Available conservation techniques are presented in this review: non inbred colonies and control strains, farm populations, in vitro methods. In all conservation programs it is necessary to manage genetic variability in some small living population. Two general recommendations are made: the variance in the number of male (or female) progeny for male (or female) parents should be as low as possible; the population should be split up in breeding groups. Developments of the tools of molecular biology will increase the efficiency of conservation methods. Two questions have still no clear answers: what is the actual efficiency of the various rules proposed to maintain genetic variability? Is there really any need to preserve genetic variability?

INTRODUCTION

Conservation of genetic resources must be considered in the light of their potential use. Improvement through selection, cross-breeding, or introduction of a major gene needs that living animals be available. Presently gene transfer is limited to special cases, with foreseeable applications rather restricted to the elaboration of pharmacological products than directed to livestock improvement. This future technique might become useful for introducing some specific gene, such as one responsible for disease resistance, and it may take the place of backcrossing a population with carriers of the specified allele. However present technical knowledge does not allow to define what should exactly be the DNA sequences to transfer. As a consequence it seems that conservation of gene libraries -as already possible in bacteriophages, cosmids or yeast artificial chromosomes- would not be surely useful for future use; rather, if DNA from peculiar breeds is to be conserved, it seems preferable to store it within cells -sperm, or embryos- where its full chromosomal organization is preserved.

Therefore, it seems that conservation of genetic resources should be thought as conservation of full functional genotypes, either found in living populations, or in frozen embryos. In both cases, the problem is managing a population made up of a small number of animals. In the case of a living population two conditions must be fulfilled at the same time: the maintenance of a sufficient number of animals to prevent extinction due to demographic fluctuations, and the limitation of genetic drift. When considering conservation of frozen sperm or embryos, a similar approach must be taken to define management rules of a population derived from a small number of founder animals.
Presently, available methods to define rules aimed at maintaining the genetic variability are mainly based on the probabilistic approach of genes segregation in an unselected finite population.

MEASURES OF THE CHANGE WITH TIME OF GENETIC VARIABILITY.

The genetic variability of a population may be defined by lists of alleles and their frequencies at many loci in the various subsets that can be found in the population (herd, age classes, sexes,...). Today allelic frequencies are not observed on a large scale in domestic animal species. However it is possible to infer the change in genetic variability over some period of time. There are various tools to predict the change of genetic variability. These tools consider one locus with no selection, no mutation and no migration. This locus is not linked with any selected locus.

Simulation of the Mendelian segregation process permits analysis of the random fluctuation of gene frequencies, and estimation of various criteria that have been introduced in theoretical population genetics. These criteria cannot be easily calculated in actual breeds. These criteria include the rate of loss of alleles and the joint distribution of allelic frequencies. For example EGGENBERGER (1973) has done a computer simulation on one diallele one-locus model to describe the changes in gene frequency in relation to mating system and population size. To summarize the results, it is necessary to define synthetic criteria like the diversity criterion, the effective number of allele, the homogeneity criterion (CHEVALET and ROCHAMBEAU, 1986).

Inbreeding and kinship coefficients are the most common tools from which one can derive the changes of variance and covariance of allelic frequencies. The mean inbreeding coefficients and the mean kinship coefficients, within and between the subsets of the population are derived as a linear function of those defined in a preceding generation interval (COCKERHAM, 1967 ; ROCHAMBEAU and CHEVALET, 1985).

One can characterize the genetic make-up of a population at time \( t_1 \), as compared to that at time \( t_0 \), by calculating in any subset defined at time \( t_1 \) the proportions of genes contributed by the various founder groups or founder genes that made up the initial population (JAMES, 1972).

There are various definitions of the effective population size (KIMURA and CROW, 1963 ; FELSENSTEIN, 1971 ; KIMURA and OHTA, 1971 ; HILL, 1972). Based on a derivation of LATTER (1959), HILL (1972) has given a formula for a population with two sexes for constant size and sex ratio.

\[
Ne = \frac{1}{16\ ML} \left\{ 2 + \sigma_{mm}^2 + \frac{2M}{F} \text{cov} (mm, mf) + (\frac{M}{F})^2 \sigma_{mf}^2 \right\} \\
+ \frac{1}{16\ FL} \left\{ 2 + (\frac{F}{M})^2 \sigma_{ff}^2 + \frac{2F}{M} \text{cov} (fm, ff) + \sigma_{ff}^2 \right\}
\]

where M and F are the numbers of males and females that reach adult age each year. From male parents, the variance in the number of male progeny reaching adult age is \( \sigma_{mm}^2 \), of female progeny \( \sigma_{mf}^2 \), and the covariance of these numbers is \( \text{cov} (mm, mf) \). From female parents, the corresponding quantities are \( \sigma_{fm}^2 \), \( \sigma_{ff}^2 \) and \( \text{cov} (fm, ff) \). L is the generation interval (average age of parents along the four pathways for gametes). This formula for populations
with overlapping generations is very useful to study the effects of variances and covariances of the numbers of progeny reaching adult age. However the significance of the effective population size is asymptotic. In the first generations, the changes in genetic variability are not well described by the effective population size. ROBINSON and BRAY (1965) have given a good example of this remark. It must be noticed that a lot of authors used the effective population size to study the changes in genetic variability. Others criteria are seldom used. For further informations on these criteria see the review by VU TIEN KHANG (1983).

The development of new technologies in molecular biology gives new informations. Within a few years it would be possible to estimate the frequencies of various alleles at many loci in one domestic species. Then biological polymorphism could be used to characterize the changes in genetic variability. By measuring temporal changes in allele frequency, it is possible to estimate the effective population size indirectly (WAPLES, 1989). Furthermore by measuring allele frequency, it is possible to greatly improve the actual methods to preserve genetic variability. When available, polymorphisms can also be useful for mating, for culling the dams and for selecting the sires (BODO, 1987). We will illustrate this point later.

AVAILABLE CONSERVATION TECHNIQUES

OLLIVIER and LAUVERGNE (1988) have made a distinction between conservation and general resource management. Animal genetic resources are made by various classes of populations: wild populations, traditional populations, standardized breeds and selected strains (RENARD et al., 1983). We do not discuss in this paper reasons for preserving genetic resources. We are only trying to describe how to preserve some genetic variability. BODO (1987) gave for several species an estimate on the minimum number of females necessary to avoid extinction.

Table 1 Minimum number of breeding females to prevent the danger of extinction (BODO, 1987).

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of breeding females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>750 to 1000</td>
</tr>
<tr>
<td>Sheep</td>
<td>500 to 1500</td>
</tr>
<tr>
<td>Pigs</td>
<td>150 to 200</td>
</tr>
<tr>
<td>Horses</td>
<td>200 to 500</td>
</tr>
<tr>
<td>Goats</td>
<td></td>
</tr>
</tbody>
</table>

These numbers are only a coarse estimation. The number of breeding males is also significant. MAIJALA et al. (1984) have introduced a further distinction: prevention of loss of populations and prevention of loss of genes within populations. The methods of conservation will differ in the two cases (SMITH, 1984).

Various methods are now available to preserve genetic variability: (1) maintain populations of each breed or strain to be conserved; (ii) create gene pools which combine the genes of a number of breeds and strains and then breed the populations containing the gene pools deliberately to avoid genetic change; (iii) set up stores of frozen semen or frozen fertilized ova; (iii) create genomic libraries.
We may also deliberately reduce selection differentials to allow us to keep the genetic base broad. GODDARD (1987) has given an example of such a policy. The choice of sires to breed bulls in dairy cattle populations offers opportunity for setting a compromise between high selection response and low genetic drift. This point deals with exploitation of genetic variability and will not be discussed here. We will study more deeply the preservation of genetic variability.

A lot of works were done on this topic since the paper about systems of mat- ing written by WRIGHT (1921). ROBERTSON (1964) has given some useful principles: (i) reduction of genetic drift to a minimum requires the formation of permanent sublines. (ii) Any degree of sublining will reduce the final rate of decline in overall genetic variability. (iii) Circular mating systems are a special type of sublining in which the mating system follows the same plan in each generation. These principles were illustrated by YAMADA (1980). When a population is subdivided, the chance of extinction of one allele decreases as the number of sublines increases. However, the larger the number of sublines the higher the rate of inbreeding. By mixing these sublines at a certain interval, the coefficient of inbreeding remains at a rather low level. Nevertheless the rate of inbreeding does not increase steadily. This method does not seem of practical value.

ROBINSON and BRAY (1965) have studied the expected effects on the inbreeding coefficients of two restrictions on the mating system: (i) no matings are allowed between full or half sibs; (ii) each parent shall be represented by one offspring of each sex in the subsequent generation. The imposition of the first restriction holds the inbreeding coefficient at zero for one additional generation. Despite this initial effect, the second restriction soon gives rise to a lower inbreeding coefficient. The effect of this second restriction decreases as population size increases, whereas there is little difference in the effect of the first restriction on population size. The system with both restrictions gives rise to a higher inbreeding coefficient than the system utilizing only the second restriction. Taking \( N \) as the population size in a random mating monoecious population, the effective population sizes are: \( N+1/2 \) without restriction; \( N+2 \) for the first restriction; \( 2N-1 \) for the second restriction; \( 2N-2 \) for both restrictions.

Non inbred colonies and control strains

Non inbred laboratory animals colonies are numerous. One can find in the literature various methods to breed these colonies. EGGENBERGER (1973) has evaluated some mating systems using a simulation by the Monte-Carlo Method. There were four mating systems: POILEY, ROBERTSON, FALCONE, HAN. The investigations show that population size is a decisive factor in maintaining the genetic structures of such populations. The homogeneity of the genetic structures is substantially different between rotation systems. CHEVALET and RO- CHAMBEAU (1986) have proposed to select the individuals according to an index when it is possible to know the genotype of each individual at some loci. This index is the inverse of the product of the frequencies of the alleles carried by the individuals, the frequencies being computed at each generation. This selection delays the decrease of the genetic variability. For any population one may find the optimum values of the selection intensity and selection rate in relation to the size of the population, the numbers of loci and alleles, and the cost of the typing of the genotypes.
Various methods have also been tested for control populations. Gowe et al (1959) have studied two alternatives for poultry control populations: the random-breeding flock and the pedigree flock. In the first one, chicks are randomly chosen within each sex from the population available, and the required males and females to be used as parents are taken at random from the survivors available for breeding. In the second one, each sire shall contribute exactly one male and a fixed number of females, one from each of the dams to which he is mated. After a theoretical discussion the following conclusions are drawn: the pedigree flocks have sufficient advantages to justify the extra labor of pedigree mating and wing banding. Flocks with equal numbers in both sexes are the most efficient. Environmental conditions should be optimal to reduce any effect of natural selection. Matheron and Chevalet (1977) have compared several designs for a control strain of rabbits. Two types of circular matings between eleven groups are compared to the random mating scheme with the same renewal rules. For practical purpose, emphasis is put on the behavior during the first generations and on the influence of initial relationships between founders. These points cannot be taken into account through the effective size. Comparisons are made during ten generations with respect to both mean values and fluctuations of inbreeding coefficients. The best scheme is an application of a proposal of Cockerham (1970) that combines the properties of the circular subpopulation mating (Kamura and Crow, 1963) and of the third degree circular system. It allows a permanent reduction of the mean inbreeding coefficients. It makes all coefficients equal after a few generations, avoiding any random appearance of highly inbred individuals.

For some control strains, it has been possible to measure the efficiency of the mating system used. Havenstein et al (1988) have studied six replicate strains of a random bred control, three pedigree and three nonpedigree. Replicate strains in the pedigree system had larger effective population sizes, lower rates of inbreeding, and smaller average family sizes than those in nonpedigree system. Farid et al (1986) have studied eight control strains of mice of different origins each maintained in two different environments. The realized effective sizes were 33% to 66% smaller than the expected in different populations. Unequal contributions of different families to the next generation resulted in the variance of family size being larger than zero in almost all generations in all strains. The genetically heterogeneous strains had smaller variance of family size and larger effective sizes in both environments compared with those which had longer history of inbreeding.

A gene pool is a large population resulting from crosses between various strains. The principles to be applied in establishing and maintaining gene pools have been discussed during the second European Poultry Conference held in 1966. Maijala (1970) have summarized some of them. One should not combine more than two or three populations in a pool in order to keep the frequencies of most alleles at a useful level. It is recommended to have 60 pairs of parents per generation and at least ten offspring for each pair. Matings should be randomized and the effects of natural selection should be avoided.

Farm populations

Rochambeau and Chevalet (1985) have proposed a method for controlling inbreeding rates in small populations. This method takes into account usual breeding constraints: all the males and females do not have the same probability to give one progeny, generations overlap, the demographic structure of the various subsets of the population change from one year to the other or
from one farm to the other. A population is divided into breeding groups. The demographic structure of each group is defined by parameters stating the rates of renewal and the reproducing capabilities. At any time, the genetic description is made up of the set of mean inbreeding coefficients of the subsets (sex x breeding group x age) and of mean kinship coefficients between these subsets. General conditions that minimize inbreeding rates over the first few generations are given: (i) the number of breeding groups should be at least ten; (ii) the mating schemes should involve the circulation of males from any group over females of all other groups; (iii) the number of males used per year, and their rate of renewal should be maximized. These conditions lead also to a reduction in the variance of inbreeding coefficients, and induce a genetic structure independent of the initial relationships between founder individuals. ROCHAMBEAU and CHEVALET (1989) defined another criterion - the effective number of founder genes - to study the changes in genetic variability. The decrease of this effective number confirms that the number of breeding groups must be greater than ten (Table 2). However, the mating scheme has no influence either on this effective number or on the mean kinship coefficient, whereas it has a strong effect on mean inbreeding coefficients. The effect of the number of breeding groups can be explained by the effect of group size, on the variance of progeny number per breeding animal. With a fixed total number of breeding animals, the larger the number of breeding groups, the smaller the size of each breeding group and the smaller the variance of the number of offspring per breeding animal.

Table 2 : Effective number of founder genes (A), inbreeding coefficient (F), and kinship coefficient (T) after 15 years in a population of 616 she goats and 44 males. In the first mating scheme the males of one group are always mated with the females of the same group. In the second mating scheme they are mated each year with the females of a new group.

<table>
<thead>
<tr>
<th>Number of breeding groups</th>
<th>5</th>
<th>5</th>
<th>11</th>
<th>11</th>
<th>23</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating scheme</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>77</td>
<td>75</td>
<td>87</td>
<td>88</td>
<td>104</td>
<td>103</td>
</tr>
<tr>
<td>F</td>
<td>0.63</td>
<td>1.18</td>
<td>1.49</td>
<td>0.67</td>
<td>1.70</td>
<td>0.44</td>
</tr>
<tr>
<td>T</td>
<td>1.24</td>
<td>1.24</td>
<td>1.06</td>
<td>1.07</td>
<td>0.88</td>
<td>0.89</td>
</tr>
</tbody>
</table>

ROCHAMBEAU (1983) discussed how to make the breeding groups. When the population is split up into various farms, the best way is to make some breeding groups with the same demographic structure. There will be females from one or two farms in one breeding groups. In this case, it is not useful to study the pedigrees. On the contrary when the population is in the same farm, and when pedigrees are available, it is possible to use this information to build the breeding groups. Some statistical methods like an ascending hierarchical classification, a factorial analysis of a distance table or a clustering analysis can build the breeding groups using information from the pedigrees. ROCHAMBEAU (1987) has given an example with a kinship coefficient table between 74 bulls of a cow breed.
In vitro methods

Storage of frozen semen or embryos is the best way of preserving genetic stocks for various authors (SMITH, 1984; MAIJALA et al, 1984). OLLIVIER and LAUVERNÉ (1988) have agreed with this point. For them the advantages of the cryogenic conservation may be expressed by the number of years above which it become cheaper than keeping living animals. However the advantage for frozen semen may be considerably reduced if the objective is to regenerate the initial stock, since this would require a back-crossing period.

SMITH (1984) studied the problem of sampling. The aim is to get a representative and adequately sized sample of the population to be conserved. Relationships among sampled individuals should be avoided. A maximum level of in-breeding might be set at about 2%. This would be also the percentage loss in genetic variation in forming the store, due to limited numbers. SMITH (1984) recommended to use 25 unrelated sires with frozen semen or 25 parental pairs with frozen embryos. The number of frozen embryos or semen doses to store from each mating or each sire depends on the reproductive success with the frozen material. Then with frozen semen, no inbreeding is generated by using sires rotationally on each others's daughters until the circle of sires is completed. Inbreeding could be avoided in the same way with frozen embryos by rotating over the original embryo strains.

However, CHEVALET and ROCHAMBEAU (1985) studied such a mating plan with frozen semen. A conservation programme was initiated several years ago for the French cattle Bretonne Pie Noire breed (QUEMERE, 1978). Eight unrelated bulls were chosen among offspring of old cows. The population is split up into eight reproduction groups of about 40 females. The semen of each bull is frozen; a bull is mated during two years with cows of one group: the bull is rotationally mated with cows of all the other groups, and then, is replaced by a son. After 20 years the mean inbreeding coefficient is rather low (less than 2%), but 87% of original genes were lost. Most of the genes of the female founders had disappeared and had been replaced by the genes of the eight male founders.

DISCUSSION

MAIJALA et al (1984) have summarized the usefulness of main conservation methods in one table (table 3). Estimated costs of each method were more deeply studied by SMITH (1984). It appears quite clearly that a method have some advantages and also various handicaps. When it is possible living populations should be combined with frozen material.

Table 3 Usefulness of main conservation (method after MAIJALA et al, 1984 ( + advantageous, 0 moderate, - disadvantageous)

<table>
<thead>
<tr>
<th></th>
<th>Semen</th>
<th>Embryos</th>
<th>Gene pool</th>
<th>Farm population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease of genetic variability</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Inbreeding depression</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Contamination by other populations</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Adaptation by natural selection</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Conserved genes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Conserved gene combinations</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Use for cross breeding</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
In all conservation programs it is necessary to manage genetic variability in some small living populations. Indeed after a storage of frozen material, it is necessary to start a new population with a small number of founders. We could make two general recommendations to reduce the decrease in genetic variability: (i) the variance in the number of male (or female) progeny for male (or female) parents should be as low as possible; (ii) the population should be split up in various breeding groups.

The foregoing proposals are based on probabilistic calculus of genetic changes at one locus. This approach will be progressively replaced by actual genetic methods. Within a few years, developments of the tools of molecular biology will result in the establishment of gene maps for the main domestic species (cattle, sheep, goat, pig, poultry, rabbits...), then in the description of many polymorphic loci. Further, the chromosomal regions responsible for production traits variation will be discovered. Methods of conservation will have to be reevaluated, as well as methods of selection: this new knowledge will provide a better description of various breeds, and of their actual genetic differences. The choice of genotypes to conserve will be based on their bearing marker alleles linked to specific traits: disease resistance, meat quality, reproductive characteristics, etc... More directly, any available polymorphism may be used in a conservation scheme, without knowing the linkage relationships between marker loci and quantitative trait loci. It will be possible to measure the actual genetic variability at some loci, to monitor the evolution of allele frequencies, to control the efficiency of a conservation programme.

Finally two questions have still no clear answers: what is the actual efficiency of the various rules proposed to maintain genetic variability? Is there really any need to preserve genetic variability? Few data are available to answer the first question. In several instances, a posteriori estimations of population effective sizes have suggested that the predictions had been optimistic (FARID et al., 1986; HAVENSTEIN et al., 1988). On the contrary, OLLIVIER and LAUVERGNE (1989) have reported that in two old small populations (Merinos Rambouillet sheep in France, and a Large-White pig strain in Spain), the residual genetic variability estimated from a few biological polymorphisms was greater than expected from inbreeding calculations. Present methods are only based on a simple one-locus model without selection, so that they may give an unrealistic picture of the change with time of genetic variability. A realistic modelling of the problem should take into account a completely integrated genome structure, including the effects of recombination, of mutations and of natural selection.

REFERENCES


