GENETIC CONSERVATION OF LIVESTOCK AND FACTOR ANALYSIS

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Abstract. It is necessary to keep diversity in livestock as well as in wild life. Decreased variety in wild life leads to extinction of species whereas in the aspect of livestock it may lead to hunger. This can be because of inbreeding depression or lost genes that are still needed. Different lines of livestock should be kept to prepare for a future that requires attributes not needed today. The different lines can be determined using factor analysis. Separate lines then can be merged by random mating; making way to a herd that has the variance of the original herd. Alternatively, biological material can be stored cryogenically. Live animals can adapt to environmental changes in time and may prove more useful though keeping live animals may cost more than cryogenic storage of biological material.

Keywords. cryogenic storage, livestock, biotecnology, biodiversity, sperm, oocites

Introduction

Humans like old things and try to keep old structures and memories, such as old buildings and photographs. People bring children into the World and want them to follow their footsteps. They try to reach out in time backwards by keeping old things and forward by having children. Humans like to see old things from their early life to forget the fact that their time is passing.

The same reason "trying to stop the time" might be true for the people who try to keep rare breeds or wild species that are about to vanish. There is certainly an emotional drive in keeping those animals in the World. Wild animals are widely regarded as part of the World's heritage and it is the duty of present generation to keep them for the future ones. We did not inherit those from the past generation; we borrowed them from the next generation (an old-Native-American-saying).

Another chief reason to keep those animals may be their possible benefits to the industry in the future. It is a well known fact that much genetic improvement in plants has been obtained by introducing genes from wild, "unproductive" species into cultivated crop plants today. A gene preventing flounder from freezing is introduced to a domestic tomato breed [1]. Another example of a useful character would be the isolated population of feral pigs found on Ossabaw Island, a coastal island off Georgia, USA [2, 3]. Those animals have a unique lipid physiology that makes them useful in medical and nutritional research [4]. The animals can either be kept alive or biological material can be collected to be recovered when needed.

Methods

Live Animals

The animal preservation used to be a hobby and some zoos existed keeping a few animals. Lately, some governments have started paying owners for keeping and breeding an endangered breed, such as the prolific Taihu sheep in China. In 1950's, the US Congress passed a law to ensure the conservation of the Texas Longhorn cattle breed as part of the country's living heritage. Today there is a revival of interest among farmers and the breed is no longer in danger [5]. The advantage of keeping live animals is that a breed can respond to the changes in external circumstances progressively and a performance evaluation is possible. However, because of high costs, only small populations can be kept; therefore genetic variance declines [5].

It was clearly shown by Sewall Wright in the 1930s that inbreeding depression causes fitness problems and extinction in small, isolated populations [6]. In addition, danger of losing a unique herd due to disease is high, especially in poultry. Smith [7] estimated minimum size of a breeding unit and number of animals that should be replaced to keep inbreeding levels to about 2 % a year (Table 1). The main strategy used to achieve a maximum genetic variation in most conservation programs is to set up a breeding system that maximizes the effective population size [8]. These kinds of programs have been shown by Bodo [9] using Hungarian Grey Cattle and by Alderson [10] using Portland and British Milksheep, to be effective in maintaining genetic variation at least when the populations were in a growth phase. According to Alderson a rapid population growth can maintain genetic variability even in the presence of intensive selection. However, when breeds with less than 100 females and a few males are taken into consideration, keeping the variance at a high level can be a serious problem. Lacy [11] showed by computer analysis that a random change by genetic drift may override all changes arising for other reasons in such populations. Borlase [12] reported that keeping the family variance at a low level (equalizing the family size) is recommended in captive breeding programs as it increases the effective population size, reduces inbreeding and slows down the loss of genetic variation. Berger [13] concluded by analyzing 122 populations of mountain sheep that populations of fewer than 50 animals were subject to rapid extinction within 50 years. Krausman [14] reported six populations – ranging in size from 8-46 – persisted for 34 or more years using data from Arizona.

	Cattle		Sheep		Pigs		Poultry	
	Male	Female	Male	Female	Male	Female	Male	Female
Size of breeding unit	10	26	22	60	44	44	72	72
No. of breeding animals entering/ year	10	5	22	12	44	18	72	72

Table 1. Minimum number of animals required for conservation by management (Smith, 1984a).

Maintaining Genetic Diversity and Factor Analysis

A breed, which is in danger of extinction, may possess characteristics that could be useful in the future. Chiefly disease resistance and adaptation to stressful environments such as drought and temperature stress are more likely to be some of those [2], as well as anything preparing future animals to eat novel types of feed and crop or industrial byproducts [5]. However, it is very hard to determine exactly what those attributes might be. Therefore, one might have to keep lines with different attributes with the ones that are not economically valuable today. The various lines of live animals can adapt to environmental changes in time and may prove to be more useful than storage of biological material cryogenically. Keeping live animals may prove beneficial because they can be used immediately without complicated laboratory techniques of biotechnology.

The lines to be kept can be determined using factor analysis. By defining covariance relationships among many variables and by describing those in terms of some underlying but unobservable random quantities called factors [15], one could separate all the animals into well defined-groups. This enables the owner of the herd to keep different lines of animals for the future. Animals that are resistant to specific diseases or perform better in various environments can be obtained using these lines. The idea in using factor analysis is that one can make groups of animals according to an underlying factor. This can be e.g. a disease resistance gene that gives way to a group of animals that are resistant to hunger, or bad nutrition. This can be an enzyme or protein or some other biological factor that makes the animals, for example, behave calmly and also induces higher milk production. Whatever the underlying factor is, grouping animals using factor analysis may lead to a better separation, increasing the overall variance. Keeping different lines of animals will cause some genes to be fixed and some genes to be lost due to genetic drift. The subpopulations will have smaller variances, but the original variance will be restored when all subpopulations are merged back together, assuming that all circumstances are optimal and there is no selection, migration or mutation [16]. This may be a good way to keep the original variance intact since keeping the whole herd together is costly and results in mating of relatives, which increases inbreeding.

Spearman [17] invented the common factor analysis. Kim and Mueller [18, 19] presented a basic discussion of the common factor model. Mulaik [20] is a good general reference on factor analysis. Usually, the term factor is a source of confusion in factor analysis. It refers to a hypothetical, unobservable variable, as in the phrase common factor. In this sense, factor analysis must be distinguished from component analysis since a component is an observable linear combination. A common factor is an unobservable, hypothetical variable that contributes to the variance of at least two of the observed variables. The unqualified term "factor" often refers to a common factor. A unique factor is an unobservable, hypothetical variable that contributes to the variance of only one of the observed variables. The model for common factor analysis posits one unique factor for each observed variable [21].

Simon [22] reported that animals kept for conservation can be used in the future to overcome possible selection limits within the present breeding populations and within the prevailing environment. Simon wrote that based on Robertson's [23] theory on selection limits and on experimental results with laboratory animals, animal breeders have to face the hazard that the response to selection "will cease sooner or later, after a continuous decline in magnitude" [24].

Cryogenic Storage

By using cryogenic methods now it is possible to store a variety of cells for a long period. Living material is stored at -196 Celsius in a liquid nitrogen tank and the length of storage time seems to be indefinite. Most disadvantages that apply to live animal preservation can be avoided by storage of frozen cells at lower costs. Brem [25] compared the costs of the methods (*Table 2*). Techniques of cryogenic storage can be summarized as deep-freezing of sperm and oocytes, deep-freezing of embryos and storage of genes as DNA.

Method	No. of animals needed	Costs (DM)			
		To establish	Per annum		
Small population	5m 25f	50000	15000		
Frozen semen (500 doses)	25m	2500	500		
Frozen embryos (100)	25f	40000	500		
And semen	25m	2500	500		

 Table 2. Comparison of the different methods of genetic conservation for cattle

 (m= male, f= female; DM= Deutsche Mark)(Brem, 1984).

Sperm and Oocytes

Deep-freezing semen is possible in all domestic animals, including poultry. The techniques are well documented. One difficulty is that a relatively complex breeding system is needed to regenerate a purebred population from semen alone [5]. Brem [25] wrote that at least 5 generations of backcrossing are required to achieve 97 % genes of the rare breed. Brem added that inbreeding and genetic drift has to be avoided which can be done by using a rotational breeding system. Smith [26] estimated that 25 sires per breed are needed to prevent inbreeding when males are used rotationally on each other's daughters. Loskutoff [27] reported that in vitro fertilization (IVF) is already proving to be a powerful tool for rescuing gametes (sperm and oocytes) directly from gonads after death or gonadectomy. Coulter [28] reported that manipulation of spermatozoa provides opportunities for the predetermination of sex of resulting offspring, the introduction of foreign DNA into oocytes and formation of transgenic individuals. The cryopreservation of oocytes of most animal species remains a challenge due to their complex structure [29]. Using these techniques and new ones, one can control the structure of a newborn herd in the future and direct it to the needs of that future time.

Embryos

Cryopreservation of mammalian embryos has been successfully used in cows, sheep, goats and horses [5]. The entire genetic information is stored in a single diploid embryo and no complicated backcrossing programs are necessary. Once the embryos are obtained -which can be done non-surgically now- storage costs become very low. On one hand, Sciewe [30] reported that transportation of embryos can reduce/eliminate the need

for shipping live animals. On the other hand, the same authors also stated that efficient worldwide movement of germ plasm requires established cryobanks. In addition, Loskutoff [27] wrote that traditional approaches of superovulation and non-surgical embryo recovery have been hampered in non-domestic ungulate species by inconsistent responses to commercially available gonadotropin preparations; requiring IVF which is apparently a more expensive procedure than the non-surgical method.

Storage of DNA

If a breed becomes extinct, one can bring the stored DNA into the active gene pool of the species by insertion of DNA into the embryos of another breed of the same species. DNA is a chemical and not viewed as biological material by animal health and quarantine authorities, so there should not be any problems with international transportation. The costs of DNA collection is lower than collecting semen, embryos etc. since this is possible simply by taking some blood samples [5].

Conservation of rare breeds or vanishing species is necessary. It is clear that one day humans will need those animals. In a group of bacteria, some individuals carry disease resistance genes in their plasmids. Those individuals are heavier and slower than the others and they require more energy. However, the other bacteria in the group do not leave behind those who carry the resistance genes. In case of a new disease, the bacterium carrying the resistance gene for that specific disease passes along the gene to the others and thus, the whole group is saved. In conclusion, it is necessary to keep the animals, which may not be useful today, but can be beneficial in the future.

Conclusion

Among all of the conservation methods, the easiest and cheapest one is taking and storing DNA/cell samples. Although wild animals are meant to be kept alive continuously, rare livestock breeds can be kept as DNA/cell and regenerated when needed. In the near future, it can be possible to generate a herd from one cell using the cloning technique (first one animal from one cell and then a herd from that animal's cells). Keeping different lines of animals requires extension of resources, not only for the costs of these animals, but also on management and monitoring the attributes. Various kinds of animals should be kept to make sure that the gene pool is available when needed, and keeping different lines. Live animals can adapt to environmental changes in time and may prove to be more useful though keeping live animals may cost more than cryogenic storage of biological material.

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