]) \\ &\quad \cdot \frac{1}{2}(E[x_E] + E[x_F]) \\ &= \frac{1}{4}(V[x_E] + V[x_F] + 2\text{cov}[x_E, x_F]) + E[\delta_{EG}\delta_{EH}] \\ &\quad + E[\delta_{FG}\delta_{FH}] \\ &= \frac{1}{4}(\theta_{EE} + \theta_{FF} + 2\theta_{EF})q(1-q) + E[\delta_{EG}\delta_{EH}] + E[\delta_{FG}\delta_{FH}] \quad (2) \end{aligned}$$

where θ_{ij} is the coancestry between individuals i and j , and q is the frequency of an arbitrary neutral allele (A) in the base population.

The first term in the final equation in (2) is the term following the ordinary computation rule of the coancestry [5], and the second and the third terms are the additional terms that are responsible for the application of the cell manipulation technique. Since $E[\delta_{EG}\delta_{EH}] = E[\delta_{FG}\delta_{FH}]$, we will derive $E[\delta_{EG}\delta_{EH}]$ as an example. Using the inbreeding coefficient of individual E (F_E), the probability of individual E being the heterozygote (Aa) is $2q(1-q)(1-F_E)$. According to Santiago and Caballero [10], combinations of the value of δ_{EG} and δ_{EH} , and the probabilities they arise when E is a

heterozygote can be expressed as shown in Table 2. With these values and probability, $E[\delta_{EG}\delta_{EH}]$ can be computed as:

$$\begin{aligned} E[\delta_{EG}\delta_{EH}] &= 2q(1-q)(1-F_E) \\ &\cdot \frac{1}{16} \left(\frac{1 - e^{-\frac{3m}{2}}}{6} - \frac{2 + e^{-\frac{3m}{2}}}{6} - \frac{2 + e^{-\frac{3m}{2}}}{6} + \frac{1 - e^{-\frac{3m}{2}}}{6} \right) \\ &= \frac{-1 - 2e^{-\frac{3m}{2}}}{24} (1-F_E)q(1-q), \end{aligned}$$

where m is the mean number of crossovers which occurs between the centromere and the locus site.

Therefore, coancestry between full-sibs in generation t can be computed by adding

$$\begin{aligned} 2 \times \frac{-1 - 2e^{-\frac{3m}{2}}}{24} (1-F_{t-1}) &= 2 \times \frac{-1 - 2e^{-\frac{3m}{2}}}{24} P_{t-1}(0) \\ &= 2M_{t-1} \end{aligned}$$

to the ordinary coancestry. Here, F_{t-1} is the inbreeding coefficient in the parental generation. Using M_{t-1} , the recurrence equation of the panmictic indices for the population where the cell manipulation technique is applied can be expressed as:

$$h_t = Gh_{t-1} - 2m_{t-1}, \quad (3)$$

where $m'_{t-1} = [0 \ 0 \ \dots \ M_{t-1}]$.

Table 2. Mendelian sampling terms (δ_{EG} and δ_{EH}) of a heterozygote parent and their probability when two gametes are sampled from the same meiosis but the different cells after meiosis I.

δ_{EG}	δ_{EH}	probability
1/4	1/4	$\frac{1 - e^{-\frac{3m}{2}}}{6}$
1/4	-1/4	$\frac{2 + e^{-\frac{3m}{2}}}{6}$
-1/4	1/4	$\frac{2 + e^{-\frac{3m}{2}}}{6}$
-1/4	-1/4	$\frac{1 - e^{-\frac{3m}{2}}}{6}$

m : mean number of crossovers between the centromere and the locus site.

3. Results

To assess the effects of the cell manipulation technique on the accumulation of inbreeding, numerical computations with equations (1) and (3) were carried out. Figure 3 shows the inbreeding of the population with $N=8, 16$, and 32 . “original” is the results computed by (1). Since genetic drift due to Mendelian sampling is completely suppressed when $m=0$, as indicated by Santiago and Caballero [10], inbreeding coefficient remains 0, irrespective of the population size (N).

Without the cell manipulation technique, inbreeding of the population with $N=8$ reached 0.48 at generation 20, however, inbreeding can be suppressed to 0.19 for the locus site of

$m=0.5$ if the cell manipulation technique is applied. The increase of m inflates the inbreeding, but its effect diminished as m increased. This tendency was also observed for the population with $N=16$ and 32 . From these results, the cell manipulation technique seems to substantially improve MAI in terms of the restriction of inbreeding.

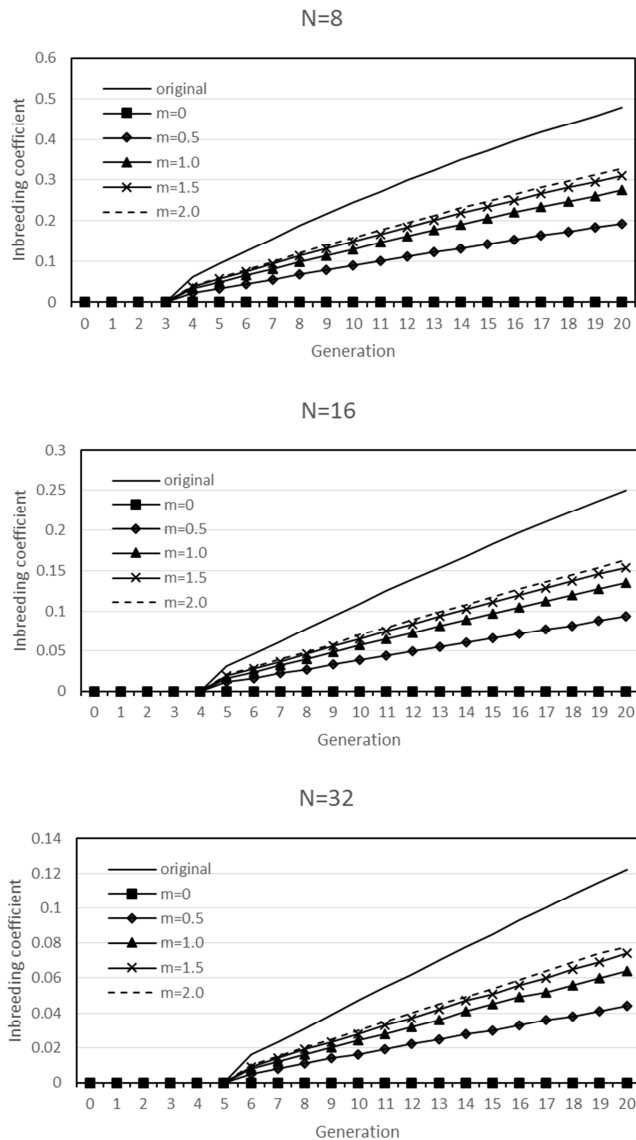


Figure 3. Inbreeding coefficient of the populations under MAI, with the size of $N=8$, 16, and 32 individuals. "original" indicates MAI without cell manipulation technique. m is the mean number of crossovers between the centromere and the locus site.

4. Discussion

MAI is the mating system, which can be applied to the population with 2^x individuals ($x \geq 2$), and many researchers theoretically studied its features in the past [15-17]. Because MAI is more useful for diminishing the inbreeding coefficient in the initial generations than the other mating systems (e.g. circular pair mating, circular mating), it seems to be suitable for the practical use in genetic management of a small population. Indeed, it is introduced in a famous textbook of

conservation genetics [1] as one of the reproductive tools for the endangered species.

In the field of management of captive endangered animals, various schemes to select individuals for breeding using pedigree or demographic data have been proposed to maintain genetic variability of the population [18, 19]. Among these schemes, minimization of average coancestry of the population is theoretically proved to be the best [5, 20, 21], because it corrects unequal contributions from ancestral generations, leading to maximization of effective size in multiple generations from the past [5, 21]. Therefore, the mating system considered in this study will be of serve in a situation where pedigree or DNA marker information for estimating relationships among individuals is lacked.

Although this study and that of Santiago and Caballero [10] assumed the population of equal sex ratio, we revealed that the cell manipulation in the gametogenesis effectively suppresses the genetic drift even in the population of unequal sex ratio (Results not published). In that study, we assumed that the cell manipulation technique was applied to the population under the mating systems proposed by Gowe et al. [7] and Wang [8]. Although the mating system of Gowe et al. [7] was always superior in restricting the genetic drift due to Mendelian sampling, total genetic drift was always suppressed more effectively by the mating system of Wang [8].

5. Conclusion

In this study, the idea of restricting genetic drift by the use of cell manipulation technique was applied to one of the most famous and classical mating systems, i.e., maximum avoidance of inbreeding. The recurrence equation of the inbreeding coefficient of the population under the mating system adopting the cell manipulation technique was derived. Based on the numerical computation with the recurrence equation, we concluded that the cell manipulation technique in gametogenesis considerably improves the maximum avoidance of inbreeding, and recommended this improved mating system as a conservation scheme of an endangered wild species when pedigree or DNA marker information for estimating relationships among individuals is lacked.

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