Genebanks and the management of farm animal genetic resources

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On the cover: *upper left:* Reggiana cow *upper right:* Groningen horse

lower left: Iberian pigs *lower right*: Finnish Landrace sheep

(Photos courtesy of G.C. Gandini (Reggiana) and Oklahoma State University).

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Preface

Economic, social and environmental developments were the driving force for the selection of high productive breeds to be used in the intensive animal production systems. This selection decreased the contribution of locally developed breeds with lower-input, lower-output levels to food production and threatened the existence of these breeds. For good reasons the society and the breeding organisations can not afford and will not accept the non-reversible disappearance of breeds including the loss of genetic variation within the important farm animal species. World-wide genebanks for plants in which genetic variation is conserved do exist already for more then 30 years and only in a few countries farm animal genebanks exist. A scientific foundation for the set up of a farm animal genebank, its role in conservation for future use and its integration in *in situ* conservation programs is highly needed for the European Union.

So far, several organisations were active in the field of conserving animal genetic resources, e.g. the European Association for Animal Production (EAAP) and the Food and Agricultural Organisation of the United Nations (FAO). This book can be seen as an addition to the work published by EAAP and FAO, especially as an addition to the "Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans" of FAO published in 1998.

Within the Concerted Action BIO4-CT96-0197 three meetings were held in 1997 and 1998 in which several aspects of conservation of genetic variation in farm animal populations in Europe were presented and discussed in order to develop guidelines for the cryoconservation of farm animal genetic diversity. Much attention was paid to the integration of the *ex situ* conservation in a genebank in programs for *in situ* conservation in EU circumstances. Geneticists from Scotland, Norway, Finland, France, Italy, Spain, The Netherlands and from FAO participated in these meetings. In a fourth meeting in 1998 they synthesised these aspects and discussions for publication in this book.

The readers of the book might be people working in education, in research, in animal breeding and in governmental and non-governmental organisations. This book will help them to stimulate awareness for the problems resulting from the extinction of breeds and create

awareness for the opportunities of conservation of genetic diversity within species by the creation and use of genebanks and management of farm animal genetic resources in the EC. The contents of the book will help people, who should make decisions on conservation activities for farm animals, to found their viewpoints. Such activities aim at the future use of conserved genetic material in European animal breeding programs and will secure future food production and future market demands, e.g. a diversification of production systems or consumer demands for specialised foods.

Chapter 1. Introduction

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1.1 Challenges for food production and developments in animal production systems

1.1.1 Food for a growing global population

World-wide the human population is expected to grow by more than 50% until the year 2030. In the past the demand for an increased food production has been realised by a combination of genetic improvements, greater farming inputs and cultivation of more land for agriculture. It can hardly be expected that in the future the agricultural inputs can still be increased and that more land can be cultivated for intensive food production systems. Therefore, on the one hand the genetic improvement of farm animals is the most viable approach to meet the increasing demand for food from animal origin in intensive systems. On the other hand rural areas might be used for food production with low-input, low-output breeds which are locally developed and adapted to these areas.

1.1.2 Growing demand for diversification of animal production

One of the major reasons for the domestication of animals was to supply food for the family of the owner. In the last centuries, animal food supply of mankind in the developed world is

concentrated on specialised farms and farm animals are bred and managed to express production and quality traits in an efficient way. Besides, in the developed world it is observed that an increase in income leads to rise in the demand for specialised foods generated by a diversification of animal production systems. To make the genetic approach successful in intensive and extensive circumstances and in the demand for a diversification of animal products, the genetic variation must be maintained or must be enlarged in our selection activities for farm animals.

1.2 Developments in farm animal populations used for food production

1.2.1 Driving forces

In Europe breeding organisations strongly influenced the composition of farm animal populations used for food production. Economic, social and environmental developments were the driving force for the selection of high productive breeds to be used in intensive animal production systems. This selection decreased the contribution of low-input, low-output breeds to food production and threatened the existence of these breeds.

1.2.2 Effects of technology

Applying genetic science in the improvement of the genetic ability of farm animals and the use of artificial reproduction techniques led to the development of advanced breeding schemes and advanced methods to identify the genetic superior animals within the selected high productive breeds. In these breeds a high genetic improvement is obtained for only a few traits. The other traits, which were ignored in the selection, were compensated by increasing management efforts. This way of selection widened the gap in productivity between the developed high productive breeds and the original low-input, low-output breeds, which was a further step towards the extinction of locally developed breeds.

1.3 History of conservation in Europe

1.3.1 Increase in erosion and start of conservation

World-wide the discussion on conservation of genetic resources in animal production started much later than in plant production. However, already at the start of artificial insemination of

cattle in the fifties, Swedish AI-studs conserved semen from each bull used for breeding. In the sixties, scientific and farmer communities draw attention to the high rate of erosion of animal genetic resources. In Europe, farmers were leaving the rural areas where much breed diversity was present and many local breeds were replaced by a few highly promoted and highly selected breeds. These breeds were also exported to developing countries outside Europe and replaced breeds, which were well adapted to circumstances and management systems deviating sharply from those in Europe. In 1972 the first United Nations Conference on the Environment in Stockholm recognise these developments and problems. Ultimately, the first Global Technical Consultation on Genetic Resources was held at FAO-headquarters in Rome in 1980.

1.3.2 FAO-activities for the management of farm animal resources

In 1985 FAO introduced under the responsibility of the Commission on Genetic Resources for Food and Agriculture an expanded Global Strategy for the Management of Farm Animal Resources. In 1992 FAO launched a special action program for the Global Management of Farm Animal Genetic Resources with a framework to stimulate national participation in the global effort to implement conservation activities. National and regional focus points play an important role in stimulating and co-ordinating these actions. The Domestic Animal Diversity Information System (DAD-IS) is used to collect information on breeds and conservation activities and it offers the opportunity to retrieve guidelines for conservation activities. In 1998 this program got a new impulse in the first session of the Intergovernmental Working Group on Animal Genetic Resources for Food and Agriculture. This working group recommended the Commission on Genetic Resources for Food and Agriculture that FAO continue to shape more clearly the framework and further develop the framework and that FAO co-ordinate the development of a country-driven Report on the State of the World's Animal Genetic Resources.

1.3.3 Convention on Biological Diversity stimulates awareness for farm animal genetic variation

In 1992 the second United Nations Conference on the Environment in Rio de Janeiro recognised the importance of farm animal genetic resources in Agenda 21 and in the Convention on Biological Diversity. Nearly all countries have signed this convention, which resulted in political and social awareness of national animal genetic resources and activities to conserve them in several countries also within the European Community. The CBD considers

farm animal genetic variation as a component of the overall biological diversity. The CBD recognises the sovereignty of each country over his own genetic resources, which implies also the obligation to conserve these resources.

1.3.4 Initiatives of the European Association for Animal Production

Concerted activities on farm animal genetic resources started in Europe in 1980 when the EAAP established a working group in this field. The main activities were to organise regular surveys of breeds of farm animals in different European countries and to integrate the genetic science in conservation activities. From 1988 to 1994 FAO and EAAP managed the Global Data Bank for farm animal genetic resources at the Hannover Veterinary University in Germany. In 1993 the data were transferred to Rome to DAD-IS of FAO. Since then, the European national co-ordinators regularly update the databank in Hannover, which send the information also to FAO in Rome. According to DAD-IS there are 332 cattle, 407 sheep, 123 goat, 156 pig and 213 horse breeds maintained in 37 European countries. In the Western (EU) part of Europe the decline in farm animal genetic resources in the intensification of animal production system has token place. In the Central-Eastern European countries, where development of animal production is still the first priority, the strong decline in farm animal resources may be continued.

1.4 What is farm animal genetic variation?

1.4.1 Genetic variation expressed as differences in phenotype

Between animals differences in production and quality traits can be observed as well as in health, reproduction and conformation traits. These phenotypic differences are the result of differences in genetic ability and differences in management. Statistical techniques and knowledge about relationships between animals unravel the phenotypic variation in quantitative and qualitative traits in an environmental part caused by differences between animals in management and the genetic variation caused by differences in coancestry. In general 50% of the total genetic variation within a species is estimated to be between breeds. Therefore, loss of breeds can substantially reduce the genetic variation within a species. In statistical term genetic variation is described as the variance within and between breeds.

1.4.2 Genetic variation measured as DNA-polymorphism's

The development of molecular biological techniques for the detection of DNA-polymorphism's in coding and non-coding regions of chromosomes facilitates to describe the genetic variation more accurately. Such information can be used to calculate the resemblance between animals within breeds and the resemblance between breeds, which might be used for the development of conservation activities.

1.5 Why should farm animal genetic variation be conserved?

1.5.1 Opportunities to meet future market demands

In the prosperous countries of the European Community the demand for specialised food from animal origin increases. This results in a diversification of animal production systems and of animal products. Besides, prosperity increases the use of animals for other goals like hobby farming and the use of animals for sports (horses). These developments request a large variability in the genetic variation of the species used. The breeds used in the present agricultural systems can not meet all these future market demands.

1.5.2 Insurance against future changes in production circumstances

The high-input high-output systems are characterised by the high use of fertilisers and concentrate and within these systems veterinary treatment with drugs for preventive and clinical use are sometimes practised at high levels. Agricultural pollution and resistance against drugs can create conditions for animal production in which higher levels of respectively feed intake and disease resistance are required. The conservation of genetic variation is necessary as an insurance against changes in production circumstances or the threat of a new disease.

1.5.3 Present socio-economic value

In many countries local breeds are used by a small group of farmers sometimes for special reasons (e.g. biological farming or grazing of marginal lands) or purposes (e.g. local products for niche markets). The development of breeding programs for these local breeds is too costly for breeding organisations and the absence of a breeding program is a threat for the existence of the breed. However, present socio-economic value, which creates income for a small group of farmers, justifies the establishment of a conservation program.

1.5.4 Opportunities for research

World-wide in animal production, molecular geneticists are searching for genes, which influence production, quality of products, and health and reproduction traits of animals. In this search, crosses between breeds with extreme characteristics play an important role. They guarantee a high degree of heterozygosity and linkage disequilibrium, which is required to detect associations between highly polymorphic marker loci and polymorphism's at quantitative and qualitative trait loci.

1.5.5 Cultural and historic reasons

Many breeds are the result of a long domestication process and a long period of adaptation to local circumstances. They reflect a long history of symbioses between mankind and farm animals and can help to clarify adaptation processes, which can still be worthwhile for the management of animals in present production systems.

1.5.6 Ecological value

Within the community the awareness is growing for the ecological value of regions as a result of landscape, nature and farm management. Within this complex the presence of animals from native origin which interact with parts of this complex is of great ecological importance. Besides, these animals can contribute to the development of local products with an ecological image.

1.6 Who should conserve farm animal genetic variation?

1.6.1 Role of national governments in an international context

In accordance to the principles of the Convention on Biological Diversity each country is responsible for the management of their own national farm animal resources and for the implementation of conservation strategies. However, many breeds are spread over several (neighbouring) countries and exchange of genetic material between the subpopulations of such an international breed is very common. Therefore, collaboration between countries in conserving breeds is often the most efficient strategy. For example, EU-regulations 1467/94 and 2078/92 aim respectively at an efficient development of conservation plans over countries and at uniform stimulating procedures for practical farmers to keep animals of breeds at risk

within conservation plans. A general strategy for the establishment of databases in which relevant breed information is stored and of the outline of conservation plans might serve conservation activities world-wide. FAO developed a frame work for a global strategy for the conservation of animal genetic resources with the development of a network of co-ordinating institutes and focal points as a backbone and the development of a database and of guidelines for conservation activities as major products.

1.6.2 The responsibility of scientists, breeders and breeding organisations

Scientists should develop monitoring and signalling schemes to help governmental organisations to watch over populations, which might become at risk or are threatened with extinction. Besides, genetic science should be translated in guidelines for conservation plans. Individual breeders and breeding organisations may help in conservation activities by offering genetic material to a genebank and in performing breeding programs for small populations at risk. In collaboration with organisations initiated by governments and with national subsidy breeders and breeding organisations play a key role in the success of conservation activities.

1.7 How should farm animal genetic variation be conserved?

1.7.1 Selection schemes of breeding organisations

To avoid inbreeding and to minimise random drift in populations under selection breeding organisations should pay a lot of attention to the number of founder animals and their relationship and to the mating scheme and the effective number of breeding animals in each generation. A large effective population is a guarantee for the conservation of a large amount of genetic variation. However, costs of a breeding plan and the genetic gain, which is necessary to justify the costs, require high selection intensity and a short generation interval. Both might be a threat for the genetic variation in a population of a small effective size.

1.7.2 *In situ* conservation

When a breed is at risk or is threatened by extinction *in situ* conservation is to be preferred. Then, all objectives of conservation can be reached the best. Besides, the development of the breed can continue, which means selection for economic profit as far it is possible within the limits of a small population and it facilitates adaptation to changing circumstances. However,

the risks of inbreeding and random drift have to receive full attention in the breeding schemes for small populations.

1.7.3 *Ex situ* live conservation

Sometimes the production of breeds may be uneconomic and management may be demanding. Nevertheless, a cultural, historic or ecological value may exist. Such breeds can fulfil a role in zoo's, farm parks or natural parks. The costs of *ex situ* live conservation are low, but the breed is kept outside its production environment to which it is adapted.

1.7.4 *Ex situ* conservation

For most farm animal species it is possible to cryo-conserve semen and realise high or acceptable levels of conception after thawing the semen and inseminating females. For some species frozen embryos can be used to create live offspring. Also, recent developments have been made in freezing techniques for oocytes. For all animal species DNA-storage and storage of somatic cells is a well-known technology. However, techniques like nuclear transfer should be developed further in order to use these types of storage to regenerate animals. Storage of genetic material in genebanks is not in line with all objectives of conservation. Conservation of a breed in a genebank does not contribute to its socio-economic value, its contribution to culture and history and to its ecological value. So, the main value of a genebank for farm animals is decreasing the risk of *in situ* conservation schemes. Conservation in genebanks might be less costly compared to *in situ* conservation when it is expected that it will take many years before an objective of conservation has to be fulfilled with the stored genetic material.

1.8 Background to decision making in conservation farm animal genetic variation

1.8.1 Aspects of conservation

In chapter 1 some general aspects of conservation farm animal genetic variation and the recent history are briefly outlined. For economic reasons it is impossible to keep all low producing breeds of farm animals alive. Therefore, in chapter 2 the strategies, which can be chosen for conservation, are outlined. Artificial reproduction techniques, including cryo-conservation of DNA, gametes, embryos and somatic cells, facilitated the establishment of genebanks for farm animal species, in which genetic variation threatened by extinction of

breeds can be stored for possible future use. The weighting of criteria to be used in order to make a choice between strategies are discussed.

1.8.2 Selection of breeds and choosing animals within breeds

Several objectives of conservation favour the live conservation of breeds not used anymore for economic reasons in animal production systems. However, both types of conservation are costly.

Therefore, scientific knowledge should be used to make proper choices between and within breeds. Many breeds were used over different countries of Europe in subpopulations and many breeds originate from common ancestor breeds kept in past centuries before breeding programs started. In chapters 3, 4 and 5 of this book the aspects of selection for conservation among and within breeds and the set up of conservation schemes are described.

1.8.3 Operation of conservation activities

In chapter 6 the operation of conservation schemes is outlined and discussed and in chapter 7 the development of an expert system for conservation is described.

Chapter 2. Choosing the conservation strategy

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- ***Preface***
- ***Objectives and techniques***
- ***Options for self-sustaining local breeds***
- ***Costs***
- ***Making the decision***
- ***Legal issues***

2.1 Preface

Techniques for conservation of animal genetic resources (AnGR) are generally divided in *in situ*, i.e. the maintenance of the breeds within their production systems, and *ex situ*. *Ex situ* techniques are further divided, according to FAO (1998), in cryoconservation of genetic material, which includes haploid cells (semen, oocytes), diploid cells (in vivo and in vitro embryos, somatic cells) and DNA, and *ex situ* live, i.e. the maintenance of live animals of a breed outside its production system (e.g. herds kept in natural protected areas, in experimental and show farms, in zoos).

Until recently, *ex situ* techniques were often advised for their high potential as a reliable conservation strategy. Today, there is wide consensus on conservation by maintaining populations within their production systems (*in situ*):

- the Convention of Biological Diversity (CBD) (art. 8) emphasises the importance of *in situ* conservation and advises (art. 9) *ex situ* conservation as an essential activity complementary to *in situ* measures,
- FAO underlines in its "Guidelines" (1998) the priority for *in situ* conservation,
- the Common Agriculture Policy (CAP) of the European Community incentives farming of local endangered breeds in their production systems (EU reg. 2078/92).

Ex situ, however, continues to provide powerful and safe tools for conservation of AnGR. It seems therefore reasonable to make efforts to build a framework for the effective integration of *in situ* and *ex situ* techniques, in which *ex situ* conservation is complementary to *in situ*.

In this chapter we first see how *in situ* and *ex situ* techniques differ in their capacity to reach the various conservation objectives. *In situ*, because it implies the maintenance of the breeds in their production systems and it achieves the widest spectrum of objectives. However, it can be associated with high costs. It is useful to evaluate the different options for self-sustaining populations *in situ*. Economic incentives may be important in the period needed to reach self-sustainability: current EU incentives and propositions for additional incentives are discussed. In reviewing the scarce literature on costs of conservation techniques, we underline the need for more research. Finally, we propose some criteria to choose the most appropriate conservation technique for a specific breed and for the breeding context. A short discussion on some legal issues associated to the management of genome banks concludes this chapter.

2.2 Objectives and techniques

The objectives for AnGR conservation, as stated in the introduction of this book, are:

- opportunities to meet future market demands,
- insurance against future changes in production circumstances,
- present socio-economic value,
- opportunities for research,
- cultural and historical value,
- ecological value.

Different techniques are available to achieve the different conservation objectives (table 2.1.). *In situ* conservation is effective to reach all objectives. The *ex situ* live method excludes the present socio-economic value, because in this conservation strategy the breed is removed from its socio-economic context. For the same reason the cultural and ecological objectives cannot be effectively pursued. Cryoconservation is an option, when socio-economic, cultural and ecological values are missing or are of no concern.

In table 2.1, the different techniques are compared on the basis of factors associated to the conservation objectives: i) the opportunity for breed evolution and genetic adaptation, ii) the opportunity to better characterise the breed, iii) the exposure of the breed to random genetic drift and inbreeding. Beside the ontogenic process, cryoconservation 'freezes' also the evolutionary process of the breed and impairs its genetic adaptation, i.e. its future production value. Nowadays, very little is known on the performances of most endangered breeds; there is a need to increase our knowledge on local breeds in order to better define their conservation value (see paragraph 2.3.). Populations of small size are exposed to random genetic drift, which is measured as effective population size and is established as inbreeding. The rate of genetic drift (see chapter 6) can be controlled by accurate selection and mating of sires and dams. *Ex situ* live populations are expected to be smaller than *in situ* populations and consequently they are more exposed to genetic drift. However, *ex situ* live conservation might facilitate better genetic management. For QTL studies, semen is the first option. For studies on genetic variation, purified DNA is sufficient.

In table 2.2, *ex situ* techniques are compared for their effectiveness to achieve the different aims of cryoconservation. All techniques available or in development are discussed. Oocytes differ, in terms of efficiency to achieve the various aims of cryoconservation, from embryos because with oocytes it is still possible to choose the desired mating. Nevertheless, oocytes here are not distinguished from embryos. Embryos are the first option for breed re-establishment, followed by somatic cells (cloning), assuming this technique will be soon available for this purpose. The breed can be re-established by using semen for backcrossing females, but then four generations are required to achieve over 90% of genes of the endangered breed. With semen or somatic cells cytoplasmic effects of the endangered breed will be lost or altered. Semen is the genetic material of choice for creation of synthetic breeds, for gene introgression and as aid to the genetic management of *in situ* or *ex situ* live

Table 2.1. Conservation objectives, factors associated and conservation techniques.

	Technique		
objective	**cryoconservation**	***ex situ* live**	***in –situ***
meet future - insurance	yes*	yes *	yes *
socio-economic value	no	no	yes
research and education	yes **	yes	yes
cultural-historical value	no	poor	yes
ecological value	no	poor	yes
factors associated			
breed evolution/genetic adaptation	no	poor	yes
better knowledge of breed characteristics	poor	poor	yes
exposure to genetic drift	no	yes (+)	yes

* several differences among the three techniques. See factors associated.

** based on crossings with other breeds.

Table 2.2. Conservation aims with different *ex situ* techniques.

	***ex situ* technique**			
aim	**semen**	**embryos**	**somatic cell**	**DNA**
breed re-establishment	yes* but < 100%	yes	yes*	no
creation of synthetic breeds	yes	poor	poor	no
gene introgression	yes	poor	poor	no
cryo-aided live scheme	yes	poor	poor	no
QTL studies	yes	poor	poor	no
DNA studies	poor	poor	poor	yes

* no extra-chromosomal DNA.

programmes (cryo-aided live schemes. See chapter 6). For that purpose embryos and somatic cells are less efficient.

2.3 Options for self-sustaining local breeds

The technique of maintaining endangered breeds in their production environments covers the widest spectrum of conservation objectives. Then, it becomes important to analyse the options we have for an efficient *in situ* conservation.

An analysis of the dynamics of the erosion of farm animal genetic resources in Europe in the last decades will probably reveal a complex picture. It may shows cultural, social and food demand changes, transformation of the food production chain, country regulations and technological changes affecting in various ways the decline of local breeds. In most cases it is likely that these factors result in a lack in economic profitability of the local breed compared to other breeds or other economic activities in that region. The absence of a breeders association or a breeding programme may also play a role.

The fall in population size is the first result of these facts. Other effects are the decrease among breeders in enthusiasm for the promotion of their breeds, which impairs performance recording and development of breeding schemes. All these aspects might trigger a further decline of the breed, finally leading to extinction.

It is likely to assume that in conservation of AnGR prevention of breed decline can be more effective than a conservation therapy. Therefore, it is important to counteract the processes of breed decline before the population size will be too small. This will impair the probabilities of success and will increase the costs for recovering.

Then, several questions can be raised:

- Which options are available to stop and reverse the decline process into a self-sustaining programme?
- What can we learn from recent experiences?

- Does the European context offer particular opportunities to make local endangered breeds self-sustainable?

These questions will be answered by considering five general options:

- establishing the economic performance of the breed,
- improving infrastructures and technical assistance,
- genetic improvement,
- optimisation of the production system,
- developing activities to increase the market value of breed products,
- developing incentives.

2.3.1 Establishing the economic performance of the breed

For most of the local European breeds we have no reliable data on their performances. Most often:

- performances are estimated on small samples,
- available information refer only to phenotypic data, with no estimates of genetic parameters,
- information is not available on secondary traits, such as longevity, fertility, mortality, feed and management requirements, i.e. on those characters, which might significantly contribute to breed profitability.

In many areas on the world comparisons of performances between crossbred and indigenous breeds have been based on poor experimental designs, which often produce misleading results (FAO, 1998).

It is likely that better evaluations of the economic performances of local breeds may change the results of comparisons among local and exotic breeds and it may correct possibly erroneously perceived differences or may assess possible strong points of the local breed.

Breed comparisons should first be based on a good assessment of breed performances. When the breeds participate in a national recording scheme for production traits, the biological information for the comparison can be gathered much more accurately and to some extent an economic comparison is feasible in combining performances with market prices of products.

More accurate comparisons require:

- awareness for interactions between the farm management and the characteristics of the breed, which requires comparisons of breeds in different management systems. For example, the composition of the diet may interact with breed performances,
- additional trials at experimental stations or at practical farms under controlled conditions. Despite possible high costs, these trials offer the opportunity to compare breeds accurately for input and output factors, which is essential for a proper economic comparison,
- studies on farms and in experimental stations including evaluation of crossbreds to better understand the potential use of the local breed in different crossbreeding production systems.

The relative economic advantage or disadvantage of a breed is a function of the relative prices for the different animal products. A breed, which is not used in high-input, high-output systems, can be profitable in a low-input system through a high feed intake capacity, longevity, fertility, hardiness, quality of the products or a niche market for its product.

2.3.2 Improving infrastructures and technical assistance

Most often local breeds are producing in areas characterised by a low socio-economic development. This might result, with respect to a breeding programme, into a lack of infrastructure and technical assistance, including networks for milk collection and processing, slaughterhouses, networks for commercialisation of products, performance recording and services for technical assistance. Genetic improvement is an important aspect and will be discussed in the next sub-paragraph. It is likely that the lack of these elements impairs the economic performance of local breeds.

2.3.3 Genetic improvement

In general local breeds do not benefit from modern breeding techniques. Selection programmes may increase the genetic ability for productivity and consequently the profitability of local breeds.

Two major considerations have to be forwarded:

- the definition of breeding goals should take into account the conservation value of the breed. Traits proposed for selection should be accurately evaluated for their genetic correlations with those traits that determine the conservation value of the breed, in order to

avoid the deterioration of its conservation value. These might include adaptation to a harsh environment or to low-input production systems or traits like longevity, fertility, meat and milk quality ;

- selection schemes should take into account the maintenance of genetic variation within the breed and risks associated with a high rate of inbreeding. To reach these goals, a theoretical framework has been developed in the last years and software are available for field use (see chapter 6).

Box 2.1. Reggiana cattle

In the 1940's the Reggiana, a local dairy cattle farmed in Northern Italy, counted more than 40,000 cows, then progressively dropped to a minimum of 500 cows in the early eighties. It was a typical process of displacement of a local breed by the cosmopolitan better-promoted and more productive Friesian breed. Since the late eighties several activities were begun to support the breed (shows, performance recording, valorisation of products, etc) leading to a progressive increase of the number of cows up to 1,200 in 1998. A discussion was recently opened among breeders to develop some selection programmes. Breeding goals will probably include udder morphology followed by kg. of protein, while monitoring several aspects of milk characteristics (associated to cheese production), longevity and fertility. Constraints on rate of inbreeding have been included (Gandini et al, 1998). It is felt that, besides increasing profitability, the introduction of a breeding programme might also increase the interest of farmers for their breed.

2.3.4 Optimisation of the production system

Increasing economic performance of local breeds might require re-organisation of their production systems, such as seasonal planning of the production, changing age or weight at slaughter or introducing some crossbreeding.

Attention should be given to the conservation of the local breed. Taking the introduction of crossbreeding as an example:

- breeding schemes should guarantee the maintenance of viable populations of the local breed through a sound pure breeding scheme,
- the breed might be used for the production of commercial crosses with a high performance breed. The commercial crosses might benefit of higher input production systems, while the

local breed should be maintained in its original production environment to maintain its adaptation characteristics,

- the use of the local breed as a female population (instead of as male population, which might be more profitable) may be advisable to guarantee the maintenance of a large population of the local genotype adapted to the production environment,
- the use of a high performance breed that will produce crosses which cannot be distinguished from the local breed is not advisable, because of the risk of non-voluntary introduction of exotic genotypes in the local breed.

Crossbreeding schemes are of interest also for indigenous breeds in developing countries (FAO, 1998).

2.3.5 Developing activities to increase the market value of breed products

2.3.5.1 Links between products and breeds

The starting point is the question: is the relationship between breed and product helpful to diversify its products and to sell them at higher prices, i.e. to improve the economic profitability of the breed?

It is known that diversification is an opportunity to grade products. Generally the control and the enhancement of agricultural products' quality is a combination of the raw material (meat or milk) and the processing. Once a certain quality level is achieved, it can be promoted by advertising, which let the consumer perceive some elements of the product. Some recent experiences (Gandini and Giacomelli, 1997), summarised in the examples in Box 2.2, support the approach of a marketing link between products and local breeds.

Only a few breed-product links have been reported in the literature. In this respect, it is advisable that all experiences in the future will be reported. Some general conclusions can be drawn from known experiences:

- the link between product-breed can improve breed's economic profitability,
- building this link offers several options: e.g. the link can be part of a protected designation of origin (PDO) or can be used to further differentiate a product within a market already differentiated (e.g. within a PDO),

- the overlapping of an exotic breed in the farming area of the local breed might hamper (e.g. difficulty of separate milk collection) the creation of a link between the local breed and the product,
- in some cases, the link between product and the breed-environment seems to be more appropriate than the link between product and breed.

Box 2.2. Niche marketing

In France, since 1986 the Tarantaise and the Abondance cattle breeds are associated to the production of the Beaufort and together with the Montbéliarde breed to the Reblochon and Abondance cheeses. The positive economic valorisation of milk is documented (Verrier, 1995). On the Italian side of these Alps there is a similar case: the Fontina cheese is produced manufacturing milk only of the Valdostana breed. Other examples, in France, of links between cheeses and breeds includes: the Ossau-Iraty cheeses and the Manech and Basco-Bearnaise sheep breeds, the Comté cheese and the cattle breeds Montbeliarde and Simmenthal, the Laguiole cheese and the cattle breeds Simmenthal and Aubrac. In Italy, the Parmigiano Reggiano cheese since 1955 has a protected designation of origin (PDO). In 1991 a consortium of breeders started the marketing of a brand of Parmigiano Reggiano made only with milk of the Reggiana breed (the original breed, before the Friesian, used to produce this cheese). Since 1991 this brand is sold at a price 16% higher than the common Parmigiano Reggiano. The recent increase of the number of cows, from 500 of the 1980's to 1,200 in 1998 is thought to be strictly associated to this operation.

2.3.5.2 Ecological and cultural breed products

In this respect we may consider that (Gandini and Giacomelli, 1997):

- in Europe, before the intensification and industrialisation process in the last decades, livestock farming was closely linked to the use of farmland and was in general extensive. Most of the areas which are recognised nowadays as natural areas are in fact agro-ecosystems created and maintained by farmers and their local breeds. The decline of local breeds and of their production systems are raising concern for the maintenance of these agro-ecosystems and cultural landscapes;
- when grazing ceases, bush encroachment follows, which makes it more difficult to use the lands for recreation. Farmers maintain landscapes of great beauty, which are rich in culture.

Examples in this respect are the Alpine pastures, which attracts large amounts of tourists in summer;

- the reduction of livestock grazing is known to increase risks associated to natural fires, especially in the Mediterranean regions, and to floods in the alpine areas;
- local breeds may be considered, from a cultural point of view, to be testimonial of the farming civilisation of the specific area.

Based on these and similar considerations, several countries and the EC developed specific agriculture and environment policies, including subsidy systems directed to the development and maintenance of rural landscapes and agro-ecosystems management. However, subsidies are not expected to be available in the long term. Then, the question arises: is it possible to develop a market value for the ecological and cultural services from local breeds?

Recent experiences allow some optimism:

- in Southern Europe, cheese producers and breed associations started to envisage an ecological role for their local breeds (see Box 2.3.);
- in Austria, some Tyrolean communities and tourism enterprises observed that summer tourists expected the presence of a farming community with their breeds and the availability of local food products, the presence of animals and a farming culture on tracking areas. Since a few years, they subsidise the farmer community to avoid the decline of farming activities. In doing so, they recognise a market value of the cultural and ecological value of farming;
- in several parts of Europe horses are recognised as a proper help to harvest the wood under rough conditions. This may facilitates the conservation of the original heavy European horses.

Box 2.3 Ecological use

From 1993, in Savoy in France, herd milk production (Tarantaise and Abondance cattle breeds) for making the Beaufort cheese is limited to 5,000 kg per lactation in order to maintain the optimum stocking rate of .7 head per hectare. On the Southern side of the Alpes, in the Italian Valle d'Aosta, the production of the Fontina cheese implies that milk comes from Valdostana cattle taken to alpine summer pastures.

2.3.6 Incentives

During the time needed to increase the economic profitability of the endangered breed, incentive payments can effectively halt the decline of the breed. Incentives may be necessary to improve the economic profitability of the breed.

2.3.6.1 Current incentives

- The EU regulation 2078/92 provides incentive payments to farmers keeping endangered breeds of several species. The incentive payments are thought to compensate farmers for the lower profitability of the local breeds compared to substituting these breeds with more profitable exotic breeds. Local experiences have proved the efficacy of incentives payments. Among the different forms of incentive payments (FAO, 1998), the most effective ones for the short and medium term support of the breed should be used.

- EU Council Regulation 1467/94 ("European Community Programme on the conservation, characterisation, collection and utilisation of genetic resources in agriculture") provides grants to the State members for conservation activities in agriculture. Until now, much emphasis has been put on the plant sector. It is to be expected that farm animal genetic resources will get more attention in the coming years.

- The 3rd Conference of the Parties of the CBD (held in Buenos Aires in 1996) drew the attention (in the document "Conservation and sustainable use of agricultural biological diversity") of both the international funding agencies and the CBD to support the conservation and sustainable use of biological diversity important to agriculture.

2.3.6.2 Proposition for additional incentives

Considering that CAP supports local breed conservation, it is worthwhile to investigate the introduction of new measures aimed to AnGR conservation into present EU Regulations. This might include:

- subsidies to buy milk quotas by farmers with endangered breeds to extend their herd size;
- subsidies to buy milk quotas by farmers with non-endangered cattle breeds, willing to extend their herds with cows of endangered breeds;

- suckling cows subsidies for endangered breeds producing some milk for the market and in mixed (suckling and dairy) herds (Institut de l'élevage, 1992);
- permission to store semen and embryos of endangered breeds that cannot fulfil the present sanitary rules of the EU. Before this genetic material is used, it has to be tested for the relevant diseases and cost might be paid by EU;
- to include in reg. EU 2078/92 species presently not-considered, primarily the pig;
- to develop a legal framework for the promotion of breed-product links, taking into account for example EU regulation 2081/92.

2.4 Costs

The future economy of animal production is a major argument for preventing erosion of AnGR. Then, costs for their conservation should be seen as an insurance against uncertainty of future market and production circumstances. In a shorter time horizon, costs can be seen as an investment in the development of animal production, as a contribution to environmental, landscape and cultural conservation and as support to scientific progress.

From the scarce literature on costs of conservation techniques (Brem et al., 1984; Ollivier and Lauvergne, 1988; Lomker and Simon, 1994; Ollivier and Renard, 1995) some considerations can be stated:

- in comparing costs of different techniques, aims should be well defined. Cryoconservation with semen can be a very costly method when breed re-establishment is the aim (Lomker and Simon, 1994),
- in comparing costs of different techniques, the conservation time horizon should be taken into account. With cryoconservation, costs of maintenance are a function of the number of years before use. The advantage of cryoconservation over *in situ* conservation can be expressed by the number of years of conservation above which it becomes cheaper than keeping live animals (Ollivier and Lauvergne,1988),
- cryoconservation costs of the different methods vary among species, because differences in efficiency of techniques do exist. For example, in the rabbit embryobanks are cheaper than semen banks (Ollivier and Renard, 1995),
- costs are expected to differ significantly among regions and countries,

- *in situ* costs strongly depend on the economic competitiveness of the endangered breed, i.e. on the subsidies necessary to compensate for the gap in profitability with the high producing breeds (Lomker and Simon, 1994),
- all the literature refers to simulated costs; no real data from field projects are available in the literature,
- *ex situ* conservation is in general considered to be less expensive than *in situ* conservation.

More research is needed to compare conservation techniques on an economic basis. Some general considerations can be proposed separately for *in situ*, *ex situ* live and cryoconservation techniques:

- *In situ*

Our approach is to try to take the breed to self-sustainability, therefore:

- incentive payments to farmers should cover the gap in economic return between the endangered breed and the average commercial breed. Breed comparisons (see paragraph 2.3) are useful to determine the amount of the incentive payment;
- the length of economic incentive payments depends on the period necessary to take the breed towards self-sustainability;
- taking the breed towards self-sustainability may imply costs for technical assistance, for the development of a breeders association, for performances control and breeding schemes, and costs to identify, qualify and market products linked to the breed and to market the cultural and ecological value of the breed.

It should be noted that, when self-sustainability is reached, conservation costs become zero. Costs of cryo-aided live schemes should be accounted for, where applicable.

- *Ex situ* live

Costs of *ex situ* live conservation are equal to the difference between the profit from farming the average commercial breed and the endangered breed. Market strategies promoting tourism (herds kept in natural protected areas, in show-farms) and high quality products (e.g. "biological" products) can be used to increase profitability. Costs of cryo-aided live schemes should be accounted for, where applicable.

- *Cryoconservation*

All costs should be accurately accounted for.

  - Considering semen, costs include:
    + finding donor males or producing matings to obtain males when necessary to widen the genetic variation in the semen to be stored (see chapter 5),
    + raising males to puberty necessary in some situations when it may be difficult to find boars because males are usually castrated at early age,
    + taking males to special centres for collection and freezing. (because of health regulations, it can be difficult in Europe to use regular A.I. facilities),
    + semen collection and processing,
    + semen storage and maintenance at two locations (costs of tanks and liquid nitrogen),
- If the aim is breed-reconstruction, add costs for backcrossing.
  - Considering embryos, costs include :
    + finding donor females, identifying optimal matings and producing embryos,
    + embryo collection and processing,
    + embryo storage and maintenance at two locations (costs of tanks and liquid nitrogen).

2.5 Making the decision

Most often, because financial and human resources are limited, breeds cannot be given the same priority for conservation. Chapter 4 will discuss criteria and methods for selecting breeds for conservation. When a breed or a set of breeds has been selected, the question arises: which *in situ* or *ex situ* technique or which *in situ* and *ex situ* combination should be used?

In paragraph 2.2., the capability of each technique to reach the conservation objectives was analysed. Costs of techniques were discussed in paragraph 2.4. Now we present some criteria to choose the most appropriate conservation technique. The following nine steps, illustrated in figure 1, should be considered:

1. **Evaluate the current conservation values of the breed**. An accurate evaluation of the conservation values of the breed is expected to be done in selecting breeds for conservation (chapter 4). Two aspects require further consideration:
   - to estimate the genetic variation of a breed and its uniqueness we developed objective criteria (e.g. genetic distances, genetic parameters). Conversely, we do not yet have appropriate and standard tools to evaluate the cultural, ecological and socio-economical value of a breed. For example, it might be said that because breeds are products of domestication as such, they have a cultural value. This is not necessarily true, in general a breed will have a cultural value when it played in the past an important role in the creation and development of local agricultural techniques, food products, gastronomy, landscape, local handcraft techniques, costumes etc.;
   - conservation values of the breed must be present in a form that allow their conservation. For example, a breed will have a high cultural value worth considering conservation when expressions of this culture are still present in the farming area. If not, it will be only a pale testimony of it.

2. **Identify the conservation objectives for the breed**. Not all the conservation values of the breed may be taken as conservation objectives. The choice may vary among countries, because the different interest and priorities of Government and human societies. E.g. nowadays, cultural and ecological values are more eligible as conservation objectives in Europe than in other regions of the world.

3. **Select the techniques, which allow reaching the conservation objectives previously identified**. Not all techniques allow reaching the same conservation objectives with equal effectiveness. Following tables 1 and 2, select techniques (*in situ, ex situ* live, cryconservation) or combinations of these techniques that could be used to reach the conservation objectives previously identified. If the socio-economical value of the breed is an objective, maintenance of the breed within its productive context (*in situ*) is the only technique available. If cultural value or ecological values are among the conservation objectives, *in situ* or *ex situ* live techniques can be used. In all these cases, cryoconservation (a genome bank) can be used in addition to *in situ* and *ex situ* live to reduce the risk of a breed loss. When objectives are limited to the opportunities to meet

future demand, the insurance against future changes in production circumstances and the opportunities for research, all techniques and their combinations are eligible.

4. **Rank techniques for risk.** Analyse the techniques or combinations of techniques on the basis of the risk of failure and exclude those with a non-acceptable level of risk. Risk of failure is a function of:
   - the degree of endangerment of the breed (EAAP Working Group on Animal Genetic Resources, 1998). When *in situ* or *ex situ* live are the techniques of choice and the population size is very small, health conditions are critical and the socio-economical stability of the area is low, the risk of breed loss can be high,
   - the possibility of controlling genetic drift (availability of skilled technicians to perform mating schemes),
   - the success of past conservation: the success of removing factors that in the past limited conservation success.

5. **Rank techniques for costs.** For all techniques (all *ex situ* techniques, i.e. semen, embryos, somatic cells) or for combinations of techniques that guarantee acceptable risks of failure, costs should be evaluated. Rank all techniques on the basis of costs.

6. **Rank techniques for the opportunity of breed evolution/adaptation and of a better knowledge of breed characteristics.** When we prefer to continue the evolution and genetic adaptation of a breed, then the *in situ* technique should be chosen. This will also guarantee the opportunities to increase our knowledge of the breed.

7. **Rank cryoconservation techniques for efficiency of conservation aims.** Not all the *ex situ* techniques, semen, embryos and cells, allows to achieve the different cryoconservation aims (see table 2.2).

8. **Rank techniques for efficiency of conservation of cultural and ecological values.** If cultural value or ecological values are among the conservation objectives, *in situ* or *ex situ* live techniques can be used; however *ex situ* live has poorer performances (table 2.1).

9. **Choose the technique.** Consider the different rankings for 1) for risk of failure, 2) for costs, 3) for opportunity of breed evolution/adaptation and of better knowledge of breed characteristics, 4) for efficiency of conservation aims 5) for efficiency of conservation of cultural and ecological values. The weighting of each of these factors will depend upon the amount of resources available, interests and priorities, strategy preferences.

2.6 Legal issues

The choice of *ex situ* techniques implies the creation of a genome bank and the discussion of some associated legal issues that, because they have been barely investigated until now, deserve some attention in this paragraph.

FAO recently pointed out the need to develop a legal framework for access to genome-banks (FAO, 1998). Here, it is underlined that a discussion should be opened with some urgency, considering that:

- indistinct present situations of *ex situ* collections of plant germplasm, built before the Convention on Biological Diversity (CBD) entered into force, should be avoided in farm animals as much as possible. Animal genome-banks are still at an early stage, which minimise possible difficulties due to pre-existing situations;
- the debates on related issues such as Intellectual Property Rights (IPRs), Farmers' Rights (FRs) and General Agreement on Trade and Tariffs (GATT) are ready to start.

2.6.1 State of the debates

- The CBD clearly states among its objectives (article 1) the "equitable sharing of the benefits arising out of the utilisation of genetic resources, including by appropriate access". It recognises "the sovereign rights of States over their natural resources" (article 15). It invites each Contracting Party "to create conditions to facilitate access to genetic resources by other Contracting Parties" (article 15). Within the CBD framework, access to genetic resources, including genome-banks, are subjected to national legislation, which should promote equitable sharing within and between countries.

- Farmers' Right (FRs) have been developed by FAO under the International Undertaking for Plant Genetic Resources (Resolution 5/89) and are defined as "rights arising from the past, present and future contribution of farmers in conserving, improving and making available plant genetic resources. These rights are vested in the International Community, as trustees for present and future generations of farmers, for the purposes of ensuring full benefits of farmers and supporting the continuation of their contributions".
- IPR is a framework of laws that provides monopoly rights for the products of human activity, agriculture and technology. It include patents, copyrights, Plant Breeders' Rights, trademarks, etc. The CBD discusses the protection of intellectual property rights in article 16 "Access to and Transfer of Technology". It recognised that patents and other IPRs may have influenced the implementation of the Convention and stated that Contracting Parties "shall co-operate in this regard subject to national legislation and international law in order to ensure that such rights are supportive of and not run counter its objectives".
- Patenting of biotechnological inventions in the European Union is subjected to the Directive 98/EC of the European Parliament and of the Council.

2.6.2 Developing a framework

The development of a legal framework in EU should consider, among others:

- the development of FRs for animal genetic resources in order to i) allow farmers to fully participate in the benefits derived from the improved use of AnGR, ii) assist farmers in the conservation of their breeds, iii) ensure that sufficient funds are available for these purposes;
- a full definition of intellectual and commercial properties that may arise from the use of stored material, both cells and DNA;
- the ownership, if any, of genome-banks, to which contributed farmers, governmental and private organisations, private companies, national and international funding agencies.

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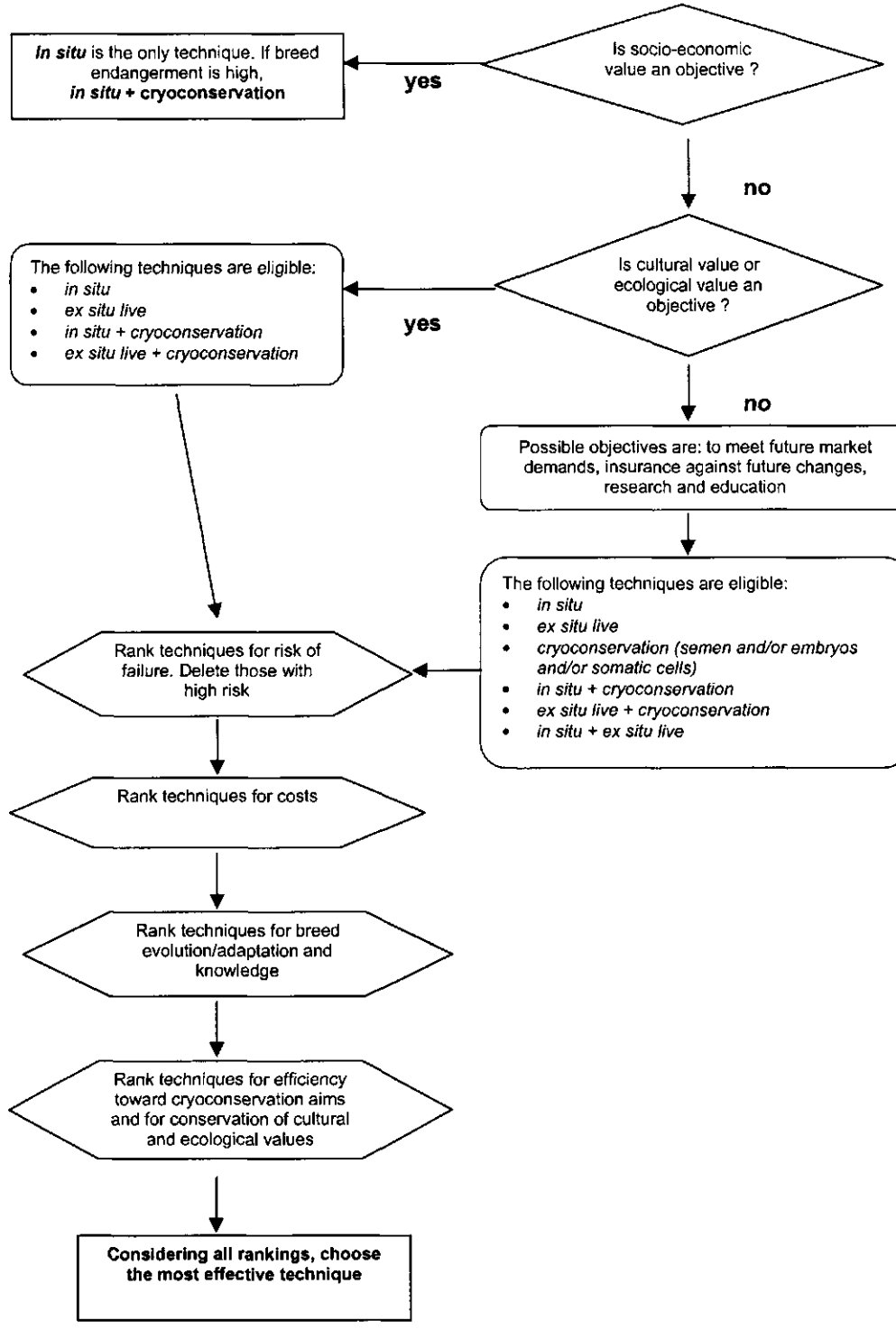
Figure 1. Choosing the technique



Chapter 3. Measuring genetic uniqueness in livestock

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- ***Introduction***
- ***What kind of genetic information should be used?***
- ***What is the basic theory behind genetic diversity?***
- ***How are genetic distances interpreted?***
- ***What are the practical considerations?***

3.1 Introduction

3.1.1 Uniqueness of breeds

In Europe there is a large interest in the uniqueness of local breeds (meaning the extent to which a breed is different to all others). To know the uniqueness of a breed one must study the diversity in a set of breeds. Over the last few years the number of studies into diversity and distances between European breeds has grown, with many large-scale projects under way. These projects include studies into cattle (Roslin, Scotland; combined Scandinavian effort with regard to Nordic breeds), pigs (INRA, France. A project that when finished should cover 50 different breeds), poultry, horses, sheep, goat and rabbits.
Because of the increased interest in this kind of project, it might be of interest to look more closely into the mechanisms underlying genetic distances and genetic diversity.

It should be stated here, that the uniqueness of breeds is just one criterion among others for prioritising breeds for conservation. For a further discussion on all relevant criteria we refer to chapter 4.

3.1.2 How can diversity be defined?

Diversity can be defined in a number of different ways on a number of different levels. Looking at nature, there is an enormous amount of diversity. This diversity we can observe and measure directly is *phenotypic*. On the other hand the largest part of diversity is hidden, because it is *genetic* diversity, embodied in the chromosomes of each cell of an animal. Modern technology used in genetics enables us to measure this type of variability.

3.1.3 Measuring diversity

Variability between populations (species, breeds) can be assessed using mathematical tools, which translate the differences to a measure of distance between a pair of populations. Given the distinction between phenotypic and genetic variability, it follows that distances can also be divided into phenotypic and genetic distances. Which of these tools is preferred, depends on the objectives of the user.

3.1.4 Phenotypic diversity

If the interest is in phenotypic diversity only, one should use phenotypic distance measures. An example of the possible use of phenotypic distances is prioritising of breeds for adaptation criteria. Great importance is placed on adaptation of breeds to their environment, since the trend is toward more sustainable agricultural systems and well-adapted animals will be an essential part of such systems (See also chapter 2 for details on breed comparisons).

It should be noted that measuring phenotypic distances does not necessarily give the same results as genetic distances, because they are basically different measures. Phenotype is determined by genotype and environment (and their interaction). In relation to measuring diversity van Hintum (1994) discusses the merits of particular phenotypic traits (to be precise: quantitative traits) as a means to assess genetic diversity. He suggests that distances based on quantitative traits are more indicative of adaptation to environmental factors. This is supported by a study by Burstin and Charcosset (1997). They found a triangular relationship between phenotypic and marker genetic distances: Low genetic distances are associated with low phenotypic distances, high genetic distances, however, are associated with a range of phenotypic distances, meaning that two populations which are genetically distant need not be phenotypically different. In fact, phenotypically they might resemble each other closely. In other words, two breeds can show the same phenotypic characteristics without being closely related genetically, which means that breeds can arrive at a similar phenotype along different genetic routes.

The results of van Hintum suggest that phenotypic distances could possibly be used as a quantitative measure for adaptation of breeds to particular environmental aspects. Phenotypic diversity may be seen as 'expressed genetic diversity', i.e. genetic diversity at the coding regions. Whereas the 'neutral genetic diversity' is measured at non-coding loci, like microsatellites or other genetic markers. The difference between 'expressed' and 'neutral' diversity becomes clearer if we consider the example of a large population that is split into two large subpopulations. Some generations of strong selection can make the expressed traits substantially different and thus creates a large 'expressed genetic' distance. However, because of the large population sizes and therefore small inbreeding (see 3.3), the 'neutral genetic distance' will be small. Hence, the population will be considered almost identical from a neutral genetic distance point of view, while they show substantially different traits.

Most literature concentrates on 'neutral genetic diversity', which will therefore be treated in detail in this chapter and will be shortly termed 'genetic diversity'. The field of phenotypic or expressed diversity is new. Some attempts to quantify this diversity have been made by principal component analysis (Morrison, 1967). However, much more research is needed in this area.

3.1.5 Genetic diversity

FAO defines diversity as genetic diversity in types of Animal Genetic Resources, *i.e.* breeds of a species (Henson, 1992; see also chapter 4). Another definition is on the level of genes, where as much alleles as possible should be conserved (Crossa et al. 1993; Smith, 1984). The prime reason for conservation efforts in the EU is the concern that without intervention whole species might lose the flexibility to deal with changing circumstances (market demands, production systems, etc.). Therefore, in the EU context the approach is towards traits, meaning the genetic variance in traits (known traits but also unknown traits of possible future use) should be conserved. This implies that the conservation effort will be focussed on the total genetic diversity of a species, without preferring certain traits to others. The latter point of view shifts the conservation effort from allelic diversity to diversity in genotypes. Since most traits dealing with adaptability, production and reproduction are polygenic (influenced by multiple genes) it is clear that it is unnecessary to conserve every known allele per locus (which is impossible in any case). For instance, a trait that is coded for by 10 diallelic genes will have a potential of 59,000 different genotypes. In a situation with three alleles per locus this number is approximately 60.5 million different genotypes. Furthermore, calculations

show that rare alleles don't contribute substantially to the genetic variance of a trait (Falconer and Mackay, 1996). For polygenic traits, diversity is in the genotypes and not in the diversity of alleles.

In light of the developments in the EU as described above (and in the first paragraph) we have chosen to concentrate on describing the tools (genetic and mathematical) necessary for the conservation of the total of genetic diversity, without emphasising certain (groups of) traits.

3.2 What kind of genetic information should be used?

The most widely used genetic distances use differences in frequencies of alleles in different populations. In principle any gene that shows polymorphisms, *i.e.* has 2 or more alleles, can be used. In the past a number of different types of markers have been used to designate differences in those frequencies. Those are:

- Biochemical markers
- Bloodtypes
- Allozymes
- Genetic markers
- Random Amplified Polymorphic DNA (RAPD)
- Restricted/Amplified Fragment Length Polymorphisms (RFLP and AFLP)
- Microsatellites

The field of molecular genetics is still in a stage of rapid development, with many new, promising techniques under way. The development of DNA-chips is an example (see Box 3.2).

Biochemical marker polymorphisms are based on differences in protein molecules. These differences occur because the genes coding for those proteins show polymorphism. Biochemical markers are therefore indirect indicators of genetic differences. However, differences in the amino acids making up the protein do not necessarily lead to detectable differences in proteins. A part of genetic diversity will therefore stay hidden.
Polymorphism of marker loci is of importance when studying the genetic relations within and between breeds (Bretting and Widerlechner, 1995). Genetic diversity is mainly a function of genetic relationships between animals. To correctly assess these relationships on the basis of

Box 3.1 Literature

All these types of genetic markers have been used in the many studies on genetic diversity. For instance, the use of microsatellites has been studies, amongst many others, by Moazami-Goudarzy et al. (1997) in European cattle breeds and by Estoup et al. (1995) in populations of honey bee.

Bretting and Widerlechner (1995) give a very useful review on the types of markers available and their utility.

marker data, ideally one would want to have a marker gene with 2N alleles divided evenly over N founders. After a number of generations the allelic frequencies for that gene would reflect the contribution of each founder to the group of animals under investigation. Although this will not be encountered in real life, using markers that exhibit the highest degree of polymorphism can best approximate this situation.

This is the reason why functional genes (i.e. genes that code for functional proteins as opposed to noncoding loci like microsatellites) and biochemical markers are less favourable for estimating genetic distances. Both functional genes and biochemical markers have roles to fulfil in the physiology of an animal. A major mutation in a functional gene or gene coding for the biochemical marker will in general have a deleterious effect.

Box 3.2 DNA-chips

It is estimated that every 100-300 nucleotides in the genome are polymorphic. There are now methods under development to identify such single nucleotide polymorphism (SNP). DNA chips are a novel and still developing technology that allows identification of every nucleotide in a studied DNA sequence in a single operation. A set of oligonucleotides is set on to a solid glass or silica support so that there is an oligonucleotide for every possible sequence variation. The studied sequence carries a fluorescent dye and is allowed to hybridise with the set of oligonucleotides. It hybridises preferentially to the exactly matching sequence. When up to 100,000 oligonucleotides can be attached to a chip of 1.7 cm^2, a very large number of SNP sites can be quickly detected by automatic readers.

This opens new possibilities for the use of marker loci in diversity studies. It will be relatively easy to screen an individual for hundreds of loci, increasing the precision of estimates based on this data.

Such a mutation will probably become extinct after some time, leaving those mutations with more favourable effects. Expressed polymorphisms of functional genes or biochemical markers will never exhibit the level of polymorphism found in microsatellites (with the possible exception of genes located on the Major Histocompatibility Complex).
Furthermore, on the request of FAO, a panel of experts has constructed a set of microsatellite loci for each species to be used as the standard set to calculate genetic distances and genetic diversity. Similar activities have been undertaken by the EAAP on the request of the European Union.
For the reasons stated above, we will concentrate here on the use of microsatellites, although most principles outlined in this section will apply to other types of markers as well.

3.3 What is the basic theory behind genetic diversity?

Measuring genetic diversity between breeds is usually done by estimating genetic distance. These distances have a base in population genetics. Before we discuss these measures into detail it might therefore be useful to introduce some key terms used in population genetics theory (For more detailed discussion of the subject see: Malecot 1969, Falconer and Mackay, 1996).

3.3.1 Kinship and inbreeding.

Central notions in population genetic theory are *inbreeding* and *kinship*. Individuals are related if they share one or more ancestors. The level of relatedness between individuals can be expressed in the *coefficient of kinship*, or Θ (Malecot, 1948 and 1969). The coefficient of kinship is defined as the probability that two alleles, drawn from two individuals are both unmutated copies of the same allele from a common ancestor. *Inbreeding* means mating of related individuals, and can be quantified in the offspring of such a mating as the probability that two alleles, drawn from the same individual, are unmutated copies of one allele from a shared ancestor. Generally this probability is denoted as F and for an individual F will be equal to the kinship of its parents.

In population genetic theory there are four evolutionary forces, which cause genetic differences between populations: Random drift, selection, mutation and migration.

3.3.2 Random drift

Random drift means the random fluctuation of frequencies of alleles due to random sampling processes in a finite population. A gene or locus is said to be neutral if the different alleles of the gene do not give the individual a selective advantage over others. Microsatellites are genes that generally reveal a large number of different alleles that are selectively neutral. If we consider two populations stemming from the same ancestral population the extent of the divergence in allele frequencies for such selectively neutral loci is a function of the level of isolation of one population from another.

Within populations

Given enough time, say t generations, every allele of every locus in a finite population will drift to a frequency of either 1 (fixation) or 0 (loss). After these t generations the population is completely inbred. This probability will be larger as the relationship between the parents of this offspring will be larger. In random breeding populations with equal numbers of males and females there exists a direct relationship between inbreeding, the number of generations (t) and the population size (N), which is:

$$F_t = 1 - \left(1 - \frac{1}{2N}\right)^t$$

The probability of an allele neither being lost nor fixated in a population is approximately proportional to 1-F (Falconer and Mackay, 1996). From this it follows that an increase in F will increase the number of loci that have been fixated.

The calculation of F is dependent on which generation we define as the first generation. Starting from different generations we obtain different results. That is why population geneticists are often more interested in the rate of increase of F per generation (ΔF). The relation between the size of a random breeding population with equal numbers of males and females and ΔF is:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}} = \frac{1}{2N}$$

Effective population size

However, such an idealised population of equal numbers of males and females breeding in separate generations, is a special case, which will not be readily encountered in real life. For more general cases, expressions exist to relate the size of a real population to the size of this idealised population in such a way that parameters (like ΔF) in the idealised population are those observed in the real population (*cf.* Lynch and Walsh, 1998; Falconer and Mackay, 1996). This size is called the *effective population size*, referred to as N_e. Inbreeding coefficients of more complicated populations can be calculated from the former expressions by substituting N_e for N. For a random breeding population with unequal numbers of males and females the relation between actual and effective population size is:

$$\frac{1}{N_e} = \frac{1}{4}\left(\frac{1}{N_{males}} + \frac{1}{N_{females}}\right)$$

From this expression we can see that effective population size is largely determined by the sex with the least number of breeding individuals. When there is large inequality in the numbers of both sexes, the effective population size will be significantly smaller than the actual population size.

Looking at the effective population size over a number of t (separate) generations we arrive at an analogous expression:

$$\frac{1}{N_e} = \frac{1}{t}\left(\frac{1}{N_{e,1}} + \frac{1}{N_{e,2}} + \frac{1}{N_{e,3}} + \ldots + \frac{1}{N_{e,t}}\right)$$

One generation where the effective size has been reduced will have a relatively large downward effect on the effective population size over generations. When we follow a population over 10 generations, during 9 of which it had an effective size of 100 and one *bottleneck* generation, where the effective size was 25, the resulting effective size is 77. A population, which has gone through a bottleneck, will experience a marked increase in the amount of inbreeding and as a result an increased amount of fixation of alleles (and loss of other alleles at the same locus).

Recapitulating: The state of a population can be expressed in terms of effective population size, which in turn determines (the rate of) inbreeding and the amount of fixation occurring in the population.

Between populations

Between populations, it is likely that random drift will cause loss of different alleles. Furthermore, random drift causes differences in allele frequencies between populations for alleles that are not lost. This means that the effects of drift within and between populations are in opposite direction. Within populations genetic diversity will be lost. The genetic diversity between populations, however, will increase as a result of these effects.
This can be illustrated by the following expression (Wright, 1969):

$$(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$$

where F_{IT} is the inbreeding coefficient of an individual relative to the whole set of populations (We assume that different populations can be thought of as sub-populations of one population). F_{IS} is the inbreeding coefficient of an individual relative to its sub-populations. F_{ST} is the inbreeding coefficient of the subpopulation relative to the entire population. If F_{IT} stays constant, an increase of F_{IS} will be compensated for by a decrease in F_{ST}.

Apart from random drift, described above, there are three other evolutionary forces that, to a greater or lesser extent, influence genetic differences between populations. These three are: selection, mutation and migration.

3.3.3 Other evolutionary forces

If *selection* occurs, meaning that certain, non-neutral alleles give the carrier a selective advantage, divergence between two populations does not necessarily reflect the extent of isolation, because both populations might have been bred in the same direction, favouring the same alleles. Generally, selectively neutral genes are preferred as a tool to study divergence.

In general, *mutation* is a force that increases the genetic differentiation between populations. This means mutation is a force, which can create genetic diversity. However, because

mutation occurs with a low frequency, the influence of this factor is only measurable over a large number of generations.

Migration (individuals moving from one population to another) is a homogenising force: It lessens the genetic differences that exist between populations. Migration is a well-studied phenomenon, with many models proposed to quantify and clarify its effect in populations.

3.4 How are genetic distances interpreted?

In classic population genetics theory a population (or breed) may be defined in terms of the frequencies of alleles segregating in that population. We will therefore concentrate on a class of genetic distances that use allele frequencies as a basis of information. Genetic distances have mathematical properties and biological significance.

Mathematically speaking a function must have some properties to be a distance. First, the distance between a population X and itself must be zero, or: $d(X,X)=0$. Second, the distance between two population X and Y must be symmetrical, or $d(X,Y)=d(Y,X)$. If a distance satisfies these conditions it is called a semi-metric distance. If a distance also satisfies the triangular inequality, $d(X,Y)\leq [d(X,Z)+d(Y,Z)]$, it is called a metric distance (Katz, 1986. See Box 3.3).

In biological terms, genetic distances must have other properties to be meaningful. In fact genetic diversity results from a particular historic development of the population(s) considered. Therefore, the biological interpretation of genetic distances depends largely on the divergence model used.
As mentioned before, in population genetics there are four forces: 1) Random drift, 2) mutation, 3) selection and 4) migration. Since the models used to study divergence between two populations descending from one ancestral population were originally designed with species in mind, these models assume independent evolution of each population. After speciation (the moment when two populations become two distinct species) there is by definition no migration between populations: In the models, migration is ignored (This assumption is too strong where breeds are concerned. However, if migration should occur, it will be reflected in the distance). Since we assume the use of selectively neutral

microsatellites, selection is assumed not to affect changes in allele frequencies of these markers. In short, the observed genetic diversity is determined by two parameters: Random drift and, over long periods of time, mutation. The classic model of random drift and mutation was primarily designed for the study of relationships between species. This means that the time period under study is long by definition (thousands of generations). When studying breeds we are dealing with much shorter time periods (most of the divergence in European breeds occurred about 200 years ago) so that the evolutionary effect of mutation can arguably be ignored.

3.4.1 Classical mutation-drift model

For a good understanding what genetic distances mean under the classical random drift/mutation model, it is necessary to describe the assumptions generally used. The first assumption is that populations are assumed to be in equilibrium with regard to random drift and mutation. Therefore, after a large number of generations the inbreeding coefficient F reaches a steady state, given by the expression:

$$F_{\infty} = \frac{1}{1+4N\mu}$$

Where N is the population size and μ is the mutation rate, expressed as the number of mutations per individual per locus per generation. This means that divergence between populations mainly depends on mutation events occurring over a large number of generations.

Historically, the first studies dealing with genetic diversity used biochemical markers (like bloodtypes or allozymes), that have a low mutation rate. Therefore, these types of studies were constrained to populations, which had been separated from each other for a long time (*i.e.* species). In this case the most widely used measure is Nei's standard genetic distance D (Nei, 1972). This distance has an expected value linear with divergence time. Trees constructed with this genetic distance were expected to draw a reliable phylogeny of species. With microsatellite loci and their particular mutation model (high mutation rate), D looses its linearity with time. Goldstein and others (like Shriver) have sought to remedy this situation. Their goal was to use the high mutation rate of microsatellites to compare very closely related populations with new types of genetic distance, named $(\delta_{\mu})^2$, Average Squared Difference

(ASD) and D_{sw} which restore the linearity with time (Goldstein et al., 1995a; Goldstein et al., 1995b and Shriver et al., 1995, resp.).

However, Takezaki and Nei (1996) showed by simulation that the chord distances (D_C) constructed by Cavalli-Sforza (1967) and D_A (Nei, 1987), which are not linear with divergence time when using microsatellites give better results in terms of topology of the phylogenetic or distance trees (see 3.5.2) drawn than Goldsteins $(\delta_\mu)^2$ or Shrivers distance (D_{sw}). This may be explained by the fact that the former distances have smaller variances than the latter. Apparently sacrificing linearity is preferable to the decrease in precision using linear genetic distances (On the other hand, D and $(\delta_\mu)^2$ give a better estimation of branch length).

This classic model should be used if time since divergence is large (for instance, between distant breeds or species) when mutation has an effect that can not be ignored.

Box 3.3 Mathematical properties of genetic distances

The natural distance between two vectors, X and Y, in a k-dimensional space is the Euclidean distance:

$$d(X,Y)=\sqrt{\sum_{i=1}^{k}(x_i-y_i)^2}$$

It's easy to show that this distance satisfies the three mathematical properties mentioned in the text. Therefore, Rogers's (1972) genetic distance,

$$D_{Rogers}=\sqrt{\tfrac{1}{2}\sum_{i=1}^{k}(x_i-y_i)^2}$$

which is derived from the Euclidean distance, satisfies these properties, despite the factor $\sqrt{\frac{1}{2}}$.

Note that the squared Euclidean distance $d^2(X,Y)=\sum_{i=1}^{k}(x_i-y_i)^2$ satisfies the two first conditions stated in the text but doesn't satisfy the third (triangular inequality). Therefore, distances derived from the squared Euclidean distance (Nei's minimum distance, Reynolds distance, etc., see Box 3.5) do not satisfy the triangular inequality. Neither does Nei's standard genetic distance. For a further discussion on mathematical properties of genetic distances see Nei et al. (1983) and Katz (1986).

3.4.2 Pure drift model

During short times since divergence (for instance between breeds in Europe) the amount of mutations appearing will be negligible. When we want to compare closely related populations the main factor to describe genetic variability is random drift.

Under the pure drift model, contrary to the former model, the inbreeding coefficient F will not reach an equilibrium value, but its dynamics is given by the expression given earlier:

$$F_t = 1 - \left(1 - \frac{1}{2N}\right)^t$$

In this model the expectation of the usual genetic distances (like Nei's standard distance) is a function of $(F_1 + F_2)$, the coefficient of inbreeding in population 1 and 2, respectively. This means that this type of genetic distance actually measures inbreeding. For example, when we look at Nei's minimum distance (Nei 1973), the expectation is:

$$E(D_m) = E\left(\tfrac{1}{2}\sum_i (x_i - y_i)^2\right) = \tfrac{1}{2}(F_1 + F_2)\left(1 - \sum_i p_{0i}^2\right)$$

where p_{0i} is the frequency of the i-th allele in the founder population. The most important aspect of this distance is the expression (F_1+F_2). The preferred distance then, should be a distance, which has an expected value equal to (F_1+F_2), without being clouded by 'left-over' terms. Reynolds (1983) introduced a measure of genetic distance which is Nei's minimum distance normalised by an estimation of heterozygosity in the founder population $(1-\Sigma_i[x_i y_i])$, effectively removing this part of the former equation. The expected value of Reynolds distance is therefore equal to $(F_1+F_2)/2$ (For more information about expression of genetic distances see Box 3.5).

A cautionary remark: Under the assumption of a pure random drift model, distance estimates do not reflect the exact phylogeny of the populations under study (the distance is now influenced by t and population size, rather than t only). However, since the main interest will be for conservation value of breeds rather than the exact phylogeny (which can not be known

in any case), this type of distance should be used for relatively closely related populations, like breeds in Europe.

Thusfar, a method was outlined that measures directly neutral genetic variation. Implicitly is assumed that conservation of neutral diversity also conserves non-neutral variation, because ultimately genetic distances use the notion of alleles *identical by descent* and therefore measure kinship. In principle, kinship is a parameter valid for the entire genome. Therefore the assumption of conservation by association of non-neutral variation through conservation of neutral variation seems reasonable. This implies a very general approach towards conservation. All of genetic variability, known and unknown is assessed and conserved.

Box 3.4 F_{ST} as a genetic distance

Wright's F_{ST} statistics (Wright, 1969), the inbreeding within a subpopulation relative to the whole population, is a popular measure of genetic diversity in animal breeding. In this approach breeds are considered to be subpopulations of a large population comprising all breeds under study. F can be expressed in terms of heterozygosity through (Nagylaki, 1998):

$$F = 1 - H = 1 - \sum_{i \neq j} p_i p_j$$

where p_x denotes the frequency for the x-th allele of a locus in the population under study.

If finite subpopulations are isolated from each other, each will separately experience inbreeding, with fixation of alleles. Which alleles are fixated will differ between populations. Therefore progressing inbreeding means increasing diversity between breeds. Calculating F_{ST} for a set of populations can be done through:

$$H_{ST} = 1 - F_{ST} = \frac{\overline{H}_S}{H_T}$$

where the bar above H_S denotes that this is the average expected heterozygosity in the subpopulations. H_T denotes the expected heterozygosity in the total population calculated from frequencies in the total population (Nagylaki, 1998).

There are alternative ways to calculate F_{ST}. Weir and Cockerham (1984) and Robertson and Hill (1984) give different estimators of F_{ST}. The estimator of Robertson and Hill gives extra weight to rare alleles for conservational purposes. However, the variance on the estimator is greater and both estimators agree only when all alleles have equal frequencies).

Nagylaki argues that F_{ST} will only be an appropriate measure of divergence between populations if the genetic diversity is low to begin with. For example: If we have n subpopulations of equal size, which do not share alleles, the expression for F_{ST} becomes:

$$F_{ST} = \frac{(n-1)(1-H_S)}{n-(1-H_S)}$$

Except when the populations are fully inbred ($H_S = 0$) F_{ST} will always be smaller than 1, even though the populations are fully differentiated. Moreover, if we have K populations fixed for a locus with L(<K) alleles, average heterozygosity within populations will be 0, $F_{ST} = 1$. F_{ST} indicates complete differentiation between lines. However, L<K means complete differentiation is only possible for L populations (Examples from Nagylaki, 1998). F_{ST} is not to be recommended as a measure of genetic diversity.

Classical genetic distances can not account for migration explicitly (see text). It may be of interest to gain some insight into this aspect of population evolution. F_{ST} can be used for the calculation of migration rate between populations.

Under the assumption of equilibrium between genetic drift and migration, the inbreeding coefficient, when in steady state, takes a form very similar to the inbreeding coefficient in case of equilibrium between drift and mutation. The formula is:

$$F_{eq} = \frac{1}{1+4N_e m}$$

where m is the migration rate.

It is evident that an increase of migration rate causes a decrease of inbreeding coefficient. These two factors, mutation and migration, maintain genetic diversity within natural populations. But in terms of genetic diversity between populations the migration allows an exchange of genes: *gene flow*. This gene flow tends to homogenise the genetic constitution of the set of populations. Then, migration rate causes a decrease in the observed genetic diversity between population. Therefore genetic distance values are smaller than those in the case, where there is no migration. The expression given above also permits to estimate the parameter, $N_e m$ (effective size multiplied by migration rate). Generally programs which give an estimation of F_{ST} also give an estimation of $N_e m$.

Box 3.5 Some distance formulae

For notation convenience x_i and y_i are frequencies of the i^{th} allele respectively drawn in population X and Y. For simplification reasons, distance formulae are given for one locus. To extend those expressions for several loci, one has to sum over loci and divide by the number of loci where summations over alleles appear in the expressions.

Nei standard Genetic distance (D):

$$D = -\ln\left(\frac{\sum_i x_i y_i}{\sqrt{\sum_i x_i^2 \sum_i y_i^2}}\right)$$

Goldstein's distances:

$$(\delta\mu)^2 = (\mu_X - \mu_Y)^2$$

where μ_X ($=\Sigma_i i x_i$) and μ_Y ($=\Sigma_i i y_i$) are average allelic sizes in each population.

Average Squared Distance (ASD):

$$ASD = \sum_{i,i'} (i - i')^2 x_i y_{i'}$$

where i and i' are the sizes of the alleles of a microsatellite locus.

Shrivers distance (D_{SW}):

$$D_{SW} = W_{XY} - (W_X + W_Y)/2$$

where

$$W_X = \sum_{i \neq i'} |i - i'| x_i x_{i'}, \quad W_Y = \sum_{i \neq i'} |i - i'| y_i y_{i'} \text{ and } \quad W_{XY} = \sum_{i \neq i'} |i - i'| x_i y_{i'}$$

The chord distance of Cavalli-Sforza (D_c):

$$D_c = (2/\pi)\sqrt{2\left(1 - \sum_i \sqrt{x_i y_i}\right)}$$

Nei's distance (D_A):

$$D_A = 1 - \sum_i \sqrt{x_i y_i}$$

Nei's minimum distance (D_m):

$$D_m = \tfrac{1}{2}\sum_i (x_i - y_i)^2$$

Reynolds distance ($D_{Reynolds}$):

$$D_{Reynolds} = \tfrac{1}{2}\frac{\sum_i (x_i - y_i)^2}{1 - \sum_i x_i y_i}$$

In Table 3.1 advice is given on which genetic distance should be preferred in different situations.

Table 3.1 Advice on the choice of appropriate genetic distance measure.

	Divergence time (generations)		
	Short (breeds within Europe) (Random drift)	Intermediate (breeds world-wide) (Mutation and drift)	Long (species) (Mutation and drift)
D_c	-	+ topology	+ topology
D_A	-	+ topology	+ topology
D	-	+ branch length	+ branch length
$(\delta\mu)^2$	-	+ branch length	+ branch length
D_m	+/-	-	-
$D_{Reynold}$	+	-	-

3.5 What are the practical considerations?

In this section we will discuss some practical implications of the theory outlined above.

3.5.1 Sampling

Since estimating genetic distance is a statistical method, the sampling process is of importance. Sampling occurs hierarchically, in that first individuals are sampled. Then, loci are sampled within individuals. Before hand, the set of loci sampled should be decided on, and this set should be equal for all populations under investigation.

The individuals sampled should be randomly drawn, to reflect the actual composition of the population. Generally, N=25 sampled animals are taken to be a minimum requirement (FAO, 1998). With that 2N=50 drawings of alleles per locus are performed, which should give a reasonably reliable estimate of allele frequencies. In the case of very small populations it might be worthwhile to sample the whole population. In that way the true gene frequencies are known. Often we will have different sample sizes between populations. It is possible to correct estimates of genetic distance for unequal sample sizes (Nei, 1987).

The sampled loci should preferably be unlinked to one another. Here, unlinked is meant in the statistical sense, meaning that if loci are located on the same chromosome, they should preferably be at least 50 cM apart. Using linked loci introduces a covariance between loci, which enlarges the variance on the estimate of the genetic distance. However, more loci always add precision to the estimations of distances. As a rule of thumb one should choose microsatellites that are as evenly spaced along the genome as possible.

Furthermore, loci differ in their information content. Loci used in genetic distancing should be informative, meaning they should display sufficient polymorphism. As stated before, genetic distances are dependent on inbreeding. Inbreeding means individuals will posses two copies of an allele descending from the same ancestor: the alleles are *identical by descent*. However, alleles can also be indistinguishable from one another without descending from the same individual, in which case alleles are said to be *alike in state*. For a correct estimation of genetic distances it is important that the probability of two alleles being alike in state is minimised. This is achieved by using loci with as much polymorphism as possible. In the set of microsatellite markers proposed by FAO, the rule of thumb was adopted that loci should have at least 4 different alleles.
One of the prerequisites of markers used to estimate genetic distance is they should have simple, mendelian inheritance (Bretting and Widerlechner, 1995). Therefore the use of sex linked loci should be avoided or used with caution.

3.5.2 Models and reality

In practice there is exchange of genetic material between breeds. This can happen through migration and crossing of breeds. The models, which assume isolation and independent evolution of populations after divergence, are by definition valid for species. In the case of breeds, however, the true representation of relationships between breeds will look more like a network than like a simple binary tree. Moreover, in the case of hybrid breeds, where two populations were combined to give the hybrid, the development is contrary to what is assumed in the models, where one population acts as a founder of two daughter populations.

However, these aspects which diminish diversity between breeds will result in a diminished distance estimate. Closely related breeds, irrespective of the nature of their relation, will be recognised as such. For conservation purposes it is not necessary to pay extra attention to

Box 3.6 Using specific regions of the genome

It might be preferred to concentrate on regions of the genome associated with certain traits deemed important (now or in the future). The Major Histocompatibility Complex for instance, plays an important role in health and might therefore receive extra attention. Specialised distance estimates based on loci in this region could be employed. Caution should be taken, however, since this type of distance concentrates on a relatively small, closely linked group of loci, which causes the distance to lose correlation with the remaining genetic diversity.

A major consideration in using genetic distances is the alleged objectivity in ranking breeds for importance to conservation. We would like to stress that this is only true if one considers total genetic diversity. Choices of certain regions of the genome over others are by definition based on subjective considerations. For a further discussion on weighing this type of subjective considerations can be found in chapter 4.

aspects like migration separately. However, if one is interested in migration, F_{ST} can be used to calculate the rate of migration between populations.

3.5.3 Distance trees

Distance trees are graphical representations or mappings of the distance matrix (*i.e.* the matrix with distances between populations). Under a number of conditions, described in the previous section, this representation can be taken as the phylogeny. However, under European circumstances the trees are not phylogenetic, since differences in effective population size and migration between breeds have a distortive effect.

There are different methods of drawing distance matrix trees. Nei et al. (1983) discuss and compare the different methods. The most widely known methods are Neighbour-Joining (NJ) and UPGMA (Takezaki and Nei, 1996), which generally give good results. NJ is superior when different rates of evolution have to be assumed. Evolution rate is among others governed by the effective population size. Since it can be expected that this will differ from one population to the next, this should be accounted for, NJ is the preferred technique.

Bootstrapping is a statistical technique of resampling data on the loci (Weir, 1990; See Box 3.7). This gives the possibility to draw multiple trees and estimate reliabilities for the different

nodes in the tree and a level of confidence of that tree. Evidently, properties of the sampling methods used will have an effect on the confidence levels of the nodes.
A remark: Bootstrapping uses the available data in an efficient manner. It can not improve on the data. The quality of the bootstrap is therefore limited to the quality of the data used in the bootstrap.

Trees can be drawn with or without a root branch. However, since trees drawn for breeds of livestock are not phylogenetic, unrooted trees should be preferred. Use of the NJ method is advisable. Apart from the reason stated above, NJ also gives higher bootstrap values in most cases.

Box 3.7 Bootstrapping

Bootstrapping is a technique to gain information about the distribution of parameters in a limited dataset. It does this by creating 'new' observations, the bootstraps. When a dataset consists of n observations, a bootstrap is a sample of n random drawings, *with replacement*, from the original data. All observations in the original dataset have an equal chance of being drawn. The value of the new bootstrap is the average of the sampled parameter (Weir, 1990; Tivang *et al.*, 1994).

Typically, this process is repeated a large number of times (≥ 1000), to create a large number of 'new' data. These can be used to estimate averages and standard deviations of an estimator calculated with the data in question.

In the case of tree construction, bootstrapping can be done for allele frequency data of multiple loci. Bootstrap sampling is usually done over loci. The frequencies of the loci in the bootstrap sample are then used to calculate a new distance matrix after which this matrix is used to construct a tree. The topology (or layout) of the bootstrap tree is then evaluated for the nodes, or clusters (see Box 3.8) that appear in it. The bootstrap value of a particular node is the fraction of times this node (or cluster) is found in whole set of the 'bootstrapped' trees.

Box 3.8 Constructing trees

In the following we give an example of the construction of a tree using UPGMA. Although NJ on the whole gives better results, UPGMA illustrates this aspect of assessing diversity more clearly.

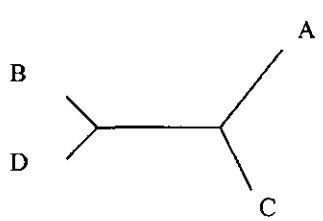
Suppose we have four breeds A, B, C and D. The distances between them are given below.

	B	C	D
A	.400	.300	.500
B		.200	.100
C			.300

We start with the pair of breeds that is closest to one another. The closest pair is (B, D). Therefore we next calculate the distances between the cluster (B,D) and A and C as the average of the distances of B and D. Example: (B, D), A = ½(.400 + .500)= .450

	(B,D)	C
A	.450	.300
(B,D)		.250

Again we find the smallest distance (between (B, D) and C) and recalculate the distance between this cluster and A (giving (B, D, C), A = .375). The resulting unrooted tree is drawn below. The branches are drawn in such a way the lengths of the branches between two breeds sum up to the distance given above.



3.5.4 How do we choose breeds using genetic distances?

Genetic distances and the tree construction methods described above give insight into the genetic uniqueness of breeds under investigation. Using another tree construction method, based on a diversity function suggested by Weitzman (1992), it is possible to assess the genetic diversity which will be lost when some breeds are excluded from the initial tree.

Thaon d'Arnoldi et al. (1998) give an example of this using the Weitzman function of diversity to construct a 'diversity tree'. Using only the distances between populations, Thaon d'Arnoldi et al. use branch length as an estimation of genetic diversity. However, Thaon d'Arnoldi et al. suggest including some measure of within breed diversity (N_e, expected survival time) to have a clearer indication of how much diversity can be preserved when certain breeds are excluded. An alternative to exclusion might be pooling of closely related breeds. Thaon d'Arnoldi et al. suggest this preserves diversity more efficiently, although it would mean disappearance of two breeds into a new synthetic. Objections to this suggestion might be formulated, in that this could mean loss of adaptability traits and other unique traits, through the loss of breed specific combinations of genotypes. Furthermore, if a breed possesses a high cultural value (see chapter 4), its loss will be deemed unacceptable.

Another way of maximising genetic diversity is calculating the optimal relative contributions of each breed to a genome bank. This method might lead to contributions smaller than required for breed survival. Constraints as to the minimal population size or number of contributions per breed should therefore be included. Alternatively, one could consider pooling related breeds to lift the contribution over the critical level. But here also the objections stated above apply.

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Chapter 4. Selecting breeds for conservation

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- ***Background to criteria for selecting breeds for conservation***
- ***Degree of endangerment***
- ***Adaptation to a specific environment***
- ***Traits of economic importance***
- ***Unique traits***
- ***Cultural or historical value***
- ***Genetic uniqueness of a breed***
- ***Species a breed belongs to***
- ***How can we weight the different criteria?***

4.1 Background to criteria for selecting breeds for conservation

Resources, both in terms of finances and manpower, are scarce in the area of breed conservation while the need is great. On a world-wide basis, it is estimated that about one third of mammal and poultry breeds are endangered, i.e. with ≤ 1000 breeding females or ≤ 20 breeding males (Scherf, 1995). In Europe, the combination of the development of herdbooks and the use of specifically defined breed standards led to a special period of subdivision of many domestic animal populations into large numbers of breeds, which lasted from the end of the 18th century to the first world war. The proportion of breeds lost this century in Europe is however, highest of all continents (Hall and Ruane, 1993) and over 40% are currently estimated to be endangered (Scherf, 1995).

In Europe, some countries have developed national strategies for management of animal genetic resources (Martyniuk and Planchenault, 1998). In addition, organisations such as the European Union (EU) and the Nordic Council of Ministers have already taken responsibility

for the development of funding programmes for co-ordinated activities. Apart from documentation and promotion of animal genetic resources, some of the available funding could be used for the establishment or support of conservation programmes directed towards specific breeds. This could include *in situ* or *ex situ* programmes for endangered breeds; supporting farmers willing to use low-input, low-output breeds in today's economic climate or supporting genetic improvement programmes and managing inbreeding for breeds not currently endangered, but which may become so in the near future. The question addressed here is which ones should be selected ? In a situation with several hundred endangered breeds, this question is very pertinent, as breeds given support today are more likely to survive into the future while those not chosen are more likely to become extinct.

In this chapter seven criteria (4.2-4.8), which might be used in the selection of breeds for conservation will be presented and the weighting of these seven criteria will then be discussed.

4.2 Degree of endangerment

From a purely conservationist point of view and because of the large uncertainty about the production environment of the future, the major goal may be to maintain and conserve as many breeds as possible. In addition, the number of endangered breeds is currently extremely high, so action on this front is especially important. Note that genetic variation is best conserved on the species level by the retention of separate pure breeding populations rather than the use of large populations without reference to breed (Hall and Bradley, 1995). This is primarily because the power of market forces can push large populations to select for a very narrow breeding goal while reproductive techniques (which may become far more efficient in the future) also make it possible for individual animals or families to have a major influence on the genetic make-up of the population. The narrow effective population size of the global Holstein dairy cattle population (e.g. Wickham and Banos, 1998) demonstrates that the use of a single, large population is not necessarily a good way of conserving genetic variation. The breed should thus be the key unit in conservation of animal genetic resources.

Note that the degree of endangerment of a breed does not depend only on its current population size. It also depends on a range of factors such as the rate of change of population

size, the degree or risk of crossing with other breeds, the degree of organisation of the farmers and the distribution pattern of animals over herds (EAAP Working Group on Animal Genetic Resources, 1998).

In the situation where we wish to conserve as many breeds as possible, the degree of endangerment is thus a key criterion. Based on demographic and population statistics, time to extinction or time to a pre-determined critical population size can be estimated for each breed (EAAP Working Group on Animal Genetic Resources, 1998). Breeds with large population sizes and in no obvious danger would obviously not be prioritised. At the other extreme, breeds that are almost extinct might not necessarily be selected for conservation because of the fact that the population size is too low and, in addition, large investments might be required to rescue them and bring the population size back up to safe levels. Resources might be better used to prevent other breeds coming to a similar end.

4.3 Adaptation to a specific environment

Through natural or artificial selection, breeds can genetically become adapted to specific environments and many such examples are known (see Box 4.1). Within continents or large countries, the number of breeds (expressed per million people) seems to be highest in remote and peripheral countries or states, which may indicate that adaptation to geographically isolated areas has been an important factor in breed development (Hall and Ruane, 1993).

Box 4.1 Adaptation

- The Kuri cattle's adaptation to the aquatic environment around the islands and shores of the Lake Chad Basin, and their inability to thrive in other environments, is one of the clearest examples of adaptation to a specific environment (Tawah et al., 1997).
- The feral Soay sheep is adapted to the very tough conditions of the St. Kilda archipelago, off the coast of Scotland, and is thought to have existed there since Neolithic times (Hall and Bradley, 1995).

Although difficult to express in purely economical terms, adaptation of breeds to specific environments is very important. Currently, indicators and criteria for the adaptation of a breed to its environment are being developed by FAO. There is an ever-increasing awareness of the importance of sustainable agricultural production systems, and well-adapted animals are central to these schemes.

The value of adaptation is acknowledged by the fact that when conserving vulnerable habitats as national or state parks, domestic breeds traditionally associated with the environment are in some cases also included in the programme (Henson, 1990). Breeds with adaptation to a specific environment should thus be prioritised for conservation, especially if the environment itself is of conservation interest.

4.4 Traits of economic importance

Breeds would be of special interest for conservation purposes if they had:
a) one or several traits of economic importance today (e.g. high fertility, efficient feed conversion, high quality products, disease resistance) and/or
b) one or several traits that will be of economic importance in the future.
While the first part is quite clear and could be documented, the second part is hard to quantify as it is obviously difficult to envisage what the production environment may be like in 20, 50 or, indeed, 100 years time.

However, we can predict that in the world as a whole there will be increased demand for animal products as a result of increased population growth and urbanisation. In developed countries the emphasis is likely to be increasingly on food quality and food production under less-intensive conditions (Upton, 1997), which could favour breeds of smaller population size that are less competitive today. In addition, we know that the world's environment is changing, with increasing CO_2 concentrations, increasing global temperatures and declining ground water levels (e.g. Brown, 1998). Our societies may also be radically different in the future as a result of changes in energy supplies, as it is predicted that a permanent decline in oil production is virtually certain to begin within the next 20 years (Hatfield, 1997). Factors such as these will affect the production environment in the future, although it is hard to say now which breeds might be favoured by such changes. The one thing that is sure, is that the future is uncertain.

Breeds with valuable traits of economic importance in today's production environment are obviously important. However, it is worthwhile remembering that traits of economic importance which provide a short-term profit today may be of little value in the future due to changing production circumstances and market requirements. Changes in the European dairy

cattle system over the last 30 years show this quite well where dual-purpose goals (meat and milk) that originally were favoured, were abandoned in favour of goals focusing only on milk production while the emphasis later moved to milk quality and secondary traits (Cunningham, 1992). Political decisions, such as the possible removal of subsidies in the EU, could also have rapid and dramatic consequences on the economic values of certain traits.

When considering this criterion, it is therefore important to assess whether the traits of current economic importance are likely to remain so in the future. Regarding traits of future importance, it is almost impossible to consider which breeds might be favoured for these hypothetical traits. However, this does not mean that they are not important. The best way to account for this uncertainty is to ensure that as many existing breeds as possible survive into the future.

4.5 Unique traits

Some breeds could be prioritised for conservation efforts because they have special behavioural, physiological or phenotypic traits, which distinguish them from all others of the same species. These may be due to single genes of large importance or polygenic effects. Some examples are given in Box 4.2.
Apart from their curiosity value or their potential economic or adaptive significance, the traits may also be of scientific interest, allowing us to increase our scientific knowledge in a variety of different areas, such as the genetic mechanisms behind human diseases or a range of physiological and adaptive characters. As with adaptation and traits of future economic importance, it is difficult to quantify the benefits of this scientific knowledge but again, this does not mean that they are negligible.

4.6 Cultural or historical value

In much the same way as paintings or buildings, breeds can be appreciated for their cultural or historical merits and can be of value regionally or nationally. As with works of art, the cultural importance of a breed lies in the eye/mind of the beholder. The cultural value of particular breeds for societies in developing countries has been discussed recently by Köhler-

Rollefson (1997). In European countries, farming was once the traditional working activity but now, as is typical for developed countries, the proportion of the population employed in agriculture is low. In some European countries then, the cultural or historical values of the breeds may be considerable as they represent a strong link to the past.

The cultural/historical value of a breed is another criterion that is hard to quantify but would obviously depend on how long the breed had existed in the region, the historical importance of its products, whether it was strongly linked with a particular tribe or people and how strongly the inhabitants of the region identified with the breed. Although it may generate income from tourism through its association with a given region (Gandini and Giacomelli, 1997), its value is generally abstract rather than purely economical. Provided that financial resources are available most countries are, in general, willing to support aspects of their

Box 4.2 Examples of unique traits

- The Meishan pig breed from China is extremely fertile and has thus been imported to Europe and North America for use in commercial stocks, as well as for study in a wide range of research projects to understand the genetic basis of fertility, and indeed, of other traits (e.g. Janss et al., 1997).
- Study of the unique musculature of the Belgian Blue cattle breed has led to an increased understanding of the genetic mechanisms behind muscular development in mammals (Grobet et al., 1997).
- The North Ronaldsay sheep breed is unique in that it feeds only on seaweed for large parts of the year and, in addition, has very efficient copper absorption and high salt tolerance (Ponzoni, 1997).
- The rare Gulf Coast Native sheep has high natural resistance to internal parasites, a characteristic which led to flocks of the breed being established at the Universities of Florida and Louisiana for research purposes (Henson, 1990).
- Many other breeds have documented resistance to specific diseases, such as the N'dama cattle which survive in areas infested by the tsetse fly, due to their high resistance to trypanosomiasis (the cattle equivalent to sleeping sickness, transmitted by the fly) and are consequently at the centre of a large research programme (FAO, 1992).

culture and history. In prioritising breeds for conservation, the relative weighting of this criterion is therefore likely to be higher for developed than developing countries.

4.7 Genetic uniqueness of a breed

Mankind has domesticated only a few species. Of these, only a handful again are of primary significance for food production in global terms. Almost all of the milk produced in the world comes from two species (cattle and buffaloes) while 90% of meat comes from pigs, poultry and cattle (FAO, 1996). Genetic variation *within* domesticated species is therefore especially important because of the reliance on a small number of species. Breeds can accumulate significant genetic differences over time through the processes of genetic isolation, genetic drift, selection and mutation.

Since many of the breeds in developed countries are only of recent origin (often within the last 200 years), their genetic histories (how they were established and if they were later crossed with animals of other breeds) are often well described in the literature. This information can be used to identify breeds that are likely to be genetically divergent from others of the same species. For example, the cattle and goat populations in Iceland are known to have arrived with the first settlers in the 9th-10th centuries and to have been effectively closed ever since (e.g. Sigurdsson 1995). Where documentation of genetic history is limited or non-existent, genetic uniqueness of breeds can be estimated using genetic distance studies based on neutral genetic loci, such as allozymes or microsatellites (see chapter 3 for more details).

Genetic uniqueness (taxonomic distinctiveness) is considered important when prioritising species in conservation biology, and it has been argued that it should also be used when prioritising domestic breeds within species for conservation purposes (e.g. Hall and Bradley, 1995). Genetic uniqueness is of value when prioritising breeds because those that are genetically divergent from each other are more likely to possess different alleles and gene combinations affecting a range of traits that may be important for adaptation, for future production environments and for scientific purposes. Conservation of breeds that are genetically different is therefore a good way of ensuring that these species will have sufficient genetic variation to adapt and to respond to selection in future generations. The limitations of using neutral loci to estimate genetic uniqueness for traits that have been under natural or artificial selection are discussed by Ponzoni (1997).

4.8 Species a breed belongs to

Only a few of the estimated 30-50 million species of the animal kingdom have been domesticated. Almost all are mammals, predominantly large and herbivorous, while the remainder are primarily birds together with a couple of insect species (Diamond, 1997). The different domesticated species carry out a multitude of purposes, providing mankind not only with food (meat, milk and eggs) but also fibre, leather, fuel, fertiliser, transport and draught power. Some species are of greater importance than others. Cattle supply 87 and 25 % of the world's supply of milk and meat respectively, buffaloes are responsible for 10% of all milk produced while pigs and poultry cover 40 and 25 % of global meat production (FAO, 1996).

While most of the 6 previous criteria might be used for prioritising breeds within species, some effort at prioritising particular species is also necessary since decisions on which breeds to conserve are normally made at the country or across-country (e.g. EU) level and not merely within species. Assigning a specific amount of resources to each species would entail that a greater number of conservation programmes could be established or supported for smaller, less-costly species, such as poultry or rabbits compared with larger mammals such as cattle or horses. For example, Ollivier and Renard (1995) showed that setting up a genebank would be much cheaper for rabbit than cattle, sheep or goat breeds. On the other hand, selecting an equal number of breeds per species for conservation would mean that the majority of resources would go towards the larger, more-costly species.

When FAO chose 12 populations for a major indigenous breed conservation programme (Cunningham, 1992), these included 5 cattle and 3 sheep breeds with only 1 population each from goats, buffaloes, pigs and camelids and none from poultry. This involved prioritising cattle and sheep at the expense of the other species, a viewpoint that might not be shared by all. Thus, when prioritising breeds it will always be necessary to use some subjective judgement regarding the importance of various species.

When prioritising species, one thing that must be considered is the present and estimated future value of each species for the country or region. Conservation efforts in other countries should also be considered and preferably decisions on what to conserve should be made in co-operation on a regional (e.g. EU) or global level. This ideally should lead to conservation efforts being concentrated not only on related breeds of one species but on breeds from many

species. Another point worth considering is whether species, not important for food production, such as cats and dogs should be included, because they can be of interest for cultural/historical reasons or for the fact that they may have unique traits (of potential scientific value). For this reason in Norway, following the establishment of genebanks for cattle, sheep and goats in the 1980's, a programme to set up a genebank for the 7 national dog breeds, the majority of which are threatened, was initiated in 1992.

4.9 How can we weight the different criteria?

The seven criteria just outlined can be used to prioritise breeds for conservation. They cover a wide range of different aspects of breed characterisation and breed value. A given breed could be of interest for conservation purposes due to one or more of these criteria. Bodies providing funding for conservation of specific breeds must in some way, whether consciously or subconsciously, weight them, while keeping in mind the different conservation objectives outlined in chapter 1.

Firstly, some decisions regarding which species are of interest and their relative importance must be made, so that an initial framework for decision-making is in place. Then, it is probably fair to say that the degree of endangerment is the single most important criterion for breed prioritisation. This criterion may even be used across species so that a country or region could try to maximise breed survival, regardless of species. By ensuring that as many different breeds as possible survive into the future, one would also be indirectly weighting traits, as yet unknown, that might be of future economic importance. Screening of breeds based on the degree of endangerment should thus be the first step. It would allow us to identify a group of breeds, which would include those currently with low population sizes, as well as those which, although current sizes may not be extremely low, might soon be endangered.

Then, in the second step, the remaining five criteria - adaptation, traits of economic importance, presence of unique traits, cultural/historical value and genetic uniqueness - should all be considered for selecting breeds within the group identified in the first step. It is difficult to say exactly how they should be weighted and some common sense would be required. Adaptation to an environment which itself is an ecological system of special interest would

make a breed even more important. Traits which are currently of economic importance due to temporary market conditions should not be considered of priority. Some attempt might be made to rank the relative importance of the unique traits - for example, which ones are likely to have the greatest practical importance for mankind ? In a similar fashion, the relative importance of the cultural/historical values of different breeds might also be compared. Estimates of genetic differences between breeds would only be of value if they also reflected breed differences for important traits that might previously have been under selection, such as fitness or adaptation.

All of these five criteria are important. One method which could be recommended for weighting them is the use of 'independent selection levels' i.e. breeds which score exceptionally highly even for only one criterion should be prioritised regardless of how they rank for the other criteria. Thus, a breed with a very high cultural/historical value would be preserved even if it had limited adaptation to a specific environment and did not score highly for the criteria of unique traits, traits of current or future economic importance or genetic uniqueness. Similarly, a breed with highly unique traits should be prioritised, regardless of how it ranked for the other four criteria.

In the above, it has been assumed that the choice of breeds for conservation has been made by national or across-country organisations that value and wish to take care of all aspects of breed characterisation and the different roles that breeds can satisfy for mankind. However, we should also consider what might happen if other bodies have the responsibility for choosing breeds. They might not include all five criteria, but might only choose those which reflect their motives or objectives for conservation (see chapter 1). For example, if breeding organisations were responsible for choosing breeds then they might only include criteria which would match their objectives for conservation i.e. to meet future market demands and to adapt to potential changes in production circumstances. The cultural and historical value of breeds would thus not be considered. If scientists were to choose the breeds, they might focus on opportunities for research, and again the historical/cultural value of breeds would not be included as a criterion. Some national governments if responsible for choosing breeds might only wish to conserve breeds for cultural/historical and ecological reasons. They might thus use adaptation to a specific environment and cultural/historical values to select breeds and ignore the other criteria.

Table 4.1. Five criteria used to select breeds for conservation and how they relate to the objectives for conservation (chapter 1).

Criteria for selection	**Objectives**					
	Future market demands	Changes in production circum-stances	Socio economical value	Opportunity for research	Cultural historical reasons	Ecological value
Adaptation	No	Yes	Yes	Yes	No	Yes
Traits of economic importance	Yes	Yes	Yes	Yes	No	No
Unique traits	Yes	Yes	Yes	Yes	No	No
Cultural historical value	No	No	Yes	No	Yes	Yes
Genetic uniqueness	Yes	Yes	No	Yes	No	No

Given the motives for conservation, the criteria used by the decision-makers for breed selection might thus differ. The way in which the five selection criteria might match the different objectives for conservation is outlined in Table 4.1.

Since the process of weighting the different criteria is complicated, as is obvious from the above discussion, it has frequently been suggested (e.g. Thaon d'Arnaldi et al., 1998) that genetic distancing should have a key role in breed selection since, in contrast to some other criteria, it can provide objective, unbiased information for separating breeds. However, objectivity can not be the sole determinant of the value of a criterion and the relative importance of the genetic uniqueness of breeds, as measured by genetic distance studies, must also be considered. This is especially valid since the correlation between genetic distances and genetic adaptation or uniqueness of traits is rather low (chapter 3). There is no way of avoiding the fact that some of the key criteria for breed prioritisation require a subjective evaluation and weighting. This is obviously difficult, but necessary.

At the time that decisions are being made concerning which breeds to select, concrete plans of action (for example, whether they involve direct support to farmers using rare breeds, *ex situ* programmes etc.) may also be proposed. In this case, the costs of the proposed plans, as well

as their likelihood of success, should also be considered as two additional criteria for breed selection. For example, if the decline in population size of a breed could be halted by some simple, inexpensive measures to support or establish a breeding programme, then this plan might be supported.

The complexity of the breed prioritisation process outlined in this chapter shows how important it is to have good information in order to make the proper decisions. However, data is often missing or documentation may be poor. In this case, some subjective judgements may be required to estimate some of the criteria or to gauge their importance. At the other extreme, it is important that the limited resources in the field of animal genetic resources should not only be used for detailed breed characterisation. If resources were only to be used for a single criterion, then it should be the degree of endangerment.

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Chapter 5. Establishing a conservation scheme

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5.1 Introduction

In the previous chapter we dealt with different criteria why a breed is included in a conservation program. Depending on the reasons why the breed was chosen and the resources available, we then can proceed in few alternative ways. It is very important to have as little compromising as possible in carrying forward the existing variability in the breed. This is mainly concerned with the size of the exercise, which could be adjusted by choosing individuals for conservation action from different families and by carrying out planned matings between the chosen animals. The variability in the conserved sample should be maximised. Therefore in selecting the individuals to be the founders themselves or to produce them, the target should be to minimise the overall kinship. This could be most efficiently achieved if the pedigree information is available in the population. If there are no pedigree records available, any other possible information, such as geographical or historical accounts, could be used to avoid redundant use of families, unnecessarily increasing average kinship.

A sound conservation scheme involves also setting up the basic infrastructure needed for recording (FAO, 1998), data analysis and responsible use of the individuals for breeding. If necessary, skilled people should be hired or educated to run such a program. The most

sustainable strategy for conservation is to utilise self-supporting, productive populations. In those cases it could be relevant to establish a well-functioning selection program for the breed. The genetic improvement can concentrate on maintaining or increasing the profitability of the production in those traits for which the breed still possesses a competitive edge.

We have seen in chapter 3 how modern molecular genetic methods provide us with very powerful tools to quantify the relationship between breeds and populations. Typing of individuals for a set of molecular genetic markers can be exploited in assessing the relationships between animals within the population. We are going to see that the molecular genetic information can be expressed in the same compact way as the pedigree records. The marker information could also be used for decision making, in filling the gaps in pedigree information or in differentiating family members or chosen chromosomal regions. The phenotypic information on individuals is the relevant criterion when the conservation is driven by the interest in specific heritable traits. The records on simple inheriting exterior traits could also be utilised in maximising the variability between individuals.

This chapter starts with assessing what kind of program is appropriate to arrive at a satisfactory result when different conservation purposes are considered. The next step is about appropriate schemes for such programs. The main alternatives are living populations in a production setting or kept in a more protected environment and cryo-preserved samples of gametes, embryos or somatic cells improve the sustainability of such operations. Because molecular genetics offers a whole range of methods to quantify the genetic variation, it is also important to store DNA from a sufficient number of individuals of the breed. At the end, we review the methodology that can be used in choosing the animals with the aim of maximising the variability in the initial set-up.

5.2 Requirements due to conservation purpose

The breed would survive on a self-supporting manner if it were used for profitable production. When the breeds are not in active production, the conservation programs can be very costly, especially when there are several breeds involved or where the maintenance costs are high, like in cattle. Therefore we have to find out the minimum requirements for the volume of the operation under different conservation purposes. Although the space

requirement in cryo-preservation is very small, the production of preserved samples can be very expensive and should be done in a cost-effective way with sound genetic potential in mind.

5.2.1 Re-establishment

The conservation scheme would always benefit from a well-designed cryo-preserved genebank to allow successful re-establishment of the breed in the future, even if the current interest is in individuals exhibiting a desirable breed specific trait. The option for a successful *re-establishment* sets the most demanding requirements for the scheme. The main cost factor is the size of population. The minimum population size is about 50 individuals, 25 of each sex. If the existing population is very large, only 1% of the genetic variation is lost by the starting generation when it is put through the bottleneck. Although this might in some cases mean more losses than those taking place in the original population, the compromise made in entering the breed in a conservation program is still very reasonable. It is also important that the individuals that form the basis of the conservation scheme are chosen so that the variability amongst them is maximised. The criteria to meet these refinements are discussed below. When the pedigree records are missing or scarce, more than 25 males and 25 females should be sampled if the resources allow it.

5.2.2 Selection program

It has been customary to consider live populations and cryo-preservation schemes as exclusive alternatives. We would like to encourage usage of different methods in a mutually supporting manner. The purpose where a large volume is required, is a scheme to support an active selection program. Here the cryo-preserved semen doses can be used as a reserve to guarantee the success of the breeding program even in the case of sudden fatal wide-spread disease or loosing elite breeding animals. Therefore, in a fast developing population, animals should be systematically sampled for cryo-preservation. Also when a breed that has an important socio-economic role in providing firm source of living in extreme (arid, cold or mountain) conditions is getting rare, it is important that a sufficient back-up system is quickly set up to prevent the breed from loosing a vital genetic variability. The breed may have an important role in maintaining local ecological and environmental characteristic. Although the agro-biodiversity function may have only local interest, a rare breed would require safely preserved sources to restore the variability.

5.2.3 Cultural value

In Europe, there are dozens of breeds that have fallen out of the most popular use in production. However, there are very attractive because of the cultural value attached to them. In many cases they symbolise the development of local agricultural heritage and are threatened to disappear due to lack of any wider interest in the country. In some cases such breeds have been revived with the promotion of breed specific products or tourism (chapter 2). The maintenance of such landrace populations requires sufficient backing via conservation schemes.

5.2.4 Desirable traits

There are breeds that are interesting only because they are exhibiting a desirable trait or bearing a gene with potential use. Although the breed is not competitive for production traits, it may carry valuable features such as disease resistance or distinctive product quality. If the variation in the trait were polygenically mediated, a larger sample would be required.

5.2.5 DNA samples

Molecular genetics provides us with very remarkable tools to analyse the variation between and within breeds. The within breed analysis would also include characterising the genetic variation in a trait or isolating a gene grossly affecting the variation. For such purposes, DNA samples are required. A sufficient number for such analyses would be obtained with a random sample of 25 males and females.

5.3 Requirements due to the conservation method

We have two different methods of conservation. Either we keep living populations or cryo-preserve samples for future needs. Whatever method is used, we have to consider the design and the attached costs. As was mentioned above, the methods could be used side by side where the frozen material is supporting the genetic resources of a selected or *ex situ* population.

5.3.1 Selection program

5.3.1.1 Animal identification system

The conservation scheme can be self-supporting when the breed has a recognised commercial value. The breed can maintain its competitive edge if there is a well-organised selection program built for improving the best traits. Therefore if there is no such scheme for the breed this should be quickly set up. This would involve decisions on strategy, logistics and funding.

A minimum requirement for any scheme where genetic variability is monitored is to have individuals tagged with unique identity codes. This is now a norm in many species within EU, but where it is not the case; a coding system should be designed. This would pave a way to a data bank where each animal appears along with its parents. The pedigree information is used in accomplishing the genetic comparison between parent candidates and in forming sound mating pairs between selected animals.

Next we have to choose the traits which are recorded. The traits should be simple and cheap to measure.

5.3.1.2 Recording scheme

The records on relevant traits are regularly collected and linked to the pedigree files. We can then quantify to what extent the differences between animals are due to genetics and how selection on one trait is affected the performance in the other one.

If the trait is poorly heritable, a large body of data on relatives is needed, before we can have a reliable way to pick up the genetically superior animals from the population. The importance of the traits and their genetic correlations would be assessed together to compute a weight for each trait in the overall selection policy.

5.3.1.3 Genetic ranking system

The animals are genetically ranked by adjusting the records for both production environment effects and the information from relatives. This could simultaneously carried out by BLUP (best linear unbiased prediction) systems for which there are several computing packages available. Similar kind of package programs can be used to quantify the variation in the trait and to compute the selection index weights.

5.3.1.4 Selection and mating plan

Until quite recently the animals were chosen to be parents for the next generation solely on their genetic superiority. When the population is small and selection intensity is very high, this leads to long-term increase in kinship among individuals. Some of the consequences are decrease of genetic variability, increased risk of achieving predicted progress and inbreeding depression. There are now methods developed (e.g. Meuwissen, 1997) which could be used along the genetic ranking systems that provide selection of mating pairs to arrive at sustainable selection rationales.

5.3.1.5 Cryo-preservation scheme

We can improve the efficiency of selection by resorting to developments in reproductive technology. Fewer males are needed and higher intensities of selection can be practised if artificial insemination is exploited. The best cows can have dozens of offspring more if embryos or oocytes are collected from them. Each selection program should be supported by a cryo-preservation scheme for a genome resource bank. The cheapest way to do this is to store semen or embryos.

5.3.1.6 Infrastructure

A breeding scheme involves setting up the basic infrastructure needed for recording, data analysis and genetically healthy use of the individuals for breeding. This would in many cases mean that skilled people should be hired or educated to run such a program

5.3.2 Living populations

In many cases the conservation scheme with a large actively producing population is the most cost-effective. Therefore, when the population is not in production, a smaller gene bank population should be established. When population is actively promoted for production purposes, it should have an organised breeding program to maintain its competitiveness amongst other commercial breeds. This would involve the setting up of a recording scheme, genetic evaluation system and a proper policy on the use elite animals either in artificial insemination or embryo transfer schemes. In initiating the breeding scheme, a large number of founder animals with maximised variability should be chosen. A genetically sound selection program would then involve monitoring the development of coancestry in the population.

When the interest in the breed has a fairly narrow basis, especially if the performance in any production traits is not profitable, a small live population is still needed. Such a population would guarantee that public would stay aware of its existence and research opportunities are kept available. Such a live genebank population with maximal retention of its genetic variability should be contemplated although the current interest in the breed is only on a specific trait or a gene. A living bank of live animals has to be based on genetically sound founders and should be supported by sufficient cryo-preserved material. The combined use of living and cryo-preserved material has to be guided by rationales that minimise genetic variability.

5.3.3 Cryo-preservation

When the population has little function in current production, in many cases the cheapest conservation method is cryo-preservation. Even in this case, however, a small living population should be considered as a norm so that the breed receives sufficient attention. Although frozen material does not require much space, its creation involves costly steps. Therefore also the cryobank should be properly designed. The best design is achieved by again maximising the variability. The individuals that are contributing to the preserved material are chosen so that the group coancestry is minimised.

Semen

Semen could be used in supporting the existing genebank populations or populations that are going to be re-established from cryo-preserved oocytes or have been re-established from embryos or somatic cells. Whatever the purpose is, the variability will be maximised if the average coancestry among males is minimised. The female population should also be taken into account and the semen producers can be obtained from matings where the whole population coancestry is considered.

A simple rule to constrain the increase in coancestry is a design where each male has a son and each female has a daughter. To meet this requirement in a cost-effective way can be accomplished when semen sexing is feasible with reasonable cost.

Semen and oocytes

The semen production is almost unlimited whereas only recently it has become possible to obtain reasonable number of oocytes. Oocytes can be collected from the ovaries of slaughtered animals or with less restriction about numbers by repeated aspirations of follicles from ovaries of living cows. The animals producing the gametes should be chosen to minimise the average coancestry. When both types of gametes can be cryo-preserved much more opportunities are left in re-establishing the population in the future by factorial mating in *in vitro* fertilisation. The usual practise is to mate each male with several females (hierarchical) but in terms of maintenance of genetic variability it is better to mate females with several males (factorial).

Embryos

The advantage of embryos compared semen is the possibility to store the entire genetic composition. The parents should be chosen to minimise the coancestry and factorial mating should be favoured when the embryos are produced. Factorial mating would be most cost-effectively done by *in vitro* fertilisation. Further savings in resources could be made if the embryos are sexed and subsequently appropriate numbers of male and female embryos are stored.

Somatic cells

Within the last two years embryo research has made long leaps in developing technology to produce undifferentiated cells from cells taken from adult individuals. When nuclei from such cells are transferred to enucleated eggs, the genetically identical individual could be reconstructed. Although the cytoplasm is that of the recipient egg, we have a very powerful technique to produce a copy of the genetic composition of individuals.

When the techniques become more feasible and consequently are not too expensive, all the animals in the breed could be sampled. At the moment the high production costs look very limiting and therefore minimisation of costs and maximisation of variability should be considered together.

The technology to produce undifferentiated somatic cells has been developed to improve the steps in gene transfer procedures. If that methodology advances as fast as promised, it could

mean that we soon will have unlimited sources of genetic variation available when targeted mutagenesis and gene transfer is extensively used.

DNA

So far we have talked about methods to restore the breed genetic composition as cheaply as possible. Because the assessment of breed diversity is a continuous process and more information is accrued along time either on techniques or on useful genome regions, we need enough DNA from each breed to carry out appropriate analyses. For that purpose, DNA from 25 randomly chosen individuals of each sex should be stored.

In conclusion, the best and least costly conservation method is to utilise the profitability of the animals in the breed and further improve the self-supporting elements by a well-designed selection program for the competitive traits. In breeds that are less profitable, a living population is politically very important. In establishing a live and cryobank, the following issues should be considered: the number of founders, with special attention to the number of living founders, against the cost of a conservation scheme and a maximum genetic variability of the genome resource bank.

5.4 Criteria to choose the founder animals

The first decision in setting up a conservation scheme is to decide about the founder animals that will contribute gametes either to the cryopreserved gene-bank or to the first generation of a live conservation scheme.

There is consensus that the initial sampling of animals should maintain the maximum genetic variability present in the breed. Genetic variability comprises allelic diversity (the number of alleles coexisting in the population at a given time), observed heterozygosity (the proportion of heterozygous individuals) and expected heterozygosity defined as the heterozygosity that would be expected under the Hardy-Weinberg equilibrium, $1- \Sigma p_i^2$, where p_i is the frequency of allele i at a given locus (Nei, 1973). The last one seems to be the criterion of choice in the conservation literature (Caballero and Toro, 1998) and therefore the one that will be considered here.

The way to calculate expected heterozygosity depends on the type of information available, as is explained below.

5.4.1 Pedigree information

If genealogical information on candidates is available the basic tool is the kinship coefficient that reflects the expected homozygosity by descent. Therefore, we should use as founders those individuals that minimise the average group kinship including reciprocals and self-kinship among individuals. Let us consider that S males and D females are available as potential donors of gametes and we also assume that kinship coefficients among all candidates are known. The problem will be to find the s sires and d dams that will be the actual donors such that the average kinship between them is minimal.

The average kinship will be calculated as:

$$k = \tfrac{1}{4}k_s + \tfrac{1}{2}k_{sd} + \tfrac{1}{4}k_d$$

with k_s, k_{sd} and k_d being the mean kinship between sires, between sires and dams and between dams.

The reason for recommending this criterion is that besides maximising expected heterozygosity by descent it will minimise the number of individuals with common ancestors and in simple cases it would result in the classical criterion of equalising family sizes (Caballero and Toro, 1998).

The situation is essentially the same as the one that arises when optimising selection response under restricted inbreeding (Toro and Pérez-Enciso, 1990; Brisbane and Gibson, 1995; Meuwissen, 1997).

The problem of finding the optimal way of producing a fixed total number of embryos will be solved in a similar way. First, we calculate the optimal number of embryos that each individual, either sire or dam should contribute and afterwards we organise matings in such way that will also minimise inbreeding of future embryos. The optimal solution would require to implement a factorial mating design that, obviously, will not be always technically feasible

and additional restrictions taken account of the reproductive limitations should be included in the optimisation problem. If only a semen bank is available it could be convenient to set up some matings beforehand in order to transfer the genetic variability present in the female population to new born males. On the other hand if somatic cells technology were available it would greatly increase the proportion of candidates that could be saved.

Box 5.1 Group kinship

If x_i denotes if individual y is selected ($x_i = 1$) or not ($x_i = 0$) the optimisation problem will be to minimise:

$$\sum \frac{x_i x_j k_{ij}}{s^2} + \sum \frac{x_i x_j k_{ij}}{sd} + \sum \frac{x_i x_j k_{ij}}{d^2}$$

subject to the restriction: $\Sigma x_i = s$ and $\Sigma x_i = d$

Gamete contributions of selected individuals could also be made unequal and the problem to solve will be the same although now s will be the number of total semen doses, d the total number of oocytes doses and x_i the number of doses contributed by individual i. The problem can be solved by integer programming techniques or by using an heuristic algorithm such as simulated annealing (Fernández and Toro, 1998).

A more complete description of the founder population can be done using the gene dropping technique (MacCluer et al., 1986). Two hypothetical alleles are assigned to each individual of the base population of the pedigree. Then the genotype of descendants is constructed working down the pedigree by simulating the mendelian segregation. The entire procedure is repeated many times and the information from the current population, either gamete donors or the founders of living population, are summarised over iterations. In that way a complete description of the joint distribution of the founder allele survival can be obtained and to infer the probability that a mendelian gene present in the base population at a given frequency will be represented in the foundation stock (Gandini et al., 1994). An additional information that can be obtained is the variance of kinship coefficients. This requires to simulate many loci (1000 or more) in each iteration reflecting the linkage pattern of the species (number and length of chromosomes).

Box 5.2 Relationship between pedigree and marker kinship

The Gudyerbas strain of Iberian pig is conserved as a closed population with complete pedigree records since 1945. For 10 animals of the actual population, a similarity matrix was calculated based on 63 AFLP polymorphic bands and it was compared with the coancestry coefficient matrix calculated from pedigree. The correlation coefficient between both values was 0.592 (Ovilo et al., 1998).

5.4.2 Marker information

When pedigree information is lacking, kinship can be estimated from molecular markers. If f_m is the marker kinship in a biallelic locus, defined as the probability that two genes taken at random are alike in state, and f is the coancestry by descent there will be related trough:

$$(1-f_m)=(1-f)(1-\sum p_i^2)$$

where p_i is the frequency of allele i.

Therefore the previous criterion of minimising coancestry could, in principle, be implemented. However, there are points that should be taken into account. First, estimation of kinship requires the use of codominant markers such as microsatellites. When the information came from dominant markers (RAPD, AFLP) a similarity matrix can be calculated that will also be correlated with the true coancestry (Lynch and Mulligan, 1994). Second, markers with alleles at intermediate gene frequencies will be the most informative ones. Third, the estimation error could be high and at least between 30-50 markers would be required. Fourth, sophisticated methods have been developed that properly weight information provided by different loci (Ritland, 1996). An alternative use of markers could be just to detect the most important relationships such as to detect the candidates that are full-sibs, half-sibs or parent-offspring. The marker requirements to distinguish between these types of relatives are considerably more easy to fulfil.

As the number of markers increases, molecular information will give a better knowledge of the true genetic diversity. However, in practise it will probably not be the case in most of the practical situations and therefore other considerations such as partial pedigree information or historical information should be taken into account.

5.4.3 Pedigree and marker information

When both pedigree and marker information exist, the optimal way to combine them would be to calculate kinship coefficient conditional on the marker information and then apply the criterion of minimum coancestry (Toro et al., 1998). However this calculation would be computationally very costly involving Monte Carlo Markov chain methods. In practise a simpler criterion could be preferable. This simple criterion would be to minimise kinship calculated from markers but within the classical framework of optimal within family selection (Wang, 1997): each sire contributes one son and among the r dams mated with each sire, one is selected to contribute a son and the remaining r-1 contribute one daughter each. In other words, marker information would be used mainly to select within families. Other criteria such as frequency-dependent selection can also be considered (Toro et al., 1998).

5.4.4 Other marker information

An interesting and special marker information can be obtained from paternally (Y-chromosome) or maternally (mitochondrial) inherited DNA. They provide information on male or female lineage. In the founder population of the conservation scheme the different haplotypes should be represented.

Box 5.3 Mitochondrial DNA polymorphism

Variation in mtDNA is extremely useful in studying genetic diversity. There are a number of reasons for this:

- mtDNA is maternally inherited with no recombination. Hence the number of nucleotide differences between the mitochondrial genomes is a direct reflection of the genetic distance that separates them,
- each cell has thousands of copies of mtDNA,
- regions of mtDNA mutate 5-10 times more than nuclear DNA.

The usual way to analyse mtDNA is sequencing of the D-loop that shows greater variation than the rest of the mtDNA molecule.

Y chromosome

In the same way as mtDNA could be used to identify maternal lineages in the populations, Y chromosome sequences provide similar information on paternal lineages.

5.4.5 Phenotypic information

Some additional phenotypic information could also be considered. In some breeds there is phenotypic variability for exterior traits (different colours or conformation types, presence and absence of horns, etc.) that could be interesting to assure its maintenance in the conservation scheme.

5.4.6 Other information

Sometimes there is also information on the herd structure of the breed. Geographical or historical information will permit to know if some herds have been maintained isolated. Furthermore, many traditional breeds are subdivided in local varieties that have been maintained isolated and show different morphological appearances. This information would be valuable when choosing the founders of a conservation scheme. For example, when the conservation program of Iberian pig was created in 1943, 4-5 sires and 17-24 dams were chosen from each one of four isolated herds (historical information) that represent local varieties (black hairless strains and red, golden or pied varieties). Obviously, in cases of breeds with a clear subpopulation structure some of the considerations made in chapter 3 and 4 would be applicable.

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Chapter 6. Operation of Conservation Schemes

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- ***Introduction***
- ***Live conservation schemes***
- ***Cryo-conservation schemes***
- ***Integrating live and cryo-conservation schemes***

6.1 Introduction

6.1.2 What issues are important?

The operational issues of conservation schemes depend on which kind of conservation plan was chosen in chapter 2 and 5. The main distinction is between pure live conservation schemes, pure cryo-conservation schemes, and a combination between live and cryo-conservation schemes. Within the live conservation schemes, one can distinguish *in situ* and *ex situ* live conservation, but this distinction is not very relevant for this chapter because the issues that are important for *in situ* schemes are also important to *ex situ* live schemes.

The issues that are important for live schemes are:

- the effective population size at which the breed is maintained,
- selection of animals within the breed,
- mating structure of the selected animals,
- the genetic improvement that needs to be achieved,
- monitoring of traits and pedigree.

These issues will be addressed in section 6.2. With respect to the issue of the selection of animals, note that some selection is possible, when sires produce more than one son and dams more than one daughter and the population is not increasing in size, but the selection may well be *at random* (instead of for a trait).

The most relevant operational issue for a cryo-conservation schemes is the replenishment, i.e., replacement, of retrievals from the genome bank, since retrievals will deplete the genetic materials in the genome bank. The operation of the genome bank is described in section 6.3.

With respect to the combination of live conservation schemes and cryo-conservation schemes there are two aims:

- A live conservation scheme is conducted while cryo-conservation serves as a back up in case the live population runs into genetic problems. If old 'back-up' genetic material is retained, the genome bank will keep track of the full history of the evolution of the population.
- Cryo-conservation can be actively used to increase the effective population size of a small live breed, and reduce genetic drift.

The former aim is an extension to a pure live conservation schemes, and can reduce risks substantially in these schemes. The latter aim implies a judicious use cryo-conservation, which will obtain special attention in this chapter.

The combination of live and cryo-conservation can result in very potent conservation strategies because:

- It can achieve all the objectives for conservation, namely future economic potential, present socio-economic value, cultural and historical value, agro-biodiversity conservation, risk reduction, research and education (see chapter 1).
- It can reduce the genetic drift substantially, and resembles in that respect a pure cryo-conservation scheme where genetic drift is very small.
- In the combination of an *in situ* live and cryo-conservation scheme the population will still evolve and adapt to the environmental circumstances.

In the combination of an *in situ* live and cryo-conservation scheme we have to find a balance between the latter two aspects: reducing genetic drift by using much old cryo-conserved stocks and promoting genetic adaptations by using little cryo-conserved stocks. How to find this balance will be described in section 6.4.

6.2 Live conservation schemes

6.2.1 The effective population size

From conservation biology theory, effective population sizes should exceed 500 animals, otherwise the accumulation of slightly deleterious mutations will deem the population to extinction (Lynch et al., 1995). Recently, however, there has arisen a controversy over the mutation rate that was assumed. Lynch et al. assumed a mutation rate of 0.5 mutations per genome per generation (mutation model A), while new estimation methods resulted in much smaller estimates of 0.03 mutations per genome per generation (Garcia-Dorado, et al., 1998) (mutation model B).

Also the mean selective advantages of mutations differed between the mutation models A and B and are estimated at 0.02 and 0.2, respectively, in *Drosophila*. Hence, under the model B, mutations are more rare and have larger effects than under model A. Note that deleterious mutations with large effects are less likely to drift to high frequencies in the population, because natural selection will prevent this. Hence, both these changes in the mutation model B result in much smaller effective population sizes that are needed to prevent a build up of mutational load. Further research is needed to assess the critical effective population size, but it will be much smaller than 500 animals.

Meuwissen and Woolliams (1994) balanced the drift of current deleterious mutations against natural selection, which purges deleterious mutants. Hence, they avoided the use of inaccurate estimates of mutation rates. Consequently, their results apply only in the evolutionary short term (say up to 20 generations), where the effects of a build up mutational load are still negligible. In practice, these results may be more relevant than the evolutionary long term results since 20 generations comprises 20 – 100 years for most species, and may be even longer in cryo-aided live conservation plan (see section 6.4). If at some point in the distant future a consider load of mutations happened to have accumulated, still an action plan can be devised to keep the population temporarily at a higher effective size in order to purge the deleterious mutants. When varying the assumptions of their model, the authors concluded that the critical effective size, i.e., the size below which the fitness of the population steadily decreases, is between 50 and 100 animals.

Although more research is needed on this subject we will assume here that the minimum effective size of a live population is 50 animals per generation, which yields a rate of

inbreeding of 1% per generation. Note however that the actual size of the population may have to be substantially larger than 50 because of unequal numbers of males and females or selection (see Table 6.1 for a comparison of actual to effective sizes).

Effective population sizes when generations overlap

Most livestock populations have overlapping generations, i.e., the parents of new born animals are not strictly of the same age such that some may be 2 and other 3 years old. We will assume here that drift, and thus effective size, is to be controlled per generation. The alternative is to control drift per year. The difference between controlling drift per generation or per year becomes clear when we consider a cryo-conservation scheme, where a population of 50 animals is re-established after 100 years of cryo-storage. In this scheme, the drift per year is small but that per generation is as large as usual for a population size of 50. Also, the cryo-conserved population did not achieve any genetic adaptation during the last 100 years. Hence, the minimisation of drift per generation can result in no genetic adaptation, whereas the minimisation of drift per generation allows for the fact that the population has to evolve. Because genetic adaptation is one of the main aims of an *in situ* live conservation plan, the genetic drift should be minimised per generation, i.e., the effective size should be maximised per generation. For *ex situ* live conservation plans also some genetic adaptation will be desirable in most cases and the same arguments holds.

Since the effective size per generation is relevant in populations with overlapping generations, also the numbers of sires and dams selected has to be expressed per generation. The number sires used per generation is the number of newly introduced sires per year times the average generation interval (averaged over sires and dams). Similarly, the number of dams used per generation can be calculated.

Monitoring of effective population size

When pedigree is recorded, the coefficient of inbreeding can be calculated for every animal (Falconer, 1989). Hence, the increase of the average inbreeding coefficient can be calculated per year. However, the increase of inbreeding is due to the increase of the average kinship in the population, which makes that the mating of completely unrelated is not possible anymore. Thus, the future average inbreeding is described by the current coefficient of kinship (see chapter 3). Hence, more up to date results are obtained, by calculating the average kinship across all pairs of animals as:

$$K_a = \frac{1}{4}K_a(m) + \frac{1}{4}K_a(f) + \frac{1}{2}K_a(mf),$$

where $K_a(m)$, $K_a(f)$, and $K_a(mf)$ are the average kinships between all pairs of males, females, and male-female pairs, respectively (excluding pairs of an animal with itself). Thus, the yearly increase of the average kinship coefficient can be calculated $\Delta K(yr)$. If further, the average age of the sires and dams at birth of their offspring is recorded, i.e., the generation interval (L) is recorded, we can calculate the increase of the inbreeding per generation as $\Delta K(gen) = L\ \Delta K(yr)$. The effective population size is now $Ne = 1/(2\Delta K(gen))$ animals per generation. It is important to monitor effective population sizes, because they can be smaller than expected due to any effect that increases the variance of the family size of the animals (e.g., selection, unequal survival rates). **Because of this and because of the following sections, it is very important to keep records of the pedigree in live population schemes, i.e., to store the sire and dam identification number of every animal in a data bank.**

Table 6.1 Numbers of Sires and Dams needed to achieve an effective population size of 50.[1]

Random selection		**Phenotypic selection**[2]		**Within family selection**[2]	
Sires	Dams	Sires	Dams	Sires	Dams
25	25	35	35	13	13
20	34	30	45	12	14
16	56	25	65	10	50
14	116	20	300	9	1000
smaller numbers of sires are not possible					

1 From FAO, 1998.

2 To be described later in the text.

6.2.2 The selection of animals

Which animals should serve as parents for the next generation?

6.2.2.1 Minimise genetic drift

Within family selection

Genetic drift may have to be minimised because we would like to maintain the genetics of population as close to that of the original population as possible, except for the traits that are under natural or artificial selection. Alternatively, population size may be small and thus drift

will be too large if we would not minimise it. The following selection process will minimise drift, where numbers of sires and dams are equal:

- Step 1: When a sire has to be replaced, select his replacement from his own sons, i.e. one of his sons replaces the sire.
- Step 2: Select the replacement of a dam from her own daughters, i.e. one of her daughters replaces the dam.

The above scheme yields strict within family selection, which keeps family sizes constant. The effective population size is twice the actual population size, i.e.

$$N_e = 2(N_s + N_d),$$

where N_e is effective population size, and N_s and N_d are the number of sires and dams, respectively, with $N_s = N_d$. If the number of sires is smaller than the number of dams, the minimisation of drift needs extra step (Wang, 1997):

- Step 3: Undo the selection of the replacement of a dam that had a son and a daughter selected under steps 1 and 2, respectively. Replace the latter dam with a daughter from another dam, which did not have a son selected under step 1.

The effective size under this selection rule is:

$$N_e = 16\, N_d^2\, N_s / (3N_d^2 - N_s\, N_d + 2\, N_s^2).$$

Random selection

In situations where the selection of sires and dams is less well controlled, random selection of sires and dams minimises drift. The effective population size is then (Wright, 1931):

$$N_e = 4\, N_s\, N_d / (N_s + N_d). \qquad [6.1]$$

When the numbers of sires and dams are equal, this reduces to the actual population size, i.e., under random selection and $N_s = N_d$:

$$N_e = N_s + N_d.$$

In cases where the use of bulls may be non-random due to their differences in breeding value, a simple rule may be to market only an equal number of doses of semen from each bull, to prevent preferential use of some bulls.

Prolonged generation intervals

Note that the above numbers of sires and dams are per generation, i.e., if the generation interval is 4 years, N_s (N_d) is the number of sires (dams) selected during 4 years. The generation interval is defined as the average age of the sires and dams at birth of their replacements / offspring. **Thus, prolonging the generation interval can be a very important method to increase N_s and N_d, and thus to increase effective population size and reduce genetic drift.** However, we also want to turn over generations in order to achieve natural and artificial selection response, and a judicious use of long generation intervals is recommended.

Minimum Kinship Selection

When the live population has gone through a recent severe bottle neck, the family structure can be very unbalanced and within family selection as described above does not minimise the genetic drift. In this case minimum kinship selection will minimise the genetic drift. With minimum kinship selection, a group of animals is selected that minimises:

$$K_a = \Sigma_i \Sigma_j c_i c_j K_{ij},$$

where Σ_i (Σ_j) denotes summation over all selection candidates; K_a is the average kinship of the selected animals; K_{ij} is coefficient of kinship between animals i and j; c_i is the contribution of animal i to the next generation, i.e., $c_i = ½ n_i / N$, n_i is number of offspring from animal i, and N is total number of offspring (the ½ is because a sire (dam) contributes only half of its genes to the offspring). The optimal contribution c_i, that minimises K_a is given in Box 6.1. When the family structure is balanced, minimum kinship selection will result in within family selection.

Box 6.1 Minimum Kinship Selection

It is useful to rewrite the minimum kinship selection problem, as described in the text, in matrix notation:

$$K_a = \mathbf{c'Kc},$$

Where **K** is (q*q) matrix of coefficients of kinships (q = number of selection candidates); and **c** is vector of contributions. It can be shown that K_a is minimised when the contributions are:

$$\mathbf{c} = \tfrac{1}{2}\,\mathbf{K}^{-1}\,\mathbf{Q}\,(\mathbf{Q'}\,\mathbf{K}^{-1}\,\mathbf{Q})^{-1}\,\mathbf{1},$$

where **1** is a column vector of ones; **Q** is a (q*2) incidence matrix of the sex of the candidates where the first column contains a one for male and a zero for female candidates, and the second column contains a one for female and a zero for male candidates. The contributions of the male and those of the female candidates will sum to ½.

In the case of a very small population, the genetic drift can be controlled at the DNA level by calculating the kinship conditional on genetic marker information (Toro et al., 1998). The markers are used to improve the coefficient of kinship between the animals. Improved in the sense that the kinship is assessed at the DNA level, whereas kinships that are calculated from the pedigree alone are average coefficients of kinship of the DNA segments. Optimisation of the contributions is again as described in the Box 6.1, where now the coefficients of kinship are based on marker information. The computation of the kinships conditional on marker information requires Monte Carlo Markov Chain methods and is very computer intensive. Alternatively, the kinships can be calculated without using the pedigree and the inaccuracy due to ignoring the pedigree can be compensated by applying the within family selection rules of Wang (1997). This high tech control of genetic drift may be useful in some situations where the size of the population has been reduced to very few animals (say a couple of males and 10 females).

6.2.2.2 Selection in small populations

The conserved live population may be well above the minimum effective size of 50 animals (see section 6.2.1) and some improvement of the genetic adaptations of the breed may be desired as described in chapter 2. In this case two selection methods will be suggested:

- Phenotypic selection, i.e., select for own performance records of the animals.
- Optimal contribution selection (Meuwissen, 1997; Grundy et al., 1998).

The first selection method is very easy to implement, whereas optimal contribution selection is a rather high tech method. Note that selection for BLUP (Best Linear Unbiased Prediction) – breeding value estimates is not mentioned in the above list, because it can severely reduce the effective population size below the actual size and thus increase genetic drift (without people being aware of it).

Phenotypic selection

Phenotypic selection is the simplest method of selection, but, at equal rates of inbreeding, it can outperform BLUP-selection. Hence, at equal rates of inbreeding, phenotypic selection is a quite competitive method of selection. It is very easily implemented when the traits can be measured at both sexes, for example, simply select the animals with the highest growth rate. If the animals are kept in different herds, which hampers a direct comparison of animals across herds, selection can be for the deviation of the animals from the herd mean (or herd-year-season mean).

When the trait is only recorded on one of the sexes, e.g., litter size, selection could be at random in the unrecorded sex. Alternatively, a number of female offspring could be obtained from the male selection candidates and selection could be for the phenotypic mean of the offspring of the males. The latter requires, however that the population is of a quite large size.

Often selection will be for more than one trait, i.e., several traits need to be improved. As before phenotypic selection will only be for the own performances of the animals, but the own performances have to be combined into a selection index such that the population mean will change into the right direction. This involves three steps:

1. Determine the optimal direction of the selection, i.e., picture the animal that is optimally adapted to its environment (and market niche). The picture of this optimal animal should not be too optimistic such that it can be reached within a reasonable time horizon.

2. Obtain a desired gains selection index to select the animals in the optimal direction using only own performance records (see Cameron, 1997). See Box 6.2 for a brief description of the desired gains index.
3. Calculate the selection response that will be achieved within the time horizon using Cameron (1997). If the selection response deviates substantially from the original goal of step 1, the goal of step 1 should be made more realistic and steps 2 and 3 repeated.

From step 2 a selection index can be calculated for every animal by weighing the own performances of the traits by the index weights. Selection proceeds as with single trait selection, but with the individual trait replaced by the selection index.

The above desired gains index avoids determining economic values for every trait. This seems useful, because the calculation of economic weights can be very complicated for traits in which local breeds often excel: fertility, disease resistance, longevity and quality of special products. Selection for over-simplified breeding goals can make the characteristics of the local breed equal to those of the introduced breed, which has usually been very intensely selected for a simple breeding goal. In situations where the calculation of economic weights is rather straightforward, the traditional optimal selection indices should be used, which are also described by Cameron (1997).

It should be kept in mind that phenotypic selection will reduce effective population sizes below those for random selection, i.e., the effective size will be smaller than calculated by equation [6.1]. The reduction in effect size increases with the heritability of the trait and the intensity of selection, but typically a reduction of 30% should be expected. Hence, when phenotypic selection is practised, the effective size calculated by Equation 6.1 should yield at least 70 and, more safely, 100 animals.

Optimal Contribution Selection

Optimal contribution selection maximises the genetic level of selected parents, while controlling the increase of the average kinship in the population. Note that the increase of the average kinship (= probability that 2 random alleles are identical by descent) equals the increase of the inbreeding (= probability that the 2 alleles of an individual are identical by descent) under random mating. Hence, optimal contribution selection maximises the genetic gain while controlling the rate of inbreeding.

Box 6.2 Desired Gains Selection Index

Let the x_i denote the change that is needed for trait i to move from the current population mean to the desired optimal population mean. Further let **x** denote a vector of these changes. The genetic variance-covariance matrix of the traits is denoted by **G**. It is assumed that all traits that are to be improved are also measured, otherwise the calculation of the index weights is more complicated. The optimal desired gains selection index weights can now be obtained from the vector:

$$\mathbf{b} = \mathbf{G}^{-1}\,\mathbf{x}.$$

The vector of selection responses of the traits over the time horizon, T, is:

$$\Delta\mathbf{G}_T = \frac{\mathbf{x}\, i\, T}{L\sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}}}$$

where: i is intensity of selection (see Falconer, 1989); L is the generation interval; and **P** is the phenotypic variance-covariance matrix of the traits.

We will describe optimal contribution selection in its simplest form. The genetic merit of the selected parents weighed by their contributions is:

$$G = \Sigma_i\ c_i\ EBV_i,$$

where Σ_i denotes summation over all selection candidates, EBV_i is the BLUP breeding value estimate; c_i is the contribution of the i-th selection candidate (as defined in section 6.2.2.1). Note that c_i is zero for non-selected candidates. The average kinship of the selected parents is:

$$K_a = \Sigma_i \Sigma_j\, c_i\, c_j\, K_{ij},$$

which should increase by no more than ΔF per generation, where ΔF is the desired rate of inbreeding. Thus, we restrict the average kinship to:

$$K_a = t\,\Delta F,$$

where t is the generation number.

Meuwissen (1997) described an algorithm that optimised the contribution of each candidate, c_i, (and thus the number of offspring that each candidate should get), such that the genetic merit of the parents, G, is maximised, and such that the average kinship does not exceed $t\,\Delta F$. In a simulation study, this selection method realised the desired levels of inbreeding and yielded about 30% more genetic improvement than selection for BLUP breeding value estimates (at the same rate of inbreeding).

If levels of inbreeding are high, we should account for the non-linear increase of the inbreeding and replace $t\Delta F$ by $1-(1-\Delta F)^t$, i.e., the desired level of inbreeding in generation t (see chapter 3), or alternatively, replace K_{ij} by $\frac{1}{2}A_{ij}^*$, where A_{ij}^* is the augmented relationship coefficient (Grundy et al.,1998). Optimal contribution selection has been extended to breeding schemes with overlapping generations by Meuwissen and Sonesson (1998) and Grundy et al. (1998).

Although it is more difficult to implement, optimal contribution selection is definitely favoured over selection for BLUP breeding value estimates. This is because optimal contribution selection actively controls the rate of inbreeding, whereas BLUP selection can result in rates of inbreeding that are much higher than expected based on the number of selected sires and dams.

6.2.3 Mating structures

Given the selected sires and dams, which sire should be mated to which dam?

6.3.2.1 General

Mating structures mainly affect the level of inbreeding of the offspring, not the rate at which the level of inbreeding increases over the generations. The latter is mainly determined by the selection of the animals and the population size (section 6.2.2). The inbreeding level is mainly important to avoid inbreeding depression.

Box 6.3 Random mating with exclusion of sib matings

Suppose the selection process of section 6.2.2 resulted in:

Optimal no of offspring from sire i = m_i

Optimal no of offspring from dam j = f_j

where the sum of the m_i (as well as is f_i), N = the total number of offspring.

And a (natural) mating between a sire and a dam yields n offspring. Then the number of matings needed with sire i is $M_i = m_i/n$, and with dam j is $F_j = f_j/n$, where some rounding[1] of these figures will be required to get the desired total number of offspring (N).

The following steps may than be used to assign the matings:

Step 1: Consider every sire in turn, denote the current sire by sire i.

Step 2: Sire i needs Mi matings: sample at random M_i dams for sire i. If a sampled dam j is a full or half sib of sire i, another dam is sampled instead of dam j, and this process is continued until none of the sampled dams are a full or half sib of sire i.

Step 3: Decrease the number of matings needed for the selected dams j with 1, i.e., F_j is decreased with 1. If F_j becomes zero for some dams, this dam is excluded from the set of dams from which the mates of the sires are selected in Step 2.

Step 4: Continue with the next sire i in Step 2 until all sires have been considered.

Sometimes, only full and half sibs of the sires will be left as dams eligible for sampling in Step 2. In this case we should re-start the entire sampling process. If after several attempts, we could not find a valid mating scheme, it seems that the number of sires and dams is too small, and we should allow half sib matings in Step 2.

[1] For example if $m_i/N = 4.459$, we need to round this figure to $M_i = 4$ or $M_i = 5$. The usual truncation point for rounding up or down is 0.5. But this truncation point can result in a sum of all M_i that is not equal to the total number of matings that we desire, i.e. not equal to N/n. However, by trial and error we can find a different truncation point for rounding that <u>does</u> result in a sum of the Mi that equals N/n. E.g., if the truncation point for rounding is 0.4, the above figure 4.459 is rounded to M_i=5. Note that the truncation point is always between 0 and 1, and a lower truncation point yields a larger total number of matings and *vice versa*. The same holds for the rounding of the F_j.

6.2.3.2 Non-sib matings

The most straightforward method to mate the individuals is assigning the matings at random, e.g., when a sire should get 10 offspring from 10 dams, the dams are sampled at random to the sire. The effective population sizes will be as described in section 6.2.2. However, some of the matings will be between close relatives, i.e., full-sib and half-sib matings. This should be avoided since the offspring from these matings will be highly inbred and will thus show much inbreeding depression. The latter can result in reduced fertility of the inbred offspring, which is undesirable because it reduces the scope for further selections. Box 6.3 shows a general sampling strategy for the matings, where sib matings are avoided.

6.2.3.3 Minimum inbreeding mating

Especially when population size is small and inbreeding levels are high, such that the depression due to inbreeding is high, it may be useful to minimise the average inbreeding level of the offspring. Note that minimum inbreeding matings per se, can result in large full sib families, because a sire reaches only with one dam the minimum degree of kinship, and thus the least inbred offspring. Minimum inbreeding matings should therefore be devised such that also the number of full sibs per family is as small as possible. Integer programming algorithms are useful to obtain the minimum inbreeding mating structure (Fernandez and Toro, 1998).

6.3 Cryo-conservation schemes

Users of cryo-conserved genetic stocks should replenish the genetic material as far as possible. To what extent genetic material can be replenished will depend on the kind of use of the genetic material:

- Embryos used for re-establishing the breed can be replenished by storing embryos from the re-established breed into the genome bank. Special care has to be taken with respect to the maintenance of genetic variation: minimum kinship or within family selection should be used to get the population that results from the thawed embryos out of the bottle neck, and the same selection should be used to select the embryos that replenish the cryo-bank.
- Semen used to re-establish the breed should also be replenished by the re-established breed. Again the maintenance of genetic variation is important. A problem is here that the stocks used for replenishing are not 100% pure bred, even after repeated back crossing

with the conserved breed. However, the genes of the replenished semen should come for more than 90% from the conserved breed. This would be obtained by 4 generations of repeated back crossing.

- A genome bank of somatic cells can easily be replenished, since the retrieval requires thawing and further culturing of the cell line. The cultured cell line can be cryo-conserved again.
- Semen used to create a 'synthetic' breed from several founder breeds can not be replenished by semen from the original breed.
- Semen used for genetic linkage analysis studies can also not be replenished.
- Semen used for introgression of genes from the genome bank breed into another breed can not be replenished.

Fortunately, the latter three uses of the genome bank require rather small amounts of semen, but still the bank has to be replenished. Therefore, these uses of semen should provide funds for a full replenishment of the breed in the genome bank. The latter involves re-establishing the breed from embryos (or semen) to replenish the bank. Also, the information that is obtained from genetic studies, crossbreeding and re-establishing the breed (and any breed comparison involved in that) are very valuable and should be entered into the data bank.

6.4 Integrating live and cryo-conservation

- General Aspects
- Cryo-back-up live conservation
- Cryo-aided live conservation

6.4.1 General aspects

Integrated live and cryo-conservation schemes resemble very much the live schemes of section 6.2 and hence the operational issues of section 6.2 also apply here. There are two kinds of integrated live and cryo-conservation schemes:

- The cryo-back-up live scheme. A live scheme where cryo-stored old genetic material is used as a back up in case (genetic) problems occur.
- The cryo-aided live scheme. A live scheme with prolonged generation intervals which are achieved by cryo-storage.

It seems risky to run a live conservation scheme without any back up in case of emergencies. Hence, the cryo-back-up live scheme is recommended for any live scheme. Especially, if the effective population size is only 50. Although, an effective size of 50 is considered to be safe, still inbreeding depression can lead to poor fertility at some point in the future, or genetic defects can become frequent. Also, diseases and other catastrophes can easily wipe out or severely reduce the small population. Continuous cryo-storage of semen and embryos seems therefore a very useful tool to minimise these risks.

The cryo-aided live scheme is useful when the (financial) resources are not sufficient to achieve the minimum effective size of 50 in the live population. With the cryo-conserved stocks the generation interval can be increased until the effective size of 50 animals per generation is reached. The increase of the generation interval reduces the rate of genetic adaptation (and improvement) of the breed and should therefore be minimised.

6.4.2 Cryo-back-up live conservation schemes

Back up in case of physical disaster

In case of a catastrophe (disease, fire, etc.), we might need to re-establish (part of) the population. The risks of physical disasters are substantially reduced by keeping the population at several herds (also the males). If a large part of the population dies, the remainder of the population will go through a genetic bottleneck. Minimum kinship selection may be used to get the population out of the bottleneck (Section 6.2.2.1). If the use of sires across the separate breeding herds is limited, the storage of semen of sires from the previous generation can alleviate the bottleneck further.

Some natural disasters spread over a large region (floods, diseases, tornadoes) and may kill the entire population. For these cases we have to be prepared to re-establish the breed. Re-establishing a breed is easier with stored embryos than with stored semen. Therefore some cryo-storage of embryos seems to be the preferred method. The following strategy seems sufficient to achieve a reasonably recent back-up for re-establish the breed:

1. At the beginning of the conservation scheme, cryo-conserve embryos from the founder population. The numbers needed are the same as those for a pure cryo-conservation scheme that aims at re-establishing a breed (chapter 5).
2. Cryo-conserve semen from every sire that is used in every generation of scheme. This will cost little effort in a AI based scheme, but more in natural mating schemes. A reduction in

the number of sires to be stored can be achieved by storing only unrelated sires (no full- or half-sib sires).

3. Repeat step 1 every 5 – 20 generations. The figure of 20 generations seems reasonable for a scheme without any selection, i.e., we only want to capture new genetic adaptations of the breed. The figure of 5 generations seems reasonable in a scheme with strong selection, where we do not want to lose much genetic progress.

In case of an emergency, we implant the most recently stored embryos into recipients and mate the offspring with the most recent semen stored in step 2. Hence, if it helps reducing the cost, we can store only female embryos. The re-established population will show a genetic lag to the deceased population of at the most 3 (=½(5+1)) or 10.5 generations, if we store embryos every 5 or 20 generations, respectively. On average the lag will be 1.75 or 5.5 generations, respectively.

In order to avoid problems due to natural disasters, the cryo bank should be kept at a different place than the live animals or, better, at two different places.

Back up in case of genetic problems

Despite an effective size of 50, a deleterious mutation can drift to high frequency in the population. In the case of a recessive genetic defect, the frequency of the mutation is rather high before the defect is discovered in the homozygote animals that show the disease. This is because as long as the defect is at low frequency it will be mainly present in heterozygote form in the population and the defect will not show. However, when we discover a genetic defect, we will not know its mode of inheritance (single gene or several genes; recessive or dominant). It seems most robust to select for BLUP breeding value estimates to reduce the prevalence of the genetic defect (possible in combination with optimal contribution selection as described in section 6.2.2.2). This is because BLUP breeding values estimates are reasonably good estimates of the true genetic value under many distributions of the true genetic values (Henderson, 1984). The use of BLUP breeding values will decrease the effective population size substantially and this can be a problem in small populations. The back up storage of semen and embryos as described in the previous section may be used to increase the effective size of the population. Note also that the older animals will have the highest breeding value estimates since the population was deteriorating over time. How much cryo-storage is needed to reduce the genetic defects is difficult to pin point, but the storage strategy that was described in the previous section seems to suffice.

In the previous paragraph we discussed selection against a genetic defect. The same method can however be applied to remedy genetic problems that are due to many genes. For example, poor reproductive performance of the animals can be remedy by selection for reproductive performance. Section 6.2.2.2 describes how to select for any trait. If the poor reproductive performance is considered to be due to inbreeding depression, the use of old cryo-stored genetic stocks can be useful to reduce the levels of inbreeding in the population. Section 6.2.2.1 describes how to minimise the kinship of the animals and thus the levels of inbreeding. Again the storage strategy as described in the previous section seems to produce sufficient amounts of cryo-stored stocks. For the purpose of reducing the kinship of the population, it is however important to retain old cryo-conserved material because the older the material the lower the kinship coefficients.

6.4.3 Cryo-aided live conservation schemes

Storage of embryos

In order to demonstrate the principles, we will first consider a cryo-aided live scheme that has a generation interval of 25.5 years. The time from birth to the production of the embryos is 1 and 2 years, for sires and dams, respectively, the embryos are frozen for 23 years, and the embryos take 1 year to develop into a new-born offspring (a total 25.5 years on average). This scheme involves in year t:

1. Thaw the embryos that were frozen 23 years ago and implant them into a recipient female.
2. Get embryos by mating the 1-year-old sires and 2-year-old dams and freeze these embryos.

It is important that the sires and dams used in step 2 are from different ages such that the generations overlap. Overlapping generations can also be obtained by using for 50% of the embryos 1-year-old sires and for 50% 2-year-old sires, and similarly for dams: 50% 1-year-old dams and 50% 2-year-old-dams. In practice, we should choose the most convenient way to achieve the overlap between the generations.

It will take some time to set up a cryo-aided live scheme, because initially there will not be 23-year-old embryos available. The scheme can be set up by freezing many embryos from the founder population, such that these embryos can be used in step 1 until they are actually 23-year-old.

If step 2 involves one male and one female, i.e., every year one male and one female is raised, the scheme involves 25 males and 26 females per generation, since the male and female generation interval is 25 and 26 years, respectively. The effective size is thus approximately 51 animals per generation. Hence, in this cryo-aided conservation scheme a zoo-sized population reaches an effective size of 50.

Storage of Semen

The cryo-aided live scheme with semen storage resembles that with embryo storage. For example in the following scheme, 1 and 2-year-old females are inseminated with 23-year-old semen which was produced by 1-year-old males, to obtain offspring when 50% of the dams are 2 and 50% are 3-year-old. The average generation interval is 13.25 years (=½(23+1) + ½(½*2+½*3)).

This scheme involves in year t:

1. Inseminate the 1-year-old (50%) and 2-year-old (50%) females with the 23 year-old-semen.
2. Obtain semen from 1-year-old males and cryo-conserve it.

The use of females from different ages is again to ensure that the generations overlap.

If we raise 6 males and 6 females per year in this scheme, and also use them in step 2, the number of males and females per generation is 24*6=144 and 2.5*6=15, respectively. Using Equation [6.1] this yields an effective size of 54. It should be noted that the extension of the male generation interval alone is far less effective in increasing effective size than extending both generation intervals (see embryo storage section).

After some number of years of extending the male generation interval, it hardly helps to extend it any further. Plotting the number of years of storage of the semen in a graph against the effective size, will show where the effect of increasing the male generation interval starts to level off. It is deleterious to the conservation scheme to extent the male generation interval beyond this number of years, because every extension of the male generation interval slows the rate of genetic adaptation of the population down.

It is very important to take great care when setting up a semen based cryo-aided conservation scheme. If we would simply store semen from the founder males and use that for the first 23 years (analogous to the embryo storage case), we would greatly increase the

genetic contribution of the founder males over that of the founder females. This could **halve** the size of the founder population. There are three methods to remedy this problem:

1. Make sure that the size of the male founder population is twice as large as initially intended.
2. Set up the semen cryo-aided scheme with embryos, as described in the embryo section, and start to run the semen aided scheme when the founder population is 23 years old in the presented example. In practice this number of years may be much smaller.
3. Start breeding from the founder population using minimum kinship selection (section 6.2.2.1) and store semen from the sires. Note that within the minimum kinship selection procedure also the 'stored' males are selection candidates, i.e., even dead males can be selected because their semen is stored. Again, as soon as the population is '23 years' old the original semen cryo-aided scheme can be run, where '23 years' will be much shorter in most practical situations. It is not useful to keep on running the minimum kinship selection scheme, because it will try to prevent all genetic drift and thus evolution when the frozen ancestors become more and more old.

Because of the high tech selection involved in option 3, the options 1 or 2 may be more practical. However, options 1 and 2 require more resources in terms of embryos or founder animals, which can be more costly than hiring a geneticist to apply a high tech selection method.

As mentioned before, cryo-aided schemes can be very useful to increase the effective size of small populations, but care should be taken to keep on turning over the generations such that the population can evolve. The actual size of the population will often be determined by the available (financial) resources, and the generation interval follows from the actual size and the effective size that needs to be achieved. The cryo-storage involved in the cryo-aided schemes serves automatically as a genetic back up that is needed as described in section 6.4.2.

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Chapter 7. Development of an expert system for conservation

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- ***The need***
- ***The components of the expert system***
- ***Conclusions***

7.1 The need

There is no right way to conduct a conservation scheme. Therefore there are no prescriptions for a successful conservation scheme that can be written down like a recipe, with each step automatically following on from the previous step, with clear objectives that are unambiguously achieved. Nevertheless successful conservation will not happen without (i) sensible decisions being taken on a large number of critical points, and (ii) knowing how to implement these decisions effectively, and (iii) experience in predicting the outcome of finely balanced decisions.

Despite the lack of a recipe for a conservation scheme there is still a process that has to be managed with a beginning, and with an ultimate objective of achieving a sustainable conservation scheme for breeds identified as being of potential benefit to mankind. Therefore we still need to know where the beginning of this process is and at each point in the process we need to know in which direction to move. This requires knowledge acquired from a wide variety of disciplines, ranging from genetics through agronomy to applied animal husbandry, and at any point this expertise may not be on hand to guide the planning and the decision making process. There is then a requirement for expertise to be shared and made available to those in need. For the expertise to be useful it must be in a form that can be assimilated and understood.

The recognition of this has led to one of the objectives of the FAO's programme for the Global Management of Farm Animal Genetic Resources being the development of guidelines for use by countries. The Primary Guideline Document (FAO, 1998a) is mainly targeted towards policy makers, and is designed to help countries get started by identifying the main elements and objectives of an animal genetic resources management plan, and outlining the strategic policy directions required to fulfil these objectives. The Primary Guideline Document is complemented and supported by secondary documents targeted mainly at those that implement policy, administratively and technically, covering a range of issues. One of the secondary documents is the FAO Guidelines for the Management of Small Populations at Risk (FAO, 1998b), which covers the topic of cryoconservation.

Together, these guidelines represent an advance in the management of animal genetic resources and it is recognised that such guidelines need to be regularly reviewed (as is partly done in the previous chapters of this book) to ensure that: (i) knowledge is updated, (ii) the best tools are referenced, and (iii) practical experience gained can be fed into the decision process.

Nevertheless there are arguments that such guidelines need to be developed into an expert system that is based upon a PC.

7.1.1 Keeping on track

The decision process for choosing among conservation strategies has multiple paths with many branching points, with previously distinct paths converging and joining together, perhaps only temporarily. It would be desirable to make the required path as linear as possible, with each step following on naturally from the last one. It is difficult to present such multiple paths cohesively in a book. It can be achieved using a computerised system.

7.1.2 Assimilation

Even though a book may contain all the necessary information, it is daunting for non-specialists to assimilate all the information. Not all parts of the book may be relevant to the application being considered. There is a danger that a deluge of technical terms (even though defined in the book), or continual cross-referencing, hampers progress. A computerised expert system may be designed to allow definitions to be read, if desired, when and where they are encountered simply by clicking on the text, with an automatic return to the point where the definition was requested. The

computerised decision pathway can avoid techniques and definitions not relevant to the application being considered.

7.1.3 Information at the point of conservation

Whilst the major planning of a conservation programme may be done in an office, many decisions can only be made on farms or in temporary facilities. A computerised PC with the reference information stored in an easily retrievable form has some advantages over carrying the actual reference books, although the disadvantage is the need to power the PC in some way.

7.1.4 Recording and data storage

During the collection of samples there will be a need to record and store information on donors and samples. An expert system could prompt for the necessary information, and store it electronically. This would facilitate the transfer of accurate and complete information to larger databases at some point in the future.

7.1.5 Sampling and mating protocols

There may be a need to choose animals for sampling based upon pedigrees, or fragments of pedigrees (see FAO (1998b) Section 5.3 and chapter 5 of this book), or to decide upon the mating design for an embryo recovery based upon the success of previous recoveries (see FAO (1998b) Section 5.4.4). Whilst relatively simple-to-use algorithms are given for these procedures, an expert system could compute these algorithms. In live-animal conservation schemes the expert system may include selection and mating tools to help meet target rates of inbreeding based upon available pedigrees and genetic evaluations (see chapter 6).

7.1.6 Customising

Guidelines need to provide some insight into numbers of samples, numbers of parents etc. This has been achieved in the FAO (1998b) guidelines by considering a set of minimum requirements for effective conservation, determined by defining objectives (chapter 6). However whilst this provides a good starting framework, on many occasions these may prove insufficient.

- In some cases breeders organisations may wish to go beyond these minimum requirements.
- In some circumstances the funding available and/or the numbers of the endangered breeds available from which to collect samples, may make it impossible to collect of the minimum number of samples envisaged by the FAO (1998b).

In both cases there will be a need to reconsider the objectives and re-calculate the numbers required. Whilst the principles behind the FAO (1998b) guidelines may be applied with any number of parents from the endangered breed, the presentation of the requirements for semen and embryos in Tables 5.3 and 5.5 in FAO (1998b) makes some assumptions upon the numbers of parents. For the collection of embryos in particular it is not easy to apply the guidelines for different numbers of parents without the aid of a computer (Meuwissen, 1997, see also chapter 6). Nevertheless changing the number of parents could easily be achieved with the expert system.

7.1.7 Benefiting from experience

The experience gained within a conservation scheme can be collected into a database classified by keywords identifying the decision pathway e.g. outlined in chapter 2 (i.e. what was the species? was live-animal conservation chosen, or cryoconservation? were embryos collected as well as semen?). When the decision process is being considered these examples can be triggered to provide information upon outcomes and people to contact for further information.

7.2 The components of the expert system

The considerations above identify several components for the expert system: (i) to provide a knowledge-based decision support system for the entire process; (ii) a database of conservation experience classified according to the decision framework; (iii) to provide tools for the active conservation process (i.e. computerized record sheets for sample collection, tools for pedigree recording, evaluation and selection tools). These components may be developed separately (the software 'engines' for some of these tasks may already have been developed) providing the whole system and the need for integrating the components is recognised.

It is necessary to prioritise these components and it is the first of these that appears most in need, to help guide the policy and decision-makers through a long-term and complex process in a cohesive way. Without the basic guidance the need for the other tools will not be recognised.

7.2.1 Suggested requirements for a basic decision support system

One of the initial questions is what kind of system should be designed. Since the initial FAO (1998a and b) guidelines are accessible on the Internet and on CD-ROM it would seem a sensible first step to build a system that operates through these media. The decision on whether or not the

tool should be intelligent is critical since this will affect the design of the tool. However a simple perspective on this question is that the development time and testing of an intelligent system may prove lengthy, whereas a more basic system would be available more quickly and may prove almost as effective.

The interface with the user should be graphical at all times to help the user, with pop-up menus. An initial step would be to develop the FAO guidelines by mapping out some of the key steps in the FAO (1998b) Figures 1.1, 2.1, 3.1, 4.1 and 5.1, and those described in chapter 2 and Figure 1 of this book. However these sketches of the decision-making process will need to be refined and developed.

The information available from the system should include: explanations and definitions; what ifs for each decision option; expected outcome(s) from executing the particular decision option chosen; recommended parties responsible for implementing any action emanating from the decision; and the next decision point(s) in the process. Decision points also need to link to relevant technical and operational protocols (e.g. recommended protocol for freezing bull semen).

7.2.2 Database of conservation experience

The basic technical specification of the database is that it should be relational, with user-friendly data retrieval. It may be possible to develop the requirements for classifying conservation schemes from the revised DAD-IS database. However there would be a need to link the classification into the decision-support system.

7.2.3 Algorithms for managing genetic resources

Some basic algorithms already exist. For example, the selection tools of Meuwissen (1997), Grundy *et al.* (1997a and 1997b) which maximise progress whilst maintaining a constant effective population size (these algorithms would include BLUP evaluation as a component; the algorithms from the different authors are very similar) are available. A number of other tools may be included: simple generation of timetables (e.g. in superovulation and embryo recovery); simple generation of checklists. More complex tools may also be developed: aids for minimising kinship selection when only a limited number of animals may be used for cryoconservation of gametes; determining optimum mating when collecting embryos for cryoconservation in order to minimise the variance of contributions from donors. Others could be developed according to need.

7.3 Conclusions

A clear case for an expert system can be made. The most pressing need is for a decision-support system, and a conservation database. Whilst a long-term perspective on such a system might include a collection of tools for genetic management.

Given the expertise on the management of genetic resources that is available within the EU, ranging from conservation practice, through technical protocols to the development of software tools to aid in their management, the EU is well placed to take the lead in the development of a flexible expert system. The provision of such a system, compatible with FAO objectives on domestic animal diversity, would make a significant contribution to global conservation and the Convention on Biological Diversity.

References

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