Marker-Assisted Mating Applied in *In-Situ* Conservation of Indigenous Animals in Small Populations : (1) Choosing Mating Schemes for Maximum Heterozygosity[a]

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ABSTRACT : Maintaining maximum genetic variability is of critical importance with *in-situ* conservation of animal species in small populations. Marker-assisted mating (MAM) was suggested to achieve maximum heterozygosity in offspring populations. The aims of this research was to investigate and decide the effectiveness and promising types of MAM to achieve this goal. Analysis of variance with simulation data revealed that the heterozygosity in offspring populations was significantly determined by sire heterozygosity from mating of non-inbred parent animals, and significantly by sire heterozygosity and percent parental difference in offspring reproduced by inbred parents. Seven types of marker-assisted mating schemes were examined, in which offspring exhibited heterozygosity that was -0.01 to 7.37% below or above that from random mating of non-inbred parent animals, and 0.00 to 16.39% above that from random mating of inbred parent animals. The great increase in offspring heterozygosity was observed with mating by tandem maximizing sire heterozygosity, percent parental difference, and dam heterozygosity. Random mating resulted in fluctuation of offspring heterozygosity. These results suggested that MAM was a promising method for maintaining maximum offspring variability in *in-situ* conservation of animal species in small populations. (***Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 4 : 431-434***)

Key Words : Marker-Assisted Mating, *In-Situ* Conservation, Indigenous Animals, Heterozygosity

INTRODUCTION

Maintenance of indigenous or endangered breeds of livestock or poultry acts as insurance against loss of genes and gene combinations that may have value for currently unforeseen uses. It is estimated that at least 40% of all breeds of domestic livestock and fowls have been lost since 1970 (Alderson, 1989). Loss of livestock breeds is of great concern today, particularly with the advance of molecular technologies which enables the manipulation of individual genes.

Mating systems are of critical importance in *in-situ* conservation of animal genetic resources (Yamada, 1988). Present mating systems for endanger animals focused on reduction of the rate of loss of genetic variation through minimization of kinship in the short run. Papp (1992) described a procedure in which selection decisions were based on the rarity of blood group genotypes. Lamberson (1998) proposed that selection on an index measuring rarity of alleles identified by genotyping anonymous markers across the genome could be used to increase the efficiency of maintaining genetic variation.

Because one of the major tasks in *in-situ* conservation of animal species is to maintain as great as possible heterozygosity in the target populations (Takeda et al., 1998), and because it is now possible to identify a limited number of anonymous markers that give coverage of the genome, and screen animals within the population for those markers (Hillel et al., 1992), marker-assisted mating (MAM) could be a method of choice in directed mating for maximum heterozygosity in offspring populations. Theoretically, when all animals in a population have been screened for all known markers alleles, selection and mating decisions can be made based on that information.

The objective of this simulation work was to investigate: (1) the effectiveness of marker-assisted mating (MAM) for maintaining maximum heterozygosity in offspring populations, and (2) which types of MAM scheme was the most effective to achieve this goal.

MATERIALS AND METHODS

Simulation animals

120 parent animals, i.e., 60 sires and 60 dams, were derived either from non-inbred or inbred parentage. The inbred parental animals were half-sibs derived from 3 unrelated sires and 15 unrelated dams, with 1 sire mated 5 dams. At breeding season, a complete pairwise mating (60×60) was arranged

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among all 120 parental animals, with each pair gave birth to 8 effective offspring, which was defined as the offspring that lived to breeding age and were able to produce the next generation.

Simulation markers

Molecular markers which followed the inheritance of co-dominance (e.g., microsatellites) were assumedly applied in this approach. It was also assumed that a gamete had 10 linkage groups, each with a length of 100 cM. These markers were randomly and evenly distributed throughout the genome. It was also assumed that there was one locus at every 10 cM, and a total of 100 loci for the whole gamete.

Stochastic models of marker transmission

Marker transmission was examined with a method of stochastic simulation based on modification of the theory of Bernardo (1992), with new models constructed for measuring molecular marker heterozygosity and percent parental difference, which is described as below. For simplicity, linkage disequilibrium was not considered in this simulation.

Let k (k=1, 2, 3,....., n) be the number of marker loci, and S_i and D_j represent the i^{th} sire and the j^{th} dam, respectively, from the simulated population, then, the molecular marker heterozygosity (MMH) at the K^{th} molecular marker locus for S_i and D_j are:

$$MMH(S)_{iik}=(P_{ik}+M_{ik} - 2P_{ik}M_{ik}), \text{ and}$$
$$MMH(D)_{jjk}=(P_{jk}+M_{jk} - 2P_{jk}M_{jk}),$$

where: P_{ik}=frequency (0 or 1) of the +allele at the K^{th} locus in the gamete of paternal origin in sire S_i; M_{ik}=frequency (0 or 1) of the +allele at the K^{th} locus in the gamete of maternal origin in sire S_i. P_{jk} and M_{jk} can be similarly defined for dam D_j. Obviously it can be seen that:

$$MMH = \begin{cases} 0, & \text{for homozygous + + (P=M=1);} \\ 1, & \text{for heterozygous + - (P} \neq \text{M);} \\ 0, & \text{for homozygous - - (P=M=0).} \end{cases}$$

The percent parental ($S_i \times D_j$) difference (PD) based on molecular markers at the K^{th} locus is:

$$PD_{ijk}= \mid P_{ik}+M_{ik} - P_{jk} - M_{jk} \mid \div 2.$$

Accordingly, PD could be:

$$PD= \begin{cases} 1, & \text{for neither of the 2 alleles (+ or -) was identical between parents;} \\ 0.5, & \text{for 1 of the 2 alleles (+ or -) is identical between parents;} \\ 0, & \text{for both of the 2 alleles (+ or -) are identical between parents.} \end{cases}$$

When S_i mated D_j, the marker heterozygosity at the K^{th} locus for the ij^{th} offspring is:

$$MMH(O)_{ijk}=(P'_{ik}+M'_{jk} - 2P'_{ik}M'_{jk})$$

where:

$$P'_{ik}=P_{ik}\ BRND+M_{ik}\times(1 - BRND);$$
$$M'_{jk}=P_{jk}\ BRND+M_{jk}\times(1 - BRND),$$

where BRND is a random number (1 or 0) generated from the random process of Bernulli. Accordingly, we have:

$$P'_{ik} = \begin{cases} P_{ik}, & \text{for BRND = 1;} \\ M_{ik}, & \text{for BRND = 0.} \end{cases}$$
$$M'_{jk} = \begin{cases} P_{jk}, & \text{for BRND = 1;} \\ M_{jk}, & \text{for BRND = 0.} \end{cases}$$

Means are obtained by summing across all loci, and divided by the number of loci (n).

$$MMH(S)_{ii}=(\sum MMH(S)_{iik})/n;$$
$$MMH(D)_{jj}=(\sum MMH(S)_{jjk})/n;$$
$$MMH(O)_{ij}=(\sum MMH(O)_{ijk})/n.$$
$$PD_{ij}=(\sum PD_{ijK})/n.$$

Statistics

Random numbers were generated by Bernulli process to simulate the generation of parental individuals and reproduction of next generation with an author-defined program in codes of Foxpro 2.6 for Windows. Analysis of variance was conducted with the following linear model using the GLM procedure (SAS Institute Inc., 1990).

$$MMH(O)_{ijk}= \mu +MMH(S)_i+MM(D)_j+PD_{ij}+e_{ijk}$$

where is e_{ijk} the random error.

RESULTS AND DISCUSSION

Analysis of variance indicated the existence of significant effect of sire heterozygosity (MMH(S)) on offspring heterozygosity (MMH(O)) ($p<0.01$), but no significant effect of dam heterozygosity (MMH(D)) on MMH(O) was detected ($p>0.05$). Percent parental difference was of significant importance to the heterozygosity of offspring reproduced from inbred parents ($p<0.01$), but of no significant importance to the heterozygosity of offspring when non-inbred parentage was involved ($p>0.05$). The results suggested that greater sire heterozygosity would most probably result in increased offspring heterozygosity. Mating of inbred parental animals that exhibited larger difference at anonymous marker loci would also produce offspring

Table 1. Analysis of variance for the general linear models and model effects[1]

Statistics[2]	Degree of freedom	Sum of squares	Mean square	F value	Pr > F
		from a non-inbred parental population			
General linear model					
Model	3	0.1329**	0.0444	32.01	0.0001
Error	3596	4.9855	0.0014		
Model effects					
MMH(S)	1	0.1329**	0.1329	95.87	0.0001
MMH(D)	1	0.0002	0.0002	0.16	0.6929
PD	1	0.0000	0.0000	0.00	0.9919
		from an inbred parental population (half-sibs)			
General linear model					
Model	3	1.3112**	0.4371	280.47	0.0001
Error	3596	5.6039	0.0016		
Model effects					
MMH(S)	1	1.2893**	1.2893	827.31	0.0001
MMH(D)	1	0.0028	0.0028	1.79	0.1805
PD	1	0.0192**	0.0192	12.31	0.0005

** $p<0.01$.

[1] Mating was arranged between complete mating pairs of 60 sires and 60 dams (60×60), each pair producing 8 effective offspring. Mean heterozygosity across all 8 offspring per dam was used in this analysis.

[2] MMH(S)=molecular marker heterozygosity of sires, MMH(D)=molecular marker heterozygosity of dams, PD=percent parental difference of molecular markers.

Table 2. Effect estimation of marker-assisted mating on mean heterozygosity of offspring[1]

Mating schemes[2]	MMH(S)	MMH(D)	PD	MMH(O)	Increase (%)[3]
		from a non-inbred parental population			
By MMH(S)	0.680±0.000	0.395±0.027	0.371±0.032	0.515±0.021	2.59
By MMH(D)	0.589±0.049	0.700±0.000	0.291±0.037	0.509±0.039	1.39
By PD	0.439±0.047	0.416±0.059	0.514±0.017	0.499±0.031	-0.01
By index 1	0.672±0.017	0.655±0.029	0.291±0.025	0.525±0.024	4.58
By index 2	0.677±0.007	0.425±0.049	0.404±0.014	0.531±0.022	5.78
By index 3	0.677±0.007	0.639±0.042	0.307±0.033	0.524±0.021	4.38
Tandem	0.680±0.000	0.429±0.047	0.390±0.009	0.539±0.022	7.37
R-mating	0.491±0.069	0.506±0.070	0.377±0.046	0.502±0.038	-
		from an inbred parental populational (half-sibs)			
By MMH(S)	0.680±0.000	0.511±0.060	0.302±0.032	0.416±0.018	13.66
By MMH(D)	0.496±0.054	0.720±0.000	0.343±0.022	0.366±0.044	0.00
By PD	0.433±0.041	0.455±0.073	0.492±0.018	0.372±0.066	1.64
By index 1	0.647±0.039	0.681±0.045	0.261±0.050	0.417±0.027	13.93
By index 2	0.649±0.026	0.432±0.038	0.419±0.029	0.419±0.022	14.48
By index 3	0.629±0.047	0.673±0.048	0.317±0.037	0.398±0.032	8.74
Tandem	0.680±0.000	0.459±0.038	0.341±0.019	0.426±0.016	16.39
R-mating	0.498±0.067	0.501±0.070	0.342±0.062	0.366±0.044	-

[1] Mating was carried out between 1 sire and 1 dam, with each pair giving birth to 8 effective offspring.

[2] MMH(S)=molecular marker heterozygosity of sires, MMH(D)=molecular marker heterozygosity of dams, PD=percent parental difference, Index 1=MMH(S)+MMH(D), index 2=MMH(S)+PD, index 3=MMH(S)+MMH(D)+PD, Tandem=deciding mating pairs with maximized MMH(S), and PD, and MMH(D) in tandem order.

[3] Percent increase of mean offspring heterozygosity over random mating across 30 times of experiments.

of greater marker heterozygosity (table 1).

Seven schemes of marker-assisted mating were simulated, that is, mating decisions were made by maximizing: (1) MMH(S), (2) MMH(D), (3) PD, (4)

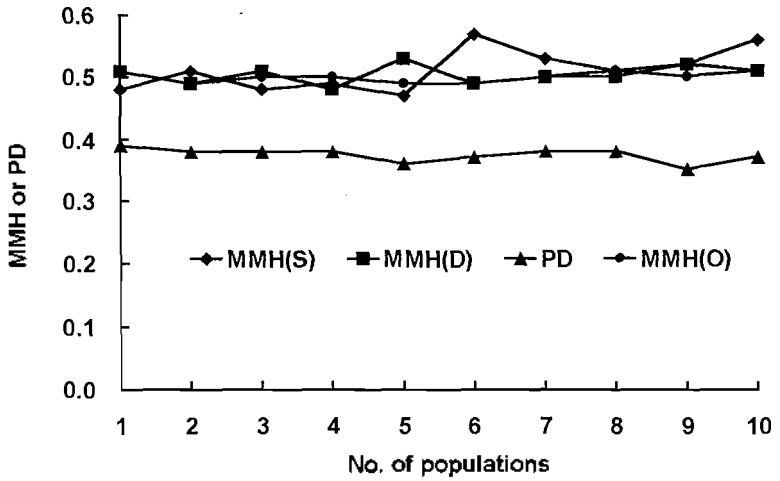

Figure 1. Fluctuation of offspring heterozygosity under random mating in a non-inbred population (MMH= molecular marker heterozygosity; PD=percent parental difference)

MMH(S)+MMH(D), (5) MMH(S)+PD, (6) MMH(S)+ MMH(D)+PD, and by (7) MMH(S), PD, and MMH(D) in tandem order. Thirty times of random mating were conducted. Corresponding means were as well calculated for a random mating control. Most MAM schemes were observed with positive increase of offspring heterozygosity over random control, large or small (table 2). The greatest offspring heterozygosity (7.37% and 16.39%) was realized by mating with maximized MMH(S), and PD, and MMH(D) in tandem, irrespective of which types of parent animals were involved, inbred or non-inbred. The second greatest offspring heterozygosity (5.78% and 14.48%) were observed when mating was directed by maximized MMH(S)+PD. This result suggested that scheme 7 and 5 were promising marker-assisted matings for maintaining greater offspring heterozygosity.

Ten times of random mating were simulated to investigate variation of offspring heterozygosity, which revealed that the fluctuation of molecular marker heterozygosity in offspring populations due to random mating, as illustrated in figure 1. This could be explained by the process of gamete sampling in transmission of alleles. As the result, MMH(O) varied between 0.49 to 0.51, when MMH(S) varied from 0.47 to 0.57, and MMH(D) from 0.48 to 0.53, and PD from 0.35 to 0.39.

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