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# SURVEY OF VARIANCE AND INBREEDING BY COMPUTER SIMULATION

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# Introduction, Objectives

Simulation methods, such as the Monte Carlo method, may be used effectively for studying difficult genetic structures, for substituting and supplementing expensive and time wasting experiments. These advantages make the utilization of simulation methods even in animal breeding possible. Certainly, the simulations cannot replace the biological experiments completely. Known regularity is formulated simply, a few connections are derived from the biological environment – this is both an advantage and a disadvantage of computer simulation. I highlight the results of my investigation of three topics in detail by using computer simulation in my dissertation. The common feature of the three topics appears in the objectives, I wished to reveal the factors effecting genetic variability, the suggestibility of genetic variability, the rate of inbreeding, as well as opportunities for preventing inbreeding. These topics focus on relevant issues for animal breeding, such as factors effecting genetic variability, the ability to modify genetic variability, inbreeding and its prevention.

Methods presently used to estimate breeding value estimate the genetic value by near $r \cong 1$ precision from the phenotype. Thus, the selection of parents occurs approximately on the basis of their real genetic value. Few animals are selected from the top of the rank, and there are probably more similar such animals among them due to the precision of breeding valuation. Selecting parents of limited number used in breeding reduces not only genetic variance, but even increases inbreeding, which increases the homozygosity of lethal and sub-lethal alleles, causing inbreeding deterioration. With selection, genetic variance declines in animal stock. The diminishing variance generates differential selection, and, therefore, a decrease in genetic advancement. The genetic value of related animals, partly due to the identicalness of their gene stock, is nearly the same, thus animals akin to each other may be found among the animals selected for over-breeding.

Genes will be fixed over the long term, the genetic reserves will be run down, and the competitiveness of a given species will fall. One of the basic conditions of effective animal breeding and animal keeping utilizing industrial methods is to exhaust genetic opportunities maximally. Computer simulation makes the change of more genetic

factors possible, i.e. genetic changes may be studied through generations without reference to the species.

My objective was to investigate the effects of genetic factors modifying genetic development and variability and inbreeding considering the size of the population, the number of the gene places, allele interactions and the mating system. I wished to compare genetic development on the basis of the average sum of the allele values, genetic variability on the basis of the time when the population became uniform homozygotes.

Presently, great attention must be paid to preserve our values, especially the genetic stock of our fauna. Certain species of domestic animals are endangered, because a species meets the particular requirements of a specific market, and changing demand will cause a decrease in the size of a species. The old naturalized domestic animal species, however, possess unique values, for which demand is growing (MIHÓK et al., 1999). Accordingly, it is relevant for breeders to keep presently unpopular species, and to preserve their genes (BODÓ-MIHÓK, 2002). Rotational line mating is used in the bronze turkey stock maintained for gene-preservation at the University of Debrecen, Centre of Agricultural Sciences.

The objective of my second investigation is to analyze the long-term effect of the present mating system on the genetic variability and to work out mating plans (breeding community dependent from size and sex), which may be more favorable for gene preservation without changing the population size.

In a traditional way, excellent performance cows may be produced using traditional breeding methods and breeding selection techniques only over a long period, because only one calf is born and the duration of a pregnancy is long. Among biotechnical and biotechnological methods, cloning has and may have a significant future in animal breeding. Several methods for cloning are presently known. By its general utilization, exceptional animals might be produced in an optimal number. There are other advantages of the multitudinous use of new biotechnological methods than the quick spread of genotype favorable from the point of view of production. The sex of descendents might be influenced in the required rate. Its economic effect is unquestionable: the birth of bull calves is favorable in meat production, the birth of a heifer is necessary for milk production. However, there is a disadvantage to using this new technology, as due to the mass production of animals of the same genetic make-up, genetic variance would fall, and inbreeding would increase in the stock. A decrease in

genetic variance would cause difficulties in later selection activities. The rise in inbreeding might contribute to the accumulation of genetic depression and to the decline of fitness characteristics.

My aim was to investigate the effect of different cloning technologies by their simulation on the change of genetic value and on inbreeding in a dairy cow population.

## Investigating Several Genetic Factors

### Preliminaries and the Utilized Research Methods

During my examination, I analyzed the effect of different genetic factors with respect to genetic development, genetic variability and inbreeding. I wished to compare genetic development on the basis of the average sum of allele values, and genetic variability on the basis of the time when the population became uniform homozygotes. The modified genetic factors were the following: population size (1000 animals, 2000 animals), number of loci (5 and 10). Allele interaction (additive effect, partial dominance and complete dominance), the method of mating (assortative mating, random mating).

I investigated 24 experimental sets in 15 replications, by changing the factors mentioned above. I defined the animals of the basic population by determining their alleles in every locus. The starting value of the two alleles per locus at 50-50% probability level was -1 or +1 in the case of five loci, and was +0.5 or -0.5 in the case of ten loci. The allele couple of the genes were independent; there was neither any epistatic effect, nor any connection between the loci. I used an over-lapping population; every animal took part in three reproduction periods and the ages of the animals were the same. Every female animal had to have had only one descendent per mating. The sex ratio was 1:4. I ranked the animals produced from these copulations on the basis of their phenotypes, and I kept as many animals of the best category as were necessary, in order not to change the size of the population and the sex ratio. In the computer simulation, I call the period from mating through the birth of descendants until the sorting out of the animals the reproduction period, and I measure the time that passed in these reproduction periods.

At mating, I selected the parent couple meeting the selection rules, and then I determined the alleles of the descendent from the alleles on the proper loci of the parents, so that the descendent should get either the one or the other set of alleles from the parents by 50% probability. After summing up the value of the alleles, where I took even the dominance effect into consideration, I determined the phenotype. Modeling the environmental effect, I attached a random number of a nil expected value, having a certain standard deviation, and of normal distribution to the genetic value. The phenotype was the sum of the genotype by loci and the environmental effects. In order to calculate the inbreeding coefficient, I applied the Wright (1921) formula. I fixed the pedigree in the reproduction periods, the allele value of the animals, as well as the generation number where every animal of the population first became the most favorable homozygote on every locus.

The reason why I chose the allele value of the animal instead of the genotype was the fact that I could compare the allele effects. I used the SCILAB 2.7.2 (DRAKOS, 1997) mathematic software for the simulation program, and I ran a part of the programs through the supercomputer of the National Informatical and Infrastructural Development Program. I analyzed the results of the experiments by variance analysis (SVÁB, 1981), non-parametric Kruskal-Wallis (VINCZE and VERBANOVA, 1993), as well as by using Gehan test (MCGRADY, 2005) and the methods of survival analysis – Kaplan-Meier valuation (BOLLA and KRÁMLI, 2005), log-rate model (VERMUNT and MOORS, 2005).

**Main Conclusions**

The different versions were compared by variance analysis. Figure 1 indicates the main effects and the most significant interactions in the analysis of allele values.

The sum of allele values of the population was correlated with each of the observed factors – population size, the number of loci, the mating system and the additive and dominance gene effects. Among these factors, the existence of complete dominance was the most determinig. Heterozygotes and the more favourable homozygotes were not discernible in terms of phenotypes with complete dominance. The disadvantegeous allele was not eliminated, thus genetic variance was not reduced to zero. With additive gene effect and partial dominance, the disadvantegeous allele was eliminated within 20 reproduction periods, the genetic values reached the maximum and genetic variance was reduced to zero.

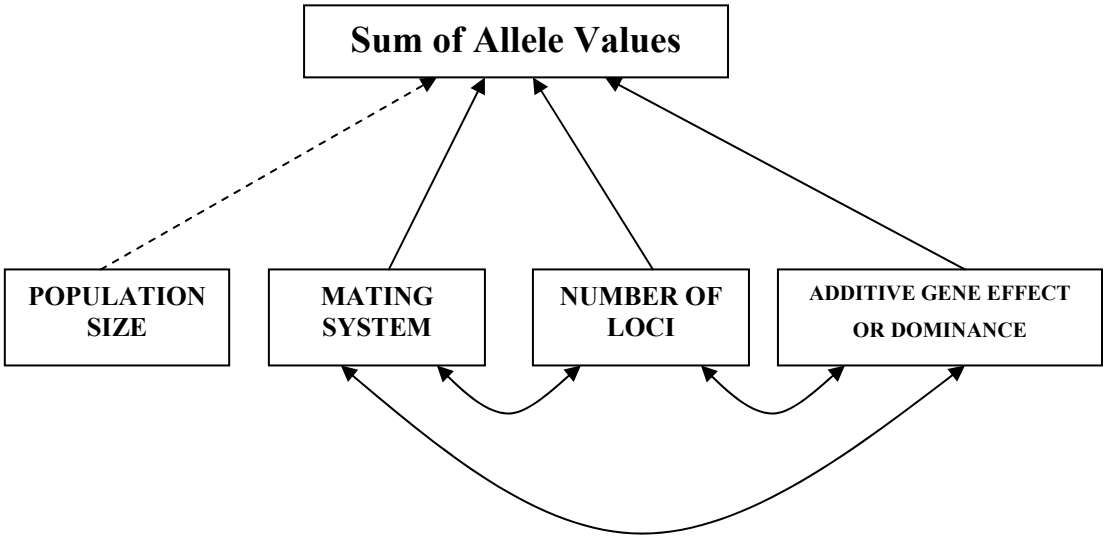


**Figure 1. Significant Factors Affecting the Sum of the Allele Values in the Model**

It was the number of loci that influenced allele frequency in the second place after dominance. When a characteristic was determined by 10 genes, the sum of the allele values was significantly smaller in the tenth reproduction period, than with 5 loci. The increase of the genetic values was slower with 10 genes than with 5. The differences in terms of effects between 5 and 10 loci were reduced by complete dominance.

The third determining factor was the mating system; genetic values were growing faster with assortative mating. This accelerating effect of assortative mating on the growth of genetic values was reduced by complate dominance. As a result of the interaction between mating and the number of loci, the effect of assortative mating was stronger with 10 loci than with 5 loci.

The differences between the population sizes were significant with variance analysis, however, the effect in itself proved to be weak. The population size had only a weak impact on the performance level of the population, which is also confirmed by the differences not being significant with a non-parameter test. Table 1 indicates the variance components and intraclass correlation values belonging to the particular factors.

Apart from performance level, the inbreeding was the other subject of the study. Figure 2 outlines the main effects and the significant existing interaction concerning inbreeding. Table 2 contains variance components and intraclass correlation values belonging to the particular effects.

| Effects | Intraclass correlation (r) | Variance-components | What percentage of the whole variance can be attributed to the particular effect |
|---|---|---|---|
| **Allele interaction** | 0.99 | 1.6681 | 63.68% |
| **Number of loci** | 0.97 | 0.5246 | 20.03% |
| **Mating system** | 0.95 | 0.2963 | 11.31% |
| **Number of loci-gene allele effect interaction** | 0.81 | 0.0698 | 2.66% |
| **Mating - allele effect interaction** | 0.71 | 0.0418 | 1.60% |
| **Mating-number of loci interaction** | 0.53 | 0.0187 | 0.71% |
| **Population size** | 0.15 | 0.0002 | 0.01% |

**Table 1. Variance Components and Intraclass Correlation Values Belonging to the Factors Determining the Sum of Allele Values**

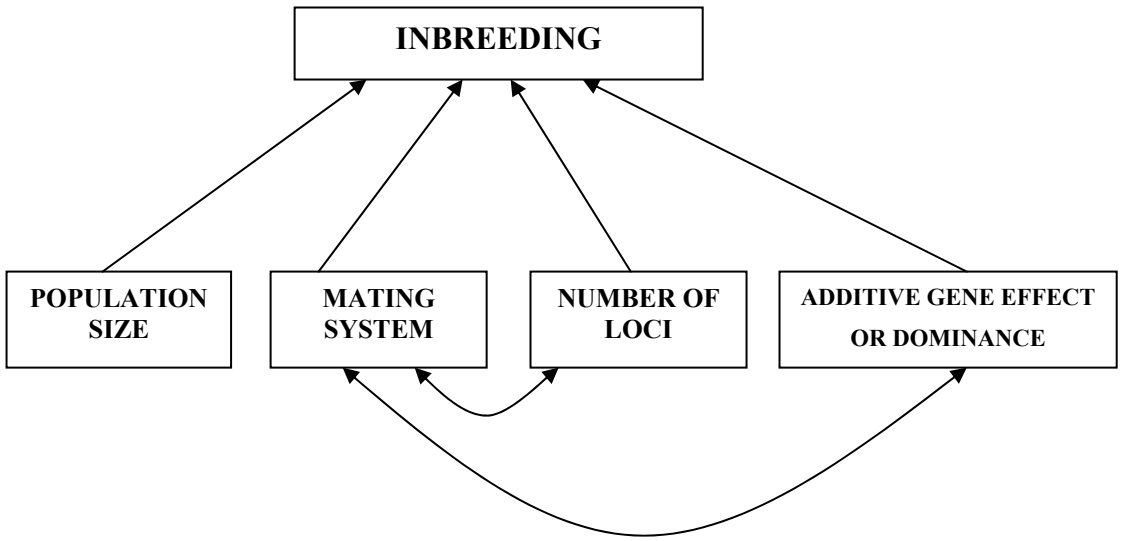


**Figure 2. Significant Factors Determining Inbreeding in the Model**

The determining factors concerning their effect on inbreeding can be ranked as follows: the mating system, gene effects, population size and the number of loci. Primarily, it was the mating system that affected the value of the inbreeding coefficients. Inbreeding was greater with assortative mating than with random mating. This can be explained by the fact that individuals with phenotypic similarity in a number of cases proved to be similar in terms of genotype as well, and are therefore relatives.

Similarly to what was observed in the case of the sum of allele values of the population, among gene effects it was the the complete dominance that made a

significant difference from both the additive gene effect and the partial dominance. There were no significant differences between the last two. Inbreeding proved to be smaller with complete dominance.

| Effects | Intraclass correlation (r) | Variance components | What percentage of the whole variance can be attributed to the partivular effect |
|---|---|---|---|
| **Mating system** | 0.62 | 0.0302 | 32.67% |
| **Allele interaction** | 0.53 | 0.0209 | 22.65% |
| **Population size** | 0.51 | 0.0193 | 20.85% |
| **Mating - allele effect interaction** | 0.38 | 0.0116 | 12.52% |
| **Mating-number of loci interaction** | 0.22 | 0.0054 | 5.83% |
| **Number of loci** | 0.21 | 0.0051 | 5.48% |

**Table 2. Variance Components and Intraclass Correlation Values Belonging to Factors Influencing Inbreeding**

A significantly different effect of complete dominance was even more absolute with assortative mating than with random mating. In other words, the increasing effect of assortative mating concerning inbreeding was significantly reduced with complete dominance, and with random mating it was not so much determining that more favourable homozygotes phenotypically could not be discerned from heterozygotes with complete dominance.

The third determining factor was the population size. With small population size, the mating probability for relatives will grow and so will the inbreeding.

The number of loci affected inbreeding only with assortative mating; with random mating there were no significant differences in the inbreeding coefficients with 5 and 10 genes.

The time of the loss of genetic variability was studied using survival analysis. The cases of complete dominance are not included in this analysis as it was not possible due to the applicability terms of the system, that is, in no instance was the genetic variance reduced to zero. The results returned confirm the results from the sum of allele values tests with variance analysis. This is not surprising, as the greater the sum of the allele values of the population in the tenth reproduction period, the sooner the loss of alleles occurred, i.e. the two examined factors were correlated. Using the log-rate model of

analysis, the probability of the loss of an allele was 5.58 times greater with 5 loci than with 10 loci, and 3.52 times greater with assortative mating than with random mating.

## Gene Preservation

### Preliminaries and the Utilized Research Methods

Rotational line mating is used in the bronze turkey stock maintained by gene-preserving at the University of Debrecen, Centre of Agricultural Sciences. The breeding population included 211 animals at the time of investigation; the distribution of the sex was 38 toms and 173 hens. The size of the population is near the same through the generations. The animals are kept in 11 lines. One line consists of 3 to 4 toms and 12 to 16 hens. Hens hatching from the line supplement the female animals of the line as breeding animals, the toms serve as breeding supplement of the toms in the adjacent line. Hens lay approximately 40 eggs. Animals are kept in breeding for 2 years in average. During the reproduction period, the animals within the lines mate randomly. All the eggs are hatched. After hatching, toms being appropriate for breeding are taken into the next line; the toms of the last line get into the first one. Breeders strive to maintain the genetic variability by this cyclical movement.

### Experiment I.

The magnitude of the genetic variance has extremely relevance in small populations. In this way, I investigated that whether the mating algorithm presently used promotes the maintenance the genetic variability or not. The present condition was revealed together with the Agricultural Biotechnological Research Centre in Gödöllő, on the basis of DNA gained from blood with the help of microsatellite markers (KOROM et al., 2003). We examined the genetic condition of the population. I gained data of genetic varieties located on 8 loci of 144 animals from the results of DNA testing. From these, I could conclude the genetic variability. Then, with the help of computer simulation, I studied the situation of the succeeding 100 generations. I started from the data originated from the DNA sample, and then I followed the stock change through 100 generations by programming the used mating and selecting methods. I repeated the simulation for 400 times, and then I compared the realized stocks with the

present ones. I fixed the frequency number of the alleles in every locus and in every generation, the allele frequency, and the Shannon index originated from the allele frequency.

$$H_i = -\sum_{j=1}^{5} p_{ij} \ln p_{ij}$$

where $i$=1,…,7 means the number of the loci, $j$=1,…,4 relates to the 4 different alleles, and $p_{ij}$ means the proper allele frequency. Additionally, I made an adjustment if either of the alleles of a locus disappeared or its frequency became nil.

**Experiment II.**

I used another simulation program to determine how fast a rear allele is eliminated from the population. I studied 9 cases on this question. The frequency of the rear allele was 0.0035, 0.007 and 0.0105 in the starting stock. This means that one, two or three animals of the 144 total bore rear alleles. Within this number, the carrier animals might have different sexes. The rare allele might be carried by only hens, by only toms, and by both as well. I modified the input data of the first experiment only to make the mentioned 9 cases that were available possible. Every experimental set was investigated through 100 generations, and every of them were replicated 400 times.

I made computer simulation belonging to the experiment I. and II. by using the parts of MATLAB 4.2 mathematical software package dealing with simulation (MATLAB, 1992). I carried out statistical analysis by $\chi^2$ test (FALCONER and MACKAY, 1996), Kaplan-Meier valuation (BOLLA and KRÁMLI, 2005), Gehan test (MCGRADY, 2005), and log-rate model (VERMUNT and MOORS, 2005). I used Microsoft Excel, SPSS and LEM programs for all these.

**Experiment III.**

In a further experiment, I looked for the answer to what line size, and what families of how many animals and reproducing communities, would result in the greatest chance to maintain the rare genes without changing the whole population size.

The division of sex was 72 toms and 288 hens. This equals a 1:4 sex ratio. I counted with a discrete population in the simulation. The genetic structure of the starting population could be of three types. In the first case, extremely rare alleles can be found, in the third case the allele frequencies are the same, while in the second case

the allele frequencies are between the previous values. Table 3 shows the three-type-line classification.

The number of the lines could be 4, 12, and 36, thus the size of the families might be 90, 30 or 10 animals. I carried out 360 replays in this experiment in every nine basic population, thus I analyzed the results of 3240 runs. The program fixed the Shannon index in every voliere and in the whole population, as well as the first occurence of when one gene variety disappeared.

| Cases | Alleles | Loci | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1. | A | 0.9750 | 0.9750 | 0.9750 | 0.9528 | 0.9528 | 0.9528 | 0.9806 | 0.9806 |
| | B | 0.0111 | 0.0111 | 0.0111 | 0.0250 | 0.0250 | 0.0250 | 0.0194 | 0.0194 |
| | C | 0.0069 | 0.0069 | 0.0069 | 0.0222 | 0.0222 | 0.0222 | | |
| | D | 0.0069 | 0.0069 | 0.0069 | | | | | |
| 2. | A | 0.2917 | 0.2917 | 0.2917 | 0.4167 | 0.4167 | 0.4167 | 0.7500 | 0.7500 |
| | B | 0.2917 | 0.2917 | 0.2917 | 0.4167 | 0.4167 | 0.4167 | 0.2500 | 0.2500 |
| | C | 0.2917 | 0.2917 | 0.2917 | 0.1667 | 0.1667 | 0.1667 | | |
| | D | 0.1250 | 0.1250 | 0.1250 | | | | | |
| 3. | A | 0.2500 | 0.2500 | 0.2500 | 0.3333 | 0.3333 | 0.3333 | 0.5000 | 0.5000 |
| | B | 0.2500 | 0.2500 | 0.2500 | 0.3333 | 0.3333 | 0.3333 | 0.5000 | 0.5000 |
| | C | 0.2500 | 0.2500 | 0.2500 | 0.3333 | 0.3333 | 0.3333 | | |
| | D | 0.2500 | 0.2500 | 0.2500 | | | | | |

**Table 3. Starting Allele Frequencies Belonging to Experiment III.**

**Experiment IV.**

I studied by changing the sex ratio the fact that how the variance changes as well as during how many generations the rare alleles eliminate. The size of the starting population was again 360 animals. I classified the animals into 12 lines, in this way a line consisted of 30 animals. The rate of toms and hens was 1:2, 1:4 and 1:9.

I changed the allele frequency, which equaled with that used in the experiment IV. I carried out 360 replays again, with 9 basic cases through 15 generations, thus I made conclusions according to the results of 3240 runs with respect to genetic variability and to allele elimination.

I used the SCILAB 2.7.2 (DRAKOS, 1997) mathematical software for experiments III. and IV., and I ran a part of the programs through the supercomputer of the National Informational Infrastructural Development Program. I carried out the statististical analysis in both experiments using the Kolmogorov-Szmirnov test

(LUKÁCS, 1996), Kaplan-Meier valuation (BOLLA and KRÁMLI, 2005) and Gehan test (MCGRADY, 2005). I created the statistical analysis by SPSS program.

The population was in Hardy-Weinberg equilibrium concerning each of their loci. The calculations were made using the $\chi^2$ test. The estimated inbreeding is 1.17% $\left(F = 1 - \frac{H_0}{H_e}\right)$, where $H_0$ is the observed heterozygosity and $H_e$ is the expected heterozygosity derived from Hardy-Weinberg equilibrium.

Calculations concerning entropy confirm that the rotaitonal line algorithm is suitable in terms of genetic equilibrium and inbreeding, provided the breeding object is to preserve genetic variability. The entropy became stable at close to the initial value. It must be emphasized that this is true in terms of statistics and individual differences are large. In this process, chance events can have a marked impact, which can inevitably result in the loss of an allele.

During the computer simulations, it was recorded how many generations it took for a rare allele to be eliminated. We observed how many generations it took for the frequency of an allele to become zero if just one, two or three turkeys in the population had a rare allele. The sexes had to be analyzed separately as males and females have different probabilities of transmitting their genes. Table 4 contains the results of the simulation.

| | | Average allele-loss period | Percent of cases the allele was lost in the first 5 generations | Percent of cases the allele reamined after 25 generations | Percent of cases the allele remained after 100 generations | Standard deviation |
|---|---|---|---|---|---|---|
| **Initial allele frequency: 0.0035** | **1 female** | 1 | 85.5% | 2.8% | 0.0% | 11.2 |
| | **1 male** | 23 | 21.5% | 48.5% | 6.5% | 37.1 |
| **Initial allele frequency: 0.007** | **2 females** | 3 | 71.0% | 6.8% | 0.3% | 14.4 |
| | **1 female 1 male** | 31 | 19.8% | 53.3% | 7.8% | 38.1 |
| | **2 males** | 59 | 5.5% | 72.0% | 1.3% | 34.4 |
| **Initial allele frequency: 0.0105** | **3 females** | 7 | 43.5% | 30.5% | 1.0% | 30.8 |
| | **2 females 1 male** | 39 | 15.3% | 60.0% | 5.3% | 34.9 |
| | **1 female 2 males** | 75 | 6.3% | 76.0% | 24.0% | 36.6 |
| | **3 males** | 96 | 1.0% | 88.0% | 33.8% | 29.9 |

**Table 4. Results from Calculations Concerning the Loss of Alleles after 400 Runs**

For a more thorough survey of allele losses, two methods of survival analysis were used: the Kaplan-Meier estimation and the log-rate model. There were significant differences in the time of the allele loss between any two instances (Gehan test, P<0,001). Survival here means the preservation of a rare allele.

Using an exponentional log-rate model, I calculated what the correlation was between the probabilities of losing a rare allele in certain comparable cases. Table 5 contains these quotienses. The numbers in the cells indicate how many times greater the probability of losing a rare allele is with gene owners of a definite number and sex (upper row) compared with gene owners of another number and/or sex (far left column). For example, the probability of allele loss is 4.392 times greater in a single female owner than with a single male owner. Another example - in the base population an allele was owned only by two individuals, a male and a female – is compared with the case of two male owners. In this case, the number 1,269 in column 5 and row 5 is the multiplicator of the probability of losing the rare allele with mixed owners.

| | **1 female** | **1male** | **2 females** | **1 female 1 male** | **2 males** | **3 females** | **2 females 1 males** | **1 females 2 males** |
|---|---|---|---|---|---|---|---|---|
| **1 male** | 4.392 | | | | | | | |
| **2 females** | 1.646 | | | | | | | |
| **1 female 1 male** | | | 2.758 | | | | | |
| **2 males** | | 1.312 | 3.501 | 1.269 | | | | |
| **3 females** | 3.075 | | 1.868 | | | | | |
| **2 females 1 male** | | | | | | 1.54 | | |
| **1 female 2 males** | | | | | | 2.187 | 1.42 | |
| **3 males** | | 1.899 | | | 1.447 | 2.712 | 1.761 | 1.24 |

**Table 5. Proportions of Probabilities of Losing Alleles**

The genetic variability was observed after dividing the same base population of 360 individuals into 4, 12 and 36 subpopulations. The Shannon indices were higher, that is, the preservation of genetic variability was more efficient with a population divided into more mating subpopulations of a smaller size (36×10 individuals/line), however, these diffrences were not significant with allele frequencies smaller than 0.01 in the base population. In general, if there were no striking differences in the base values of

allele frequencies, 36 small populations were more efficient than 4 large mating populations. This allowed us to draw the conclusion that with rotational line mating, cyclic moving of males into a next line was a more efficient means concerning gene preservation than random mating. As relates to the time of loss of very rare alleles of frequency smaller than 0.01, significant differences appeared only between the smallest and the biggest family sizes (10 and 90 individuals/line). The families of the bronze turkey population of the Agricultural Centre of Debrecen University are of efficient size (15-20 individuals/line).

The effective population size was changed by changing the sex ratio. Naturally with larger effective population size the Shannon indices used for the measurement of genetic variability produced higher values as well. However, significant differences appeared only when base allele frequencies were the same. Consequently, the genetic variability cannot be increased significantly only by increasing the number of males if the population size is not increased. However, concerning the preservation of very rare genes, there did appear significant differences between the cases of different sex ratios. In these instances the sex ratio of 1:2 was more favourable. Concerning gene preservation, 1:4 is the efficient sex ratio in the applied breeding method.

## The Effects of Mass-Utilization of Cloning in Dairy Cow Population

### Preliminaries and the Utilized Research Methods

I compared the potential effect of mass utilization of somatic cell cloning and embryo splitting technology regarding a simulated dairy cow population being similar to the domestic cow number by computer simulation. I investigated the change of the genetic values, the change of the genetic variability as well as the average inbreeding level of the dairy stock.

### Generating the Basic Population

The size of the basic population is 200,000 cows, 500 or 667 or 1,000 bulls depending on the experimental set, and the calves. I applied the infinitesimal model during the cloning experiment. I did not take dominance and epistatic effect into consideration, thus the total genetic value equaled with the additive genetic value. I

characterized the animals with their genetic values and their breeding value. The genetic values in the starting population were random numbers from standard normal distribution. The breeding value was also a probability variable showing a normal distribution; I added a random number having an expected value of zero to the genetic value. This random number also originated from normal distribution, its deviation was dependant on the sex, it was 0.05 in bulls and 0.2 in cows. The quantity of data used for estimating breeding value and the rate of breeding are different in the two sexes in this way I handled them separately. The genetic value had role in realizing descendents: descendents got the average of the genetic value of the parents and a random number having an expected value of zero, this last one modeled the Mendel variance. I ordered the animals on the basis of breeding values.

I compared three different cloning methods in several varieties with a control population in the experiments. I studied overlapping population in the experiment. The length of a simulation period equaled with the period between two calvings (410 days). I will use the term reproduction period for this from now on in this thesis. Mating, calving and selecting the next parent population occur within one reproduction period. I used assortative mating in every experimental set. I mated bulls of the highest breeding value with cows of 200, 300 or 400 breeding value. The second best bull was mated with the next female animal in the order. The age structure of the dairy stock was stable (Table 6.), I worked out the rate according to the table of lifetime index (BÁDER et al., 2002).

| Age in Years | Age in Reproduction Period (1 reproduction period=410 days) | Number of Animals |
|---|---|---|
| 0 | 0 | 60 000 |
| 1.1 | 1 | 60 000 |
| 2.2 | 2 | 60 000 |
| 3.4 | 3 | 48 000 |
| 4.5 | 4 | 34 000 |
| 5.6 | 5 | 25 000 |
| 6.7 | 6 | 17 000 |
| 7.8 | 7 | 10 000 |
| 9 | 8 | 5 000 |
| 10.1 | 9 | 1 000 |

**Table 6. The Age Structure of the Dairy Stock in the Simulation Program in Every Experimental Set**

The age structure of bulls was differing, as the breeding animals are kept in breeding till they are among the best animals under at least 75% reliability and at the age of five (PRINCZ, L., personal communication, 2005).

The variety *A* was the control population, where I did not apply cloning. In variety *B*, I modeled the nuclear transfer cloning with the help of embryonic cell breed. During mating, I established 9-9 clones by using the embryos of cows of the best breeding value, the genetic values of the clone descendents were the same. (The three twins of the variety *D* give reasons for the number 9, I wished to establish population being comparable with that.)

I modeled somatic cell cloning in the variety *C*. I created 9-9 clones of cows of the best breeding value. Their genetic values equaled with that of the donor. The somatic cell cloning in the present practice is the less successful method; on the other hand, it has great significance in animal breeding, as animals of known performance might be cloned in this way.

The variety *D* studies the third type of cloning. It is known as embryo splitting and aims at producing twins of one ovum. An embryo is kept till its eight-cell-condition, when it is divided into four parts. Division of the embryo into more parts than this has not resulted in viable descendents, yet. I devoted one of them in the model in order to determine the sex, as I wished to clone only female animals. To sum up, triple twin descendents were born having the same genetic values. I originated more triple twins from one mother and from one mating. The genetic difference between triple twins from the same parents was thanked to the Mendel variance.

Another animal is necessary for bearing the embryo, usually called second mother or nurse. I chose nurses from animals of the lowest breeding value, thus within a reproduction period cows of the best breeding value could take part even in sexual reproduction. I did not calculate the effect of the second mother to the embryo. All of the clones born were female animals.

I changed two parameter values during the experiments. At one hand, I changed the number of bulls; on the other hand, I changed the number of the clones born in a cycle. The number of the bull animals might be three types. When determining the number of clone animals in a cycle, I chose a number, which can be divided by 18, in order to make the comparison with case of the triple twins possible. In this way 10,080 or 5,040 or 1,008 clones were born in every cycle. The table below contains the experimental sets (Table 7.).

I applied the 9 sets found in the table separately with all three numbers of bull animals, thus I investigated altogether 9 x 3 = 27 experimental sets. I turned the program into 18 reproduction periods, by which I studied 20.2 years. I recorded the average genetic value of the dairy stock, the deviation of the genetic values and the inbreeding coefficient to every animal. During one experimental program, more than 1.34 million animals were born under 18 reproduction periods. I carried out the 27 experiments with 20 replays. Because of the high number of animals, I needed great performance and hard disc, thus I ran the programs in SCILAB language through the supercomputer of the National Information Infrastructure Developing Program. For counting the inbreeding coefficient for 1.43 million animals the SCILAB language was not appropriate, it was not able to handle so much data at the same time. In this way, on the basis of a pedigree file done by the SCILAB I counted the inbreeding coefficient to every animal on the basis of the Wright formula with the help of a program in JAVA language. This program was established by an IST engineer (Information Sciences and Technology) using my algorithm and guidelines.

| Letter mark | Type | Number of mothers/donors | Number of clones total | Rate of clone in comparison with the dairy stock |
|---|---|---|---|---|
| **A** | Control population, no cloning | 0 | 0 | 0% |
| **B1** | Nuclear transfer cloning | 1120 | 10080 | 5% |
| **B2** | | 560 | 5040 | 2.5% |
| **B3** | | 112 | 1008 | 0.5% |
| **C** | Somatic cell cloning | 1120 | 10080 | 5% |
| **D1** | Embryo splitting method | 1120 | 10080 | 5% |
| **D2** | | 560 | 5040 | 2.5% |
| **D3** | | 112 | 1008 | 0.5% |
| **D4** | | 560 | 10080 | 5% |

**Table 7. Describing the Experimental Parameters**

**Main Conclusions**

The average genetic value of the base population was 0, the standard deviation of genetic values was 1. The table below indicates how these two factors have changed over 18 reproduction cycles, i.e. over 20.2 years (Table 8.).

Concerning the increase of genetic values, determining factors were the proportion of the cloned progeny in the first place, and teh number bulls in the second

place. The three cloning methods observed in the simulation tests (nuclear transfer from a cell culture, somatic cell cloning and embryo splitting) were compared concerning the increase of genetic values: the worst values were produced by the somatic cell method. The main reason for this may be the fact that the zoogamy determining the genome took place several reproduction periods (years) before the cloning in the case of the donor of the somatic cell, whereas in the cases of the other two methods, cloning and insemination were carried out in the same reproduction period. Between the two moments, the population was improving genetically, thus the expected genetic values of the clones derived by somatic cell cloning were lower than those of the clones derived by the other two methods. The population cloned by somatic cell method reached the same level of performance later than the populations cloned by embryo splitting or nuclear transfer. The average genetic value of a population was significantly increased if the same cloning proportion was produced using less mothers/donors.

| Type of test setting | With 500 bulls | | With 667 bulls | | With 1000 bulls | |
|---|---|---|---|---|---|---|
| | Average of genetic values | Standard deviation of genetic values | Average of genetic values | Standard deviation of genetic values | Average of genetic values | Standard deviation of genetic values |
| **A** | 7.44 | 1.12 | 7.23 | 1.11 | 6.91 | 1.06 |
| **B1** | 9.97 | 1.84 | 9.82 | 1.85 | 9.58 | 1.86 |
| **B2** | 9.17 | 1.73 | 8.94 | 1.70 | 8.66 | 1.70 |
| **B3** | 8.03 | 1.37 | 7.82 | 1.36 | 7.52 | 1.34 |
| **C** | 9.42 | 1.56 | 9.24 | 1.56 | 8.97 | 1.54 |
| **D1** | 9.97 | 1.84 | 9.81 | 1.86 | 9.53 | 1.84 |
| **D2** | 9.09 | 1.70 | 8.85 | 1.68 | 8.64 | 1.70 |
| **D3** | 8.03 | 1.38 | 7.86 | 1.37 | 7.54 | 1.35 |
| **D4** | 10.31 | 1.99 | 10.22 | 2.02 | 9.96 | 2.03 |

Abbreviations applied in the types of test settings: A – control population; no cloning. B1, B2, B3 – nuclear transfer cloning; 5%, 2.5%, 0.5% of the dairy population are clones. C – somatic cell cloning, 5% of the dairy population are clones. D1, D2, D3 – embryo splitting; 5%, 2.5%, 0.5% of the dairy population are clones. Case D4 – embryo splitting cloning, 5% of the dairy population are clones. Case D4 differs from case D1 in the number of mothers being the half of D1 case

**Table 8. Genetic Values and the Standard Deviation of Genetic Values of Dairy Cattle (average of 25 runs)**

The prime factor influencing the inbreeding was the type of cloning. The lowest inbreeding values were produced with embryo splitting after the control population. With this method only three clones can be derived from the same embryo, so e.g. if nine clones of a mating are needed, they can be produced with three triplets. The degree of relationship between triplets is only 50%, wich decreases the inbreeding significantly. The next lowest values were produced with somatic cell cloning. In this case, the

relatively low values result from the retardation described in connection with the genetic values. The genom of a clone is identical with that of the donor's; real parents were conceived two generations earlier than the cloned progeny. This distance decreases the probability of the mating of relatives as well. The highest inbreeding values were produced with nuclear transfer method that uses nuclei from a cell culture. Another effect that influenced the inbreeding coefficients was the number of the cloned individuals: the higher the proportion of clones compared with the rest of the dairy population was, the greater the calculated F value was. The number of bulls was only the third important influencing factor.

The average values of the inbreeding coefficients after the 18. reproduction period are described in Table 9.

| Types of Experiment | Inbreeding co-efficient | | |
|---|---|---|---|
| | 500 bulls | 667 bulls | 1000 bulls |
| **A** | 0.50% | 0.35% | 0.28% |
| **B1** | 2.48% | 2.26% | 1.90% |
| **B2** | 2.33% | 2.12% | 1.84% |
| **B3** | 1.49% | 1.31% | 1.24% |
| **C** | 1.39% | 1.26% | 1.12% |
| **D1** | 0.72% | 0.62% | 0.49% |
| **D2** | 0.61% | 0.58% | 0.44% |
| **D3** | 0.58% | 0.53% | 0.41% |
| **D4** | 0.97% | 0.75% | 0.59% |

Abbreviations applied in the types of test settings: A – control population; no cloning. B1, B2, B3 – nuclear transfer cloning; 5%, 2.5%, 0.5% of the dairy population are clones. C – somatic cell cloning, 5% of the dairy population are clones. D1, D2, D3 – embryo splitting; 5%, 2.5%, 0.5% of the dairy population are clones. Case D4 – embryo splitting cloning, 5% of the dairy population are clones. Case D4 differs from case D1 in the number of mothers being the half of D1 case

**Table 9. Averages of Inbreeding Coefficients Concerning a Dairy Population after the 18th Reproduction Period**

## New Findings Produced through this Dissertation

### Investigating Several Genetic Factors

The following have effects on the average and from generation to generation growth of allele values:

– the type of allele interaction in *additive - partial - total* dominance order (63.68%),

– the number of gene locations in *more (5) – less (10)* order (20.03%),

– the type of mating in *assortative mating – random mating* order (11.31%),

– the size of the population in *greater (2000) – smaller (1000)* order (0.01%).

The remaining percantages of the total variance come from the examined effect interactions, altogether in 4.97%.

The followings have effects on the average and from generation to generation growth of inbreeding:

– the type of mating in *assortative mating – random mating* order (32.67%),

– the type of allele interaction in *additive - partial - total dominance* order (22.65%),

– the size of the population in *smaller (1000) – greater (2000)* order (20.85%),

– the number of gene locations in *less (5) – more (10)* order (5.48 %).

The remaining percantages of the total variance come from the examined effect interactions, altogether in 18.35 %.

The following modify the time genetic variance is ceased:

– the type of mating (aim maitng, and random mating). (The probability of the fact that the genetic variance becomes nil is 3.52 higher in assortative mating than in random mating.)

– The number of gene locations (5, and 10). (The probability of the fact that the genetic variance becomes nil is 3.52 higher in case of 5 loci than 10 loci.)

The following did not have any effects:

– the population size (1000, and 2000),

– the type of allele interaction (additive, and partial dominance).

**Gene Preservation**

During my investigations, I concluded the following:

- The bronze turkey maintained for gene preserving by the University of Debrecen, Centre for Agricultural Sciences was in Hardy-Weinberg balance at the time of the experiment on the examined locus.
- The presently used rotational mating system ensures well the maintanance of the genetic variability. During the simulation experiment, the average of the Shannon indexes measuring the genetic variability was not lower in comparison with the starting population.
- When using rotational random mating, toms have a greater opportunity to pass on their genes, than hens. In order to preserve rare alleles, not only the allele frequency should be considered, but the sex of the carrier animal, as well.

In the case of a population of a given size, the maintanance of gene variability is better ensured if the population is divided into a subpopulation, instead of using random mating, and the migration between subpopulations is monitored regularly. When establishing a subpopulation, as high an effective population size as possible must be guaranteed.

**Effects of Mass Utiliyation of Cloning in Dairy Cow Population**

The genetic values increased in the following order with respect to the type of cloning:

- control group,
- somatic cell cloning,
- embryo-splitting and nuclear transfer cloning. (The genetic values were the same in embryo-splitting and nuclear transfer cloning.)

The genetic values increased by the reduction of breeding bulls. The growth of the ratio of cloned animals caused an increase in genetic values, as well.

The inbreeding co-efficients increased in the following order with respect to the type of cloning:

- control group,
- embryo-splitting cloning,
- somatic cell cloning,
- and nuclear transfer cloning.

The reduction of the breeding bulls occurred with the growth of the inbreeding co-efficient. The growth of the ratio of cloned animals caused an increase in inbreeding, as well.

During my experiements, I concluded that, in the case of mass utilization of cloning, it is not practical to apply somatic cell cloning. Rather, clones gained from embryos of animals of highest performance may be used, either by embryo splitting technology or by nuclear transfer method. In this case, the growth of inbreeding may be significantly reduced if fewer clones originating from one mating are utilized, instead of many clones gained from one embryo.

I highlighted that in case of regular use of cloning in not higher than 5%, the growth of the average inbreeding coefficient of the population is not higher than 0.0248 over 20 years.

## The Practical Use of the Results

### Investigating Several Genetic Factors

The presence of complete dominance influenced considerably the sum of population alleles and the inbreeding. This effect should be noticed when constructing a model.

### Gene Preserving

To sum up my experience, I determined that the presently used rotational line mating is an appropriate method for gene preserve poultry population. It is expedient to complement this by regular DNA testing, from which exact data may be gained with respect to the genetic composition of the population. Its frequency depends on many factors, such as financial opportunities or the genetic state of the population. More precise evaluation may only be planned for a population after analyzing the result of a given DNA test, in order to determine the recommended frequency of DNA test. On the basis of my results, I recommend this DNA test in a population of such a structure in every fourth generation, if rare alleles having 0.05 or lower frequency are found in the population and the carriers are significantly or wholly hens. According to my calculations, intervening, i.e. keeping more descendants than usual from these hens, is only necessary if the allele frequency falls below 0.03.

### Effects of Mass Utilization of Cloning in a Dairy Cow Population

The cloning experiment highlighted that, from the point of view of the growth of genetic values and inbreeding, the most favorable method is the use of embryo splitting technology. The number of clones in every generation might be high as well, although the rate of inbreeding still remains moderate, due to the fact that, in many cases, the connection is fraternal from a genetic aspect among clones originating from one mating. Considering this result, it is not the application of the embryo splitting method which is stressed, but the fact that it is practical to produce more embryos by utilizing the ova and sperm cells of a selected female and male animal, and only few clones should be kept from one embryo under the optional technological solution.

According to the results of the experiment, it is worth thinking over the utilization of somatic cell cloning in animal breeding. This method has an unquestionable

advantage, i.e. a breeding animal of known performance may be produced in an optional number, but the genetic values lag behind those of the other two methods. In other words, the high costs of cloning make profit questionable.

According to the simulation experiment, utilization of cloned stock to a small degree within a reasonable time does not lead to the growth of inbreeding to a high degree. I did not strive to avoid inbreeding purposefully, as with attention by breeders, inbreeding may be reduced. If the genetic depression is realized in time with the help of genetic investigations at the embryonic stage, inbreeding depression will be avoidable, and thus the positive effects of cloning will turn into negative ones.

# Scientific Publication List

1. **Szőke Szilvia** - Komlósi István: A variancia változásának vizsgálata szimulált populációban; IV. Magyar Genetikai Kongresszus 1999. április 11-14. Siófok. Poszter.
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7. Tóth Zsuzsanna – **Szőke Szilvia** – Bodó István – Sölkner, Johann: A lovaknál előforduló szürke szín intenzitásának elemzése, Innováció, a tudomány és a gyakorlat egysége az ezredforduló agráriumában Konferencia, Debreceni Egyetem Agrártudományi Centrum, Debrecen, 2004. április 16. Poszter.
8. **Szőke Szilvia** – Komlósi István –Mihók Sándor: Kis populációk párosítási szerkezete, Innováció, a tudomány és a gyakorlat egysége az ezredforduló

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9. Zsuzsanna Tóth – **Szilvia Szőke** –István Bodó – Johann Sölkner: Analysis of the grey colour in horses. 2nd International Scientific Symposium ”Natural Resources and Sustainable Development” Oradea, April 23-24, 2004. Poster.
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