The Third
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(MAFF)
International Workshop on Genetic Resources

Animal Genetic Resources: Efficient Conservation and Effective Use

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Welcome address

TOSHIAKI HOSODA
Research Councillor, Council's Secretariat,
Agriculture, Forestry and Fisheries Research Council
Ministry of Agriculture, Forestry and Fisheries

1. Distinguished guests, ladies and gentlemen, on behalf of the Agriculture, Forestry and Fisheries Research Council, it is my great pleasure to extend sincere greetings and best wishes to all participants in this "MAFF International Workshop on Genetic Resources".

Conservation of genetic resources is of great concern with respect to agricultural research, because genetic resources furnish the basic material for research and is the foundation of all breeding activities. This is why many national and international research centers have genebanks, and are extending their genetic resources research programs. Worldwide efforts are being made to conserve and use genetic resources in a sustainable way.

Japan, as a member of international community, will continue to make every effort in the field of sustainable conservation and use of genetic resources.

2. In addition to our effort of transferring technologies relating to genetic resources, Japan shares with the international community the responsibility for creating a coordinated global genetic resources network. Japan is actively engaged in the discussions of many international fora. We are a member of the "Commission on Plant Genetic Resources" in the Food and Agriculture Organization of the United Nations, and also Japan is a member of the Contracting Parties to the "Convention on Biological Diversity". Recently, I attended the second meeting of the Conference of the Parties to the "Convention on Biological Diversity" held in Jacarta, Indonesia, and discussed intensively with our partners how to realize the spirit of the Convention.

3. Animal genetic resources, the theme of this workshop, comprise an important component of agricultural genetic resources. Consequently Japan supported the FAO's resolution at its 28th Conference to strengthen its activities on animal genetic resources. Even before that decision, Japan had been supporting the FAO's field project
"Conservation and Use of Animal Genetic Resources in Asia and the Pacific" since its inception in 1992, not only with financial support, but also by dispatching a specialist to the project. We fully hope that the recent decision of the FAO Conference will mark the first milestone in the worldwide effort to sustainably conserve and use animal genetic resources.

4. MAFF International Workshop on Genetic Resources is held in part as an expression of Japan's commitment to contribute actively to the conservation and use of genetic resources globally. The purpose of it is to enhance international collaboration and cooperation and to increase understanding and awareness of research progress among concerned scientists in this vital field. Like the previous Workshops, Plant and Microorganisms Genetic Resources, this third Workshop will offer a chance of sharing information and experiences on Animal Genetic Resources.

I would like to conclude my address by expressing my sincere desire that this Workshop will strengthen our mutual understanding and develop warm and lasting friendships, so that it will bring us a solid basis for cooperation in the future.

Thank you very much.
Opening address

MASAHIRO NAKAGAHRA
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It is a great pleasure for me to extend a warm welcome to all participants of this workshop. I would like to particularly welcome those participants that have travelled a long way to be with us. I think Dr Bodo has travelled the furthest, from Hungary, and I hope he has overcome his jet lag. We do appreciate you all for sparing the time to join in this workshop.

I would like to acknowledge and thank the Agriculture, Forestry and Fisheries Research Council and our sister institutes who have helped to make this workshop possible.

Conservation of genetic resources is of central concern globally and seeking ways to improve global food and basic commodity security for all mankind is among the most urgent tasks facing humanity. All countries are interdependent when it comes to conserving, evaluating and using genetic resources. The speed with which the many issues related to biodiversity are becoming international issues involving government level meetings has implications for science and scientists. To help ensure barriers to germplasm exchange are not erected and cooperation on issues related to germplasm conservation are enhanced personal contacts and mutual trust and friendship are essential. Fostering international cooperation and interaction is one of the results which I hope will emerge from the next few days you all have together in Tsukuba.

Animal genetic resources conservation is the particular focus of your discussions over the next few days. The papers to be presented in this meeting cover a very wide range of species from the small silk worm to the buffalo. Birds, mammals and insects will be discussed in different sections of the workshop. Each species has its own particular characteristics. Conservation systems for each species differs. However, over the next few days I hope all participants will be stimulated by the diverse reports presented. I hope you will:

a) find differences among research which will be of interest in the research you are engaged in;

b) seek commonalities which will make the broad management of conserving animal genetic resources more efficient.
I would like to take this opportunity to tell you a little about the National Institute of Agrobiological Resources (NIAR) which you will learn more about this week. NIAR has 5 main research sections:

- Genetic Resources,
- Molecular Biology,
- Cell Biology,
- Applied Physiology,
- Radiation Breeding.

Perhaps I can explain the connection between these sections by saying that each aims to provide a foundation for improving agriculture. Conservation of genetic resources, as well as, basic research on molecular biology are essential building blocks in agricultural improvement. We are making full use of the advances in biotechnology and information sciences to make progress.

Currently our institute is reorganising and extending its research facilities so that it will be able to carry out a strong research program into the next century. One of the additions next year to our facilities will be a new genebank for animal and microorganism genetic resources.

However, an institute does not stand alone. All our research depends on partnership both within Japan and abroad. The collaborative linkages we are building and hopefully strengthening by joint research are an important component of our institutes vitality. We are looking forward to enhanced collaboration in Japan and, particularly, with colleagues from overseas in the future.

When we produce the proceedings of this workshop the questions are faithfully recorded. Questions are valuable to seek clarity and stimulate ideas. I encourage all participants, mindful that the proceedings will go to a much wider audience, to make use of the question sessions fully.

Finally, let me wish you an enjoyable time in Tsukuba, so that you may take happy memories back to your homes, to share with your families over the holiday season.

Thank you very much.
Keynote address I

Why and How to Conserve Domesticated Animal Genetic Resources

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Summary

The organized activity for maintenance of domesticated animal genetic resources has accelerated in recent decades for cultural and technical reasons.

The ancient animal breeds are products of human selection and they are important in teaching. There is also the aesthetic argument that they are elements of nature and landscape protection. Their role in tourism must not neglected either.

The future requirements for animal production are unknown. Non commercial animal breeds can be used in harsh environments, and also in cross breeding as gene resources, and as control populations. Animals world wide play an important role in organic matter production.

Conservation programmes can be organized "in situ" or "ex situ" using traditional and modern methods.

Introduction

In the second half of the 20th century the improvement of animal breeds has accelerated. As a consequence some breeds have increased worldwide while other non-commercial ones have decreased or even become extinct. The idea of the maintenance of valuable old things, "antiques", is not a new feature of mankind. In the first place old monuments were protected and the concept of protecting wildlife is not new either. Domesticated animals seem to have been neglected perhaps because they are the products of human selection and not considered "natural" in the way wild animals are. On the other hand, living beings cannot be compared to buildings or other man made monuments.

Only during recent decades has the idea of conservation of animal genetic resources become increasingly a part of animal breeding, both in theory and practice.

Conservation means the management for human use of livestock so, "that it may yield the greatest sustainable benefit to present generations while maintaining its
potential to meet the needs and aspirations of future generations" (IUCN definition).

According to this definition, conservation touches animal science and husbandry as a whole, because without this attitude the future genetic diversity of livestock for mankind would be rapidly lost.

_Preservation_ has a narrower meaning according to the FAO definition which is: "That aspect of conservation, by which a sample of animal genetic resources population is designed to an isolated process of maintenance, by providing an environment, free from the human interference which might bring about genetic change. The process may be "in situ", whereby the sample consists of live animals in a natural environment, or it may be "ex situ", whereby the sample is placed, for example, in cryogenic storage".

The purpose of preservation is to maintain farm animal populations without genetic change as far as possible.

The conservation of such domesticated animal breeds needs financial support. Therefore the case for their conservation must be made very strongly (Bodó et al. 1984).

**Arguments**

There are two kinds of arguments for the preservation of genetic resources of farm animals:

* cultural reasons and
* technical arguments.

**Cultural reasons**

** The ancient, non commercial breeds are the product of human selection and effort therefore like old monuments or buildings, they also deserve protection. Also domesticated animal breeds are a valuable part of human culture.

** Among these old breeds there are many animals with an attractive conformation. So their maintenance is reasonable from an aesthetic point of view. Many artist, painters, photographers and sculptors would understand this argument.

** Ancient domesticated animal breeds can be used in teaching history and animal breeding. They belong to both the history of human life and to the history of animal husbandry.

** A landscape with pastures is coloured with the grazing animals and so the breed is important for the picture of the landscape. If the breed changes, it involves
changing the environment as well. Thus, if there are countryside protection areas breeds of grazing animals are also important.

** Human art uses animals as subjects too. For example, the shepherds and herdsmen used to carve artistically the long horns of old Hungarian Grey cattle. Also the tools they use are decorated with the pictures of these old fashioned animals. So the ancient animal breeds and folk art are well connected.

** The people from the towns highly appreciate the unusual view of these old farm animals. Therefore the possible role of ancient, non commercial farm animal breeds in rural tourism should not be neglected. It is a potential source of revenue for conservation of domesticated animal genetic resources.

** Technical arguments

** We do not know the future requirements of mankind for animal production. It is possible, that some neglected products of today can come into fashion again.

** There are marginal areas with very harsh conditions for animal production. Some ancient animal breeds are well adapted to these areas. They are resistant to the diseases and well adapted to climatic conditions. In some cases in these areas only local breeds can be used.

** Ancient local breeds are well adapted to harsh conditions and are very useful in cross breeding systems. In most cases these local breeds are suitable for female lines and they should be crossed by a terminal male line. The results of such crossing are economically advantageous if the breeds are well selected.

** The genes of ancient breeds are very important gene resources. The use of desirable genes from these animals is not easy, because they are linked to undesirable genes. In the future, biotechnology and genetic engineering will help break these linkages or provide new means of transferring desirable single or quantitative genes.

** In order to compare the genetic progress in production or in other traits, old breeds can have the role of a control population (Crawford, 1989).

** It is not true, that only native, local breeds can be used in organic production, but the old fashioned conformation and the good adaptation and resistance of the old breeds make them suitable for this use, at least as trade marks of given special products.
The most important methods of conservation

The two basic methods are "in situ" and "ex situ" methods. In situ conservation means the maintenance of the population in its native environment under traditional management conditions. Ex situ conservation includes cryogenic storage of breeding material (ova, sperm, embryo). Specialists used to compare these two methods from the aspect of their relative costs. The general opinion is, that the maintenance of living herds is in most cases more expensive than the storage of deep frozen material. Thus, there are many arguments in favor of both methods.

Comparison of the maintenance of living herds and cryogenic storage

The most important advantage of cryogenic storage is the lack of genetic change, if the sampling was quite correct when stored. One can imagine an unexpected effect of, for example, background radiation but up to now during the first 40 years of deep frozen storage of sperm in Cambridge no such signs have been observed. In the case of living animals selection can not be avoided. The changes by selection can be minimized, but it is impossible to avoid it totally.

It is nearly impossible to compare the expenses of these two methods. The rough costs of cryogenic storage are less. Nobody can make, however, calculate the compensation received from rare breed products sold in the market. The prices of these products depends on the market and it seems there is a tendency for there to be an increased appreciation for these products in different markets. It is questionable, however, whether lower productivity of these breeds can compensated by the higher prices of special products in the market. The possible income to breeders from the tourism can not be neglected either. Therefore a comparison of costs can not be calculated globally, only in individual cases.

The advantages of the in situ preservation are that the useful traits of the animals are not forgotten, and breeders can eliminate some genetic defects, if there are any.

For the in situ herds there is some danger of natural accidents and diseases, however an evolution of resistance can be supposed against the developing bacteria, which does not happen in deep frozen situation. The most important source of danger for cryogenic storage is human negligence.

The conclusion of this comparison is that the best means of conservation is to use these two methods together, the one method can complement the other one very well.
How to select populations to be preserved?

The problem is the choice of the animal populations for preservation which is subsidized by the government or club (e.g. the Rare Breeds Survival Trust in the United Kingdom). Classification is needed between candidates. There are many methods in this respect such as the Red data book, Alderson (1989), Hanson (1990), Bodó (1990b), and the North American system (Bixby et al., 1994). I want to mention here only the most important aspects.

1st step. We have to take into consideration the grade of endangeredness i.e. the risk status of the given population (see the discussion below on the minimum number). The smaller the population size the faster conservation measures should be taken.

2nd step We should weigh the pros and cons for the task of a given country to preserve given population or not. The following questions should be answered:
* Is the given breed native to the country?
* If not, is it unique (i.e. if in the native country it is already extinct, are there any other countries where an important population of the given genotype can be found)?
* Are there close relatives somewhere?
* Has the breed some national merit (belonging to a specific landscape, or some habits of people etc.)?
* Is the breed older or younger than 200 years old?
* Is it a pure population or a mixed one? (Sometimes this is a decisive factor although a mixed cross bred population can be maintained as well, if it has other merits!)
If the answer is yes to any of these question conservation is necessary.

3rd step Ranking between the population involved into preservation based upon the following aspects:
- performance
- special products
- adaptation
- resistance against diseases
- other traits (morphological, immunological, ethological etc.)
- distinctiveness from the other breeds
- good combining ability.

Scientific problems in preservation of domesticated animals

Conservation and preservation are of increasing concern to animal scientists. Scientists are faced with an array of new research problems. Some of these research problems include:

New concepts and definitions, the pros and cons arguments, decreasing genetic variability in different breeds, the problem of inbreeding, the necessary population size (the minimum number of animals within populations to be maintained), selection in small populations, the different methods for maintaining living herds and cryogenic stored genetic material. I will discuss briefly three of these issues.

Minimum number of the individuals in preserved populations

How many animals are needed for preservation programmes, and what is the limit for an endangered status. Science has not yet provided definite answers to these questions. In the Genetic Diversity of European Livestock Breeds (Simon and Buchenauer, 1993) the use of effective population size (Ne) has been the basis. In the World Watch List of FAO (Loftus and Scherf, 1993) a category system is used, and a similar one has been elaborated in America (Bixby et al., 1994).

It is necessary to create a system accepted worldwide for the minimum number of domestic animal populations to be preserved for three reasons:

- It is important to create and keep up to date a World Watch List or Regional, National lists of domestic animal breeds in order to observe the vulnerability status and also to access the risk of genetic drift and extinction.
- It is indispensable to recommend a population size for each population needed for preservation programmes in situ or to give advices for sampling a population to be involved in preservation programmes ex situ.
- It is possible, that the scientific based grade of risk status will serve for a basis of financial support of the populations in question.

In order to avoid genetic drift and the damaging effects of inbreeding as many
animals as possible should be kept. On the other hand, to keep the costs low the population size of non-commercial animals must be as small as possible. Therefore, a compromise should be found.

There are some problems when using Ne alone as a basis for the minimum population number so scientists have recommended some other criteria besides Ne, for example, variations in family size, number of herds, existence or lack of cryogenic storage, trends in population size, crossbreeding, existence of outstanding traits and special adaptations (Majala, 1992), distribution, percentage of migration, the relationship among males (Simon, 1994).

Thus Ne must be complemented with other factors to be of practical use. The category systems (e.g. Bodó, 1990a; Loftus and Scherf, 1993) are not suitable for practical use without other considerations. Therefore, the FAO and ALBC system incorporates some of these additional factors.

My conclusion is, that the combining of a category system and Ne is the best system for determining the number of individuals to have in conserved populations. In the framework of accepted categories of endangered status i.e. between 100 and 1000 females with an adequate Ne population can be maintained with a minimal genetic loss. Within these figures it is obligatory to create a special minimum number for each breed or herd. In order to obtain a correct solution, the peculiarities of a given species, or breed, the mating system, the genetic variation within the population, the management, effect of the given environment, the possible generation interval, some practical aspects of herd size have to be taken into consideration.

The number of breeding animals, the sex ratio are only preconditions to correct preservation because the most important point is the replacement. The population age and sex structure must be maintained in its optimum proportions over time.

Recommendation for a correct replacement system

In the literature there are many opinions concerning the minimum number of breeding animals and their sex ratio (Ne) but the most important point, replacement, also a correct Ne should include conservation of replacement, is scarcely mentioned, therefore the most important aspects should be mentioned here:

- Each breeding animals must be substituted by his/her offspring.
- The male lines and female families must be maintained.
- The frequency of all possible traits (inc. immunogenetic ones) must not be changed.
- Generation interval should be adequate for a given breed.
- Priorities must not be given to a favorite progeny group in replacement.
- For higher level of safety reserve animals should be kept.
- The most important precondition for correct replacement in preserved herds is the retention of the genetic diversity of the parent population in the progeny using many parental males (narrow sex ratio, high Ne in the proceeding matings). Yamada and Kimura (1984) discuss the details and consequences of different mating systems.

Selection within the preserved populations

In order to maintain the given gene structure of the preserved populations, theoretically all the forms of selection are prohibited. In practice, however, it is impossible. If we do not keep all the males and females for the new generation it is already a form of selection. Some random selection is possible for big populations of small animals (King, 1982), but it is more difficult in the case of large animals. Simon (1982) is of the opinion that in preservation secondary traits are important and the adaptation to environments today will be significant in the future.

The main rules of selection have already been given, because at the moment of replacement the final decision for the selection is made.

Use of the preserved livestock

The main purpose of preservation of domestic animal genetic resources is to maintain valuable genes for the future. In spite of this, it is important to speak about a present use as well, because it is necessary in order to secure funding. Also there are already good examples of the practical use of animals considered to be non-commercial.

First of all the use of rare breeds in alternative farming and in organic production should be mentioned. Alternative farming is possible with modern improved breeds or hybrids as well, but the ancient breeds are better adapted to the harsh conditions. It is a good idea to use the attractive old breeds as trade marks for organic production. A good possibility presents itself in National Parks, where the use of artificial fertilizers is prohibited and the grazing animals are in harmony with the protected nature. The market of organic product is increasing and becoming more organized in Europe and North
America. Certificates from the IFOAM (International Federation for Organic Agricultural Movements) are given for these products.

Rural tourism can provide some financial help for the maintenance of rare breeds. Ancient herds can afford an attraction for visitors. Special products are more and more required in the markets of developed countries e.g. the fat produced by the Hungarian Mangalica pig breed was neglected because of the demand for lean pork until recently. Now a new market is open for old fashioned ham which is well produced by this ancient breed.

Successful cross breeding experiments have also occurred. The primitive Hungarian Grey cattle was crossed with Simmental and the F1 with a terminal breed Charolais gave good economic results compared to other cross bred animals. The use of crossing draught horses (Irish Draught, Cleveland Bay or Hungarian Noniusz) with English Thoroughbred stallions give good horses for sporting (Show jumping, dressage and three day events) purposes.

**Conclusion**

The conservation and preservation of domestic animals is of increasing concern among scientists involved in animal science and animal breeding.

The FAO category system is suitable for different Watch Lists. For individual cases, however, a special population size must be determined, all the specific conditions taken into consideration.

The maintenance of threatened populations is possible within the category "endangered", if the scientifically based methods are used.

In some cases, the use of ancient breeds has already resulted in special products being produced.

The obligation of governments is to protect and maintain populations of all national domestic animal breeds threatened by extinction.

**References**


Bodó, I. 1990a. Special problems of conservation of domestic livestock. 4th WCGALP Edinburgh


Keynote address II

History and Phylogeny of Japanese Native Animals and Strategies for Their Effective Use

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Summary

Nine animal species native to Japan or domesticated in Japan are conserved. About 10,000 years ago, the first migrants to Japan (the Jomonese) brought dogs to Japan. About 2,000 years ago, the Yayoi migrants brought silkworms, chickens and the other type of dogs to Japan. They also brought pigs which have already become extinct. In the Kofun period (1700-1400 BP), the new migrants brought horses and cattle.

In the Heian period (800-1190 AD), cats and new varieties of chickens were introduced. In the Kamakura period (1200-1270 AD) or the Muromachi period (1370-1394 AD), ducks were introduced from China. Goats were introduced in the limited parts of the West Japan after 1500 AD. Quail were domesticated in Japan 400-600 years ago from the migrating wild quail. Many associations for conservation of the native animal breeds have been established.

Many Japanese native animal species such as beef cattle, silkworms and quail have excellent quality. Japanese beef cattle (the Japanese Black) are famous for their well-marbled meat. Japanese silkworms are famous for producing high quality and quantity of silk fiber. Japanese quail have high egg production and are used as experimental animals in avian physiology and genetics worldwide.

Other animal species such as chickens, horses, goats, dogs and cats are kept for their specific characters. Japanese native chickens consist of 17 breeds, and they have beautiful external features such as long-tailed fowl breeds (the Onagadori), and bantam breeds (the Chabo), for their longer crows (the Totenko, the Taromaru and the Koeyoshi) and for delicious meat production such as game breeds (the Shamo and the Hinaidori). Japanese native horses consist of eight breeds, and kept for various special uses. For example, the Hokkaido is used as horses to carry burdens on rough and mountainous roads in Hokkaido. Recently, small sized Japanese native goat breeds (the Shiba Goat) are considered useful as experimental animals for ruminant physiology. Japanese native dogs consist of six breeds, and are becoming popular as companion animals. They are famous for their simple features and fidelity to their owners. Japanese native cats are famous for their graceful features. They are also companion animals.

Biotechnologies for keeping and multiplying animals with the aid of artificial insemination, embryo transfer, and cloning and multiplication of fertilized embryos, are now essential techniques for effective use of the Japanese native animal genetic resources. Further, improvements in genetic engineering to produce transgenic animals encourages us in adopting new strategies for animal breeding such as to transfer of the
genes controlling the resistance to specific diseases and the resistance of the stressed environments (cold, hot, arid or humid) occur in the native animals.

**Introduction**

Recent knowledge concerning the genetic constitution and evolution of animals and plants helps in understanding the importance of research in relation to conservation of native animal genetic resources. Advances in biotechnology, including gene manipulation, in recent years has further increased the importance of conserving native animal genetic resources.

Eight animal species are native to Japan, dogs, chickens, silkworms, cattle, horses, cats, ducks and goats. One species was domesticated in Japan, the quail. Introduced species, including the pig, were brought to Japan from mainland Asia. Native Japanese pigs have become extinct. Three Japanese native animal species beef cattle, silkworms and quail have excellent quality. The other six animal species, chickens, ducks, horses, goats, dogs and cats are kept for their specific characters. An outline of the history and phylogeny and the present status of these animals will be discussed in this review. A list of major domesticated animals in Japan and the time of their migration to Japan is given (Table 1).

**History and Present Status**

1. **Dogs**

   Six Japanese native breeds were assigned as Japanese natural monuments, these are the Hokkaido (the Ainu) dog, the Akita dog, the Kai dog, the Shikoku dog and the Shiba dog. Two other native dog breeds, the Mikawa dog and the Ryukyu dog are kept in Tokushima Prefecture and Okinawa Prefecture, respectively. Phylogenetic relationships of Japanese native dogs and native dogs breeds or populations in Korea, Sakhalin, Taiwan, Indonesia, Bangladesh and Mongolia, and European dog breeds were studied by using protein polymorphisms detected by electrophoresis and chemical analysis (Tanabe, 1991; Tanabe et al., 1991a; Tanabe and Hayashi, 1995). A scattered diagram of the 52 dog breeds or populations on the basis of the 1st and 2nd principal component scores is given (Figure 1). It shows evidence of gene flow to Japan in two directions. One is from Southeast Asia through the Ryukyu Islands to the main islands of Japan excluding Hokkaido. The other is from Northeast Asia through the Korean
Table 1. A list of major domesticated animal species in Japan and the time of migration to Japan.

<table>
<thead>
<tr>
<th>Domesticated form</th>
<th>Probable wild ancestors</th>
<th>Place and (time in years) of domestication</th>
<th>Time in years before and (period of their migration to Japan)</th>
<th>Main purpose of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>dog (Canis familiaris)</td>
<td>wolf (Canis lupus)</td>
<td>West Asia (30,000)</td>
<td>10,000 (Jomon)</td>
<td>hunting, watching, companion</td>
</tr>
<tr>
<td>sheep (Ovis aries)</td>
<td>Asiatic mouflon (Ovis orientalis)</td>
<td>West Asia (12,000)</td>
<td>200 (Edo)</td>
<td>meat, wool</td>
</tr>
<tr>
<td>goat (Capra hircus)</td>
<td>bozoar goat (Capra aegagrus)</td>
<td>West Asia (11,000)</td>
<td>550 (Muromachi)</td>
<td>meat</td>
</tr>
<tr>
<td>pig (Sus domesticus)</td>
<td>wild boar (Sus scrofa)</td>
<td>China (11,000)</td>
<td>2000 (Yayoi)</td>
<td>meat</td>
</tr>
<tr>
<td>cattle (Bos taurus)</td>
<td>aurochs (Bos primigenius)</td>
<td>West Asia (9,000)</td>
<td>1,600 (Kofun)</td>
<td>meat, draft</td>
</tr>
<tr>
<td>(Bos indicus)</td>
<td>banteng (Bos javanicus)</td>
<td>India (6,000)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Bos javanicus)</td>
<td></td>
<td>Southeast Asia (5,500?)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mouse (Mus musculus)</td>
<td>wild mouse (Mus musculus)</td>
<td>China (200)</td>
<td>400 (Edo)</td>
<td>pet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Europe</td>
<td>120 (Meiji)</td>
<td>experimental use</td>
</tr>
<tr>
<td>horse (Equus caballus)</td>
<td>wild horse (tarpan) (Equus ferus)</td>
<td>Southeast Europe (6,000)</td>
<td>1,600 (Kofun)</td>
<td>riding</td>
</tr>
<tr>
<td>honey bee (Apis mellifera)</td>
<td>honey bee (Apis mellifera)</td>
<td>Egypt (5,000)</td>
<td>120 (Meiji)</td>
<td>honey gathering, pollination</td>
</tr>
<tr>
<td>silk moth (Bombyx mori)</td>
<td>Chinese silk moth (Bombyx mandrina)</td>
<td>China (5,000)</td>
<td>2,000 (Yayoi)</td>
<td>silk</td>
</tr>
<tr>
<td>cat (Felis catus)</td>
<td>wild cat (Felis silvestris libyca)</td>
<td>Egypt (3,500-4,000)</td>
<td>1,200-1,300 (Nara-Heian)</td>
<td>mouse and rat control, pet</td>
</tr>
<tr>
<td>duck (Anas platyrhynchos)</td>
<td>mallard (Anas platyrhynchos)</td>
<td>China (3,000)</td>
<td>800 (Late Heian)</td>
<td>meat</td>
</tr>
<tr>
<td>Japanese quail (Coturnix japonica)</td>
<td>Japanese wild quail (Coturnix japonica)</td>
<td>Japan (400-600)</td>
<td>400-600 (Muromachi-Edo)** egg, experimental use</td>
<td></td>
</tr>
</tbody>
</table>

* Wild mouse were possible introduced twice in Jomon and Yayoi periods, respectively.
** Domesticated in Japan.
Table 2. Gene frequencies of hemoglobin (Hb) and ganglioside monoxygenase (Gmo) variants in wolves

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Hb</th>
<th>Gmo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hb(A)</td>
<td>Hb(B)</td>
</tr>
<tr>
<td>European wolf (Canis lupus lupus)</td>
<td>9</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Mongolian wolf (Canis lupus chanco)</td>
<td>16</td>
<td>0.875</td>
<td>0.125</td>
</tr>
<tr>
<td>Afghan wolf (Canis lupus pallas)</td>
<td>6</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>New Guinea Singing Dog(^*) (Canis familiaris hallstromi)</td>
<td>5</td>
<td>0.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

\(^*\)Feral dog in Papua New Guinea (Tanabe and Hayashi, 1995)

peninsula to the three Japan mainland islands of Honshu, Shikoku and Kyushu. This studies show that the two genes, Hemoglobin A (Hb\(A\)) and ganglioside mono-oxygenase (Gmo\(B\)), are present only in East Asian dog breeds and populations including Japanese native dog breeds and populations on the three islands. Interestingly, Hb\(A\) and Gmo\(B\) is found in Mongolian wolves (Canis lupus chanco) but not found either in European wolves (Canis lupus lupus) or the Indian wolves (Canis lupus pallas) (Table 2) (Tanabe and Hayashi, 1995). These results suggest that dogs were domesticated from the West Asiatic wolves (Canis lupus pallas or Canis lupus arabs), and migrated to East Asia and they possibly hybridized with the East Asiatic wolves (Canis lupus chanco). Nine million dogs are present in Japan and are kept mainly as companion (pet) animals. Japanese dogs are especially famous for their fidelity to the owners. Cranial studies of Japanese people (Hanihara, 1991) and our studies on dogs (Tanabe, 1991) support the following assumption that the Jomonese brought an ancient type of dog from southeast Asia about 10,000 years ago, and the Yayoi migrants brought a new type of dog through the Korean peninsula about 2,000 years ago.

2. Chickens

Seventeen Japanese native fowl breeds are assigned as natural monuments of Japan, these are the Onagadori (the Long Tailed Fowl) the Totenko, the Uzura-chabo, the Minohiki-chabo, the Koeyoshi, the Tomaru, the Minohiki, the Jidori (the Tosajidori, the Gifujidori, the Miejidori and the Iwatejidori), the Shokoku, the Shamo (the Japanese Games), the Chabo (the Japanese Bantam), the Hinaidori, the Ukokkei (the Silkie), the
Kawachiyakko, the Satumadori, the Jittoko (the Creeper) and the Kurokashiwa. Other native breeds the Chan (the Utaichan), and the Nagoya (the Nagoya-cochin) are kept in Okinawa Prefecture and in Aichi Prefecture, respectively. Most of them are famous for their beautiful external features such as the long tailed breeds (the Onagadori, the Totenko and the Shokoku), bantam breeds (the Chabos) and for their long crows, up to 10-20 seconds, such as the Totenko, the Tomaru, and the Koeyoshi. The length of tail of the Onagadori can reach more than 10 meters. Some of them such as the Shamo and the Hinaidori produce high quality meat. In recent years, a hybrid (the Tokyo Shamo) between the Shamo males and the Nagoya females are used for broiler production in Japan, its meat is of higher quality than previous broilers, which were a hybrid between the White Cornish males and the White Plymouth Rock females (Oishi, 1992).

Phylogenetic relationships between 20 native fowl breeds or strains in Japan and adjacent areas and five exotic (European and American) breeds or strains, the White Leghorns, the White Plymouth Rock, the Rhode Island Red and the White Cornish are studied using blood protein polymorphisms and the results are shown (Figure 2). Relatively close relationships are found among most of Japanese native breeds, suggesting that the Totenko may be the ancestor of the Onagadori (Tanabe et al., 1991b).

The oldest bones of chicken have been found in the Harunotsuji Remains (2,000 BP) on Iki island, between Japan and the Korean peninsula. The first chicken are thought to have been brought by the Yayoi migrants about 2,000 years ago.

3. Silkworms

The silkworm moth (Bombyx mori) was domesticated for production of silk fiber from Bombyx mandarina in China about 5000 years ago. Tetravoline silkworms were introduced to Kyushu, Japan, from middle China, and trivoline silkworms from Korean peninsula to Kyushu in the early and middle Yayoi period, respectively. This has been deduced because the diameter of silk fiber found in Yoshinogari and Asahikita Remains from the middle Yayoi period, is finer than those found in the remains of the early Yayoi period (Nunome, 1988). Productivity of silkworms in Japan is very high using F₁ hybrids and other excellent breeding systems including genetic engineering techniques (Tajima, 1984).
Figure 1. Relative position of 52 dog breeds or populations defined by the first (Z1) and the second (Z2) largest principal components of the distance matrix based on variance-covariance analysis of gene frequency at 16 polymorphic loci. Figures in parentheses represent the contribution to the total variation (Tanabe and Hayashi, 1995).
Figure 2. A dendrogram of the genetic distance matrix computed from the gene frequencies of 17 variable and non-variable loci using Nei's equation among 19 Japanese Native fowl breeds, five European and American fowl breeds and one Korean fowl breed, the Chejudo native fowl (Tanabe et al., 1991b).

Figure 3. A dendrogram drawn from genetic distances among the Asian and European horse populations. S, S-M and M means small-sized, small to medium-sized, and medium-sized horses, respectively (Nozawa et al., 1984).
4. Pigs

Pigs were domesticated from wild boar (*Sus scrofa*) in China about 11,000 years ago, possibly other types of *Sus scrofa* were domesticated in the West Asia about 9,000 years ago. The Yayoi migrants brought pigs with them about 2,000 years ago. Japanese pigs were not thought to be descendants of Japanese wild boar, because the domesticated pig skull especially teeth were much bigger than those of the wild boar of Japan at the period. The native wild pigs became extinct in the beginning of the historic age (shortly after the introduction of Buddhism, around 600AD) in Japan.

5. Cattle

European cattle breeds (*Bos taurus*) were domesticated from aurochs (*Bos primigenius*) about 9,000 years ago in West Asia. Two Japanese pure native cattle populations in Mishima, Yamaguchi Prefecture and in Kuchinoshima, Kagoshima Prefecture, are descendants of aurochs (*Bos primigenius*), not the Zebu (*Bos indicus*). European-Korean-Japanese breeds or Japanese native populations have a much higher incidence of HbbA (A type of hemoglobin B) than HbbB, while HbbB are predominant in zebu-type cattle (Namikawa et al., 1984). The shape of the Y chromosome of cattle descended from aurochs including Japanese pure native cattle, is sub-metacentric and that of the Zebu cattle is acrocentric. Many breeds of Japanese beef cattle have been bred for the production of high quality meat. Japanese Black cattle (Wagyu) are from hybrids between Japanese native cattle and the Brown Swiss or the Devon. The Japanese Black is famous for the production of tender meat with a much smaller size of muscle cell fiber and a very high score for marbling compared with the western beef breeds.

6. Horses

Eight Japanese native horse breeds, i.e. Hokkaido, Kiso, Misaki, Noma, Tsushima, Tokara, Miyako and Yonakuni are conserved in Japan. Phylogenetic studies by Nozawa et al. (1976) on Asian and European horse breeds and populations suggest that all of the Japanese native horse breeds are descendants of horses which came through the Korean peninsula with migrants in the Kofun period, and not through Taiwan from Southern China. A dendrogram drawn from genetic distances among the Asian and European horse populations is shown (Figure 3). A significant difference is observed between the Asian horse breeds including the Japanese breeds and the European breeds.
including the Arab, whereas no consistent trends in body size are observed among the breeds or populations examined in this study.

7. Cats

All domestic cats were derived from the African wild cat, *Felis silvestris lybica*, and domesticated to destroy rodent pests in Egypt 3,500-4,000 years ago. The first historical records of cats in Japan appeared in 889AD (the early Heian period). It is said that Japanese envoys to China in the Tung dynasty brought cats with them to protect Buddhist scriptures and cocoons of silkworm moths from rats in the ship. More than six million cats are kept in Japan mainly as companion (pet) animals. Most of them are Japanese native cats which are descendants of the cats introduced from China 1,200-1,300 years ago. They are famous for their graceful features. Blood protein polymorphisms in Japanese cats were studied by Nozawa et al. (1985). Recently, many hybrids between the Japanese native cat and the Siamese or the Persian cat have been introduced into Japan.

8. Ducks

The duck is an important domestic animal, especially in Asia. Ducks have the second largest (681 million) population among poultry species. Eighty-six percent of all ducks are found in East and Southeast Asia.

The ancestor of the domesticated duck was the mallard (*Anas platyrhynchos*) which migrates between northern and southern parts of Eurasia. Duck have been domesticated in China for at least 3,000 years. It is quite certain that the major center of domestication of ducks is the area from East Asia to Southeast Asia. The earliest evidence of domestic ducks in Europe is as recent as the 12th century AD, and ducks were introduced frequently to Europe after 1493 when ships able to navigate to and from Asia via the Cape of Good Hope (Delacour, 1964). The egg production of ducks in East Asia especially in China is very high and exceeds 300 eggs per year (Clayton, 1984).

Ducks were introduced to Japan in the Kamakura period (1200-1270) or the early Muromachi period (1370-1394 AD) possibly from China.

In Japan, there are three indigenous duck breeds, the Kairyo Osaka, the Aokubi Ahiru and the Naki Ahiru. The Kairyo Osaka originated from hybrids between the Japanese native white duck and the Pekin, which was imported from USA, and the hybrid
was backcrossed with the Pekin. Of two flocks of Mallards examined, one has been kept in a farm in Kyoto Prefecture, Japan, after a recent catch of migrating mallards (Mallard 1), and the other may have descended from hybrids between migrating mallards and the Japanese native green headed ducks (the Aokubi Ahiru)(Mallard 2).

Phylogenetic relationships of duck breeds and populations were studied using two dendrograms drawn by genetic distances among the breeds by Nei's equation. Figure 4 shows a dendrogram based on protein polymorphisms detected by using electrophoresis on 22 genetic loci (Tanabe et al., 1988; Tanabe, 1994). Figure 5 shows another dendrogram based on DNA polymorphisms detected by randomly amplified polymorphic DNA (RAPD) patterns (Okabayashi, Kawasaki and Tanabe, 1995). The dendrograms show similar results.

These results indicate that a marked difference of genetic constitution is present between the duck breeds in Southeast Asia and those of Northeast Asia, indicating that ancestral mallards are different between the two areas. It is plausible that the first site for domestication of ducks was China, and the second site for domestication of the species was Southeast Asia including Indonesia and Malaysia.

A close relationship between the Khaki Campbell and most of the Indonesian duck breeds except those from East Indonesia is consistent with the report that the Indian Runner was the one of the founders of the breed. It is possible that European breeds were first introduced from Southeast Asian ducks and or Chinese ducks, and they were mated with European mallards later.

These present studies also shows a similarity among the Northeast Asian ducks including the Kairyo Osaka, a Japanese breed and the mallard (migrating mallards). It is postulated that Japanese native duck breeds are descendants of the hybrids between the Chinese ducks, which were introduced into Japan 700-800 years ago, and mallards, which were migrating between Siberia and Japan.

9. Goats

Goats were domesticated mainly from the Bezoar goat (Capra aegagrus) in West Asia. Goats are very important animals for meat production in Asia, and adapted to both hot and arid or hot and humid climates. Goats were first introduced to Japan through the Ryukyu (Okinawa) islands after 1,500 AD. Japanese native goats are found in Okinawa Prefecture (the Okinawa goat), Tokara islands (the Tokara goat) and the
Goto islands and interior of Nagasaki Prefecture (the Shiba goat). These goats are small and are useful experimental animals for ruminant physiology.

Phylogenetic studies on goat populations in Asia show the close relationships among the Okinawa, the Shiba, native goats in Korea and Taiwan, and Japanese Saanen (an exotic breed) and among the goats in Philippines, Indonesia (the Etawa and local goat) and Bangladesh (the Black Bengal and the Jamunapari). A dendrogram based on cluster analysis of goat population data is shown (Figure 6) (Nozawa and Katsumata, 1984).

10. Quail

The Japanese quail (Coturnix japonica) was domesticated in the Muromachi period or the early Edo period (400-600 years ago) in Japan. It is the only animal species to have been domesticated in Japan. Egg production rate of the species has improved since the beginning of the Meiji era. Wild quail still inhabit Japan and migrate between Siberia or Sakhalin and mountain regions of Honshu in Japan. Kawahara (1976) caught 306 wild quail at 800-850m in grassland at the foot of Mt. Fuji from October to November between 1965-1968, and bred them in captivity. Their egg production rate during the first 60 days was 44% and age at the first laying was 110 days. After 10 generations of domestic propagation without conscious selection, the rate increased to 79%, 90% of the level of the domesticated quail. The age of first laying decreased to 60 days compared to 49 days for the domesticated quail (Figure 7). While the coefficient of variation of these traits decreased somewhat during the 10 generations, much greater variation for the traits was observed in the wild quail and semi-domesticated ones compared with domesticated quail (Figure 8). These results strongly suggest that the contribution to the improvement of productivity during the domestication of the species was due to recombination of pre-existing genes rather than due to mutations. The egg production in captivity of wild ancestral species of domesticated birds is high in such birds as mallards and Japanese quail, although the domesticated birds always produce more eggs than their ancestral wild ones. It is thought that the high productivity of domesticated birds is an expression of natural fecundity given appropriate opportunity and owes little to artificial selection. In the wild, the expression of genetic variability would not have been possible by natural selection and makes it possible for animals and birds to adapt to the new environment. The intervention of man introduces drastic
Figure 4. A dendrogram of the genetic distance matrix computed from the gene frequencies of 22 variable and non-variable loci using Nei's equation among 16 duck breeds and strains, and two mallard flocks (Tanabe, 1994).

Figure 5. A dendrogram of genetic distance matrix computed from gene frequencies of random amplified polymorphic DNA (RAPD) among 16 duck breeds and populations and a mallard flock (Okabayashi, Kawasaki and Tanabe, 1995).
Figure 6. A dendrogram showing genetic distance of 11 goat populations. The Katjang, the Etawa and local belong to the Indonesian populations, and the Black Bengal and the Jamnapari belong to the Bangladesh-India breeds (Nozawa and Katsumata, 1984).

Figure 7. Changes in egg production (○) and sexual maturity (●) of wild Japanese quail during 10 generations in captivity without conscious selection. Vertical line represents standard deviation (Kawahara, 1976).
Figure 8. Changes in sexual maturity of wild Japanese quail during 8 generations in captivity without conscious selection (Kawahara, 1976).

Figure 9. A scattered plot of quail populations (Kimura, 1994).

○ Japanese commercial strains, ● Wild quail population caught in Japan,
□ Commercial strains in Canada and France, ■ Experimental strains in Canada,
J(UBC-J), G(Giant) and F(FRA2) are populations bred for larger sizes.
environmental changes. These changes make possible the expression of genetic variability. After capture, reproductive fecundity is much improved during domestication without conscious selections in duck and in the Japanese quail.

Phylogenetic studies on wild and domestic quail indicate that some genetic differences are observed among the commercial strains for egg production in Japan, the experimental and commercial strains for meat production in Canada and France compared to wild quail in Japan (Figure 9, Kimura, 1994). As all the quail strains in these studies originated from Japanese quail (Coturnix japonica), the genetic difference observed among the groups is derived from genetic drift. Genetic variability expressed as average heterozygosity ($\bar{H}$) is higher in the commercial strains (0.098) than the experimental strains in Japan (1.189) and in Canada and France (1.176) and wild quail in Japan (0.078) (Kimura and Fuji, 1989; Kimura, 1994). The higher variability observed in the commercial strains may be due to the breeding systems to express effectively heterosis for a higher egg production. Interestingly, the quail bred for egg production in Japan weigh 100-200g, and those bred for meat production in Canada and France weigh 250-350g. Egg production rate is very high in the commercial strains in Japan reaching 300 eggs per year. Quail are also very useful as experimental animals in avian physiology and genetics.

**Effective use of native animals**

Recent progress in genetic engineering, which allows us to manipulate genes, increases the importance of conservation of indigenous breeds or species which are well adapted to the environment of various Asian regions. For efficient conservation of genetic resources, long term storage of frozen sperms and embryos of valuable animals at -196°C, and the application of artificial insemination, embryo transfer techniques, and cloning and multiplication of fertilized embryos are very useful for reducing the numbers of animals that need to be maintained. The production of transgenic animals and sexing of sperms or fertilized embryos allow us to find more effective way of using genetic resources in animals and poultry (Tanabe, 1989). Further improvements in genetic engineering to produce transgenic animals (Palmiter et al., 1982; Pursel et al., 1989) encourages us in adopting new strategies for animal breeding, such as, to transfer genes controlling the resistance to specific diseases and adaptation resistance to the stressed environments (cold, hot, arid or humid) which occur in native animals.
References


Questions and answers in Keynote address session

Q: Referring to the theme of the workshop, how efficient and effective is the conservation of animal genetic resources? (Muladno)

A: Animal genetic resources conservation was initiated only a few years ago. Time is needed for the system to be effective and results to be obtained. The leader organization in this effort is the FAO. In some countries there are good results from conservation of farm animals, for example the United Kingdom and Hungary? (Bodo)

Q. Dr. Bodo can you give your ideas on balancing conservation of animal genetic resources and national programs which encourage crossbreeding programs? (Mathius)

A. The two concepts crossbreeding and preservation are not contradictory ones. Both are important and complementary. Local adapted breeds properly maintained can be useful material for crossing with exotics? (Bodo)

Q. Dr. Bodo is the objective of animal conservation the species or the breed.

A. The most important objective is the gene. It can be found in breeds. Species are not threatened with extinction, as far as domesticated animals are concerned. If we consider preservation of breeds, it is not the name that is important, but the valuable genes in them. Therefore, the different varieties within breeds should not be neglected either.

When we preserve breeds, species will be conserved automatically, but we have to look in the breeds structure (sub-species, lines, families etc.) in order to save the valuable genes. (Bodo)

Q. Dr. Bodó, you discussed ex-situ verses in-situ conservation. Between these two approaches where do zoos fall? (Vaughan)

A. In the strict sense of the word the activity of zoos in conservation should be considered "ex-situ" conservation. The role of zoos is very important, for example the Przewalski horse, and the maintenance of special separated lines of other rare breeds? (Bodo)

Q. Dr. Tanabe, you showed that worldwide cattle are the most numerous of domesticated animals for food. However, Japan has more pigs and much fewer sheep compared to the world average. Could you explain this? (Ishii)
A. Pigs are more efficient than beef cattle in meat production. Most Japanese are lactose intolerant so cannot drink much milk, unlike Western people. The Japanese government led by Mr. Toshimichi Ohkubo imported many sheep but most of them died. Sheep are not resistant to the hot, humid climate of Japan. (Tanabe)

Q. Dr. Tanabe are there still water buffaloes in Japan? (Tan)

A. There are a small number of water buffaloes in the Ryukyu islands, less than a thousand. They do not appear on the FAO census figures because the number is so few. (Tanabe)
Economic Evaluation of Mongolian Sheep Genetic Resources

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Abstract

Mongolia is known to be one of the world's traditional centers of husbandry. The domestication and selective breeding of animals started in Mongolia some five thousands years ago. As a result of this a pure breed of animals well adjusted to the natural conditions of Central Asia was developed. The established husbandry employs inexpensive technology. This experience is an integral part of the world husbandry heritage.

It is possible to use the basic stock of Mongolian sheep for selection and breed new lines more adjusted to climatic specifics of Mongolia.

Presently there are 27 million livestock in Mongolia, of these 14 million are pasture sheep stock.

I. Mongolian Sheep Breeding Lines

For thousands of years Mongolian sheep basic stock have been used for breeding. It is possible to distinguish two basic groups of sheep: short fat tail and wide fat tail. This could be explained by the fact that sheep are use primarily for their meat and fat.

Two groups could be further divided into several sub-groups:

1. Khalkha Breed Sheep (Figure 2, 3 and 4)

The largest number of Mongolian sheep belong to this breeding line. They are strong, and well adapted to Central Asia climatic conditions. They are well adjusted to pasture husbandry. Usually they have a dark brown bald head. They are mainly used for meat and fat.

2. Bayad Breed Sheep

This breed of Mongolian sheep is raised for meat-fat production. It has a strong body and usually has white head. There are estimated to be 100,000 sheep of this group.

3. Gobi-Altai Breed Sheep

This line is grown mostly in Bugat and Tseel somons (municipalities) in Gobi-Altai province (Figure 1). They belong to the fat tail group, and compared to other
breeds are characterized by bigger bodies and better quality wool. They are well adapted to high mountain pastures and weather conditions. Presently there are about 20,000 heads of this line.

4. Darkhad Breed Sheep

This line is grown mostly in the three northern somons of Huvsgul province. Compared to Khalkha lines they belong to wide fat tail group and are adapted to mountain pastures.

5. Uzemchin Breed Sheep

This line belongs to the short fat tail group. Compared to Khalkha line they have similar body type but can gain weight in a short time. In the first year they have 60 percent of the weight that a grown up sheep have and in the second year they already have 87 per cent. They are grown mostly for meat. They are well adapted to open steppe pasture conditions.
Figure 2. Flock of Khalkha sheep line.

Figure 3. Khalkha sheep line (male).

Figure 4. Khalkha sheep line (female).
Table 1. Mongolian sheep body weight and wool output

<table>
<thead>
<tr>
<th>Breeds and lines</th>
<th>Body weight (kg)</th>
<th>Wool output (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khalkah</td>
<td>60</td>
<td>1.4</td>
</tr>
<tr>
<td>Bayad</td>
<td>73</td>
<td>2.0</td>
</tr>
<tr>
<td>Gobi-Altai</td>
<td>58</td>
<td>2.3</td>
</tr>
<tr>
<td>Darkhad</td>
<td>69</td>
<td>1.8</td>
</tr>
<tr>
<td>Uzumchin</td>
<td>74</td>
<td>1.3</td>
</tr>
<tr>
<td>Barga</td>
<td>62</td>
<td>1.3</td>
</tr>
<tr>
<td>Sartuul</td>
<td>63</td>
<td>2.0</td>
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Table 2. Allele frequency of Tf and Hb of Mongolian sheep

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alleles</th>
<th>Mountain-steppe (m=326)</th>
<th>Seppe population (m=465)</th>
<th>Desert population (m=668)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>A</td>
<td>0.212 ± 0.03</td>
<td>0.210 ± 0.03</td>
<td>0.146 ± 0.02</td>
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<tr>
<td></td>
<td>B</td>
<td>0.788 ± 0.03</td>
<td>0.790 ± 0.04</td>
<td>0.854 ± 0.02</td>
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<tr>
<td></td>
<td>D</td>
<td>0.206 ± 0.002</td>
<td>0.209 ± 0.03</td>
<td>0.121 ± 0.01</td>
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<td></td>
<td>G</td>
<td>0.302 ± 0.003</td>
<td>0.370 ± 0.04</td>
<td>0.351 ± 0.02</td>
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<tr>
<td></td>
<td>J</td>
<td>0.204 ± 0.02</td>
<td>0.141 ± 0.03</td>
<td>0.163 ± 0.01</td>
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<tr>
<td>Tf</td>
<td>M</td>
<td>0.190 ± 0.02</td>
<td>0.200 ± 0.03</td>
<td>0.303 ± 0.02</td>
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<tr>
<td></td>
<td>P</td>
<td>0.074 ± 0.01</td>
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<tr>
<td></td>
<td>S</td>
<td>0.024 ± 0.08</td>
<td>0.009 ± 0.001</td>
<td>0.004 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>-</td>
<td>-</td>
<td>0.001 ± 0.001</td>
</tr>
</tbody>
</table>

6. Barga Breed Sheep

Barga Breed sheep are well adapted to steppe pastures, and have a little bigger and longer body than Khalkha sheep. Presently there are 140,000 heads in this line developed since 1980 in Khulunbuir somon of Dornod province (Figure 1).

7. Sartuul Breed Sheep

This breed has a large strong body and are well adapted to highland pastures. They usually have a black and brown, bald head and are grown primarily for their meat. Presently there are 60,000 heads of this line in Erdenesaihan somon of Zavkhan province (Figure 1).

II. Results of Mongolian Sheep Genetic Resources

The genetic research of Mongolian animals is not complete yet for several reasons. Genetics as a branch of science, compared to other countries, was late to
Table 3. Hb and Tf Allele frequency of four sheep lines

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alleles</th>
<th>Khalkha n = 273</th>
<th>Uzemenchin n = 269</th>
<th>Gobi-Altai n = 256</th>
<th>Bayad n = 251</th>
</tr>
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<tbody>
<tr>
<td>Hb</td>
<td>A</td>
<td>0.198 ± 0.02</td>
<td>0.190 ± 0.02</td>
<td>0.172 ± 0.02</td>
<td>0.179 ± 0.01</td>
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<tr>
<td></td>
<td>B</td>
<td>0.801 ± 0.21</td>
<td>0.810 ± 0.20</td>
<td>0.828 ± 0.02</td>
<td>0.821 ± 0.01</td>
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<tr>
<td></td>
<td>D</td>
<td>0.150 ± 0.02</td>
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<td>0.189 ± 0.02</td>
<td>0.118 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>G</td>
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<td>0.262 ± 0.02</td>
<td>0.303 ± 0.02</td>
<td>0.381 ± 0.021</td>
</tr>
<tr>
<td>Tf</td>
<td>J</td>
<td>0.322 ± 0.02</td>
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<td>0.319 ± 0.02</td>
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<tr>
<td></td>
<td>M</td>
<td>0.223 ± 0.01</td>
<td>0.232 ± 0.02</td>
<td>0.205 ± 0.02</td>
<td>0.167 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.262 ± 0.01</td>
<td>0.045 ± 0.01</td>
<td>0.079 ± 0.01</td>
<td>0.016 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>-</td>
<td>0.064 ± 0.002</td>
<td>0.002 ± 0.002</td>
<td>-</td>
</tr>
</tbody>
</table>

develop. Also there is a shortage of research facilities and equipment.

Given the importance of genetic training specialists in this field have the following priorities:

a) study the specifics of Mongolian sheep genetic

b) use genetic studies for selection and breeding

Genetic Specifics of Mongolian Sheep

Presently we have studied Hb and Tf alleles of Mongolian sheep and also have identified some alleles based on wool hair color analysis. Beginning in 1970 most research was concentrated on a comparative study of the main groups of sheep divided according to climatic, geographical and other factors. Table 2 summaries the findings:

Mongolian sheep Tf and Hb structure system does not differ from sheep in other countries. However, HbB, TfG, TfG, TfD, TfJ alleles frequencies are higher in Mongolian sheep.

TfP and TfS, TfH alleles frequencies tend to be rare. TfH allele frequency for the desert populations is, for example, only 0.001. Therefore, based on the hypothesis of Harrison (1973) that gene frequency variation within 0.001 - 0.005, given sufficient numbers of animals are tested, means a new mutant allele, we can conclude that this new line may have resulted from hybridization of Mongolian sheep with other breeds.

There is not much difference in Hb allele frequency and gene structure among Mongolian sheep groups (Table3). Note there is a difference in Tf allele.

We find some differences between Khalkha groups from the Gobi-Altai line in
Tf and from the Bayad line in TfM, TfP alleles. Such difference can be explained by the long period of separate breeding and local conditions.

Mongolian sheep are an inexpensive means of wool and meat production well adjusted to conditions of a nomadic husbandry based economy. However in terms of meat and wool the output is rather low. So there is a need to improve the output by hybridization and selection of the wool-oriented line (Orkhon breed) and the meat oriented line (Touguud breed).

Currently breeding of West Mongolia long fat tail sheep with wild mountain sheep (Argali) is being conducted in order to produce a more productive meat line. Also experiments are being carried out to cross Mongolian sheep with karakul sheep.

Another aim of the institute’s research is to increase the number of births per sheep. Presently about 10 per cent of sheep give birth to two lambs and attempts to develop a special breed with a greater number of births appears to be successful.

Since 1994 a group of Japanese scholars, headed by Prof. Yuichi Tanabe, has been actively involved in research on Mongolian domesticated animals. When this joint research project is finished we will have a clearer picture of genetic specifics of Mongolian domesticated animals including sheep groups and lines.
Economic Evaluation of Pig Genetic Resources in China

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Abstract
Pig genetic resources in China are rich and diverse. This paper discusses economic evaluation of Chinese pigs and analyses their use. The genetic characteristics of Chinese pigs for prolificacy, meat quality, stress resistance and miniature body size are reviewed. The excellent reproductive performance of Chinese pigs (especially Taihu pig) has been investigated as an important project in many countries. Chinese pigs are being used to study the genetic mechanism of reproduction for improving pig breeds and are being used in crossbreeding programs for commercial pig production. Chinese pigs can contribute to improvements in future pig production worldwide.

Introduction
Pig production in China has a long history. As early as 9000-6000 years ago, local wild pigs had been domesticated by the Chinese (Zhang et al., 1990). Pig breeds diversified over thousands of years of selection and breeding. According to the book "Pig breeds in China" (Zhang, 1986), there are 48 native pig breeds which can be divided into 6 types and these are a major gene pool for pig breeding worldwide.

2000 years ago, Chinese pigs were introduced to the Roman Empire to improve their local pigs and thus bred the Roman pig. In 1770-1780, Chinese pigs were introduced to the UK and crossed with local pigs and the famous Yorkshire and Berkshire were developed. In 1816-1817, Chinese pigs were introduced into USA and the Poland-China and the Chester White were bred. Chinese pigs played an important role in the development of some world famous pig breeds.

In recent years, due to the shortage of pig breed resources, many countries have paid great attention to Chinese pigs (especially the Taihu pig) with high reproductivity. These have been introduced into many countries to study the genetic mechanisms of fertility for improving pig breeds and for use in crossbreeding programs for commercial pig production, and have achieved a great progress.

Genetic Characteristics of Chinese Pigs
Chinese native pig breeds have some unusual characteristics compared with the Western breeds.
1. Prolificacy

Most Chinese native pigs are prolific, except some breeds which belong to the South China type, the Southwest type and the Plateau type. The Minzhu has a litter size of 13.5 and the Taihu pig of 15.3. There are 7 strains of Taihu pig and they have similar reproductive performance. Erhualian pigs have the largest total number born and number born alive. The litter size of Taihu pig from Taihu region is shown in Table 1 (Zhang, 1991).

The large litter size of Taihu pig is probably due to the high ovulation rate (Wang, 1990). The ovulation rate for sows averages 28.16, and was 6.58 and 6.76 more than the mean of that of other Chinese native breeds and Western breeds, respectively, which indicated that farrowing potential is very great in Taihu pig (Zhang, 1991). And secondly, many authors (e.g. Bidanel et al., 1990; Legault and Bidanel, 1992; Terqui et al., 1992; Xu, 1992) found that the large litter size of Taihu pigs is due to a low embryonic mortality. The early embryonic mortality rate for Taihu pig and Western pigs averaged 19.99% and 28.40-30.07%, respectively, and that was 8.41-10.08% lower in Taihu pig than in Western breeds (Chu, 1995).

2. Meat quality

Chinese pigs also have characteristics of superior meat quality. According to studies of meat quality for 10 native breeds, the results indicated that they were superior in meat color, marbling, muscle fibre diameter, intramuscular fat, dry matter, crude fat, water holding capacity in muscle, flavor, juiciness and tenderness than Western breeds (Chen, 1989)(Table 2).

3. Resistance and adaptation

Investigations on resistance and adaptability indicate that Chinese pigs have high stress resistance. In particular, some breeds have cold resistance (Minzhu), heat resistance (South China type) and adaptation to high altitudes (Tibetan pig and Neijiang) (Xu, 1989; Zhang et al., 1990).

Almost all Chinese pigs are PSS (Porcine Stress Syndrome) free.
Table 1. Litter size of 7 strains of Taihu pig

<table>
<thead>
<tr>
<th>strain</th>
<th>No. litters</th>
<th>No. born</th>
<th>No. born alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erhualian</td>
<td>278</td>
<td>15.93</td>
<td>14.12</td>
</tr>
<tr>
<td>Fengjing</td>
<td>220</td>
<td>15.17</td>
<td>13.13</td>
</tr>
<tr>
<td>Meishan</td>
<td>170</td>
<td>14.79</td>
<td>13.90</td>
</tr>
<tr>
<td>Jiaxing Black</td>
<td>293</td>
<td>15.02</td>
<td>13.73</td>
</tr>
<tr>
<td>Mizhu</td>
<td>82</td>
<td>14.65</td>
<td>13.38</td>
</tr>
<tr>
<td>Shatouwu</td>
<td>78</td>
<td>14.30</td>
<td>13.49</td>
</tr>
<tr>
<td>Hengjing</td>
<td>93</td>
<td>14.28</td>
<td>13.59</td>
</tr>
</tbody>
</table>

Table 2. Meat quality of native breeds in comparison with Western pigs

<table>
<thead>
<tr>
<th></th>
<th>MP</th>
<th>HLE</th>
<th>JQH</th>
<th>EHL</th>
<th>JXB</th>
<th>JH</th>
<th>NJ</th>
<th>XP</th>
<th>LBW</th>
<th>DWZ</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>optimal density</td>
<td>+0.28*</td>
<td>-</td>
<td>+0.56*</td>
<td>-</td>
<td>-</td>
<td>+0.036</td>
<td>+0.09</td>
<td>+0.03</td>
<td>-</td>
<td>+0.23*</td>
<td>+0.20</td>
</tr>
<tr>
<td>pH value</td>
<td>+1.40*</td>
<td>+0.50*</td>
<td>+0.59*</td>
<td>+0.50*</td>
<td>+0.05</td>
<td>+0.29</td>
<td>+0.33</td>
<td>+0.02</td>
<td>-</td>
<td>+0.46*</td>
<td>+0.32</td>
</tr>
<tr>
<td>drip loss %</td>
<td>-6.04</td>
<td>-</td>
<td>-2.09</td>
<td>-2.05</td>
<td>-</td>
<td>-4.88</td>
<td>-3.07</td>
<td>-2.94</td>
<td>-</td>
<td>-3.89</td>
<td></td>
</tr>
<tr>
<td>expressible moisture %</td>
<td>-4.04</td>
<td>-4.31*</td>
<td>-0.38*</td>
<td>-3.74*</td>
<td>-3.88*</td>
<td>-1.08*</td>
<td>-2.24*</td>
<td>-0.28*</td>
<td>-1.36*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>crude fat %</td>
<td>+1.18</td>
<td>+5.58**+2.86**</td>
<td>+2.24**</td>
<td>+0.12</td>
<td>+2.10**</td>
<td>+1.45*</td>
<td>+2.01*+2.57**</td>
<td>-</td>
<td>+2.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Figures are presented for difference between the Chinese native breed and control breed (Landrace, Large White and Harbin White); MP=Minzhu, HLE=Hetao Large-ear, JQH=Jiangquhai, EHL=Erhualian, JXB=Jiaxing Black, JH=Jinhua, NJ=Neijiang, XP=Xiang pig, LBW=Large Black-white, DWZ=Dawuzi.

* P<0.05, ** P<0.01

Table 3. Goal traits and selection criteria of Chinese native pigs

<table>
<thead>
<tr>
<th>goal trait</th>
<th>selection criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>litter size</td>
<td>litter size born alive</td>
</tr>
<tr>
<td>daily gain</td>
<td>average daily gain</td>
</tr>
<tr>
<td>feed efficiency</td>
<td>feed conversion ratio, average daily gain</td>
</tr>
<tr>
<td>lean meat content</td>
<td>lean meat content of carcass, backfat,eye muscle area</td>
</tr>
<tr>
<td>meat quality</td>
<td>meat color, pH value, water holding capacity, intramuscular fat</td>
</tr>
</tbody>
</table>

4. Miniature body size

There are several mini-pig breeds in China. Xiang pig, Bama Xiang pig, Wuzhishan pig, Banna mini-pig and Small-ear pig of Province Guizhou, Guangxi, Hainan, Yunnan and Taiwan, respectively. The body weight of adult pigs is about 40kg. Chinese mini-pigs have a small body size, slow growth, low metabolic rate, early sexual and body maturity, genic purity, resistance to disease and excellent meat quality. They are an ideal laboratory animal for medical research and also an ideal raw material for
These genetic characteristics of Chinese pigs are valuable and also stable in crossbred offsprings. Therefore, they could play an important role in improving pig breeds.

**Economic Evaluation of Chinese Pigs**

1. Breeding goal for Chinese pigs

   The breeding goal for Chinese pigs is to obtain the best possible overall animal for the food industry with consideration for future commercial conditions of production. A definition of the breeding goals includes
   - economic definition of the breeding goal
   - the improvement of the animal
   - considerations related to production on the commercial level
   - future needs.

   The quantitative breeding goal for the population is performed in two steps:
   - choice of the traits in breeding goal
   - weighting traits with economic coefficients or values.

   The economically orientated breeding goal leads to establishment of the total breeding values which is a linear function of the quantitative breeding goal. In principle, all traits influencing efficiency of commercial pig production should be included in the total breeding values and weighted by their economic values:
   \[ A_T = \sum W_i A_i \]
   where \( A_T \) is the total breeding value, \( W_i \) is the economic weight for trait \( i \), \( A_i \) is the breeding value for trait \( i \).

2. Breeding goal traits and selection criteria

   All traits with a major impact on efficiency of commercial pig production should be included in the breeding objectives. However, the estimation of breeding value is more difficult and its accuracy and genetic gain of each trait decreases with increasing number of traits. Therefore the term "major" implies also that a limitation should be set to the number of traits involved in the breeding goal. The number of 10 to 15 traits may usually
Table 4. Marginal profits of the traits in the breeding goal

<table>
<thead>
<tr>
<th>Trait</th>
<th>V(Yuan)</th>
<th>$\sigma_A$</th>
<th>$V_\sigma_A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>litter size born alive</td>
<td>94.32</td>
<td>0.3687</td>
<td>34.776</td>
</tr>
<tr>
<td>daily gain</td>
<td>0.49</td>
<td>32.336</td>
<td>15.845</td>
</tr>
<tr>
<td>feed conversion ratio kg/kg</td>
<td>-108.00</td>
<td>0.2449</td>
<td>-26.449</td>
</tr>
<tr>
<td>lean meat %</td>
<td>2.86</td>
<td>2.2627</td>
<td>6.471</td>
</tr>
<tr>
<td>water holding capacity %</td>
<td>-0.35</td>
<td>1.7089</td>
<td>-0.598</td>
</tr>
<tr>
<td>pH value</td>
<td>105.00</td>
<td>0.0690</td>
<td>7.245</td>
</tr>
<tr>
<td>intramuscular fat %</td>
<td>9.13</td>
<td>0.3796</td>
<td>3.466</td>
</tr>
</tbody>
</table>

Table 5. Performance of new dam lines of the Chinese meat type pig

<table>
<thead>
<tr>
<th>Trait</th>
<th>DIII</th>
<th>DIV</th>
<th>DV</th>
<th>DVI</th>
<th>DVII</th>
</tr>
</thead>
<tbody>
<tr>
<td>litter size</td>
<td>14.08</td>
<td>13.15</td>
<td>12.50</td>
<td>13.06</td>
<td>15.67</td>
</tr>
<tr>
<td>age at finishing * day</td>
<td>178.7</td>
<td>178.0</td>
<td>176.0</td>
<td>162.0</td>
<td>178.0</td>
</tr>
<tr>
<td>feed conversion ratio kg/kg</td>
<td>3.14</td>
<td>3.01</td>
<td>3.14</td>
<td>3.30</td>
<td>3.19</td>
</tr>
<tr>
<td>lean meat %</td>
<td>58.20</td>
<td>61.28</td>
<td>60.03</td>
<td>59.56</td>
<td>54.31</td>
</tr>
</tbody>
</table>

*Body weight at finishing of line DIII, DIV and DV was 90kg, and that of line DVI and DVII was 85kg.

Table 6. Summary of crossbreeding parameters for reproductive traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>British</th>
<th>French</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>a</td>
</tr>
<tr>
<td>No. teats</td>
<td>15.85</td>
<td>-</td>
</tr>
<tr>
<td>age at puberty d.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. born alive</td>
<td>12.24</td>
<td>ns</td>
</tr>
<tr>
<td>litter birth wt. kg</td>
<td>14.08</td>
<td>ns</td>
</tr>
<tr>
<td>mean birth wt. kg</td>
<td>1.21</td>
<td>ns</td>
</tr>
<tr>
<td>survival pre-wean</td>
<td>.831</td>
<td>.061</td>
</tr>
<tr>
<td>l.s. at weaning</td>
<td>10.10</td>
<td>ns</td>
</tr>
<tr>
<td>l. wt. at wean kg</td>
<td>77.1</td>
<td>5.8</td>
</tr>
<tr>
<td>ADG post wean g</td>
<td>417</td>
<td>ns</td>
</tr>
<tr>
<td>ADG test/grower g</td>
<td>590</td>
<td>-118</td>
</tr>
<tr>
<td>FCR kg/kg</td>
<td>3.03</td>
<td>.39</td>
</tr>
<tr>
<td>pen DFI kg</td>
<td>1.76</td>
<td>-.22</td>
</tr>
<tr>
<td>killing out kg/kg</td>
<td>.758</td>
<td>ns</td>
</tr>
<tr>
<td>est. lean %</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>backfat mm</td>
<td>31.88</td>
<td>9.97</td>
</tr>
<tr>
<td>EMA cm²</td>
<td>28.13</td>
<td>-7.71</td>
</tr>
</tbody>
</table>

be enough to describe the whole performance of a population or of a breeding product (Fewson, 1993a). The pig is an economic animal of prolificacy and large litter size and high growth rate. Its economic value is expressed mainly by reproductive performance of the sow and growth performance of offsprings. Therefore, reproductive and carcass quality, growth traits are important traits in pig breeding programs.

Chinese pigs possess common characteristics, such as high prolificacy, tender and delicious meat, hardiness, but also some disadvantages, such as, low growth rate, low feed efficiency and low lean content. Consequently the breeding objectives for Chinese pigs are to improve growth rate, feed efficiency and lean meat content, and meat quality. The goal traits and selection criteria of Chinese native pigs are shown in Table 3.

3. Calculation of economic weights of goal trait

The economic value of a trait is the marginal profit obtained, through a unit change of the trait, considered above the population mean when all other traits are held constant. Thus the economic weight of a trait in the breeding goal is defined as the marginal profit which one gets from one difference between marginal return and marginal costs (Fewson, 1993b).

Using the established profit equations and the investigated coefficients and values under the conditions of production and market in Sichuan, China, marginal profit for traits in the breeding goal of Rongchang pig is shown (Table 4) (Wang, 1995). The genetic standard deviation \( \sigma_A \) shown in Table 4 is used to compare the economic importance of traits for different units. \( V_\sigma_A \) indicates selection importance for goal traits.

The results shown in Table 4 indicate that growing traits were the most important traits in the breeding goal of Chinese native pig breeds. The litter size is economically importance and has a large influence on the economic return from pig production. The carcass quality plays also an important part in economic return.

Use of Chinese Pigs

Chinese native pigs have high prolificacy, fine meat quality and stress resistance, but also have low growth rate, thick skin and low lean meat, and it is more difficult to adapt them to the requirements of modern commercial pig production. Therefore, in the future Chinese pig genetic resources can be used:

- to develop new breeds or lines
- to make gains through heterosis.

1. Development of breeds or lines

Since the founding of the People's Republic of China, many breeds/lines have been developed. Improved pig breeds have a larger body size, daily gain of about 600g and lean meat of about 50%. They are playing a great role in the pig industry in China.

In order to effectively use our pig breed genetic resources and meet the requirement of commercial production, dam lines which are prolific and have lean meat must be developed and used in cross breeding programs. Through selection over the last decade, 5 dam lines have been developed and seem to offer good prospects for use in commercial production (Zhao, 1995). Table 5 shows their performance.

2. Commercial use of Chinese pigs

Many countries have paid attention to the prolificacy of Chinese native breeds, especially Taihu pig. Since 1979 France, the UK, USA, and Japan have introduced the Chinese Taihu pig (Jiaxing Black, Meishan, Fengjing) and used it successfully to improve sow productivity. The results indicated that Taihu pig seems to offer the best prospect for use in commercial crosses. The heterosis between Meishan and Western crosses are higher than that of crosses between Western breeds. Results from France and the UK populations are broadly in agreement in the litter size of crossbreeding parameters, which are summarized in Table 6 (from Mercer and Hoste, 1994).

In the USA, Young (1994) evaluated reproductive traits of gilts and sows that are one-half or one-quarter Meishan, Fengjing, Minzhu, or Duroc. First-cross Chinese gilts were superior to first-cross Duroc gilts for nearly all reproductive traits. Meishan and Fengjing first-crosses had similar reproductive performance and were better than Minzhu first-crosses. Differences in reproduction among backcross breed groups were smaller than among first-cross breed groups. Most differences among backcrosses were not significant but were generally numerically better for backcross Meishan and Fengjing than for backcross Duroc and Minzhu.

The strategy for exploiting Chinese breeds is to develop a synthetic population using European animals which are both lean and prolific. This would minimize the loss in prolificacy, enabling the production of an F1 parent female containing 1/4 Chinese genes, which is still prolific, and may have some disadvantage in carcass quality. This
strategy has been used by NPD (National Pig Development Co., UK) in the development of a synthetic population (Upton Meishan). A synthetic population between prolific white breeds and Meishan had a clear advantage in litter size of some 3-4 piglets relative to the Large White and Landrace sows. Synthetic pigs grow slowly, are less efficient and more than 50% fatter than white breeds at present. Predictions of performance for a 1/8 Meishan slaughter pig suggest that relative to other NPD stock, these pigs will grow just as quickly, and produce an extra 1.5-2mm backfat at UK slaughterweights (Mercer and Hosk, 1994).

Meishan can be used successfully commercially, with an economic advantage in most markets. If the whole of the UK industry were to adopt this strategy, then the benefit would be about $45 per sow (Mercer and Hoste, 1994).

The above results ignore other likely benefits from the use of Meishan crosses, such as an improvement in general reproductive performance, and possible adaptability to different farrowing environment (English et al., 1990; Legault and Bidanel, 1992). There is also evidence to suggest that the Meishan may have a fibre which can be used in the diet more efficiently (Edwards et al., 1991). It is also possible that rapid improvements in the carcass can be made in synthetic populations relative to conventional white pigs (Navean et al., 1992).

Acknowledgement

I should like to thank Prof. Changshin Wu for reading the manuscript and giving suggestions.

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Characterization and Conservation of Silkworm Genetic Resources in India

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Abstract

India has the distinction of being the only country to produce all commercially known varieties of silk viz, mulberry, tasar (both tropical and temperate), eri and muga. Oak tasar Antheraea roylei, and the mulberry silkworm Theophila sp. are among the wild forms seen in the Sub-Himalayan belt. Tropical tasar variety Antheraea mylitta is found in as many as 18 eco-races in different eco-climatic regions. Philosamia ricini is a domesticated silkworm distributed in the North-Eastern States of India, whereas Antheraea assama, a semi-domesticated silkworm (muga) produces the costliest golden silk and is endemic to Assam state. Many indigenous races of mulberry silkworm, Bombyx mori are still available in different parts of India in addition to hundreds of newly evolved breeds that are commercially exploited in the hybrid form.

Steps to catalogue, characterize and conserve different silkworm breeds in their eco-climatic niches were initiated in 1980s. A Centralized Germplasm Station for mulberry silkworm established in Hosur (Tamil Nadu state) maintains a large number of indigenous and exotic breeds, and Central Tasar Research & Training Institute at Ranchi (Bihar state) conserves the tasar eco-races in different regional niches to preserve their genetic and morphological characteristics. Similarly, Central Muga Research Station at Boko (Assam state) is maintaining the muga races.

The characteristics of silkworm breeds and races are limited to voltinism, morphological features of larvae and cocoons, besides various commercial traits. For mulberry silkworm breeds, attempts are made to characterize genetically divergent populations using molecular genetic techniques, isozyme electrophoresis, DNA level polymorphism, DNA finger printing, etc. The paper provides illustration for these useful biochemical and biometric techniques for characterizing the divergence of the silkworm breeds and the approach for characterization and conservation of silkworm breeds, genotypes and important genes.

1.0. Introduction

Sericulture in India is as old as Indian culture. According to the Western history, mulberry culture spread to India by about 140 B.C. from China. But this belief has been challenged by many Indian scholars. According to Mukherjee (1919), the Aryans discovered silkworm in the sub-Himalayas beyond Kashmir. There are many references from ancient Hindu literature like the Rigveda, Ramayana, Mahabharatha on the use of silk clothes and sericulture (Nanavaty, 1990). India has many species of sericigenous insects and the flora of their food plants. India is the only country having the tradition
of using four silkworm varieties vis, mulberry silkworm (*Bombyx mori*), tropical and temperate tasar (*Antheraea mylitta* and *Antheraea roylei*), eri (*Philosamia ricini*) and muga (*Antheraea assama*). The ancestral silkworm variety, *Theophila* sp. was also found in the Himalayan belt. Prior to the 19th century, sericulture was mostly confined to Northern and Eastern India and only during a later period, it spread to the Southern peninsula (Nanavaty, 1965). Presently, 90% of the Indian silk is produced from the domesticated mulberry silkworm *Bombyx mori* and only 10% of the silk comes from non-mulberry silkworms.

Despite the long history of sericulture in India from time immemorial, nothing is known about the characteristics of the ancient races except that the reared silkworms produced white and colored cocoons. Prior to the British period (18th century), silkworm races were chiefly produced and maintained by the egg producers (Datta, 1984). The research work to characterize the races and to prepare manuals on mulberry cultivation, cocoon production etc. started in the British period. It is only in independent India, that a concerted effort was initiated for characterizing and conserving the silkworm races and their populations in different agro-ecological niches when the Government of India constituted the Central Silk Board under the Ministry of Commerce in 1949.

2.0. Non-mulberry Silkworms

The natural silks are broadly classified as mulberry and wild or non-mulberry. The non-mulberry sericulture includes tropical and temperate tasar, eri and muga. India produces over 1000 MT of non-mulberry silks every year. Being the homeland of several sericigenous insects, the Sub-Himalayan region of North-Eastern India is believed to be site of origin for various lepidopteran species including those of commercial silkworms vis, eri, muga and temperate tasar. Several ethnic groups have been are engaged in production of different varieties of silk for a long time. The availability and the exploitation of sericigenous fauna in India with brief information about these lepidopteran species is presented (Table 1).

2.1. Tropical Tasar Silkworm

Indian tasar silkworm, *Antheraea mylitta* D. is one of wild fauna of tropical India. It exists in a wide range of geographical conditions and about 18 ecological races
Table 1. Availability and exploitation of sericigenous fauna in India

<table>
<thead>
<tr>
<th>Category</th>
<th>Species/Races</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Non-mulberry:</td>
<td></td>
<td>Evidences point to production of such silk in India prior to origin of mulberry sericulture in China.</td>
</tr>
<tr>
<td>Tropical tasar</td>
<td>Antheraea mylitta</td>
<td>Wild variety exploited commercially has 18 distinct eco-races in different cc-oeclimatic regions.</td>
</tr>
<tr>
<td>Temperate tasar</td>
<td>Antheraea royeli</td>
<td>Available in wild form in Sub-Himalayan regions. Poor silk yield due to double layers.</td>
</tr>
<tr>
<td></td>
<td>Antheraea proylei</td>
<td>The synthetic breed developed from its Indian and Chinese parental species has been established as the commercial oak tasar variety in North-Eastern and North-Western regions.</td>
</tr>
<tr>
<td>Muga</td>
<td>Antheraea assama</td>
<td>Endemic to the Assam and Meghalaya states and produces the most expensive golden silk.</td>
</tr>
<tr>
<td>Eri</td>
<td>Philosamia cynthia/ricini</td>
<td>The domesticated silkworm producing non-reelable cocoons in two colours (white and brown). Commercially reared in many North-Eastern states of India.</td>
</tr>
<tr>
<td>B. Mulberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild silkworm</td>
<td>Theophila sp.</td>
<td>Exist in the wild on both non-mulberry and mulberry food plants. The different species under this genus are not commercially exploited at present but definitely constitute an important gene pool.</td>
</tr>
<tr>
<td>Mulberry silkworm</td>
<td>Bombyx mori</td>
<td>A few indigenous varieties - all hibernating - still most popular with the farmers in their respective localities and so have their own niche. At least two indigenous hibernating breeds one in Bengal and the other in Kashmir are now extinct. Hundreds of hibernating breeds imported/developed are maintained as germplasm stocks while a few of them are used as parents of hybrid seed exploited over large parts spread over the country.</td>
</tr>
</tbody>
</table>

are recorded in different parts of the tropical forest (Table 2). In order to assess the genetic potential of tasar silkworm, many surveys were conducted by the Central Tasar Research & Training Institute (CTR&TI), Ranchi since 1964 in different tasar producing states (Jolly et al., 1968). The tasar zones are located between 16° (E) latitude and 60°-88° (NS) longitude. These ecological populations/races are either univoltine, bivoltine or trivoltine and the voltinism depends on climatic conditions of the region. It may also be seen from Table 2 that the ecological races/populations are confined to the various food plants. For example; Raily, Laria, Modal and Nalia are sal (Shorea robusta) based, while others are Terminalia based and a few others like Seoni and Tira are Lagestromia based.
<table>
<thead>
<tr>
<th>Eco-race</th>
<th>State/Area (Bihar)</th>
<th>Food plant</th>
<th>Voltinism</th>
<th>Fecundity</th>
<th>CWT (g)</th>
<th>SWT (g)</th>
<th>SR %</th>
<th>FL (m)</th>
<th>Denier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daba</td>
<td>Singhbhum</td>
<td><em>Terminalia</em> sp.</td>
<td>Bivoltine</td>
<td>250</td>
<td>12.10</td>
<td>2.8</td>
<td>12.6</td>
<td>750</td>
<td>12</td>
</tr>
<tr>
<td>Sarihan</td>
<td>Santhal Pargana</td>
<td>- do -</td>
<td>Trivoltine</td>
<td>201</td>
<td>6.89</td>
<td>0.7</td>
<td>10.3</td>
<td>533</td>
<td>9</td>
</tr>
<tr>
<td>Laria</td>
<td>Hazaribagh Lohardaga (Bihar)</td>
<td><em>Shorea robusta</em></td>
<td>Bi/trivoltine</td>
<td>215</td>
<td>9.75</td>
<td>1.9</td>
<td>19.3</td>
<td>785</td>
<td>10</td>
</tr>
<tr>
<td>Barherwa</td>
<td>Simdega (Bihar)</td>
<td>- do -</td>
<td>Bivoltine</td>
<td>167</td>
<td>13.76</td>
<td>3.0</td>
<td>21.9</td>
<td>1234</td>
<td>12</td>
</tr>
<tr>
<td>Sukinda</td>
<td>Sukindagarh (Orissa)</td>
<td><em>Terminalia</em> sp.</td>
<td>Trivoltine</td>
<td>266</td>
<td>13.0</td>
<td>1.8</td>
<td>13.6</td>
<td>845</td>
<td>10</td>
</tr>
<tr>
<td>Modal</td>
<td>Kaptipada, Orissa</td>
<td><em>Shorea robusta</em></td>
<td>Bivoltine</td>
<td>299</td>
<td>14.2</td>
<td>3.6</td>
<td>25.7</td>
<td>1383</td>
<td>12</td>
</tr>
<tr>
<td>Walia</td>
<td>Sundargarh</td>
<td>- do -</td>
<td>- do -</td>
<td>182</td>
<td>11.7</td>
<td>2.6</td>
<td>22.0</td>
<td>1201</td>
<td>12</td>
</tr>
<tr>
<td>Andhra local</td>
<td>Warrangal Adilabad (AP)</td>
<td><em>Terminalia</em> sp.</td>
<td>Trivoltine</td>
<td>119</td>
<td>9.4</td>
<td>1.6</td>
<td>16.6</td>
<td>700</td>
<td>10</td>
</tr>
<tr>
<td>Raily</td>
<td>Jagadalpur (MP)</td>
<td><em>Shorea robusta</em></td>
<td>Bivoltine</td>
<td>280</td>
<td>12.77</td>
<td>2.7</td>
<td>20.8</td>
<td>1232</td>
<td>13</td>
</tr>
<tr>
<td>Bhopal- patanum</td>
<td>Jagadalpur (MP)</td>
<td><em>Terminalia</em> sp.</td>
<td>Trivoltine</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Seoni</td>
<td>Seoni (MP)</td>
<td><em>Lagestromia</em> sp.</td>
<td>Bivoltine</td>
<td>241</td>
<td>12.37</td>
<td>2.7</td>
<td>20.8</td>
<td>1232</td>
<td>13</td>
</tr>
<tr>
<td>Tira</td>
<td>Purulia (WB)</td>
<td><em>Lagestromia</em> sp.</td>
<td>- do -</td>
<td>NA</td>
<td>8.6</td>
<td>1.3</td>
<td>15.2</td>
<td>674</td>
<td>10</td>
</tr>
<tr>
<td>Bankura</td>
<td>Bankura (WB)</td>
<td><em>Terminalia</em> sp.</td>
<td>- do -</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Nowgong (Assam)</td>
<td>Nowgong sp.</td>
<td><em>Terminalia</em> sp.</td>
<td>- do -</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bhandara</td>
<td>Bhandara Chandrapur (Maharashtra)</td>
<td><em>Terminalia</em> sp.</td>
<td>Trivoltine</td>
<td>195</td>
<td>6.9</td>
<td>1.2</td>
<td>17.9</td>
<td>613</td>
<td>9</td>
</tr>
<tr>
<td>Belgaum</td>
<td>Belgaum (Karnataka)</td>
<td><em>Hardwickia</em> sp.</td>
<td>- do -</td>
<td>160</td>
<td>6.4</td>
<td>0.5</td>
<td>8.5</td>
<td>617</td>
<td>8</td>
</tr>
<tr>
<td>Munga</td>
<td>Dawaria (UP)</td>
<td><em>Terminalia</em> sp.</td>
<td>Bivoltine</td>
<td>185</td>
<td>6.9</td>
<td>0.8</td>
<td>13.0</td>
<td>550</td>
<td>10</td>
</tr>
<tr>
<td>Tesera</td>
<td>Sahabad, (Rajasthan)</td>
<td><em>Terminalia</em> sp.</td>
<td>- do -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Comparative analysis of traits in temperate tasar silkworm species

<table>
<thead>
<tr>
<th>Traits</th>
<th>A. roylei</th>
<th>A. pernyi</th>
<th>A. proylei</th>
<th>A. yamami</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg colour</td>
<td>Bluish green</td>
<td>Brownish</td>
<td>Bluish green</td>
<td>Pinkish/Brownish</td>
</tr>
<tr>
<td>Larval colour (Newly hatched)</td>
<td>Black</td>
<td>Dull black</td>
<td>Black</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Matured larval wt. (g)</td>
<td>12.0</td>
<td>12.0</td>
<td>15.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Cocoon structure</td>
<td>Double reelable</td>
<td>Single reelable</td>
<td>Single reelable</td>
<td>Single reelable</td>
</tr>
<tr>
<td>Cocoon colour</td>
<td>Inner-cream, brown</td>
<td>Light brown</td>
<td>Light brown</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>Cocoon wt. (g)</td>
<td>5.3</td>
<td>5.28</td>
<td>6.22</td>
<td>6.00</td>
</tr>
<tr>
<td>Peduncle</td>
<td>Thin, short with ring</td>
<td>Thin, short with ring</td>
<td>Weak, short with ring</td>
<td>Thin, short with ring</td>
</tr>
<tr>
<td>Shell wt. (g)</td>
<td>0.50</td>
<td>0.60</td>
<td>0.76</td>
<td>0.80</td>
</tr>
<tr>
<td>Filament length (m)</td>
<td>468</td>
<td>411</td>
<td>750</td>
<td>600</td>
</tr>
<tr>
<td>Denier</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Table 4. Seasonal variation in yield traits of A. assama (Muga silkworm)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td>102 (summer)</td>
<td>240 (Autumn)</td>
</tr>
<tr>
<td>Hatching %</td>
<td>19.0 (-do-)</td>
<td>84.0 (-do-)</td>
</tr>
<tr>
<td>Total life cycle (days)</td>
<td>50 (-do-)</td>
<td>120 (Winter)</td>
</tr>
<tr>
<td>Larval span (days)</td>
<td>22 (-do-)</td>
<td>60 (-do-)</td>
</tr>
<tr>
<td>Cocoon weight (g)</td>
<td>4.1 (Winter)</td>
<td>5.8 (Autumn)</td>
</tr>
<tr>
<td>Shell weight (g)</td>
<td>0.28 (-do-)</td>
<td>0.57 (-do-)</td>
</tr>
<tr>
<td>Shell ratio (%)</td>
<td>5.49 (-do-)</td>
<td>8.39 (-do-)</td>
</tr>
<tr>
<td>Filament length (m)</td>
<td>204 (-do-)</td>
<td>500 (-do-)</td>
</tr>
<tr>
<td>ERR (%)</td>
<td>20 (-do-)</td>
<td>60 (-do-)</td>
</tr>
</tbody>
</table>

Table 5. Commercial traits of domesticated eri silkworm variety

<table>
<thead>
<tr>
<th>Food Plant</th>
<th>Polychaegous (Caster, Tapioca, Papaya etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td>300 to 400</td>
</tr>
<tr>
<td>Larval weight</td>
<td>8 to 10 g</td>
</tr>
<tr>
<td>Larvae spotted and unspotted.</td>
<td>Tubercles with setae all over body.</td>
</tr>
<tr>
<td>Cocoon weight</td>
<td>3 - 4 g</td>
</tr>
<tr>
<td>Shell weight</td>
<td>0.4 - 0.5 g</td>
</tr>
<tr>
<td>Shell ratio</td>
<td>13 - 14 %</td>
</tr>
</tbody>
</table>
2.1.1. Characteristics

The commercial characteristics of the various eco-races, presented in Table 2, show that many commercial traits like cocoon weight, shell weight, shell percentage as well as denier vary significantly amongst different eco-races of tasar silkworm raised in different regions. High genetic coefficient of variation has been reported for these traits by various authors (Sen et al., 1976 and Chatterjee et al., 1983). The characters like larval duration, shell weight, cocoon weight and shell ratio show high heritability with low genetic advance possibly due to inter and intra-allelic interactions (Siddiqui et al., 1985, 1988, 1989).

2.1.2. Cytological characteristics

Chromosome numbers in A. mylitta D. was studied by Sinha and Jolly (1967) and the haploid number of chromosomes (n=31) was established. Interestingly, no variation was recorded with respect to chromosome number amongst different eco-races of tasar silkworm. However, variability at the cytological level was reported by Gaur (1986) and Sinha et al. (1994) in many different eco-races with respect to chiasma frequency. Gaur (1986) has also reported variation on the basis of occurrence of the B chromosome.

2.1.3. Biochemical characterization

Variability at the biochemical level was studied through gel electrophoresis for some of the major eco-races of A. mylitta. Out of 26 bands reported, the band numbers 3, 6, 17, 20 and 24 were common in all, while bands 23 and 25 were found to vary in different populations (Sinha et al., 1994).

2.2. Temperate Tasar Silkworm

An extensive survey of the sub-Himalayan belt in India conducted in 1966 by the CTR&TI, Ranchi indicated the presence of vast wealth of oak in these regions as well as the occurrence of the indigenous species, Antheraea roylei. Oak tasar plant is also found in the North-Eastern region of India covering the States like Manipur, Assam, Arunachal Pradesh, Meghalaya, Mizoram and Nagaland. CTR&TI, Ranchi successfully raised the hybrid from A. roylei and its Chinese counterpart A. pernyi. The resulting hybrid called A. proylei Jolly, proved to excel both parents in all economic characters and thus gave rise to temperate tasar culture in India. This industry is spread mainly in
North-Eastern States owing to ideal socio-economic conditions, specially in Manipur.

2.2.1. Characteristics

The distinctive morphological characteristics of different temperate tasar silkworms are presented (Table 3). *A. proylei* is more similar to *A. roylei* with respect to egg color and larval body color. But many characters of *A. pernyi* like larval size, cocoon weight, shell percentage, filament length are similar to that of *A. proylei*. The filament length in *A. proylei* was found to be longer than that of *A. pernyi* and only single shell occurred instead of the double shell structure seen in *A. roylei*.

2.2.2. Chromosome number

The haploid chromosome number of these Antheraea species of temperate zone have been determined (*A. pernyi* n=49; *A. proylei* n=30; *A. yamamai* n=31; *A. frithii* n=31; *A. sivalica* n=31 and *A. polyphemus* n=30). The chromosome number of *A. roylei* shows polymorphism in Eastern India varying from 32 to 34 and when interspecific hybridization was performed between *A. roylei* and *A. pernyi*, variable chromosome number was seen in the progeny. However, in the surviving *A. proylei*, the chromosome number was found to maintain n=49. Nagaraju and Jolly (1986) have shown how the chromosome orientation takes place in *A. proylei*.

2.3. Muga Silkworm

Muga silkworm is cultivated only in Assam because of its characteristic ecological requirement of host plants. The polyphagous muga silkworm feeds primarily on the leaves of *Machilus bombycina* (som) and *Litsaea monopetala* (soalu). The Eastern Goalpara and South-Western part of Kamrup District of lower Assam are the major seed areas and the trade is largely in the hands of the tribal community. Commercial rearing is practiced mainly in Sibsagar and Lakhimpur and to a lesser extent in Nowgang, Darang and other Districts. The naturally grown cocoons are used for seed and over 80% of the commercial cocoons produced in the plains are reeled on a large scale in the Sualkuchi area.

2.3.1. Characteristics of Muga

The range of variation in commercial traits of muga silkworms reared in different seasons is presented (Table 4). The egg number and the hatching percentage are lower
in summer compared to autumn. In winter, the total life cycle extends to 120 days compared to 50 in the summer. Similarly, the cocoon weight, shell weight and shell ratio are reduced very much in summer months compared to those reared in winter. The larval body of the muga silkworm is characterized by black inter segmental markings over the yellowish body. At maturity, the larval body weight reaches 15-16g. The body is covered by tubercles on dorsal and lateral (upper and lower) sides. The tubercle setae are pointed and blackish in color.

2.4. Eri Silkworm

Eri silkworm (*Philosamia* sp.) is the only one in the non-mulberry category which is completely domesticated and represented by two species in India today. The North-Eastern state of Assam in India is considered to be the original home of eri silkworm and its advent in India is lost in antiquity. However, the earliest reference documented in 1779 speaks of eri silk produced in Ghoraghat of undivided Bengal. Eri silk is popularly known as poor man's silk owing to its cheaper cost of production and lesser market price of the spun silk produced out of its unreelable cocoons.

2.4.1. Morphological characteristics

*Philosamia* (*Attacus*) *cynthia* is still found in wild state and performs poorly under domesticated rearing. It is either bi or trivoltine and produces compact, light brown cocoons. *Philosamia ricini* is the domesticated variety reared in many North-Eastern states including Assam, Bihar and Orissa on the castor (*Ricinus* sp.) leaves. But ericulture is being practiced in many isolated patches spread over other parts of India and exploiting its polyphagous nature, eri silkworm is also reared, though on minor scale, on Tapioca and Papaya. The cocoons are comparatively loose and mostly white but sometimes brick red. Commercial traits of this domesticated variety are given (Table 5).

3.0. Mulberry Silkworm

3.1. Mulberry Silkworm in Wild

*Theophila religiosa*, a wild species of silkworm is considered as a candidate for the ancestral form of the domesticated mulberry silkworm (Tazima, 1964). *Theophila* species are still found widely distributed in their natural habitats in India as a polyphagous silkworm. A recent survey has recorded its occurrence on mulberry in sub-Himalayan regions, especially Kalimpong (West Bengal), Sikkim and Manipur. It was
also found prevalent on *Artocarpus, Ficus bengalensis* and *Ficus religiosa* food plants (Singh and Pavan Kumar, 1995).

### 3.2. Domesticated Mulberry Silkworm

The mulberry silkworm rearing has been practiced since ancient times in India. According to historians, some indigenous races have come to India through Siam (Thailand). Unfortunately, not much is known about the descent of indigenous races in the literature. India became home land for the silkworms which were brought from China and then at a later stage spread to Northern and to some extent, Eastern India. However, the scenario of sericulture, is well documented and presents a picture of the diversity of silkworms that Indian people reared since 18th century. "Handbook of wild silk of India" describes six species from the genus *Bombyx* prevalent at that time.

#### 3.2.1. Characteristics of mulberry silkworm

The multivoltine mulberry silkworm, race Nyapau, is prevalent in India and was reared both in Burma and Bengal in 18th Century. It was often referred to as Burmese silkworm and produces multivoltine white and yellow cocoons. The races namely Madrasi, Chotopalu, Cheenapalu, Mysore, Boropalu, Lahemia and Kashmir that were reared in 18th century in Eastern, Southern and Northern India are presented (Table 6). The characteristics of most of these races are more or less the same, producing small yellow cocoons of low quality whose larval period extends from 25 to 35 days. Only the Boropalu race was found to be univoltine producing larger cocoons. The cocoons are also mostly white with floss and are pointed at one end. The Kashmir race was also univoltine producing golden yellow and white cocoons with high silk quality (Table 6).

In early 20th century, sericulture continued in Bengal. In independent India, the local indigenous multivoltine race Nistari maintained its popularity over the degenerate univoltine Boropalu race which was reared only for a limited period during spring and autumn and the synthetic breed, Nistid, which was developed in 20th century by hybridizing Nistari with Italian breed. Nistid was an improvement in terms of productivity and quality, but it was not popular in Bengal and later became extinct.

The characteristics of different multivoltine breeds including races like Nistari, Sarupat, Moria and synthetic multivoltine breeds are presented (Table 7). The cocoon weight of the races ranges from 0.8 to 1.3g and SR % ranges from 11 to 15% indicating
Table 6. Mulberry silkworm varieties in Pre-independent India

<table>
<thead>
<tr>
<th>Variety</th>
<th>Oldest report and place of existence</th>
<th>Characteristics</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyapau</td>
<td>18th century Burma, Bengal.</td>
<td>Multivoltine; white and yellow cocoons and Burmese silkworm.</td>
<td>Also referred to as <em>Bombyx arracanensis</em></td>
</tr>
<tr>
<td>Madrasí/</td>
<td>1780 - 181 Bengal</td>
<td>Multivoltine (7 to 8 broods/yr); cocoons yellow; fibres with less elasticity and brilliance; thriving well under hot weather, moths milky white; larvae milky white with conspicuous marking; larval span 25 days in summer and 35 days in winter.</td>
<td>Also referred to as <em>B. croesi</em> (Hutton) - still popular in Bengal</td>
</tr>
<tr>
<td>Canary/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nistari</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desí/Choto</td>
<td>18th century. Bengal, Assam.</td>
<td>Multivoltine (5 breeds per year); small; loose golden yellow cocoons; Moths dusky; worms blush; larval span 25 days.</td>
<td>Also referred to as <em>B. fortunatus</em> (Hutton).</td>
</tr>
<tr>
<td>palu</td>
<td>(Older than Nistari and may be the first tropical variety of India.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheenapalú</td>
<td>18th century. Bengal, Assam.</td>
<td>Multivoltine; Very small cocoons inferior to earlier varieties.</td>
<td>Also referred to as <em>B. sinensis</em> (Hutton)</td>
</tr>
<tr>
<td>Chotapat/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sina</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mysore</td>
<td>Introduced in 1785 by Tippu Sultan in Karnataka. Also Cuddappah (AP) and Coimbatore (TN).</td>
<td>Multivoltine (Introduced from China)</td>
<td>Also referred as <em>B. meridionalis</em>.</td>
</tr>
<tr>
<td>Boropalú/</td>
<td>18th century. Bengal, Assam and Manipur (May be Italian origin).</td>
<td>Univoltine; Larger cocoons; Normally white but sometimes yellow; More flossy and pointed at each end; Less gum; Larval span 30-40 days (10 - 12 days V instar)</td>
<td>Also referred to as <em>B. texor</em> Now extinct.</td>
</tr>
<tr>
<td>Lehemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kashmir</td>
<td>Kashmir. Reportedly exported to Europe around 1855.</td>
<td>Univoltine; Golden yellow or white cocoons with very good silk quality; Often dark coloured or black worms I moult.</td>
<td>Became extinct due to a bad epidemic in 1978.</td>
</tr>
</tbody>
</table>

that these multivoltine races have very low silk yield. The filament length ranges from only 384 to 527 m. The denier in races like Nistari, Sarupat, Moria is very low compared to other breeds indicating that very thin yarn is preferred by the industry. The low quality of these breeds is reflected in the neatness which is low and floss percentage which is high.

Research institutes of the Central Silk Board collected many hybrids from
### Table 7. Characteristics of some indigenous multivoltine mulberry silkworm varieties of India

<table>
<thead>
<tr>
<th>Race</th>
<th>State of origin</th>
<th>Larval pattern</th>
<th>Cocoon Shape</th>
<th>Color</th>
<th>Yield/10,000 (No.)</th>
<th>CWT (g)</th>
<th>Shell wt. (cg)</th>
<th>Silk Ratio (%)</th>
<th>FL (m)</th>
<th>Denier</th>
<th>Raw Silk (%)</th>
<th>Reel %</th>
<th>Neat %</th>
<th>Floss %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Traditional races</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nistari</td>
<td>W.Bengal</td>
<td>M</td>
<td>Spindle</td>
<td>GOY</td>
<td>8295</td>
<td>0.934</td>
<td>12.4</td>
<td>13.3</td>
<td>492</td>
<td>1.28</td>
<td>5.02</td>
<td>84.3</td>
<td>78</td>
<td>7.7</td>
</tr>
<tr>
<td>Pure Mysore</td>
<td>Karnataka</td>
<td>P</td>
<td>Spindle</td>
<td>GRY</td>
<td>8371</td>
<td>0.867</td>
<td>12.3</td>
<td>14.2</td>
<td>352</td>
<td>2.04</td>
<td>8.00</td>
<td>74.4</td>
<td>75</td>
<td>18.6</td>
</tr>
<tr>
<td>C'Nichi</td>
<td>- do -</td>
<td>P</td>
<td>Peanut White</td>
<td>White</td>
<td>8915</td>
<td>0.841</td>
<td>9.6</td>
<td>11.4</td>
<td>375</td>
<td>1.44</td>
<td>5.09</td>
<td>76.4</td>
<td>73</td>
<td>4.1</td>
</tr>
<tr>
<td>Sarupat</td>
<td>- do -</td>
<td>P</td>
<td>Spindle</td>
<td>G.W.</td>
<td>7320</td>
<td>1.116</td>
<td>16.1</td>
<td>14.4</td>
<td>432</td>
<td>2.08</td>
<td>5.45</td>
<td>87.0</td>
<td>77</td>
<td>9.8</td>
</tr>
<tr>
<td>Moria</td>
<td>Assam</td>
<td>P</td>
<td>Spindle</td>
<td>G.W.</td>
<td>8035</td>
<td>1.088</td>
<td>15.6</td>
<td>14.3</td>
<td>430</td>
<td>2.1</td>
<td>5.32</td>
<td>85.0</td>
<td>76</td>
<td>9.5</td>
</tr>
<tr>
<td>Nistid(Y)</td>
<td>W.Bengal</td>
<td>P</td>
<td>Spindle</td>
<td>GOY</td>
<td>9015</td>
<td>0.939</td>
<td>12.7</td>
<td>13.7</td>
<td>372</td>
<td>2.42</td>
<td>6.18</td>
<td>85.4</td>
<td>77</td>
<td>5.7</td>
</tr>
<tr>
<td>Nistid(W)</td>
<td>- do -</td>
<td>P</td>
<td>Spindle</td>
<td>White</td>
<td>8150</td>
<td>1.011</td>
<td>13.9</td>
<td>13.6</td>
<td>284</td>
<td>2.53</td>
<td>5.0</td>
<td>79.7</td>
<td>79</td>
<td>8.2</td>
</tr>
<tr>
<td><strong>Early breeds developed for tropical regions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-race</td>
<td>W.Bengal</td>
<td>M</td>
<td>Oval</td>
<td>GOY</td>
<td>8424</td>
<td>1.143</td>
<td>16.9</td>
<td>14.8</td>
<td>375</td>
<td>2.05</td>
<td>6.01</td>
<td>88.9</td>
<td>76</td>
<td>7.4</td>
</tr>
<tr>
<td>A4E</td>
<td>- do -</td>
<td>M</td>
<td>Oval</td>
<td>GRY</td>
<td>8052</td>
<td>0.995</td>
<td>15.5</td>
<td>15.6</td>
<td>445</td>
<td>2.22</td>
<td>7.03</td>
<td>71.1</td>
<td>77</td>
<td>9.0</td>
</tr>
<tr>
<td>A14DY</td>
<td>- do -</td>
<td>M</td>
<td>Oval</td>
<td>GOY</td>
<td>8235</td>
<td>1.128</td>
<td>16.6</td>
<td>14.7</td>
<td>359</td>
<td>2.76</td>
<td>6.36</td>
<td>85.7</td>
<td>78</td>
<td>5.9</td>
</tr>
<tr>
<td>T.White</td>
<td>T.Nadu</td>
<td>P</td>
<td>Spindle</td>
<td>White</td>
<td>8283</td>
<td>0.975</td>
<td>14.1</td>
<td>14.5</td>
<td>438</td>
<td>2.05</td>
<td>7.97</td>
<td>86.2</td>
<td>80</td>
<td>9.8</td>
</tr>
<tr>
<td>Hosa Mysore</td>
<td>Karnataka</td>
<td>P</td>
<td>Oval</td>
<td>GRY</td>
<td>8631</td>
<td>1.219</td>
<td>19.1</td>
<td>15.7</td>
<td>351</td>
<td>2.82</td>
<td>6.49</td>
<td>82.4</td>
<td>73</td>
<td>9.5</td>
</tr>
<tr>
<td>Kolar Gold</td>
<td>- do -</td>
<td>P</td>
<td>Oval</td>
<td>White</td>
<td>8439</td>
<td>1.307</td>
<td>20.3</td>
<td>15.5</td>
<td>527</td>
<td>2.23</td>
<td>7.41</td>
<td>79.4</td>
<td>80</td>
<td>5.4</td>
</tr>
<tr>
<td>MU1</td>
<td>- do -</td>
<td>P</td>
<td>Oval</td>
<td>GRY</td>
<td>7471</td>
<td>1.179</td>
<td>18.4</td>
<td>15.6</td>
<td>405</td>
<td>2.22</td>
<td>6.03</td>
<td>87.0</td>
<td>67</td>
<td>8.1</td>
</tr>
</tbody>
</table>
Table 8. Genetic diversity in the mulberry silkworm germplasm stocks (hibernating) available in India.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Available diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>Fecundity (No.)</td>
<td>251</td>
</tr>
<tr>
<td>Total larval duration (D:H)</td>
<td>23.00</td>
</tr>
<tr>
<td>V age larval duration (D:H)</td>
<td>5.06</td>
</tr>
<tr>
<td>Yield/10,000 brushed larvae</td>
<td></td>
</tr>
<tr>
<td>a. Number</td>
<td>3101</td>
</tr>
<tr>
<td>b. Weight (Kg)</td>
<td>4.197</td>
</tr>
<tr>
<td>Single cocoon wt (g)</td>
<td>0.722</td>
</tr>
<tr>
<td>Single shell wt (g)</td>
<td>0.116</td>
</tr>
<tr>
<td>Shell Ratio (%)</td>
<td>10.1</td>
</tr>
<tr>
<td>Filament length (m)</td>
<td>515</td>
</tr>
<tr>
<td>Denier</td>
<td>1.18</td>
</tr>
<tr>
<td>Raw silk (%)</td>
<td>5.5</td>
</tr>
<tr>
<td>Reelability (%)</td>
<td>65</td>
</tr>
</tbody>
</table>

Table 9. Biochemical parameters used for characterisation of mulberry silkworm germplasm stocks in India.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Existing diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>A. Quantitative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Digestive amylase</td>
<td>µg meltodextrin released/20 µl/30 min</td>
<td>22.57</td>
</tr>
<tr>
<td>2. Digestive alkaline phosphatase</td>
<td>µg alkpo4/50 µl/30 min</td>
<td>2.82</td>
</tr>
<tr>
<td>3. Protease (pH 7)</td>
<td>µg L-tyrosine released/50 µl/15 min</td>
<td>1.29</td>
</tr>
<tr>
<td>4. Protease (pH 10)</td>
<td>- do -</td>
<td>1.88</td>
</tr>
<tr>
<td>5. Invertase</td>
<td>µg glucose released/10 µl/h</td>
<td>178.0</td>
</tr>
<tr>
<td>6. Haemolymph amylase</td>
<td>µg meltodextrin/µl/30 min</td>
<td>0.276</td>
</tr>
<tr>
<td>7. Haemolymph glucose dehydrogenase</td>
<td>Activity/µl/min</td>
<td>26.2</td>
</tr>
<tr>
<td>8. Haemolymph trehalase content</td>
<td>µg trehalose/10µl</td>
<td></td>
</tr>
</tbody>
</table>

B. Qualitative

1. Haemolymph protein banding pattern by native PAGE
2. Haemolymph esterase isozymes
3. Digestive amylase (anodic and cathodic) isozymes.
different countries in the early 20th century and developed them into pure breeds to increase the genetic resources. The genetic diversity in present day mulberry germplasm stocks is presented (Table 8). It may be seen from the synthetic stocks that the variability of these breeds is very high for fecundity, fifth age larval duration, yield by number and weight (yield / 10,000 larvae). Consequently, the commercial traits like single cocoon weight, shell weight, filament length were also found to range from very low to high.

3.2.2. Biochemical characterization

During the breeding process, a large number of silkworm eggs have been isolated and stabilized whose maintenance and use in India warrants proper classification and listing. Attempts have been made to classify these races on the basis of bio-chemical parameters along with other traits used for characterization of mulberry germplasm stocks in India. Both qualitative and quantitative parameters viz., isozymes, activity of digestive amylase, digestive alkaline phosphatase, protease, invertase, haemolymph amylase, haemolymph glucose-6 phosphate dehydrogenase, haemolymph trehalose content were studied to understand the diversity of breeds. The minimum and maximum values of various bio-chemical traits used for characterization of the silkworm breeds is shown (Table 9). Both diapausing and some non-diapausing strains show negligible digestive amylase activity at pH range of 3-11, while most non-diapausing strains registered strikingly higher amylase activity at pH 3 to 9 (Abraham et al., 1992). Polyacrylamide gel electrophoretic analysis revealed the occurrence of only anodal digestive and haemolymph amylases in the diapausing strains, whereas both cathodal and anodal isoymes were seen in the digestive fluid of the non-diapausing strain.

Mulberry silkworm germplasm stocks also showed genetic variability in terms of haemolymph esterase isozyme (both a and b) patterns. A total of 12 bands with 3 bands each in the four (A, B, C and D) zones were detected, but none of the strains showed all the bands. B1 and B2 bands were present in all stocks whereas the high mobility band A1 was observed in only one and A3 in two stocks, respectively. A2 was found in all the hibernating strains and may be associated with diapause.

3.2.3. Biometrical characterization

In Central Sericultural Research and Training Institute, Mysore, attempts were
also made to classify stocks by Mahalanobis genetic divergence techniques (Rao et al., 1989; Jolly et al., 1989). In addition, a detailed analysis was undertaken to test the efficacy of hierarchical agglomerative clustering (UPGMA method) in grouping the races and strains of the mulberry silkworm, *Bombyx mori* L. The analysis was based on data from 54 selected races/strains of different geographic origins with varying yield potentials (Chatterjee and Datta, 1992). The results indicate that seven clusters can be found with yield parameters alone, whereas the inclusion of biochemical parameters in clustering resulted in only two broad groups: one having all the breeds with high cocoon weight and shell weight and the other having all the low yielding silkworm strains. Further sub-groupings under these two groups highlights genetic differences associated with the differentiation of various groups of races in temperate and tropical races as well as their significance for silkworm breeding (Chatterjee and Datta, 1992). This study represents a novel approach to analyze the relationship of different silkworm races and breeds on the basis of Squared Euclidean distance. It suggests the prospects of easy handling of a large number of silkworm races and strains and supports the observation on clustering system proposed by others (Peters and Martinelli, 1989). The results also highlighted the use of biochemical parameters in obtaining more desirable information on genetic relationships.

### 3.2.4. Characterization using DNA markers

Techniques of studying DNA polymorphism by RFLP, minisatellite DNA and RAPD markers have been recently initiated to further characterize our mulberry silkworm germplasm stocks. DNA profile by RAPD technique of 13 silkworm genotypes of diverse origin were studied by generating 216 amplified products using 40 random primers (Nagaraja and Nagaraju, 1995). Pair-wise comparison of amplified products and hierarchical clustering on the basis of Nei-Li Similarity Coefficient resulted in two distinct groups of six diapausing and seven non-diapausing genotypes which suggests that RAPD could be used for characterization of silkworm stocks. Further, DNA-finger printing of silkworm strains by first digesting the genomic DNAs with BstNI and HinHI restriction enzymes followed by hybridization with banded Krait mini satellite DNA, Bkm-2(8), minisatellite probe revealed 9-31 discrete intense bands some of which were specific to the genotype (Nagaraju et al., 1995). This once again highlights the potentials of these methods for varietal identification.
3.3. Conservation

3.3.1. Non-mulberry

At the initial stage of the establishment of the Research Institute at Ranchi, all the eco-races were brought to Ranchi and maintained in the established bush plantations of *Terminalia tomentosa*, *T. arjuna* and *Shorea robusta*. Later, it was realised that many commercial traits had deteriorated or modified due to the maintenance of different geographical populations in a new niche. Hence, the Central Silk Board decided that these eco-races are to be maintained in their respective ecological niches where they were available from time immemorial. At present, CTR&TI, Ranchi is maintaining these breeds through different Basic Seed Multiplication & Training Centres (BSM&TC) and Regional Tasar Research Stations established in different states of India. The cocoons of different ecological races are also preserved in the forest areas without seasonal collection as practiced by the tribals. It has been found that restricted multiplication of the eco-populations in different pockets have strengthened the availability of *A. mylitta* in the various regions of the country. The tribals, who are involved in tasar culture are trained periodically not to interfere in natural populations that are maintained in the forest.

For conservation of the wild *A. roylei* as well as the synthetic species, *A. proylei* in their respective eco-geographical environments, Central Silk Board has established three Regional Tasar Research Stations, one at Imphal in the North-Eastern state of Manipur; another at Batote (Jammu & Kashmir state) in the Western sector and the third one at Bhimtal in the Northern state of Uttar Pradesh.

The conservation of Muga silkworm has received attention, as this species is being endangered. The Assam State Sericulture Department has earmarked "Muga village Grazing Reserves" which are being allotted to the muga rearers with a nominal 10 % levy. This may on the one hand, save the habitat of muga silkworm from being depleted or diverted for any other purpose and on the other hand, the muga silkworm rearers are encouraged to keep up the traditional avocation. Central Silk Board had established a Regional Muga Research Station in 1981 at Boko (Assam) which has now been upgraded to a full-fledged Research Institute. It endeavors to solve technical problems related to muga sericulture and thereby aid its conservation.

Central Silk Board of India has also established an Eri Research Station at
Mendipathar in Assam which is maintaining a number of lines to fix up morphological segregants and some epigenetic variations. It is also actively concerned with the characterization and conservation of the wild *P. cynthia* species in its own habitat.

### 3.3.2. Mulberry

For conserving mulberry silkworms, Central Silk Board has established an exclusive Germplasm Maintenance Station at Hosur (Tamil Nadu state). Besides, the germplasm stocks are maintained at different centres of the Central Silk Board as indicated below:

<table>
<thead>
<tr>
<th>Centre</th>
<th>No. of mulberry silkworm stocks maintained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mulberry and Silkworm Germplasm Station, Hosur, Tamil Nadu</td>
<td>226</td>
</tr>
<tr>
<td>CSRTI, Mysore, Karnataka</td>
<td>200</td>
</tr>
<tr>
<td>CSRTI, Berhampore, West Bengal</td>
<td>24</td>
</tr>
<tr>
<td>RSRS, Kalimpong, West Bengal</td>
<td>52</td>
</tr>
<tr>
<td>CSRTI, Pampore, Jammu &amp; Kashmir</td>
<td>129</td>
</tr>
<tr>
<td>RSRS, Dehradun, Uttar Pradesh</td>
<td>24</td>
</tr>
<tr>
<td>RSRS, Coonoor, Tamil Nadu</td>
<td>30</td>
</tr>
</tbody>
</table>

### 3.4. Conclusion

To conserve commercial live stock of silkworm and genetic material, India has established the Mulberry and Germplasm Station at Hosur in Tamil Nadu state for the mulberry silkworm *Bombyx mori*. The collection at this centre exceeds more than 100 varietal stocks of commercial hybrids and nearly 200 genetic stocks. The unique distinction of India for its rich fauna of sericigenous insects of non-mulberry crop is well known. North-Eastern India including states of Assam, Arunachal Pradesh and Manipur has been identified as the natural habitat of nearly a score of sericigenous insects. Many of these fauna are threatened with extinction. It is suggested, therefore, to have an International centre for collection and maintenance of silkworm live stocks, characteristic of this region. Possibilities of having regional centers in other Asian countries especially China and Japan is proposed. A special emphasis should also be made on collection and preservation of a number of non-mulberry silkworms especially, of the *Anaphaee* genus in Africa.
References


Chicken Genetic Resources in Japan and New Evaluation Research

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Abstract
There are many chicken genetic resources in Japan. They consist of three groups. The first group consists of the native breeds which were selected before 1868. The second group are the breeds imported after 1868. The third group are the breeds derived from crosses between native and introduced breeds.

Most of the native breeds were developed for special plumage, crowing or cockfighting. Seventeen breeds have been designated natural treasures of Japan. Most of them have low egg and meat productivity. Recently some native breeds have been used to improve chicken meat quality. Japanese native breeds are different from commercial breeds based on gene frequency. In the MAFF Gene Bank Project, we are collecting semen from native breeds and preserving it in a frozen state. We have initiated research on chicken microsatellite DNA for use as genetic markers in evaluating chicken genetic resources.

Introduction
Chicken eggs and meat in Japan and many other countries today are produced from a few chicken crossbreeds. Hens for egg production are crossbreeds between some lines of White Leghorn or Rhode Island Red. Chickens for meat production are mainly crossbreeds between White Plymouth Rock and White Cornish. These factory chickens are very high yielding, but they require a carefully controlled environment.

In contrast, traditional or heritage breeds may have survival traits no longer found in industrial breeds, such as, climatic adaptation and resistance to diseases. These breeds are an important reservoir of genetic diversity.

In Japan, there are many chicken breeds including industrial breeds. However there are no indigenous chickens in Japan in the true sense of the word. Chicken breeds in Japan were introduced into Japan at various times. These breeds have broad genetic diversity and are important as genetic resources.
Japanese Chicken Genetic Resources

Japanese chicken genetic resources can be divided into three groups according to their origin and when they were introduced into Japan (Obata et al., 1994). The first group consists of the breeds developed before the Meiji Restoration of 1868. Between 1635 and 1854, Japan was closed to all countries except for China and Holland. In that period many unique breeds were developed for special plumage or crowing in Japan. These breeds are called "native breeds". The second group are the breeds introduced after 1868. Many chicken breeds have been imported from America and Europe for egg and meat production and to improve Japanese native chickens. These breeds are called "imported breeds". The third group are the breeds derived from crosses between the native and imported breeds. These chickens are called "crossbreeds".

Japanese Chicken Breeds

In Japan all native breeds and some crossbreeds are called "Japanese chicken breeds" (Mitsui and Koyama, 1979; Okada, 1991). Most of them are specialized for plumage, crowing or game. There are more than 30 distinctive breeds (Table 1). Seventeen of them have been designated as natural treasures of Japan. The estimated population size of each Japanese chicken breed is shown (Table 2). Most Japanese chicken breeds have low egg production and meat yield. These breeds are reared in small populations by a few people as a hobby. Many of such breeds are in danger of disappearing. Japanese chicken breeds retain genes of old Japanese chickens and are important as genetic resources.

Description of Japanese Chicken Breeds

Onaga-dori (Mitsui and Koyama, 1979; Okada, 1991) (Figure 1)

Onaga-dori is a native breed. It is the most famous Japanese breed because males have very long tail feathers. This breed can be found in Kochi Prefecture, Shikoku. The main tail feathers of males do not molt and can grow by 90 cm each year. The tail feathers sometimes grow longer than 8 meters, and the record is 12 meters. To grow such a long tail feather, special care in feeding and management is needed and the technique is difficult. This breed is thought to have originated from a mutation of Shokoku or Totenko breeds, in the 17th or 18th century.
Table 1. Japanese chicken breeds

<table>
<thead>
<tr>
<th>Group</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Breeds</td>
<td>Chabe*, Onagadori*, Hinaidori*, Satsumadori*, Jidori*, Shamo*,</td>
</tr>
<tr>
<td></td>
<td>Kurokashiwa*, Ukokkei*, Minohiki*, Uzurachabo*, Minohikichabo*,</td>
</tr>
<tr>
<td></td>
<td>Ingikei, Shibattori, Tokuchi-jidori, Sado-higejidori, Kurukodori,</td>
</tr>
<tr>
<td></td>
<td>Utai-chan, Iwate-jidori, Tsushima-jidori, Tokara-jidori, Okinawa-higejidori</td>
</tr>
<tr>
<td>Crossbreeds</td>
<td>Tosa-kuin, Kumamoto, Nagoya, Izumo-cochin, Mikawa, Miyajidori</td>
</tr>
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</table>

* designated as Natural National Treasure

Table 2. Estimated number of chickens

<table>
<thead>
<tr>
<th>Number</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 5000</td>
<td>Shamo, Chabo</td>
</tr>
<tr>
<td>2000 - 5000</td>
<td>Hinaidori, Satsumadori, Nagoya, Ukkokei</td>
</tr>
<tr>
<td>1000 - 2000</td>
<td>Totenko, Minohikichabo, Uzurachabo, Tomaru, Shokoku, Gifu-jidori</td>
</tr>
<tr>
<td>500 - 1000</td>
<td>Koeyoshi, Minohiki, Tosa-jidori, Utai-chan, Mikawa</td>
</tr>
<tr>
<td>500 &gt;</td>
<td>Jitokko, Kawachiyakko, Mie-jidori, Iwate-jidori, Onagadori, Shibattori,</td>
</tr>
<tr>
<td></td>
<td>Kurokashiwa, Tosa-kuin, Kumamoto, Tsushima-jidori</td>
</tr>
<tr>
<td>rare</td>
<td>Izumo-cochin, Sado-higejidori, Kurukodori, Tokuchi-jidori, Miyajidori</td>
</tr>
</tbody>
</table>

modified from Okada, 1988

Tomaru (Mitsui and Koyama, 1979; Okada, 1991) (Figure 2)

Tomaru is found in Niigata Prefecture, Honshu. This breed numbers about 1000 individuals. This breed is famous for the prolonged crowing ability of males. The cocks can crow for up to 18 seconds. Tomaru is a large breed and the adult male body weight is about 3.5 kg. This breed is thought to be derived from chickens imported from China in the 16th or 17th century.

In Japan, besides Tomaru, there are two other long-crowing breeds, Totenko and Koeyoshi. In Okinawa prefecture there is the Utai-chan with a crowing pattern very different from the long-crowing breeds.

Shamo (Mitsui and Koyama, 1979; Okada, 1991) (Figure 3)

Shamo is a typical game type chicken, and is found all over Japan. Shamo is divided into six varieties according to body size or shape. This breed is thought to be derive from a Malay-type chicken imported from Thailand in the 16th or 17th century. Recently Shamo is used for crossing to improve chicken meat quality.

The Satsuma-dori is similar to Shamo and is found in Kagoshima and Miyazaki
Figure 1. Onaga-dori. Photo by Mr. Shichiro Koyama was reproduced from Nihonkei Taikan (Mitsui and Koyama, 1979).

Figure 2. Tomaru. Photo by Mr. Shichiro Koyama was reproduced from Nihonkei Taikan (Mitsui and Koyama, 1979).

Figure 3. O-Shamo the biggest variety of Shamo. Photo by Mr. Shichiro Koyama was reproduced from Nihonkei Taikan (Mitsui and Koyama, 1979).

Figure 4. Katsura-chabo a typical variety of Chabo. Photo was reproduced by courtesy of Mr. Mitsuyoshi Tatematsu from Nihonkei Taikan (Mitsui and Koyama, 1979).
Figure 5. Nagoya. Photo was reproduced by courtesy of Aichi-ken Agricultural Research Center.

Figure 6. Gifu-jidori is a typical variety of Jidori. Photo by Mr. Shichiro Koyama was reproduced from Nihonkei Taikan (Mitsui and Koyama, 1979).

Prefectures, Kyushu. This breed was derived from crosses between Shamo and other native breeds in the 17th or 18th century. In Akita Prefecture there is the Hinai-dori which has a similar origin to Satsuma-dori.

Chabo (Mitsui and Koyama, 1979; Okada, 1991) (Figure 4)

Chabo is a bantam chicken. Its adult body weight is about 700g for the male. In Chabo there are 26 varieties with different plumage patterns. The features of Chabo are there small body size, large single comb, erect tail feathers and short shanks. This breed seems to derive from chicken imported from Indo-China in the 16th or 17th century. This breed is a hobby breed and is found throughout Japan.

Nagoya (Okada, 1991) (Figure 5)

Nagoya is a popular breed and was derived from crosses among indigenous chicken of the Nagoya area, such as Buff Cochin and other imported breeds. This breed has good egg production. Crossbreeds between Nagoya and White Leghorn were widely
used as layers until 1950's. Since then the number of the Nagoya chicken has decreased. Recently this breed has been used for meat production.

A similar breed is Mikawa. It was derived from crosses between Cochin and other imported breeds. The number of Mikawa chicken has declined because its meat quality is not as good as Nagoya chicken.

**Jidori** (Mitsui and Koyama, 1979; Okada, 1991) (Figure 6)

Jidori means an indigenous chicken. Jidori retains primitive chicken characteristics. Until the 19th century there were many local varieties of Jidori throughout Japan. Many varieties of Jidori were crossed with imported foreign breeds in the late 19th and early 20th century. Consequently many Jidori varieties became extinct. Now, there are three varieties, Gifu-jidori, Mie-jidori and Tosa-jidori. These have been designated natural treasures of Japan. Besides these varieties some other varieties can be found in various districts, but these are reared in such a small number that they face extinction.

**Use of Japanese Chicken Breeds**

Most of Japanese chicken breeds are not used for food. Recently some Japanese chicken breeds, particularly Shamo, Satsuma, Hinai-dori and Nagoya have been used to improve meat quality (Okada, 1991). They have been crossed with White Plymouth Rock or Rhode Island Red and are used to produce special regional delicacies (Fig 7). The meat from these crosses is considered better than that of the crossbreeds with White Plymouth Rock and White Cornish (Oishi, 1992).

**Present Status of Japanese Chicken Genetic Resources**

The number of breeds and lines of Japanese chicken genetic resources is estimated to be more than 200 including breeding stocks, experimental stocks and Japanese native chickens.

Most of the Japanese chicken breeds are reared by hobbyists and prefectural agricultural experiment stations. Their populations are small and usually number less than 100. Hobbyists rear these breeds in very small numbers of two to ten pairs.

Breeding stocks are reared by commercial breeding companies, some prefectural agricultural experiment stations, and two national livestock breeding stations. Most of the breeding stocks are lines of four representative breeds; White Leghorn, Rhode Island
Figure 7. Distribution of regional chicken delicacies. These prefectures are using the crossbreeds with Japanese chicken breeds and foreign breeds.
Red, White Plymouth Rock and White Cornish. In national livestock breeding stations, there are 14 White Leghorn lines, 3 Rhode Island Red lines, 9 White Plymouth Rock lines and 5 White Cornish lines.

Experimental stocks include many lines, such as lines established by selection experiments, developed for disease modelling, or having a specific gene or trait. These are reared by university laboratories, such as, Nagoya University, Hiroshima University and Tokyo University of Agriculture. Some national research institutes, such as, the National Institute of Animal Industry and National Institute of Animal Health, rear some of them also. Besides these organizations, there are some experimental animal companies.

There are also a few chicken breeds in small populations exhibited at zoos, parks and shrines.

In Japan in recent years, the number of prefectural agricultural experiment stations and hobbyists has gradually decreased.

**Japanese Chicken Breeds in MAFF Gene Bank Project**

According to some studies on blood type and protein polymorphism, Japanese chicken breeds are different from commercial breeds based on gene frequency (Hashiguchi et al., 1981; Okada et al., 1988; Tanabe et al., 1991). Japanese breeds have broad genetic diversity and are useful genetic resources. The Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) started the MAFF Gene Bank Project in 1985. In this project, the National Institute of Agrobiological Resources (NIAR) has been collecting semen of various Japanese chicken breeds and preserves samples in a frozen state. To date semen is fourteen breeds and five varieties have been collected.

**New Evaluation Research for Chicken Genetic Resources**

Blood type, blood protein polymorphisms, and morphological characteristics have been used to evaluate chicken genetic resources. These methods are insufficient because the number of genes that are involved for these traits is limited.

Molecular techniques offer many new tools to analyse genetic polymorphisms, for example, Restriction Fragment Length Polymorphisms (RFLP), Variable Number of Tandem Repeats (VNTR) which involve repeated short DNA sequences (Hillel et al., 1992). Microsatellites are a type of VNTR and are tandem repeats with between one and
four bases. Microsatellites are useful genetic markers (Hillel et al., 1992; Cheng and Crittenden, 1994). Microsatellites are evenly distributed throughout the chicken genome, they are highly polymorphic and can be assayed using the Polymerase Chain Reaction (PCR) methodology, and they are inherited as codominant alleles in a Mendelian manner.

To evaluate genetic characteristics of chicken genetic resources, we have initiated research on the chicken microsatellite. Takahashi et al. (1995) developed an efficient method to clone chicken microsatellites. Microsatellite markers can be used to assess genetic distance between breeds or between lines, and to assess inbreeding levels in populations, and for evaluation of genetic variation within lines (Hillel et al., 1992). In the future, microsatellite DNA markers will contribute to linkage analysis of quantitative trait loci (Chen and Crittenden, 1994). Research on the chicken microsatellite is expected to provide a new field for evaluation of chicken genetic resources.

References


Questions and answers in Session 1

To Dr. Zhanchiv

Q. Are there distinct differences in lines of sheep developed for different practical goals such as use of meat, skin, wool? (Bodó)
A. No, there are no big differences. (Zhanchiv)

Q. I heard about the reintroduction of animals which had died out in Mongolia? Is the reintroduction proceeding well or are there bad effects of acclimitization? Are they wild or domesticated? (Bodó)
A. They are acclimitized well in two regions. Most of them are from zoos and are domesticated. There are some from Russia which are half wild. (Zhanchiv)

Q. What is the importance of different sheep products in Mongolia? (Bodó)
A. Meat and wool (for carpets) are most important. The skin of the Karakul breed is important too. (Zhanchiv)

Q. How successful has the reintroduction of Prezwalski horses been in Mongolia? (Tan)
A. The horses from the Ukraine and Switzerland have performed satisfactorily in the wild in Mongolia. (Zhanchiv)

Questions to Dr. Nirasawa

Q. In Indonesia we have some problems with cryo-preservation of chicken semen. Could you tell us the method you use and have you tested the semen for quality after some time in storage? (Astuti)
A. We use the Lake's method for cryo-preservation. The motility is excellant. (Nirasawa)

Q. How many semen samples are taken to represent each Japanese native breed? (Li Ning)
A. For each breed the number ranges from 10 to 500. The number of birds from which semen is collected ranges from one to forty. (Nirasawa)
TECHNICAL REPORTS

Session 2

Genetic classification

Chairpersons
A. Amano
R.K. Datta
Genetic Classification of Asian Buffaloes Based on Biochemical and Molecular Polymorphisms

SOON GUAN TAN
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43400 UPM Serdang, Malaysia

Abstract
Electrophoretic analysis of 39 allozyme loci was conducted on 15 populations of phenotypically Swamp buffalo from Malaysia, Indonesia, Thailand, the Philippines, Australia, Sri Lanka and two populations of the Murrah breed of River buffalo. Nei's genetic distance estimates based upon the allelic frequencies between some pairs of genetically Swamp populations were of the same order of magnitude as those between recognized breeds of livestock in developed countries. An UPGMA dendrogram of three phenotypically Swamp Lankan buffalo populations cluster with the Murrah. Similar results were obtained when a subset of 9 phenotypically Swamp populations and two Murrah populations were typed for 21 nuclear microsatellite loci. However, there was greater differentiation among the populations. Nucleotide sequencing of a 303 base fragment of the mitochondrial cytochrome b gene revealed that the Murrah possessed a sequence that differed diagnostically from the Southeast Asian Swamp at two sites. The Lankan had the same genotype as the Murrah. Among the mainland Southeast Asian Swamp populations four genotypes were observed whereas only one of these was present in the insular populations. Nucleotide sequencing of a 158 base pair fragment of the mitochondrial D-loop from 80 animals revealed the presence of 33 genotypes. These genotypes separate into 2 major clusters. One major cluster contained genotypes found in the Swamp only and the other cluster contained genotypes that were found in the Swamp, the River (including Lankan) and in both the Swamp and the River types. Hence, both our DNA and allozyme data indicate that the Lankans are genetically River and that there is sufficient genetic variation present among the Swamp populations to warrant crossing between them as an alternative to the usual buffalo breeding practice of River (2n=50) and Swamp (2n=48) crosses.

Introduction
The Swamp buffalo *Bubalus bubalis*, is indigenous to Southeast China, Myanmar, Assam in India, Indochina, Thailand, Malaysia, Indonesia, the Philippines and Sri Lanka (Cockrill, 1987). It is much closer to the wild buffalo than are the buffaloes of India. They are different from the Indian breeds not only in appearance but also in behavior and use. In Malaysia, there is no wild buffalo and the habitat of the domestic buffalo is swamp or marshland. Hence, they had been named Swamp buffalo in contrast to the imported Indian Murrah buffalo which is called River buffalo (MacGregor, 1941) since their usual habitat is the river valley. Swamp buffalo has traditionally been used
for draught power in Southeast Asia and the River for milk and meat. However, the Swamp is being increasingly used for meat as Southeast Asia develops and in the U. S. A., Swamp buffalo meat is being promoted as a health food since its calorie, fat and cholesterol contents are lower than those of beef cattle. While the River buffalo has been selected to form improved breeds with high milk yield and differing in horn form, the Swamp has not been divided into different types. It has retained the low milk yield and primitive horn shape of the wild Arni buffalo found in Northern India and Indochina (Cockrill, 1987). Chromosomal studies showed that the morphologically Swamp indigenous buffalo of Sri Lanka (the Lankan buffalo) share the same diploid chromosome number of 50 with the River buffalo while the Southeast Asian Swamp buffalo had 48 chromosomes (Bongso et al., 1977).

Amano and his team have put in a monumental effort to study morphological, blood group and biochemical variation in several Asian livestock species over a period of more than twenty years (Amano et al., 1992 and references therein). However, since they were studying many species it is understandable that their results for biochemical polymorphism studies in buffalo were based on sampling a relatively small number of animals from each locality (ranging from 2 to 28) and from a modest number of biochemical loci (ranging from 8 in the earlier reports to 25 in the later ones). They found that the number of polymorphic loci for buffalo per country ranged from 4 to 7. Their results were published in separate reports for the livestock species of each country. However, in their report for Nepal (Amano et al., 1992) they included a UPGMA dendrogram of genetic relationships based on the genetic distances of Nei (1972) among 22 Asian water buffalo populations. This dendrogram showed that the 9 Southeast Asian Swamp populations clustered together while the 2 Lankan, 3 Nepali and 3 Bangladeshi native buffalo populations formed another cluster together with a Murrah population of River buffalo from Sri Lanka. The Philippine and Indonesian River populations formed a third cluster together with one Swamp X River hybrid population each from Indonesia and the Philippines respectively. Hence, it is likely that introgression of Swamp genes had occurred into the Indonesian (Amano et al., 1981) and the Philippines River populations.

Apart from the work of Amano and his colleagues little information is available about the population genetics of Southeast Asian Swamp and Lankan buffaloes. Consequently, we initiated this study in 1988 to elucidate the population structure of
water buffaloes through the use of biochemical and molecular techniques. We now have data on the same animals for nuclear electrophoretic and microsatellite loci and for mitochondrial DNA cytochrome b gene and D-loop control region. I shall now give a summary of the results that we currently have from our study of Swamp, River and Lankan buffaloes from Southeast Asia, Sri Lanka and Australia.

Materials and Methods

Blood samples were collected from 15 Swamp buffalo populations between 1988 and 1991 in Malaysia (three localities: Trengganu, Sabah and Sarawak), Indonesia (Bogor, Medan and Sulawesi), the Philippines (Mindanao), Thailand (Chengmai, Haadyai, Surin and Kam Paeng Seng), Sri Lanka (South, North-central and Kandy) and Australia (Northern Territory) and from two populations of the Murrah breed of River buffalo (Universiti Pertanian Malaysia, Serdang, Malaysia and Kotaliya in Sri Lanka). The samples were collected, processed and separated into plasma, red cells and buffy coat, transported in liquid nitrogen to Kuala Lumpur and stored at -70°C (Tan et al., 1991) until used.

The plasma and red cells were used for analyzing nuclear biochemical markers by cellulose acetate, polyacrylamide and starch gel electrophoresis (Tan et al., 1991) and polyacrylamide gel isoelectric focusing (Tan et al., 1993). The BIOSYS-1 computer package (Swofford and Selander, 1989) was used to calculate Nei's genetic distances (Nei, 1972) based on the allelic frequencies and to generate an Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) dendrogram (Sneath and Sokal, 1973). DNA extraction, polymerase chain reaction (PCR) and cycle sequencing for a 303 base fragment of the mitochondrial DNA cytochrome b gene were done following a user-friendly system (Lau et al., 1995). This PCR and cycle sequencing system was also used to sequence a 260 base pair segment of the mitochondrial D-loop region using primers derived from the known sequence of bovine mtDNA. However, due to unequal reading of the sequences at the terminii, only a common 158 base sequence which was obtainable for all the 80 animals typed were compared and 33 D-loop genotypes were observed. The frequencies of these genotypes were used to calculate the $D_A$ genetic distances of Nei et al. (1983) among the 11 populations (same populations with microsatellite loci data) from which the animal were obtained and these genetic distance values were used to cluster the populations by the neighbor joining method. The 33 D-loop
Table 1. Sequencing of a 303 nucleotide fragment of the mDNA cytochrome b gene of Asian Water Buffaloes form 14 populations

<table>
<thead>
<tr>
<th>Localities/Breeds</th>
<th>n</th>
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<th>4</th>
<th>5</th>
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<td>Murrah, Kotaliya, S.Lanka (n=5)</td>
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<td>Murrah, UPM, Malaysia (n=4)</td>
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<td>4</td>
<td></td>
<td></td>
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<tr>
<td>Swamp, Terengganu, Malaysia</td>
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<td>3</td>
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<td>Swamp, Haadyai, Thailand</td>
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<td>Swamp, Jawa, Indonesia</td>
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<td>Swamp, Mindanao, Philippines</td>
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<td>Swamp, N.Territory, Australia</td>
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</table>

Genotypes observed were also individually clustered using the proportion of nucleotide differences and neighbor joining with the corresponding bovine sequence as the outgroup. Twenty one microsatellite loci were typed for using the method of Moore et al. (1995) from eight Southeast Asian Swamp populations namely Surin, Trengganu, Sabah, Sarawak, Bogor, Sulawesi, Musuan and Australia; from the South Lankan population and from the Murrah populations of Sri Lanka and Malaysia. The microsatellite data were analysed in a manner similar to that used for the allozyme data.

Results

Data are presently available for 39 biochemical genetic loci in 17 populations of which two are Murrah populations (Tan et al., 1994). In addition, the three phenotypically Swamp Lankan populations were shown electrophoretically to be of River type since they clustered with the two Murrah populations on the UPGMA dendrogram presented in Figure 1 (Barker et al., 1991). Hence, there are 12 genetically Swamp populations, three from Indonesia, one from the Philippines, four from Thailand, three from Malaysia and one from Australia. In most populations, about 50 animals were typed for the polymorphic loci and 25 for the monomorphic ones. Of the 39 loci, 14 were polymorphic in Swamp populations and one diaphorase-1 zone 2 was polymorphic only
in Murrah. Of the 14 polymorphic Swamp loci, glyoxalase-1 was polymorphic in four populations and the other 13 (acid phosphatase, albumin, amylase, catalase, diaphorase-1 zone 3, group-specific component, glutamate pyruvate transaminase, malate dehydrogenase, malic enzyme, peptidase-B, peptidase-C, protease inhibitor and transferrin) were polymorphic in at least nine of the populations. The percentage of polymorphic loci ranged from 12.8 in the Northern Territory feral population to 35.9 in Trengganu. Mean heterozygosity was also lowest in the Northern Territory population, suggesting that this population probably underwent a genetic bottleneck on introduction to Australia. Of the Southeast Asian populations, those in Sabah and Sarawak showed lower genetic variability than others. Measures of genetic variability in the Murrah and Lankan populations were similar to those for the Swamp populations. The matrix of genetic distances among each pair of Swamp populations showed values ranging from 0.002 to 0.058. Within countries, the highest value was for Malaysia (0.026) primarily due to differentiation of the Sabah population. Our estimates of genetic distances among Swamp buffalo populations were of the same order of magnitude as distances among recognized livestock breeds in developed countries for example those for European breeds of cattle (Gonzalez et al., 1987). The three Lankan populations were most closely related to Murrah from Sri Lanka (D=0.011) and then to Murrah from Malaysia (D=0.028). This group of populations was distinct (D=0.052) from the Swamp populations (Barker et al., 1992).

About 20 animals from each of the 8 Swamp populations, the South Lankan population and the 2 Murrah populations were analysed for 21 nuclear microsatellite loci (Barker et al., 1995). All 21 loci were polymorphic with the number of alleles detected per locus ranging from 2 to 14 in Swamp buffalo and from 2 to 18 in River buffalo, with one or more alleles at each locus shared between the two buffalo types. The mean number of alleles per locus ranged from 2.6 (Sarawak) to 5.3 (Lankan buffalo). The mean observed heterozygosity among the Swamp populations ranged from 0.589 (Surin) to 0.400 (Sabah) while among the River group the Sri Lanka Murrah had the highest value of 0.613 while the values for the Lankan buffalo and the Malaysian Murrah were the same (0.531). The Australian population had a mean heterozygosity value of 0.409 and 3.0 alleles per locus. Nei's (1972) genetic distances (D) among the Swamp populations ranged from 0.026 between the Surin and Trengganu populations to 0.492 between the Australian and Sarawak populations. Among the River group the lowest D
value (0.041) was between the two Murrah populations while the highest D value of 0.067 was between the Lankan and the Murrah population from Sri Lanka. The highest D value among the 11 Asian buffalo populations was between the Sabah Swamp population and the Murrah population from Malaysia (1.125). In the UPGMA dendrogram based on the 21 microsatellite loci and Nei's genetic distances (D) the Lankan and the 2 Murrah populations form one cluster while the 8 Swamp populations form another cluster (Figure 2).

When genetic distances (D) based on protein data for 25 polymorphic loci were calculated for the same 11 populations with microsatellite data available, it was observed that the mean D among the 8 genetically Swamp populations was 0.098, among the 3 genetically River (which included the Lankan) populations it was 0.046 and for the genetically Swamp versus River populations it was 0.173. In comparison, the values based on the 21 microsatellite loci were 0.228, 0.056 and 0.897 respectively (Barker et al., 1995). Thus, microsatellite loci showed much greater discrimination among populations in terms of larger genetic distance values than protein loci. MacHugh et al. (1994) typed 11 microsatellite loci in 6 European breeds of dairy and beef cattle namely Aberdeen Angus, Hereford, Simmental, Charolais, Jersey and Friesian. The average of all pairwise genetic distances (D) among these 6 breeds was 0.083. Therefore, the magnitude of genetic differentiation (D=0.228) based on 21 microsatellite loci among the 8 genetically Swamp Southeast Asian buffalo populations is much greater than that among well recognized and long-established cattle breeds (Barker et al., 1995).

Our sequencing of a 303 base fragment of the mitochondrial cytochrome b gene of Swamp buffalo from the three populations from Malaysia, four populations from Thailand, two from Indonesia (Bogor in Java island and Sulawesi), one from the Philippines (Musuan in Mindanao island) and one from Australia and the two Murrah populations revealed that the Swamp and the Murrah River buffaloes differed diagnostically at two sites. The South Lankan population possessed the River genotype (Figure 3). Among the Southeast Asian Swamp four genotypes were observed in the mainland populations whereas only the most frequent one of these was present in the insular populations (Sabah, Sarawak, Mindanao, Bogor and Sulawesi). All the nucleotide substitutions observed were transitions and silent.

Comparison of the sequences obtained from 80 animals for a 158 base fragment of the mitochondrial D-loop region revealed the presence of 33 genotypes. Clustering of
Figure 1. Dendrogram of genetic distances among 12 swamp and 5 river buffalo populations. This dendrogram is based on 39 allozyme loci (from Barker et al., 1991).

Figure 2. Genetic relationships among 11 Asian water buffalo populations based on 21 nuclear DNA microsatellite loci. UPGWA tree (from Barker et al., 1995).
Figure 3. Phylogeny of 33 buffalo mtDNA D-loop haplotypes (BOV=bovine sequence as outgroup), using proportion of nucleotide differences and neighbor joining. *haplotype found in river buffalo only, **haplotype found in both river and swamp, and all others found in swamp only (from Barker et al., 1995).
Fig 4. Phylogeny for 11 buffalo populations, using mtDNA D-loop haplotype frequencies in each population (from Barker et al., 1995)

these 33 genotypes by the neighboring joining method (Saitou and Nei, 1987) resulted in two major clusters (Figure 3). One major cluster contained genotypes found in the Swamp only and the other cluster contained genotypes that were found in the Swamp, the River (including the Lankan) and in both the Swamp and River types. When the populations were clustered based on the $D_A$ genetic distances (Figure 4) there were similarities to the microsatellite (Figure 2) and allozyme (Figure 1) based clusterings. However, the Swamp population from Bogor, Indonesia clustered with the three River (including Lankan) populations. But with 33 genotypes and sequences available for only 5 to 10 animals per population (average 7.3) the data are inadequate for reliable conclusions to be made. Hence, more animals per population and more populations should be sequenced for the D-loop since our data showed the presence of a substantial amount of variation and seemed to indicate that the Swamp is the ancestral type (Barker et al., 1995).

Discussion

Both our biochemical and molecular results revealed that the phenotypically Swamp Lankan buffalo is actually of River type genetically. The geographically
separated Swamp buffalo populations of Southeast Asia are generally phenotypically similar but there is sufficient genetic variation present among them for us to suggest to buffalo breeders to consider crossing between animals from genetically distant Swamp populations as an alternative to relying on crosses between Swamp and River buffaloes in order to obtain hybrid vigour (Tan et al., 1994). Moreover, crossing among the Swamp would avoid any future fertility problem that may arise at the village level as a result of the current widespread practice in countries like the Philippines (Steane et al., 1994) of crossing between the River and the Swamp which differ in their chromosome numbers. Our results emphasize the importance of conserving Swamp buffalo populations from different geographical areas of Asia in order to preserve and use the genetic variations present in them so as to improve the Swamp buffalo herds at the village and farm levels. In addition, our data support the hypothesis of Amano et al. (1992) that the Swamp and the River buffaloes have been domesticated from different origins and that mitochondrial DNA polymorphisms could be used to distinguish between them (Amano et al., 1994, Tanaka et al., 1995).

It is proposed that large scale population genetic studies such as ours and those of Amano and his colleagues be extended to the Swamp populations of China, Myanmar, Vietnam, Laos and Cambodia and to the River populations of India and Pakistan in order to ascertain the levels of genetic variation present in them and to determine their genetic relationships to one another as well as to the buffalo populations which we have studied. Also it would be desirable if the centres of origin of the Swamp and River buffaloes of Asia could be identified. Animals from more populations in mainland Southeast Asia should be sequenced for cytochrome b and more populations as well as more animals per population should be sequenced for the D-loop region. At the Department of Biology, Universiti Pertanian Malaysia, Serdang, Malaysia, we already have stored in our deep freezers buffy coat samples from animals (random animals as well as those from complete families) and populations which ought to be, but which have not yet been, typed for DNA markers like microsatellites and mitochondrial D-loop and cytochrome b. Although they have all been typed using allozymes, we have subsequently demonstrated that DNA markers are far more informative. Our current funding to do such DNA studies have been exhausted after we analysed a subset of these animals and populations for the DNA markers. However, it should be emphasized that overall the genetic relationships obtained among the populations that we studied are similar irrespective of whether
biochemical (protein-level) or molecular (DNA-level) markers are used to obtain them. This overall similarity greatly strengthens the confidence that can be placed on the measured relationships among the Asian water buffalo populations which we have studied.

Acknowledgement

I thank the Australian Centre for International Agricultural Research for funding this work through PN 8364 and a project grant Molecular genetic analysis on Swamp buffalo populations, the Malaysian Programme for Intensification of Research in Priority Areas (IRPA) through grant 1-07-05-057 from the Ministry of Science, Technology and the Environment, Malaysia and Universiti Pertanian Malaysia through grant 50205-95-50. This report is mainly based on work done in collaboration with J.S.F. Barker (University of New England, Australia), T.K. Mukherjee and O.S. Selvaraj (University of Malaya, Malaysia), C.H. Lau and K. Yusoff (Universiti Pertanian Malaysia), S.S. Moore, R. Drinkwater and D.J.S. Hetzel (CSIRO, University of Queensland, Australia). The invaluable assistance of numerous University and Veterinary Department staff from Australia, Indonesia, the Philippines, Sri Lanka, Thailand and Malaysia during sample collection is gratefully acknowledged.

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Phylogenetic Relationships and Conservation of Bovine and Bubaline Species in Asia

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Abstract

A variety of wild bovine and bubaline species still inhabit restricted areas of South and Southeast Asia. These species are more or less related to the ancestor of domestic cattle or water buffaloes phylogenetically. However, most of these species are thought to be declining in number and endangered or threatened with extinction. Detailed biological characterization is inadequate and schemes for conservation are insufficient.

The wild species of bovine in Asia consist of, the banteng (Bos javanicus), gaur (Bos gaurus subspp.), yak (Bos mutus), and kouprey (or Indo-Chinese forest ox) (Bos sauveli). The kouprey is now very rare and little is known of its present status. No concrete genetic or reproductive data on the kouprey is available to compare with that of the related species, although very detailed morphological or osteometrical characteristics have been described (Coolidge, 1940). The other wild species of bovine can help elucidate the phylogenetic relationship of domesticated cattle, because hybrid offspring (mostly in the female) between these wild species and domesticated cattle are fertile (Figure 1).

At least four wild species are generally accepted in the genus Bubalus; the Asian wild buffalo (Bubalus bubalis), the lowland anoa (Bubalus (Anoa) depressicornis) and the mountain anoa (Bubalus (Anoa) quarlesi) endemic to Sulawesi, Indonesia, and the tamaraw (Bubalus mindorensis) endemic to Mindoro Island of Philippines (Figure 2).

In this paper, we elucidate phylogenetic relationships of bovine and bubaline species by summarizing our previous genetic or molecular evolutionary studies of them. We also discuss how these basic data are significant in relation to conservation and use of these wild species, which are closely related to domesticated cattle or water buffaloes.

Phylogenetic study on bovine species

Molecular evolutionary relationship of bovine hemoglobin-β variants

In native cattle populations of Southeast Asia, three hemoglobin-β globin variants are generally found, namely Hbβ-A, Hbβ-B and Hbβ-XBali. These variants are well known to be expressed by the allelic genes, HbbA, HbbB and HbbX, respectively (Namikawa and Widodo, 1978).

We determined the amino acid sequence of Hbβ-XBali of the Bali cattle (Bos javanicus) and compared it with two common variants, Hbβ-A and Hbβ-B, previously reported in domesticated cattle (Namikawa et al., 1983; Namikawa et al., 1987). The
lysyl residue at Hbβ-A18 was substituted by the histidinyl residue at the Hbβ-X\textsuperscript{Bali} 18. This change requires two DNA base substitutions at the triplet codon. The Hbβ-A differs from Hbβ-B in the residues of 15, 18 and 119. This finding indicates that a common ancestor of Hbβ-B of the domesticated cattle and Hbβ-X\textsuperscript{Bali} of Bali cattle first diverged from Hbβ-A of the cattle through the Lys→His substitution at the residue 18. Bali cattle has generally been accepted to be a domesticated form of the banteng (*Bos javanicus*).

The evolutionary relationship of Hbβ-A, Hbβ-B and Hbβ-X\textsuperscript{Bali} globin sequences involves different allelic frequencies of the HbbA, HbbB and HbbX explains genetic differences between native cattle populations in Southeast Asia.

**Distribution of hemoglobin β globin alleles in native cattle populations in Asia**

The geographical distribution of Hbb alleles in the cattle of Eastern Asia has been described (Namikawa, 1981)(Figure 3). All of the native cattle populations in Southeast Asia are polymorphic with the HbbA, HbbB and HbbX alleles. The humpless or northern type cattle are nearly fixed with the HbbA, and the Indian zebu cattle was highly polymorphic with the HbbA(0.64) and HbbB(0.34). The HbbX frequency was uniquely high in the Bali cattle of Indonesia(0.79) and decreased in populations distant from the Bali cattle population.

On the assumption of the three-way hybridization process in development of the Asian cattle, amount of gene-flow from Indian zebu (P\textsubscript{Ind}), the Bali (P\textsubscript{Bal}) and the northern type (P\textsubscript{Nor}) were estimated for native cattle populations of eastern Asia. P\textsubscript{Nor} values dominated in the Japanese, Korean cattle and some Thai native cattle, while the distribution of P\textsubscript{Ind}, P\textsubscript{Bal} and P\textsubscript{Nor} in Southeast Asian native cattle suggests that the Indian zebu cattle as well as the Bali cattle and northern type cattle have contributed to these native populations at various levels (Figure 4).

**Genetic relationship of cattle breeds/populations using multi-genetic characters**

Using genetic data on the nine blood group systems and three biochemical polymorphic loci, principal component analysis was conducted for many Asian cattle populations (Namikawa et al., 1984)(Figure 5). The results showed a large genetic divergence between the northern type cattle (Korean, Japanese cattle and European breeds) and southern type cattle (Indian zebu, many Southeast Asian native populations and the Bali cattle). The southern type cattle are subdivided into four major groups;
(1) the Bali cattle, (2) Indian zebu, (3) Taiwan-Philippine and (4) Thai-Malaysia-Indonesia. These genetic relationships correlate to the Hbb allele distribution mentioned above, that is, the Japanese-Koran native group is characterized with large \( P_{\text{Nor}} \) values, the Taiwan-Philippine group with large \( P_{\text{Ind}} \), \( P_{\text{Bal}} \) values and small \( P_{\text{Nor}} \) values, and Thai-Malaysia-Indonesia group with relatively large \( P_{\text{Nor}} \) values and various \( P_{\text{Ind}} \) and \( P_{\text{Bal}} \) values.

Source of genetic diversity in the native cattle populations of Southeast Asia and conservation

The above mentioned studies show the broad genetic differentiation of the native cattle populations of South and Southeast Asia. It can be said that the broad wide genetic variability of cattle is primarily provided by three distinct types of cattle or different "species" of bovine; probably *Bos primigenius* (the northern type or the humpless cattle), *Bos javanicus* (the banteng or the domesticated form Bali cattle) and the zebu cattle of which detailed ancestry remain unknown. Therefore, most of the genetic diversity of the Asian cattle will be conserved in these three representative types of bovine.

Phylogenetic study on bubaline species

*Phylogeny of Bubalus spp. by cytochrome b gene evolution*

The whole of the cytochrome b gene region of 1140 bp was sequenced for seven bubaline mtDNAs; *Syncerus caffer* (African buffalo), *Bubalus mindorensis*, *Bubalus (Anoa) depressicornis*, *Bubalus (Anoa) quarlesi*, and *Bubalus bubalis* (Sri Lankan wild buffalo, river type buffalo and swamp type buffalo). The pairwise sequence difference, considering position of codons as well as transition and transversion were compiled. These sequence data will be appeared in the databank of DDBJ and elsewhere. Only five nucleotide substitutions (all transition at the third position of the codon) were found between the Sri Lankan wild buffalo and the river type buffalo. This substitution rate is comparable to that estimated within the river type buffaloes (Tanaka et al., 1995). On the other hand, 28 substitutions existed between the river and swamp type buffaloes.

The phylogenetic tree of six *Bubalus* sequences was constructed using the neighbor-joining method, in which *Syncerus* and/or *Bos javanicus* sequences were placed as an outgroup. In the dendrogram (Figure 6), the swamp type buffalo and tamaraw made a cluster, but the river type buffalo and Sri Lankan wild buffalo were separate from this
Figure 1. Genus *Bos*. Upper left: Gaur. Indian gaur ox (*Bos gaurus gaurus*) in wild (photo by Dr. T. Shotake). Upper right: Yak (*Bos mutus*). A domestic yak bull of Nepal (photo by Dr. T. Shotake). Lower left: Banteng (*Bos javanicus*). The banteng bull at Surabaya zoo of Indonesia. The banteng is a wild form of the Bali cattle. Lower right: Mishima-ushi (bull). There are two purely native populations of cattle in Japan; one is Mishima-ushi of which population size is about 100 and the other Kuchinoshima feral cattle in a small island, Kuchinoshima of Kagoshima.
Figure 2. Genus Bubalus, Asian buffalo species. Upper left: The swamp type of the domestic buffalo \((\text{Bubalus bubalis})\), male in Okinawa. Upper right: Mountain anoa \((\text{Anoa quarlesi})\), male. The mountain anoa is endemic to Sulawesi of Indonesia. The lowland anoa \((\text{Anoa depressicornis})\) is slightly bigger than the mountain anoa and it also inhabits only Sulawesi. Lower left: The river type of the domestic buffalo \((\text{Bubalus bubalis})\). One of the dairy breeds of the river type, Murah breed, is characterized by the curly horns and back and in coat color. Lower right: The tamaraw \((\text{Bubalus mindorensis})\), male. This animal is found only on Mindoro Island of Philippines. The body-size and horn shape is intermediate between the anoa and the swamp buffalo.
According to the fossil record, divergence time between *Bos* and *Synceros* (or *Bubalus*) is approximately 10 Myr (Savage and Russell, 1983). Based on this, the divergence time between *Bubalus* and *Synceros* was calculated as 6.3 Myr (the fossil record 5 Myr). The largest divergence within the six *Bubalus* species had a mean divergence time of 2.0 Myr. All the present *Bubalus* species, therefore, diverged from each other 2.0 Myr BP or less.

Divergence time is roughly as follows: most recently 1 Myr or less between the river type and Sri Lankan wild buffaloes, 1.5 Myr between the river and swamp type buffaloes, 1.3 Myr between tamaraw and swamp buffalo, 2.0 Myr between anoa and other *Bubalus* species, and around 2.0 Myr between the two anoa species.

Molecular evolution of hemoglobin-β globins of anoa species

Kakoi et al. (1994) reported hemoglobin-β globin amino acid sequences of *Bubalus depressicornis* and *B. quarlesi*, and showed no substitution between the swamp and river buffaloes, two and five between the two anoa species and the domestic buffalo, and five between the two species of anoa, suggesting relatively large divergence time between the two anoa species compared to that between the two types of domestic buffaloes. The result agrees with the cytochrome b sequence data above.

Comparison of the chromosome set of tamaraw with other bubaline species

The tamaraw had 46 chromosomes in diploid form (2N) and 58 as the fundamental number (FN) including the sex chromosomes involved six pairs of submetacentric or metacentric autosomes, 16 pairs of acroscentric autosomes and two pairs of acrocentric sex chromosomes (Namikawa et al., 1995). In contrast, all the bubaline species of *Bubalus* and *Synceros* have FN=60 (2N=48-54) with exception of the swamp buffalo with FN=58 but 2N=48, previously reported elsewhere. The tamaraw and the swamp buffalo gave unique karyotypes which may suggest a close phylogenetic relationship between them.

Source of genetic diversity of the domestic water buffalo: the river and swamp types

The cytochrome b gene sequence analysis (Figure 6) showed that the swamp type buffalo has a closer phylogenetic relationship to the tamaraw (*Bubalus mindorensis*) than to the river type buffalo. The wide genetic diversity in the domesticated buffalo (Figure
Figure 3. Geographical distribution of hemoglobin β alleles (Hbb) of local populations (or breeds) of cattle in eastern Asia (Namikawa, 1981).

Figure 4. Estimated amount of gene-flow to cattle of eastern Asia, based on the hypothesis that three-way hybridization had occurred in establishing process of the present-day cattle populations in this area. The $P_{Ind}$ is amount of Hbb genes originated from zebu cattle, the $P_{Nor}$ from northern type (or humpless cattle), and $P_{Bali}$ from the bali cattle (see the text for the details) (Namikawa, 1981).

Figure 6. A phylogenetic tree of the eight bovine sequences constructed by using the neighbor-joining method. Numerals on internal branches are the bootstrap probabilities (%) from 1,000 trails (Tanaka et al., unpublished data).

Bubalus

Syncerus

Bos

Tamaraw

Swamp type buffalo

River type buffalo

Sri Lankan wild buffalo

Mountain anoa

Lowland anoa

African buffalo

Banteng (Bos javanicus)
Figure 7. Dendrogram drawn from the Nei's Genetic distance matrix among twenty-two water buffalo populations, based on data of twenty five blood protein loci (Amano et al., 1992)
7), therefore, comes from two species or subspecies.

**Conclusion**

Domesticated cattle and buffaloes both have polyphyletic origins from two or more subspecies or species of wild bovine or bubaline. This complexity in the domestication process is considered to be the primary cause of the very broad genetic diversity in local populations (or breeds) of the domestic cattle as well as of the domestic buffalo in the world today.

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Tanaka, K., Yamagata, T., Masangkay, J. S., Faruque, M.O., Dang, V.-B., Salundik, Mansjoer, S.S.,
The Phylogenetic Relationship and Differentiation of Wild and Domesticated Pigs (*Sus scrofa* L.) in Asia

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Abstract

Blood groups and biochemical polymorphism for the wild and domestic pigs of Asia were investigated by the serological technique and electrophoresis in order to clarify the phylogenetic relationships between them. Comparisons of erythrocyte antigens were observed. Gene frequencies for several blood protein loci in the Asian wild and domesticated pigs were found to be very different from the European wild pig and improved breeds. These differences were clearly observed at the Tf and Am-1 loci of serum protein. Tf* and Am-1A alleles were regarded as typical alleles of the wild and domestic pigs of Asia. These erythrocyte antigens and protein loci may be useful genetic markers for phylogenetic relationship studies between wild and domesticated pigs. The genetic variability for the wild pig populations tended to be rather low in comparison with that of the domesticated pig population. The analysis of genetic similarities among populations of wild and domesticated pigs substantiated the historical fact that Asian and European domesticated pigs are each derived from different wild stock.

Introduction

Pigs (*Sus scrofa* L.) were domesticated from wild populations of the same species. Wild pigs are distributed throughout the Eurasian continent and surrounding islands. It has been considered that some subspecies of wild pig, for example the Indian wild pig (*Sus scrofa cristatus*) and the Southeast Asian wild pig (*Sus scrofa vittatus*), formed the original stock for the existing domesticated native pig of Asia. However, there have been insufficient studies of phylogenetic relationships between wild and domesticated pigs. Many questions concerning the origins of domesticated Asian pigs remain unsolved.

The purpose of this paper is to clarify the phylogenetic relationships and differentiation of wild and domesticated pigs in Asia using information on blood groups and biochemical polymorphism.
Methodology

Animals investigated

According to recent concepts in zoological taxonomy (Haltenorth, 1963), wild pigs may be classified into about 30 subspecies, of which, the author has investigated 7 subspecies and 16 local populations, including wild pigs from Japan and several other countries. This paper focuses on blood group and biochemical polymorphism of the Japanese wild pig, in relation to Asian domesticated pig.

In Japan, there are two wild pig subspecies which may have come to Japan from continental Eurasia. The Japanese wild pig (Sus scrofa leucomystax) is found on the Japanese islands of Honshu, Shikoku and Kyushu, and the Ryukyu wild pig (Sus scrofa riukiuanus) is found throughout the Ryukyu islands. These islands include Amami Oshima, Kakeroma Jima, Tokuno Shima, Okinawa Jima, Ishigaki Jima and Iriomote Jima. There are several morphological differences between these subspecies (Figure 1). The Ryukyu wild pig is very small in comparison with both the Japanese wild pig, and the continental wild pig. Recently, the Ryukyu wild pig has become an endangered species, as a result of hunting (Yasuma, 1983).

Blood typing and electrophoretic analysis

Blood samples were collected from animals caught by local hunters in each country or area investigated. This study was completed with the help of researchers from various countries including Sri Lanka, Nepal, Taiwan and Russia.

Blood typing of the wild pigs was carried out by using the blood group reagents developed for domesticated pigs. The reagents used in the study were as follows: A, O (A system), Ea, Eb, Ed, Ec, Ef, Eg (E system), Fa, Fb (F system), Ga (G system), Ha, Hb (H system) Ka, Kb (K system) and Lh, Lk (L system). These reagents were produced by the National Institute of Animal Industry of Japan, and they were standardized using the international comparison test.

The genetic variation for the 22 blood proteins (Table 1), each of which encodes one locus. These genes analyzed by means of starch and polyacrilamide gel electrophoresis. This analysis was performed according to the methods described by Oishi et al. (1970, 1978) and Tanaka et al. (1992).
Table 1. List of blood protein examined

<table>
<thead>
<tr>
<th>Name of blood protein</th>
<th>Locus symbol</th>
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<tbody>
<tr>
<td>Serum</td>
<td></td>
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<tr>
<td>Prealbumin</td>
<td>Pa</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Tf</td>
</tr>
<tr>
<td>Hemopexin</td>
<td>Hp</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Cp</td>
</tr>
<tr>
<td>Amylase-I</td>
<td>Am-I</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin-α</td>
<td>Hb-α</td>
</tr>
<tr>
<td>Hemoglobin-β</td>
<td>Hb-β</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>Ak</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>LDH</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>MDH</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>Ca</td>
</tr>
<tr>
<td>Cell esterase</td>
<td>CES</td>
</tr>
<tr>
<td>Esterase-D</td>
<td>EsD</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>G6PD</td>
</tr>
<tr>
<td>6-phosphogluconate dehydrogenase</td>
<td>6PGD</td>
</tr>
<tr>
<td>Phosphohexose isomerase</td>
<td>PHI</td>
</tr>
<tr>
<td>Tetrazolium oxidase</td>
<td>To</td>
</tr>
<tr>
<td>Glyoxalase-I</td>
<td>GLO-I</td>
</tr>
<tr>
<td>Glutamic-oxaloacetic transaminase</td>
<td>GOT</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>ADA</td>
</tr>
<tr>
<td>NADH-diaphorase-I</td>
<td>Dia-I</td>
</tr>
<tr>
<td>NADH-diaphorase-II</td>
<td>Dia-II</td>
</tr>
</tbody>
</table>

Results and Discussion

Blood group reagents developed for domestic pigs can be used to detect erythrocyte antigens in wild pigs. Blood group variations in several subspecies of Sus scrofa L., including the European wild pig (Sus scrofa scrofa), the Transcaucasian pig (Sus scrofa attila), the Middle Asian pig (Sus scrofa nigripes) and the Far Eastern pig (Sus scrofa ussuricus) from the Eurasian continent have already been investigated by other workers (Buschmann, 1966; Wiatroszak, 1970; Tikhonov et al., 1972), using the same method of blood typing as was used for the domestic pig. The author also studied several populations of the Taiwan wild pig (Sus scrofa taivanus) and the Indian wild pig (Sus scrofa cristatus) including the two subspecies from Japan (Kurosawa et al., 1979, 1984, 1986).

From these results, it was apparent that the characteristic of the blood groups studied for the two Japanese wild subspecies was very different from that of the European wild pig, and was more similar to the Asian wild subspecies. The differences among
Figure 1. Two subspecies of Japanese wild pig (adult female). Animal size is shown by the measuring rod (110 cm). Upper) Japanese wild pig (*Sus scrofa leucomystax*). Lower) Ryukyu wild pig (*Sus scrofa riukiuanus*).
Figure 2. Frequency distribution of the blood group antigen Ga in different subspecies of wild pig, *Sus scrofa* L.

Figure 3. Frequency distribution of transferrin (Tf) alleles in different subspecies of wild pig, *Sus scrofa* L.
subspecies populations of the wild pig were most clearly demonstrated in the E, G, H, K and L blood group systems. The frequency of the Ga antigen in the G system is shown (Figure 2). The European wild pig characteristically had the Ga antigen at a high frequency, whereas some of the Asian wild pig, including the wild pig from Japan, did not. There was a west to east geographical cline in the frequency of this antigen. This phenomenon is useful for clarifying the phylogenetic subspeciation of *Sus scrofa*.

Of the 22 genetic loci of the blood proteins examined, polymorphism was found in the Tf, Pa, Hp, Am-1, 6PGD, G6PD and ADA loci. More precisely, of the wild subspecies studied, most of the polymorphic loci were found in wild pigs of Japan and in one subspecies from Nepal. It is particularly noteworthy that new variants which had not been found in the domestic pig were observed at a high frequency in each protein locus of the Tf, Pa and 6PGD of the wild pigs from Japan (Kurosawa and Tanaka, 1988, 1991). When comparing wild populations of Japan with those from the continent, the gene frequencies for polymorphic loci in the Japanese wild pig were shown to be very different from those of the European wild pig.
Figure 5. Dendrogram showing genetic similarities ($\bar{D}$) among wild and domestic pig populations (UWPGM).
The geographic distribution of allele frequencies at the Tf locus for various populations of wild pigs in Asia and Europe is shown (Figure 3). In addition, the data of domesticated pigs from Sumatra, Indonesia and also from Nepal which was studied by Tanaka et al. (1983a, 1992) is presented in this figure for comparison. These domesticated pigs have similar conformation to the various wild pig types (Figure 4). That is, although the Tf^B allele is commonly found in wild populations from Asia and Europe, both the wild and the domesticated pigs of Asia not only have the Tf^B allele, but also the Tf^C allele. The Tf^C allele, is found at a high frequency, especially, in wild populations of Japan at a high frequency, and moreover a new allele variant, the Tf^X allele also exists in the wild pigs of Japan. However, this was not the case with the European wild populations nor the European domestic breeds (Oishi, 1977).

Tanaka et al. (1983b) reported that the Tf^C allele is widely found in Asian domestic pig and occurs at high frequency. Therefore, it is probable that the Tf^C allele is a variant which originated in Asian wild stock. A similar result was also found for the Am-1^A allele of the Am-1 locus, that is, the Tf and Am-1 loci may be useful genetic markers for phylogenetic relationship studies between wild and domesticated pigs.

The genetic variability estimated by the proportion of polymorphic loci (P_{poly}) and the mean heterozygosity per individual (H) was compared for populations of the Indian wild pig, Taiwan wild pig and Japanese wild pig. The values of P_{poly} and H in the local populations of these wild pigs ranged from 0.0455 to 0.250 and from 0.0109 to 0.0884, respectively. The genetic variability in these wild pigs was extremely low, in comparison with that of the Asian domestic pig and other livestock. The genetic variability of Indian wild pig in Sri Lanka and the Ryukyu wild pig in Japan was the lowest, and that of the Indian wild pig in Nepal was the highest, for the wild species investigated. This difference in the wild pig populations may be attributed to the effects of isolation on island populations, whereas the continental populations typically display more genetic variability.

Genetic similarities for wild and domestic pig populations were investigated using genetic distance coefficients calculated from the allele frequencies of the polymorphic loci according to the method described by Rogers (1972). A dendrogram which shows the genetic similarities for wild and domestic pig populations was constructed by the unweighted pair-group method (Figure 5). Several wild populations from Asia are similar to the domestic pig from that area, whereas the typical wild species
of Europe are closer to the European-American breeds. The degree of genetic
differentiation between domestic pigs and their wild ancestors for each area, i.e. Europe
or Asia, is very low. From the historical and phylogenetic record, Asian and European
domesticated pigs appear to have been derived from different wildstock, results in Figure
5 supports this.

Gene frequencies and thus genetic distance can be affected by mutation, natural
selection, artificial selection or geographical isolation. As shown in the dendrogram, the
degree of genetic differentiation among the Ryukyu wild pig populations is quite large,
although these wild pigs are presumed to belong to the same subspecies, *Sus scrofa
riukiuanus*. This difference in the gene frequencies at some polymorphic loci may be due
to the effects of random genetic drift which occurred during the subspeciation process.
In addition, populations of this species are isolated on different islands of the Ryukyu
archipelago. To some degree, genetic differences may reflect the complex origin or
phylogeny of each wild pig population on the Ryukyu islands. There are different
opinions as to whether the Ryukyu wild pig is a subspecies of *Sus scrofa* or a separate
species (Imaizumi 1973). Some researchers believe wild pig constitutes the feral
descendants of semi-domesticated pigs which originated from Southeast Asian, and was
carried from island to island by ancient peoples (Naora, 1937; Hayashida, 1966). The
Ryukyu wild pigs in Ishigaki Jima or Iriomote Jima and the Southeast Asian island
domesticated pigs cluster together (Figure 5) tends to support this hypothesis. Presently
it is not possible, to substantiate this theory, therefore, further more detailed studies will
be required.

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Questions and answers in Session 2

Questions to Dr. Tan

Q. Did you find any amplified DNA products which are not polymorphic when you use cattle microsatelite primers to genotype your water buffalos? And have you any observations on the variation in repeat numbers? (Li)

A. For details please refer to our paper in Animal Genetics 26: 355-359(1995). Briefly, of the 80 bovine microsatellites tested on 5 buffaloes 56(70%) gave a single PCR product. Forty six (57%) of the microsatellites were polymorphic. A subset of 24 loci chosen based on yield and reproducibility of the amplification were then tested on about 100 animals for each locus. The number of alleles per locus ranged from 2 to 18. (Tan)

Q. According to your D-loop data, the buffalo population from Bogor on Java clustered with the Murrah buffalo group. Can you explain this? (Amano)

A. Since there are 33 D-loop genotypes found in 80 animals and we typed only 7 animals per population we attribute this anomaly to small sample size of typed animals. (Tan)

Q. When you discuss Lankan buffaloes, do you mean wild or domesticated animals? (Matsukawa)

A. We could only sample domesticated animals and not wild animals. (Tan)

Q. Your presentation showed clearly the genetic variation in Southeast Asian swamp buffalo populations. Could you tell us if there is similar variation for productive traits such as growth rate and mature body size? (Kanai)

A. Offhand I cannot quote data on such traits. However, based on morphological observations there are great differences in animal sizes among animals from different populations in the Southeast Asian region. (Tan)

Q. Lankan buffaloes are River buffaloes based upon some genetic markers, their behaviour is, however, that of the River buffalo. Is the distinctive behaviour of Swamp and River buffalo a genetically based one? (Bodó)

A. Water buffaloes wallow in order to keep cool. I presume they will wallow in whatever water is available when there is no choice. I have seen Lankans wallowing in clear water. However, when given a choice Swamp buffaloes wallow in muddy water and River buffaloes in clear water. This is probably a learned behaviour pattern. (Tan)
Question to Dr. Kurosawa

Q. Genetic variability is similar between wild and domesticated forms of animals and poultry (in the case of ducks and chickens) and it is somewhat (20-25%) higher in domesticated forms than their ancestral wild ones in the case of quail and dogs. What is the situation with respect to wild pigs and domesticated pigs? (Tanabe)

A. In Japanese wild pig (Sus scrofa lecomystax) the proportion of polymorphic loci (Ppoly) is from 0.1 to 0.25, heterozygosity (H) is from 0.03 to 0.08. In Ryukyu wild pig (Sus scrofa riukiuanus) Ppoly is from 0.05 to 0.1, H mean is from 0.01 to 0.03. In domesticated pig (Asian native and European-American pigs) Ppoly is from 0.2 to 0.47 and H ranges from 0.08 to 0.16. (Kurosawa)

Questions to Dr. Namikawa

Q. Hbb-X is found in Bali cattle. Is it found in any other cattle? (Astuti)

A. No breed or population has the X gene in so high a frequency as Bali cattle. (Namikawa)

Q. How many animals per species did you sequence for cytochrome b among the bubalines? (Tan)

A. We only used one animal from each species or group. However, we examined as many samples as possible using RFLP analysis, and then we selected samples for sequence study. (Namikawa)

Q. Lowland anoa and mountain anoa might be considered to be the same species. The difference depends on their habitat. What is your opinion on the differences between these two types? (Fukuta)

A. Molecular evolution of b-globulins of the lowland anoa and mountain anoa suggested that there are large genetic differences between them. Isolation mechanisms cannot be considered to be the cause of differentiation between mountain and lowland anoa. Perhaps immigration into Sulawesi took place at different times, the mountain anoa first and then the lowland anoa. Please refer to Groves excellant work on this topic. (Namikawa)
TECHNICAL REPORTS

Session 3

Conservation system

Chairpersons
H. Inoue
S. G. Tan
Animal Genetic Resources and conservation system in Vietnam

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I. Introduction:

There is considerable diversity of animal species in Vietnam. There are 265 species of wild animals distributed throughout the country including some rare and precious ones. There was serious damage to wild animal life during the long war period. According to recent evaluation by biologists 10 animal species are endangered, 18 species are vulnerable and 22 species are considered as rare. In December, 1993, the government of Vietnam published "The Environment Protection Law", in which the problem of animal genetic conservation is emphasised.

In this paper the author will discuss only domesticated animal genetic resources conservation.

II. Livestock population:

Vietnam is one of the first cradles of animal domestication. Various species of livestock are known to have existed in the country since the dawn of time, this include pigs, cattle, buffalo, horses, goats, sheep, rabbits, chicken, ducks, and the muscovy duck.

The present of animal population in Vietnam is shown (Table 1).

For thousands of years, under the pressure of natural and artificial selection local animal populations proved to be adapted to the ecological conditions and suited to the economic conditions of some areas of Vietnam. They possess valuable genetic potential as they can live on low quality forage and are resistant to tropical diseases (especially parasitic diseases). Some breeds have a reasonable reproductive performance and produce good quality meat. Their disadvantages is there small body size and poor annual productivity.

III. Breeding constraints and the need to carry out the project on animal livestock genetic conservation:

Poor breeding management as well as seasonal and irregular supplies of feed, particularly in the dry season, are the main constraints for the rapid genetic improvement
of local populations.

Some indigenous breeds tend to disappear due to low productivity and competitiveness with crossbred and exotic animals.

Artificial insemination in pigs is extensively practiced; the number of inseminated sows is around 60% of the population. Evidently, the use of semen of exotic breeds makes the preservation of the pure native breeds difficult. There seems to be a contradiction in the orientation towards a free marketing system and conservation of genetic resources. Farmers who keep local sows do not want to raise local litters because their market price is low. Overall poor breeding management has lead to some pure breeds becoming endangered.

The establishment and implementation of livestock conservation practices is an urgent task for the country, because it links to the development of the economy and the income of the farmers, especially those who are living in marginal areas for food production. They also contribute to the protection of the environment and will be useful for future development plans.

IV. Livestock genetic conservation programme: objectives and measures

The main objectives of the conservation programme are:

1. To collect data of animal breeds in different ecological regions throughout the country and identify certain genes that need to be conserved.
2. To recover the economic traits of the local breeds, which are in danger, in order to keep and improve the livestock genepool.
3. To assist the development of some objectives which have been neglected (such as sheep for meat) or species in the process of domestication (such as *Cervus Nippon Termeric* deer).
4. To contribute to economic, scientific, cultural and tourist development. The breeds of interest to the national conservation programme are:

*(1) Pigs:*

In Vietnam the pig population is very high. In the North, Mong Cai and "I" pigs are the two popular native stocks. The Mong Cai breed is spreading throughout country due to a good reproductive performance, while the "I" breed is less popular as these pigs are less prolific and tend to produce fatter carcasses. The project for the conservation of
Table 1. The present of animal population in Vietnam

<table>
<thead>
<tr>
<th></th>
<th>1992</th>
<th>1993</th>
<th>1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>12,140</td>
<td>14,800</td>
<td>15,570</td>
</tr>
<tr>
<td>Cattle</td>
<td>3,151</td>
<td>3,330</td>
<td>3,460</td>
</tr>
<tr>
<td>Buffalo</td>
<td>2,885</td>
<td>2,960</td>
<td>2,970</td>
</tr>
<tr>
<td>Goat</td>
<td>312.3</td>
<td>353</td>
<td>370</td>
</tr>
<tr>
<td>Sheep</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Deer (Sika deer)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>97.5</td>
<td>102.390</td>
<td>100.627</td>
</tr>
<tr>
<td>Duck</td>
<td>27</td>
<td>31.000</td>
<td>32.000</td>
</tr>
</tbody>
</table>

Units: '000 heads

"I" pigs is a high priority because they are still needed by the farmers of the poor humid areas.

The mini Co pig breed still exists in some parts of the central north area of the country but nobody likes keeping them, due to their economic inefficiency.

The Meo and Muong Khuong pigs are mostly found in the mountainous regions. In the south of Vietnam, the Thuoc Nhieu and Ba Xuyen are the most popular breeds.

(2) Cattle:

Vietnam Yellow cattle belongs to the humped type Asian breed with small body size and good hardiness. The average body weight is 160-180kg. There are larger crossbred (crosses with Red Sindhi or Zebu sires ) in Vietnam. In mountainous areas there is Muong and Moi cattle with smaller body size and strong conformation.

(3) Buffalo:

Buffalo breeds in Vietnam are of the swamp type. They are named after the geographic name of the region where they are kept, such as Yen Bai and Tuyen Quang. They are also sometimes named according to their size, namely, Ngo (big) buffalo or De (Small) buffalo. The average body weight is 320-350kg.

(4) Horses:

Horses in Vietnam are all of the local type with a small body size of about 140-170kg. They are mainly found in the mountainous areas and are kept as pack and riding animals.
(5) Goats:
   Goats are of the meat type and graze mainly in the hilly rocky mountain areas. Their development is limited. The popular goat breed is local Co breed. The dual purposes breed is Bach Thao has a high reproductive performance. 75% of the litters are twin or triple. There is a clear need for a better integration of crop production, forestry and goat grazing.

(6) Sheep:
   The Phan Rang breed is the only sheep that survives in Vietnam. They belong to the meat type. The national flock is very small (no more than 3000 heads) and is located in Ninh Thuan province. Phan Rang sheep must be considered a priority in the list of livestock genetic resources for conservation purposes.

(7) Chicken:
   The Ri is a very popular native breed. Although it has a small body, it has a high egg yield and produces tasty meat. Several fighting chicken breeds are popular with the farmers, as a hobby. The large meat type breeds that still exist are the Mia, Ho and Dong Cao. Some other breeds still survive such as the Ac (black) breed, its meat is used as tonic, the Tre breed is raised for cockfighting.

(8) Duck:
   The Co duck breed is predominant and is kept in scavenger systems; they look for their food in harvested fields. This breed is popular due to its economic efficiency (feed conversion) and good laying capacities. The Bau duck is considered a meat type.

(9) Cervus Nippon deer:
   These animals are in the process of domestication although they still bear some wild characters and are difficult to manage and control. This deer breed is not now endangered and is quickly spreading in the central north part of the country with the total herd is about 12,000 heads.

(10) Sambar deer:
   This animal can be seen in the zoo. But recently farmers began to create a Sambar deer farm, in which a hundred Sambar deer are raised for meat and velvet
purposes.

V. Conclusion

The endangering and even extinction of domestic animals in Vietnam comes mainly from serious commercial competition with exotic breeds. With the expansion of the artificial insemination techniques this process is occurring very quickly. The measures to maintain genetic diversity are as follows:

* Select a limited number of local breeds to be considered a first priority for conservation. These must have some economic value and be still be in demand from farmers in certain zones such as marginal environments. Attention must be given to the "I" pig breed, some chicken breeds such as Mia, Ho, Dong Cao and the Phan sheep.

* The in situ method should be favored for animal genetic conservation. With the renovation of the country's economy (moving towards a marketing system) we have to contract with household-farmers to keep the "genes" together by establishing open nuclear herds.

* Creating "elderly people clubs" to keep the local breeds (such as nice pet) is an economical measure for genetic conservation.

* Preliminary studies on cryogenic preservation of semen, as well as embryo transfer in buffalo and cattle, have been conducted. These are considered an expensive means of ex situ conservation and are only under experimentation at this stage.

* Experiences and know-how from outside Vietnam is needed and international cooperation should be considered. It involves the support in information and up to date methodologies; training and also equipment is needed for the ex situ methods and data processing practices.

References


Conservation Systems of Animal Genetic Resources in The Philippines

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Abstract
The increasing development and intensification of animal production has threatened the genetic variation of Philippine indigenous animal genetic resources. Concerns have been raised about the proper systems for conservation of animals. Systems should complement the balance between human development and animal genetic resources conservation.

The biological diversity of the Philippines is exceptionally rich with 2,000 fish and 960 animal species, 500 of which are birds and 167 are mammals. Out of the 500 coral species worldwide, about 488 species in 78 genera are found in the Philippines. Forest plant species of the Philippines is one of the richest in the world with 12,000 species of which 3,800 are endemic.

Recently the objectives of conservation systems have been modified, from a limited focus to a broader, multi-disciplined action programmes. For a particular animal species, e.g. Tamaraw (Bubalus mindorensis Heude), conservation efforts would include habitat conservation, protected area and community development, conservation education and information, applied research and management, policy review and support activities.

This paper argues in support of a comprehensive system approach to the conservation of animal genetic resources. An outline is given of specific animal species conservation to ensure rational genetic conservation for present and future needs.

Introduction
In the Philippines, there is considerable interest in the conservation and preservation of indigenous animal genetic resources. However, the ever-increasing meat demand from human population has threatened the genetic identity of the native rare breeds and strains due to the introduction of exotic temperate breeds. Introduction of domestic animal species from Europe probably began in the early 1600s, during Spanish colonization. Some livestock and poultry from neighboring countries in Southeast Asia and China could have entered the country centuries before.

Crossbreeding with improved breeds is mainly based on the assumption that indigenous breeds have low productivity. However, clear evidence for this is lacking. The value of native breeds in terms of sustainable production should be quantified because
native breeds have been exposed for many generations to the natural selection and have developed adaptive mechanisms to the conditions of the tropics. This special ability to survive and reproduce under minimal management conditions, is a valuable attribute.

The biological diversity of the Philippines is exceptionally rich. At least 2,000 fish species thrive in Philippine waters. Likewise, there are 900 animal species of which more than 500 are birds and 167 are mammals. Approximately, 488 coral species in 78 genera are found locally out of the 500 known coral species worldwide. The Philippine forest is one of the richest in terms of plant species totaling about 12,000 of which 3,800 are endemic. The country has a total of 61 national parks, 2 marine parks and 8 game refuges and bird sanctuaries. In addition, there are 10 wilderness areas covering an area of 1.5 million hectares equivalent to 4% of the total land area.

It is imperative that the indigenous animal genetic resources are available to present and future generations. A committed effort is necessary, involving national institutions and individuals, with support from the international community to manage these genetic resources. The systems approach to this particular undertaking should consider the immediate need for increased animal productivity for the expanding human population while considering the future needs for genetic diversity.

**Domestic Animal Genetic Resources**

Economically important poultry and livestock species such as buffaloes (carabaos), chickens and pigs have been in the country for many centuries and were probably domesticated from their wild ancestors. In contrasts, native cattle, goats, sheep, turkey and geese are introduced animals. These animals are well adapted to local conditions and have developed distinctive characteristics that merit considerations to be included in the country's animal genetic resources conservation program (Arboleda, 1987).

Philippine animal production systems are still dominated by smallholders, comprising approximately 75-80% of the total population of animals (Faylon and Roxas, 1995). Poultry and swine predominate the industry which has shown a steadily increasing growth rate within the last 10 years, 1984 to 1994 (Table 1). Sheep and goats are raised as secondary source of livelihood and animal protein. Despite this, goats have shown a positive growth rate within the same time period, from 2.4 million heads in 1984 to 2.6 million heads in 1994. The population of sheep is low at about 30 to 40,000
Table 1. Poultry, swine, cattle, carabao and goat inventory (in 000 heads)

<table>
<thead>
<tr>
<th>Year</th>
<th>Chicken</th>
<th>Duck</th>
<th>Swine</th>
<th>Cattle</th>
<th>Carabao</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>59161</td>
<td>5761</td>
<td>7612</td>
<td>1849</td>
<td>3022</td>
<td>2362</td>
</tr>
<tr>
<td>1985</td>
<td>52398</td>
<td>5221</td>
<td>7304</td>
<td>1787</td>
<td>2983</td>
<td>2191</td>
</tr>
<tr>
<td>1986</td>
<td>53006</td>
<td>5208</td>
<td>7275</td>
<td>1815</td>
<td>2985</td>
<td>2177</td>
</tr>
<tr>
<td>1987</td>
<td>53248</td>
<td>5252</td>
<td>7038</td>
<td>1747</td>
<td>2865</td>
<td>2016</td>
</tr>
<tr>
<td>1988</td>
<td>60321</td>
<td>5833</td>
<td>7580</td>
<td>1700</td>
<td>2890</td>
<td>2120</td>
</tr>
<tr>
<td>1989</td>
<td>65912</td>
<td>6501</td>
<td>7909</td>
<td>1682</td>
<td>2841</td>
<td>2212</td>
</tr>
<tr>
<td>1990</td>
<td>69528</td>
<td>7236</td>
<td>7990</td>
<td>1630</td>
<td>2765</td>
<td>2192</td>
</tr>
<tr>
<td>1991</td>
<td>65480</td>
<td>8268</td>
<td>8079</td>
<td>1676</td>
<td>2646</td>
<td>2122</td>
</tr>
<tr>
<td>1992</td>
<td>63127</td>
<td>8340</td>
<td>8022</td>
<td>1730</td>
<td>2576</td>
<td>2307</td>
</tr>
<tr>
<td>1993</td>
<td>64868</td>
<td>8506</td>
<td>7953</td>
<td>1914</td>
<td>2575</td>
<td>2562</td>
</tr>
<tr>
<td>1994</td>
<td>nd</td>
<td>8187</td>
<td>8226</td>
<td>1922</td>
<td>2559</td>
<td>2631</td>
</tr>
</tbody>
</table>

nd; no data

heads.

For large ruminants, such as cattle and carabaos, the population trend showed a decline during the past decade. In 1981, the cattle population was around 1.9 million heads and it decreased to 1.7 million heads in 1991, despite the increase in human population and increasing demand. However, since 1992, it has slightly increased (Table 1). The carabao population on the other hand, showed a steady decline within the last 10 years, from 3.0 million heads in 1984 to 2.6 million in 1994.

The Philippine livestock development plan for a productive and sustainable industry is anchored on specific commodity programs and an integrated approach is being followed.

The component programs of the development plan is basically to upgrade the quality and quantity of the present animal resources, as follows:

1. *The Breeding Cattle Development Program.* The target is to increase the beef cattle population to 3.0 million heads by 1998.
2. *The Dairy Program.* The introduction of 37,500 heads of dairy animals.
3. *The Carabao Program.* The stabilization of the present population at 2.5 million heads.
4. *The Small Ruminant Animal Program.* The target is to increase the population of sheep and goats to 3.4 million heads.
5. *The Poultry Program.* Assistance to the present industry and conservation
Table 2. Performance of Philippine carabao and Murrah buffalo

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Philippine Carabao</th>
<th>Murrah Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (kg)</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Adult weight (kg)</td>
<td>367</td>
<td>382</td>
</tr>
<tr>
<td>Milk yield (kg)</td>
<td>500</td>
<td>1,384</td>
</tr>
<tr>
<td>Lactation length (d)</td>
<td>250</td>
<td>287</td>
</tr>
</tbody>
</table>

Source: Eusebio, 1980.

Table 3. Performance record of Philippine native pig and Berkjala improved strain

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Philippine Native Pig</th>
<th>Berkjala Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight, kg</td>
<td>0.60</td>
<td>0.94</td>
</tr>
<tr>
<td>Weight at 6 months, kg</td>
<td>9.85</td>
<td>16.20</td>
</tr>
<tr>
<td>Weight at 12 months, kg</td>
<td>34.5</td>
<td>69.4</td>
</tr>
<tr>
<td>Mean age at first farrowing, days</td>
<td>370.3</td>
<td>472.0</td>
</tr>
<tr>
<td>Farrowing interval, days</td>
<td>247.3</td>
<td>231.0</td>
</tr>
</tbody>
</table>

Source: Peñalba, 1993

and improvement of indigenous chickens and native ducks.


7. Component Programs. The stock farm development, quarantine station and animal health, post production and market support programs.

The Philippine Carabao

Recent development strategies regarding the Philippine carabaos have involved reproductive efficiency improvement and crossbreeding with exotic breeds from India and Pakistan, and more recently, Bulgaria. There were reports that the carabao has been decreasing in size and this was attributed to the castration of bigger males geared for draft work and that the remaining smaller carabulls were instead used for breeding (Arboleda, 1987).

The Philippine carabao (Bubalus bubalis) is classified as a swamp-type with 48 chromosomes. The river-type buffaloes have 50 chromosomes but the crossbreeds are fertile with 49 chromosomes (Arboleda, 1987).

These animals are primarily used for draft purposes, both males and females, and an adult weighs about 350-400 kilograms which is relatively smaller than that of Murrah.
or Nili-Ravi buffaloes (Table 2). The average height at withers ranges from 124 to 137 cm for adult animals (Eusebio, 1980).

**Philippine Native Pigs**

Phenotypically, native pigs in the Philippines can be recognized by their black hair color but occasionally, animals with white and red markings can be found. The ears are small and erect, the snout is long, face straight, has large eyes, trim jowls, big neck and narrow shoulders. Characteristically, the back is sagging with paunch belly and total body conformation is short and narrow. Mature boars could be recognized by their tusks but with teats. The feet are short and strong (Peñalba, 1993b).

Limited available information suggests that the pedigree of Philippine native pigs could have come from numerous species of wildpigs in the country, namely: *Sus celebensis Philippinensis* Nehring on Luzon, *Sus celebensis negrinus* Sanborn on the Visayas and *Sus barbatus ahoenobarbus* Huet on Palawan. There are indications also that before the Europeans arrived in the 1600's, pigs were introduced by Chinese traders (Peñalba, 1993b).
Throughout the years, crossbreeding with exotic breeds has been done and this resulted in the emergence of strains well adapted to Philippine conditions. Predominantly black-colored strains are called Diani and Black Ilocos, while Kaman and Koronadal strains are colored red. Diani is the result of crossing native pigs in Batangas province with Berkshire. Black Ilocos is the result of Berkshire, Poland China and native pig crossbreeding. Red Kaman is from Duroc Jersey while Koronadal was developed in Mindanao from breeding native pigs in the region with Poland China, Berkshire and Duroc Jersey (Peñalba, 1993b).

There is the potential of native pig production in terms of additional income for farmers from a sustainable-type of production system. Another point is that they are invaluable source of genetic materials for improvement programs. The performance of Philippine native pigs compared with an improved strain, Berkjala, a crossbred between Jalajala (Luzon) native pig and Berkshire is shown (Table 3).

**Philippine Indigenous Chicken**

The progenitor of the Philippine chicken is believed to be the Red jungle fowl (*Gallus bankiva*) and they were probably domesticated before written records, as early as the 1500's (Lambio and Gay, 1993).

The female has brownish plumage and the shank is yellow to gray and has a single comb. The male's plumage is more attractive, with shiny red color and black tail feathers with brown hackle. The chicken are relatively small, weighing only 1.3 and 1.0 kg for males and females, respectively.

In the 1900's many exotic stock were introduced. However, there was no definite breeding program at that time, so indiscriminate crossing resulted in the emergence of many nondescript mongrels.

In 1994, out of the 64.9 million chickens in the Philippines, 72% are being raised by smallholder farmers. The White Leghorn breed is the most popular (more than 50% of the population), and the rest are imported breeds and mongrels. The number of Philippine native chicken is very small and there is no estimate of their population (Lambio and Gay, 1993).

Although the Philippine chicken is not popular for commercial production, a number of smallhold farmers prefer to raise them particularly due to the very low level of management required. In addition, they have the best mothering ability, are good
breeders and foragers. Health problems are almost non-existent because they are very hardy and can survive under harsh conditions.

**Systems Approach to Conservation**

The continuing depletion of biological diversity due to development has reached an alarming state. Efforts to conserve what is left entail a balance between the unrestricted human development and the need to preserve genetic resources.

As presented above, although indigenous breeds of animals could not compete economically with modern-day breeds, they are important from an economic viewpoint, and as a source of genetic variation in breeding programs and as a national identity (Peñalba, 1993a).

As a prime example, in the Philippines, unrelenting efforts have been undertaken in the past few years to conserve the Philippine tamaraw (*Bubalus mindorensis* Heude), which is a rare wildlife animal species found only on the island of Mindoro.

At present, the estimated population of the tamaraw is around 200-300 heads, and is classified as an endangered species. In the early 1900's, the number was estimated to be 10,000 heads. The reasons for its decline are human encroachment, illegal logging and the loss of grazing areas to the expanding cattle farms (Monmoñgan, 1993).

Previous conservation efforts have mainly focused on the animal itself, without much concern on other factors that directly and indirectly affect its survival for the next generations. A multi-disciplined action programme which is at the same time integrative and dynamic is necessary for this particular effort to succeed.

It should be recognized that the most effective way of conserving any animal species, breed or strain and other flora and fauna is to protect its natural habitat from further destruction and/or deterioration. The deterioration and/or destruction of the natural habitat are, by and large, man-made (Tamaraw Conservation Program, 1995).

The conservation program components include the following:

1. Protected areas, community organization and community development
   - the focal point for this component is the people, particularly those that surrounds the habitat of the conservation area. It is recognized that their interaction has a marked influence on the conservation efforts.
2. Habitat conservation
   - this component would include bio-physical resources inventory (flora and fauna) and socio-economic cultural inventory, including reforestation activities and establishment of community-based strategic centers.

3. Applied research
   - this component will handle the bio-physical and socio-economic characterization of the natural habitat and to determine optimal approaches in the population restoration and/or regeneration of endangered flora and fauna.

4. Policy studies
   - the review of existing laws and restrictions, ensuring in the future the promulgations of new laws for the conservation program.

5. Conservation education, information and communications
   - the prime objectives of this component is to raise the level of awareness, understanding and appreciation of genetic resources through an expanded education and information campaign.

6. Captive species research and development
   - to conduct research to increase the productive and reproductive functions of the animals.

7. Support activities
   - this would include trainings, information systems and monitoring/evaluation of the program.

In the interests of maintaining biological diversity, the needs of the human population and national development concerns cannot be fully sacrificed. For the program to be effective, it should integrate sustainable development of animal genetic resources which basically involved the following components:

1. Accurate inventory of available animal genetic resources
   - this is the first step in any conservation effort wherein basic information should be available in the form of an animal data bank.

2. Evaluation and documentation
   - the identification of species and breeds which could be classified as endangered or threatened is important before any conservation effort can
be implemented.

3. Appropriate conservation, in situ and/or ex situ
   - the constraint of high cost is limiting the preservation of a large number of animals but this is a very important part of the whole conservation program.

4. Evaluation and use of biotechnologies
   - future technologies would ensure a more practical conservation system that needs lesser space and financial resources.

Conclusion

Much work is still needed in order to make the present conservation efforts and systems of animal genetic resources successful. However, the necessary technology and concerns are gradually being developed to suit each specific species or breeds and the needs of national development.

The urgent need is still for sufficient funds necessary for this very important undertaking to support a comprehensive system approach in the conservation of animal genetic resources.

References


Collection and Preservation of Animal Genetic Resources in the MAFF Gene Bank

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Abstract
The MAFF genebank project on animal genetic resources in Japan was initiated in 1985. The organization of MAFF genebank consists of the central bank at the National Institute of Agrobiological Resources and four subsidiary banks. A total of 713 breeds and their lines have been collected and are been preserved as small herds, cryopreserved semen and embryos. Cryogenic storage has been widely adopted to preserve germplasm for the long-term to avoid inbreeding depression. The importance of standardized cryopreservation methods for each species, and their appropriate application to prevent mishandling and mistakes is stressed.

Introduction
Current Japanese livestock breeds and their lines can be dividing into three categories according to their origin and history (Obata et al., 1992). Firstly, there are various native livestock and domesticated fowls that either existed or were introduced into Japan before the Meiji restoration (1868), and some of which have kept up to the present time. Secondly, there are dairy cattle, pigs and poultry that were imported after the Meiji restoration. Thirdly, there are beef cattle which are crossbreeds between native and imported cattle breeds after the Meiji restoration. The increasing emphasis upon certain breeds of livestock because of their economic value and high performance is resulting in other, less popular local breeds declining in numbers. It is recognized that local breeds are not very useful in the modern animal industry. However, they are essential for the improvement of animal breeds and research development of animal genetic resources to maintain genetic diversity of animal resources in the country.

This paper reports on the MAFF (Ministry of Agriculture, Forestry and
Organization of MAFF Gene Bank Project

The MAFF gene bank was initiated in 1985 for the conservation of plant, microorganism, animal, forest species and aquatic organisms. In 1993, the DNA bank was newly established and to preserve DNA and accumulate information using a computer network. The genetic resources center of MAFF was established in the National Institute of Agrobiological Resources (NIAR) for plant, microorganism, animal and DNA genetic resources. The MAFF gene bank consists of a national center and many sub-banks located throughout Japan. The project aims to survey, collect, classify, identify evaluate and preserve those genetic resources in Japan and from overseas, and further expand the genetic resources information stored in the database. The organization of animal genebank is shown (Figure 1). The organization of MAFF gene bank consists of the central bank at NIAR and 4 subsidiary banks, which are National Institute of Animal Industry, National Institute of Animal Health, National Institute of Sericultural and Entomological Science, and National Livestock Breeding Center. National Livestock Breeding Center has seven stations in different parts of Japan. The gene bank covers a wide range of animals including livestock, domesticated birds, experimental animals, silkworm and honeybees. The genebank is carried out in a collaborative way between the central and subsidiary banks. Each member of the collaborative team shares responsibilities for conservation of designated animal species.

The procedure for collection, evaluation and the category of preservation are presented (Figure 2). When we collect animal genetic resources, we record passport data which is as like a census registration consisting of basic information such as name of species, breeds or line, the place and the date of the collection are entered. Evaluation of the characteristics of animal genetic resources is based on the animal genetic resources characteristics investigation manual (National Institute of Agrobiological Resources, 1992). Characteristics are classified into 3 classes (morphological, physiological and economic), and each class consists of essential and optional descriptors. The number of descriptors varies depending on the various conditions under which animal genetic resources are reared. An example for beef cattle is shown (Figure 2). The primary characteristics of genetic resources are morphological characters such as coat color,
presence or absence of horns. The secondary items are developmental and physiological characteristics. The characteristics in this category involve blood components and chromosome numbers, and these have important implications for the use of those animal genetic resources practically. The third class of characteristics are concerned with economic benefits, such as age of first calving and meat production. 

Preservation consists of three categories, the working, base and active collections. The working collection means temporary preservation until the evaluation results are clear and the need for preservation is clear. However, the concept of working collection was developed for plants, for animal genetic resources the concept of a working collection is different. The second category is the basic collection, which means the permanent preservation and multiplication of genetic resources. The third category is the active collection consists of germplasm which can be distributed to users. 

These activities are recorded annually in the annual reports on animal genetic resources in the MAFF gene bank project and the annual survey report for animal genetic resources. Textbooks and manuals for database development and cryopreservation have been published irregularly.

Status of Collection and Preservation in the MAFF Gene Bank

When the MAFF genebank project started in 1985 there were a total of 557 accessions preserved and the percentage of silkworm accessions represented 77% (431/557). A large number of silkworm breeds and/or lines reflected Japan’s industrial background until 1950. A plan was made to collect animal genetic resources and 5 to 23 accessions have been cumulated annually. At the end of 1995, a total of 713 animal breeds or their lines of 18 species have been preserved (Figure 3). By the year 2000 the aim is to collect and preserve about 830 accessions. The number of accessions of the main animal species, and their breakdown indicates that the number of accessions of domesticated birds, cattle and pigs are increasing, while silkworms have not increased.

In Japan, there are 8 native horses breeds (Hokkaido pony, Kiso pony, Taishu pony, Noma pony, Misaki pony, Miyako pony, Tokara pony and Yonakuni pony), and 2 native cattle (Mishima cattle, Kuchinosima wild cattle) and 4 crossbred beef cattle (Japanese black, Japanese brown, Japanese polled, Japanese shorthorn), and 2 native goat (Tokara goat, Shiba goat). There are not many Japanese native animal breeds. However, there are many domesticated native birds which consist of 17 breeds and 36
Figure 1. Organization of the MAFF animal gene bank project.

Figure 2. The collection, investigation and preservation procedure for animal genetic resources.
Figure 3. The annual progress in preservation in animal genetic resources.

Figure 4. The number of preserved animal genetic resources by species in 1995.
Table 1. Major animal genetic resources preserved by the MAFF gene bank project

<table>
<thead>
<tr>
<th>Species</th>
<th>Name of major breed</th>
<th>Preservation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Mishima Cattle</td>
<td>Live, Frozen semen &amp; Embryos</td>
</tr>
<tr>
<td></td>
<td>Kuchinoshima Cattle</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Japanese Black</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Japanese Brown</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Japanese Polled</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Japanese Shorthorn</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td>Horses</td>
<td>Hokkaido Pony</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td>Kiso Pony</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Taishu Pony</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Tokara Pony</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td>Goats</td>
<td>Shiba Goat</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td>Tokara Goat</td>
<td>Live</td>
</tr>
<tr>
<td>Pigs</td>
<td>Middle Yorkshire</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Berkshire</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Bouso Landrace Strain</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Meishan Pig</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td>Chickens</td>
<td>Hinaidori</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Koeyoshi</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Tounmaru</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Toutenkou</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Shamo</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Satsumadori</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Ukokkei</td>
<td>Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Mikawa Fowl</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td>Nagoya Fowl</td>
<td>Live &amp; Frozen semen</td>
</tr>
<tr>
<td></td>
<td>Tosa Native Fowl</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td>Tsushima Native Fowl</td>
<td>Live</td>
</tr>
<tr>
<td></td>
<td>Araucana</td>
<td>Live</td>
</tr>
</tbody>
</table>

varieties. The list of major animal genetic resources preserved by the MAFF gene bank is shown in Table 1. Many breeds and their lines remain to be collected.

Cryopreservation of Animal Germplasm

There are three ways to conserve animal genetic resources:

1. *in situ* conservation: keeping animal population in their habitat, and reproduction is natural;
2. *ex situ* preservation: animals are under artificial control for nursery and reproduction;
3. *in vitro* preservation: application of the technologies of cryobiology and reproduction to animals.
In order to multiply endangered animal genetic resources, *in situ* conservation is top priority. *In vitro* preservation should be started at the same time as *ex situ* preservation is initiated. These three ways of conservation are complementary and essential for effective conservation of animal genetic resources. *In situ* conservation is the most useful method to conserve animal genetic resources. Live animal preservation has a number of advantages. These advantages are a) cultural-historical reasons, b) the ability to investigate topical and new traits in the population at any time, and c) maintaining public awareness of the existence of the breed and interest in its frozen material (Obata *et al.*, 1994). The preservation of live animals, however, causes various problem, for example, the high cost in keeping the animals, increased homozygosity within small herds and depression of fertility through inbreeding. The MAFF genebank project aims to develop ex-situ preservation methods for animals since, in Japan, land for in-situ conservation is a constraint.

Cryopreserved germcells can be saved permanently and, not with standing any accidents in the storage system, remain available in exactly the same condition as at the time of their collections, at any time in future. Furthermore, cryopreservation of germ cells and embryos could contribute not only to increase in population size but also avoid inbreeding depression in a species. Therefore, the cryogenic storage system has been widely developed to store sperms and embryos with a low operation cost in MAFF genebank.

The ability to cryopreserve sperm and embryos in each of the main domestic animals and domesticated birds is presented (Table 2). Protocols to cryopreserve sperm has already been established. But, the freezing of the embryo is difficult. Only in cattle and sheep is it practical. In the horse, goat and pig embryos are very difficult to

### Table 2. The probability of cryopreservation of semen and embryo in each animal species

<table>
<thead>
<tr>
<th>Species</th>
<th>Semen</th>
<th>Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Horse</td>
<td>○</td>
<td>△</td>
</tr>
<tr>
<td>Goat</td>
<td>○</td>
<td>△</td>
</tr>
<tr>
<td>Sheep</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Pig</td>
<td>○</td>
<td>△</td>
</tr>
<tr>
<td>Domesticated bird</td>
<td>○</td>
<td>×</td>
</tr>
</tbody>
</table>

○: Practical method developed, △: Experimental stage, and ×: Not yet successful.
cryopreserve, so many researchers are engaged in developing cryopreservation protocols for these animals. Embryos of domesticated birds also cannot be cryopreserved, but in 1994, it was reported that cryopreservation of primordial germ cells from chicken embryos was successful. A chimeric bird was produced by injection of cells into another breed of chicken embryos (Naito et al., 1994, Naito, 1994). These recent successes may lead to a new means of preservation in domesticated birds.

Many methods of freezing sperm and embryo in animal species have been developed. However, the method of cryopreservation in same species needs to be standardized so confusion and mishandeling does not occur. There are basically 4 methods to freeze of bovine embryo at the present time. These are (1) stepwise: this is most basic method which uses 1.5 M glycerol as a freezing protectant, and after thawing, glycerol is removed step by step out of a straw, (2) stepwise in straw: bovine embryo is packed 1.5 M glycerol and both side are fuelled with 0.29 M sucrose at the time of cryopreservation, and after thawing, glycerol and sucrose are mixing by figure fillip within a straw, (3) direct method: the bovine embryo is freeze by 1.8 M ethylene glycol, and after thawing , it is possible to transfer directly without any procedure, (4) vitrification: to use a vitrification medium, bovine embryos are capable of being quickly frozen, but after thawing, vitrification medium must be removed out of the straw step by step. It is necessary to standardized cryopreservation methods for each animal species to prevent mishandling and confusion. In the MAFF genebank, a stepwise method using glycerol has been adopted for bovine embryo freezing.

The procedure of germplasm freezing is different for each animal species. Domesticated birds semen has specific characteristics which make it cold temperature tolerant within 15 minutes after ejaculation. Therefore, ejaculated semen is collected in a test tube cooled to \(5^\circ\text{C}\) and after dilution with Lake solution 4 to 10 times, it is possible to cryofreeze using evaporated liquid nitrogen immediately. While, pig semen is very sensitive to temperature change. The procedure for cryopreservation of pig semen is complex, and time consuming compared with freezing semen of domesticated birds. In the MAFF genebank, the cryofreezing procedures for each animal species is described in the "Animal germplasm preservation manual" (National Institute of Agrobiological Resources, 1994). This manual describes how to get high viability of frozen-thawed germplasm and lists standardized methods and medium for each animal species. The viability of frozen-thawed semen of these species varies between 50 to 90%. These
Figure 5. The procedure of pig semen cryopreservation
Figure 6. The percentage of accessions preserved as live, semen and embryo in each animal species.

methods are incorporated into the stock management database.

The ratio of live and/or cryopreserved semen and embryos in the main domestic animals and domesticated birds at the present time is shown (Figure 6). The percentage of cryopreserved germplasm in cattle and pig are 66% and 44%, respectively. The percentage of cryopreservation in each animal species will be increased gradually. The total number of cryopreserved semen tubes of cattle, pigs and domesticated birds are 23,000/0.5 ml tube, 1,400/5 ml tube and 2,500/0.5 ml tube, respectively (National Institute of Agrobiological Resources, 1995). These cryopreserved semen have been preserved in the central bank and subsidiary banks.

Conclusion

Animal genetic resources that are not currently in great demand may be valuable for scientific and economics purposes in future. When the MAFF genebank project was initiated, it was recommended that native animals which are endangered should be collected and preserve first. This advisory recommendation was reasonable, but it became clear that it is very difficult to collect a sufficient number of samples from a
highly inbreeding endangered herd. Therefore, it is essential to collect and preserve broadly from animal genetic resources in Japan without distinction between native and introduced animals. Usually, it is impossible to build a livestock facilities to conserve new animal germplasm, space for livestock is decreasing which number of accessions is increasing. Cryogenic systems need to be further improved. Finally, we have to cooperate harmoniously with animal genebank researchers worldwide to develop an international system for animal genetic resources preservation.

References
Evaluation and Preservation of Silkworm Genetic Resources in Japan

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Abstract
In the National Institute of Sericultural and Entomological Science, more than 450 races or strains of the silkworm, Bombyx mori L., are conserved for breeding and experimental use. These genetic resources are evaluated to better understand the differences among strains. The traits evaluated are classified into the following three groups: (1) Visual characteristics of the egg, larva, cocoon and pupa, (2) features important in sericulture, for example hatchability after overwintering, rearing duration, response to artificial diet, thermo-tolerance and (3) the cocoon features in relation to reeling and both biochemical and molecular biological characters of the silkworm. Recent topics in relation to the evaluation and preservation of silkworm genetic resources in Japan will be discussed.

Domestic silkworms in Japan
In Japan, races of domestic silkworm, Bombyx mori L., can be categorized into several groups based on their uses. All races for Japanese cocoon production are "Authorized Races" they are F₁ hybrids whose parent races and mode of cross combination were authorized by the government. Cocoon production using unauthorized races is prohibited by the Sericultural Industry Law of Japan. This law was enacted to standardize raw silk quality using Authorized Races, and the breeding of silkworm races suitable for mechanized reeling of silk. Authorized Races were bred from other Authorized Races or unauthorized silkworm races conserved solely for breeding. Various breeding stocks have been conserved by many breeders. Mutant strains have been conserved for basic science as well as applied biology. Silkworm mutants have contributed greatly to classical genetics in Japan (Tanaka, 1927).

Domesticated silkworm cannot survive in natural environments since the larval body is too heavy to move on a mulberry branch to find the leaves. Also domesticated larvae have no mimicry to escape predators, in particular birds, and the adults have mating difficulties. However the wild ancestor of the domesticated silkworm has not clearly been determined. A possible ancestor is Bombyx Mandarina Moore, which has not been used for cocoon production (Yoshitake, 1984). B. Mandarina can produce
fertile hybrid progeny when mated with domestic silkworm. Strains derived from hybridization using *B. Mandarina* are conserved in several institutes.

**Institutions conserving silkworm stocks**

**National Institute of Sericultural and Entomological Science**

National Institute of Sericultural and Entomological Science (NISES) conserves many silkworm races for a variety of uses. Many stocks have been used to produce Authorized Races. Stocks used to produce Authorized Races are conserved for future breeding purposes and continue to be used for scientific studies even after governmental authorization has been terminated. Some silkworm races have been maintained more than 200 years in Japan. The silkworm races and strains conserved at NISES are divided into 5 groups: a) Authorized Races b) Geographically fixed races c) Improved races d) Breeding resources e) Mutants. Groups b, c and e are categorized as "genetic resources of NISES", whereas groups a and d are not included in this category (Sorita, 1991). The genetic resources of NISES are distributed for both research and breeding.

**Kyushu University Institute of Genetic Resources**

Kyushu University Institute of Genetic Resources conserves more than 400 mutant strains of domestic silkworm. The mutant stocks have been used in basic genetics, physiology, biochemistry and molecular biology. All stocks in Kyushu University are multi-marker strains each containing several distinctive mutant genes located on different chromosomes. The multi-marker strains are being used to establish a linkage map of *B. mori* (Doira, 1978) and are offered to investigators on request. The silkworm is an important experimental organism like the fruit fly, *Drosophila melanogaster*.

**Private companies and an incorporated organization**

Some private companies and an incorporated organization related to sericulture have originally bred Authorized Races and stocks for breeding.

**Silkworm genetic resources in NISES**

The genetic resources of the silkworm at the NISES are conserved in the Laboratory of Genetic Resources, Kobuchizawa, Yamanashi Prefecture. These genetic resources are classified as listed in Table 1.
Table 1. The number of geographical races of domestic silkworm stocked in National Institute of Sericultural and Entomological Science

<table>
<thead>
<tr>
<th>Place of breeding</th>
<th>Status</th>
<th>Number of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Native</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Improved</td>
<td>53</td>
</tr>
<tr>
<td>China</td>
<td>Native</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Improved</td>
<td>69</td>
</tr>
<tr>
<td>Europe</td>
<td>Native</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Improved</td>
<td>19</td>
</tr>
<tr>
<td>Tropical</td>
<td>Native or modified</td>
<td>6</td>
</tr>
<tr>
<td>Korea*</td>
<td>Moltinism races</td>
<td>21</td>
</tr>
<tr>
<td>Others</td>
<td>Mutants</td>
<td>173</td>
</tr>
</tbody>
</table>

* Some but not all originated in Korea, not all.

Table 2a. The items to be checked during the conservation of silkworm stocks (Primary characters)

- Voltinism
- Egg color
- Molting
- The body color of newly hatched larva
- Old larval body color
- Larval body shape
- Larval body spots
- Haemolymph color
- Cocoon color
- Cocoon shape
- Wrinkle of cocoon
- Pupal body shape
- Pupal body color
- Other adequate genotypes

Geographic races

The category of geographic races conserved in NISES involve four groups, Japanese, Chinese, European and Tropical races. Three groups, Japanese, Chinese, and European races, have contributed to cocoon production in Japan. Tropical races were used to establish lines that can tolerate both high temperature and humidity. Each group is classified into, native races and improved varieties. Native races had been used for Japanese sericulture before the inception of the authorization law. These races are unsuitable for the production of raw silk of standardized quality, since they have defects in the mass production and in the raw silk percentage of the cocoon. Native races are used to improve and stimulate breeding resources and materials for experimental
purposes. Improved races, once authorized by the government, are used in sericulture as Authorized Races. Inter-crossing of geographic groups is an efficient way to obtain heterosis.

**Moltinism races**

Most silkworms are tetramolters, however, races from Korea vary in the number of larval moltings and are classified as moltinism varieties. There are trimolters, pentamolters and one hexamolter in races from Korea. Some trimolters are used in sericulture since these produce cocoons with a particularly fine filament. The raw silk produced by such races is suitable for high class silk products.

**Mutant strains**

Mutant strains are divided into original mutant strains with one mutant gene and multi-marker strains (most of the later have come from Kyushu University). Some original strains have contributed to experiments on breeding of commercial races, for
example oligophagous races that can be reared on an artificial diet, and races possessing sex linked body markings. Mutant genes preserved in these strains serve as useful genetic markers in, for example, linkage analysis and three point analyses (Doira, 1978).

Conservation of silkworm stocks in NISES

The conservation of geographic races and moltinism races, the maintenance of genetic diversity is important because of the possibility that these races possess unknown heterogeneous characters, in addition, to the known practical characters. Usually, fifty or more moths are used for egg production to avoid the loss of minor genes from the population. In this way races are conserved as population gene pools.

Authorized races and breeding resources are maintained under high selection pressure on the basis of phenotypes related to raw silk production. Consequently, these races can produce silk of good quality. However, this type of selection is not always performed when conserving silkworm genetic resources. Consequently, silkworm genetic resources are not in the best state for cocoon production. Mutant strains are maintained only for the preservation of genes or genotypes. Although the technology of the rearing silkworm on an artificial diet has been established and generally applied to the commercial production of cocoons in Japan, an artificial diet is not used to maintain genetic resources. This is because the silkworm is considered to be adapted only to mulberry leaves and there is the fear that dietary selection would occur if silkworms are reared on an artificial diet.

Preservation of diapause eggs

Each race is reared once a year except for the tropical races which are reared twice or more per year. In winter, silkworms are preserved as hibernating diapause eggs, since mulberry leaves can only be produced in southern Kyushu and Okinawa during winter. Rearing seasons are limited to when mulberry leaves are available. We have three major rearing seasons in Japan, spring, summer and autumn. The winter diapause stage enables silkworm eggs to survive low temperatures and low relative humidity. For the efficient preservation of silkworm eggs, the diapause phenomenon of silkworm eggs should be understood well.

The diapause in silkworm eggs is induced by the action of the diapause hormone,
which is secreted from the maternal suboesophageal ganglion and influences the ovary. The newly laid eggs enter diapause at the early gastrula stage. The eggs develop again after longterm chilling. The induction of diapause is also genetically controlled by voltinism. In univoltine races, moths always produce diapause eggs. The diapause of both bivoltine races and polyvoltine races is determined by temperature and photoperiod. In Japanese sericulture, the diapause phenomenon in most races and strains of the domestic silkworm is well controlled by artificial procedures. To induce diapause eggs, the eggs of the parental generation were incubated at 25°C under long day photoperiod, 15 hours photophase and 9 hours scotophase. As a result, the female moths produce eggs that enter diapause. The diapause eggs are preserved at natural temperatures until autumn and then transferred to a low temperature until the following spring. Diapause is terminated under cold temperatures during winter. Diapause can be broken by subjecting eggs to hot hydrochloric acid treatment. The hot acid treatment is effective against the eggs for a few days after oviposition or after chilling for more than one month. If eggs have already been kept cold for more than 150 days, then the eggs should be transferred to 25°C without acid treatment. Thus the life cycle of silkworm can be completely controlled.

Diapause eggs are difficult to obtain from tropical races in Japanese conditions. Therefore the larvae of these races are reared at relatively low temperatures and altered photoperiod. Under these rearing conditions, an adequate number of diapause eggs are obtained. However, the eggs are intolerant of preservation by longterm chilling. Thus tropical races are reared twice, in spring and autumn, in Japan.

In the case of diapause mutants, the homozygotes develop without diapause even though the eggs have received diapause hormone. The mutant strains are conserved as heterozygotes that can enter diapause.

Systematic rearing of silkworms

During a rearing cycle, for efficiency, silkworm stocks should be reared synchronously. Synchronous rearing minimizes accidental loss of genetic resources. Much information useful for the planning of synchronous rearing under standard conditions has been accumulated, for example, the larval life span, hatchability and mating combination. In tetramolters and one pentamolter that have longer life span at the larval stage, the starting day of rearing is adjusted to a few days earlier than in other
races.

**Error management during conservation**

Although there is no way to completely prevent errors during conservation, we can predict which errors are likely to occur most frequently. Therefore several strategies have been designed to minimize errors.

For all races, 50 batches of eggs, each batch from one of 50 moths, are first processed for rearing. The additional eggs are stored at a low temperature, and can be used for additional rearing if the first rearing fails. Chemical disinfection and washing of rearing rooms and instruments after the end of each rearing period is important to prevent diseases. Pebrin caused by a protozoa is the most serious disease because of the transovarian transmission. Therefore, we are obliged to inspect female moths for pebrin according to Sericultural Industry Law. In addition, good maintenance of refrigerator machinery is essential for egg preservation. To prevent the loss of silkworm stocks, the eggs of each batch, each produced by one moth, are divided into two groups which are kept in different laboratories. The location of rearing is an important factor in the conservation of silkworm stocks. In the summer season in Kobuchizawa Town, the average air temperature of around 25°C, relative humidity is low and good quality mulberry leaves available which make silkworms healthy.

**Characteristics of silkworm genetic resources**

Each race of silkworms is phenotypically distinguishable. All stocks are carefully examined during each rearing period. The subjects for checking are summarized as follows.

**Visually distinctive characters**

Visually distinctive characters are used to identify races and sometimes to indicate the occurrence of contamination. In some cases, genes which control visual characters are known and the location on specific chromosomes has been determined. Primary visual characters which are always recorded are listed (Table 2a).

**Physiological characters**

Even when the responsible genes are not understood, physiological characters inherited as prominent features, typical of each race, for example, the tolerance against
extreme temperatures, are recorded. Such features and related ones needed for the conservation of silkworm stocks are considered secondary traits (Table 2b).

**Features important for raw silk production**

Most of the races of silkworm were or are used for cocoon production. Therefore, the features which are related to reeling of silk are important and recorded periodically. These characteristics are considered tertiary evaluation characters (Table 2c).

**Biochemical evaluation**

The number of biochemical traits recorded for silkworm stocks is limited. However, several proteins and enzymes have been analyzed for their location on chromosomes and the frequency of allelic genes among the silkworm stocks. These data are sometimes important to identify the stocks for molecular markers, for example amylase (Matsumura, 1951) phosphatase (Yoshitake and Akiyama, 1965), esterase (Eguchi et al., 1965; Eguchi and Yoshitake, 1966; Yoshitake et al., 1966), proteinase (Eguchi and Yoshitake, 1967), proteinase inhibitors (Fujii et al., 1989; Eguchi and Kanbe, 1982), haemolymph proteins (Gamo and Ohtsuka, 1980; Kawaguchi et al., 1970; Obara and Watanabe, 1969), storage proteins (Tojo et al., 1980), yolk proteins (Izumi et al., 1980; Irie and Yamashita, 1983).

**Pathological evaluation**

The diseases of silkworm have been investigated in NISES and in other laboratories. The susceptibility of silkworm to several viruses has been investigated in most of the silkworm genetic resources (Furuta, 1995). A noteworthy result is the recent establishment of a silkworm race which is resistant to densonucleosis virus (Eguchi et al., 1991).

**Molecular evaluation**

Molecular techniques applied to genomic and cDNA clones of the silkworm should be undertaken to obtain new perspectives on genetic resources. One main technique for the silkworm is Southern blot analysis. Genomic clones constructed for this type of analysis (Yukuhiro et al., 1993; Tamura et al., 1993) were found to have repetitive sequences or unique sequences. Two races, C108 and p50, which are
commonly used as the standard silkworm races for genome study, were revealed to be distinctive by Southern analysis using such genomic clones. Also cDNA probes were constructed from poly(A)+ RNAs of silkworm eggs and used for Southern analysis (Hara, 1996). Some of the probes could detect inter-race differences, although other probes exhibited neither inter- nor intra-race differences. The polymerase chain reaction (PCR) methodology has been applied to most of the geographic races stocked in NISES (Yasukochi, personal communication) this has uncovered Randomly Amplified Polymorphic DNA (RAPD). These techniques are helping clarify phylogenetic relationships among the races.

Conclusion

Currently the silkworm is being used in new areas, for example, for medical uses such as producing fibroin membranes. Consequently, the evaluation of silkworm genetic resources should be updated to respond to new needs. The genetic stocks of the silkworm in Japan exceeds 1,000 accessions. The establishment of systematic and authorized information system on these stocks is necessary.

References


Furuta, Y. 1995. Susceptibility of the races of the silkworm, Bombyx mori, preserved in NISES to the


Questions and answers in Session 3

Questions to Dr. Matias

Q. Why do people like black pigs in the Philippines? (Wang)
A. In fact white pigs are more popular in the Philippines e.g. Landrace and Yorkshire breeds. We have in the Philippines a special dish for pigs, that is, roasting the whole pig, wherein white colored pigs are preferred. However in some speciality shops native black pigs command a higher price than white pigs. (Matias)

Q. What is the productivity of pigs in the Philippines, for example what is the slaughter weight? (Wang)
A. The productivity of native pigs is not as high as exotic pigs or crossbreds. The slaughter weight for native pigs (in my experience from the Ifugao) ranges from 30-40kg. (Matias)

Q. According to our direct observations the surviving number of Tamaraw is only 43. However the count is continuing. Do you have any comment, Dr. Matias? (Namikawa)
A. Unofficial reports suggest the population of the Tamaraw is 200, but the exact data is not available. (Matias)

Q. Do you have any idea what the breeding structure of the Tamaraw is? (Namikawa)
A. Tamaraws are very secretive and shy animals. Efforts are still being made to determine such factors. (Matias)

Q. Would one reason for the decrease in size of the carabao be that farmers sell off the larger animals to get a better price? Leaving only small animals for breeding? (Tan)
A. I agree with your observation. Another reason for the decline in size is that farmers castrate larger bulls for draft purposes. Smaller animals are used for natural mating with females. (Matias)

Q. Do you have any evidence or historical stories concerning the domestication of native chickens or pigs in the Philippines? (Yamamoto)
A. We do have written reports regarding domestication of native chickens and pigs. However scientific facts should be established first before we can resolve issues related to domestication. (Matias)
**Question to Dr. Izaike**

**Q.** In your genebank there are 33 breeds/lines of pig. How many are breeds and how many are lines or strains? Where are they from? (Wang)

**A.** We collected 5 breeds the rest are lines. The breeds came from Europe and China. (Izaike)
Use of Marker Information to Maintain Variability in Small Populations

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Introduction

It is essential to keep genetic variability as high as possible in order to avoid inbreeding depression or disappearance of valuable genes, when we keep animal resources in a small population. We usually use coefficients of inbreeding, relationship (Wright, 1921) or coancestry (Malecot, 1948) as criteria to measure genetic variability.

Coancestry is the correspondence probability that a pair of gametes in the population inherited from the same gamete of the same ancestry, and other coefficients can be derived from coancestry. The calculation of this probabilities based on the idea that offspring inherit one gamete from each parent. Without information about which of the pair is inherited, we can only give probability of 1/2 for each (Falconer, 1960).

Figure 1 shows a simulated distribution of proportion that the gamete of X and Y inherited the same grandparental allele at 1000 loci in the family of Figure 2. Coancestry gives us only one value of probability, 0.125, for this proportion in this family, while the simulated values are widely distributed. That means, more precise evaluation is possible with some additional information.

Fortunately, many loci on animal genome maps have been located and where we can distinguish the genotype of each individual exactly (Cheng et al., 1995; Archibald et al., 1995; Crawford et al., 1994; Bishop et al., 1994; Rohrer et al., 1994; Bumstead and Palyga, 1992). The genotype of parents and offspring sometimes gives us information about which of the parents or which gamete of the pair in the parent, the gamete of the offspring is inherited from.

We can make more precise evaluation of coancestry using information on these loci as markers.
Method

Without information about marker genotypes, the probability that one gamete of an individual X and another individual Y have the same origin, \( r(X,Y) \) is the average of the probability that the gametes of Y and the parents having the same origin,

\[
r(X,Y) = \frac{1}{2} \{ r(P,Y) + r(M,Y) \}.
\]

While, if sire(P), dam(M) and offspring(X) have genotype of AB, AC and AC at one marker locus, we can easily know that the allele A of the offspring is inherited from the sire and C from the dam. Further, at the locus which have recombination ratio \( c \) with this marker locus, the allele on the gamete having A inherited from the paternal gamete having A with probability \((1-c)\), and from the paternal gamete having B with probability \( c \). Then we can evaluate the probability of correspondence at this locus as,

\[
r(X_A,Y) = (1-c) \cdot r(P_A,Y) + c \cdot r(P_B,Y).
\]

where, subscript A and B denotes the allele on the gamete having marker allele A and B, respectively. Table 1 shows the calculation of the correspondence probability for each combination of marker genotypes of parents and offspring. Allele type A, B, C, D denotes only the pattern in this table, and it covers all the possible cases. Then some generations after the base population, we have the probability of correspondence expressed as a polynomial of the recombination rate \( c \).

Now, we have a method to evaluate the probability that a pair of the gametes are correspondent at one position around a marker locus as a function of the recombination ratio, \( f(c) \). Then integral of the function and accumulation for all the marker loci scattered on the gamete leads us to the average probability for whole gamete,

\[
r = \sum w f(c) dc
\]

where \( w \) denotes the weight for each position and

\[
\sum w dc = 1.
\]

We use ordinary coancestry instead of \( f(c) \) for the area without available marker. Usually, \( w \) is the same for all positions on the gamete. We can, however, put other values for position with special importance, such as QTL or MHC (Takeda et al., 1995).
Table 1. Calculation of the correspondence probability of offspring

<table>
<thead>
<tr>
<th>genotype</th>
<th>offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>W</td>
</tr>
<tr>
<td>AA</td>
<td>AA</td>
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<td>BB</td>
</tr>
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<td>AA</td>
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<td>AA</td>
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<td>AC</td>
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<tr>
<td>AB</td>
<td>AC</td>
</tr>
<tr>
<td>AB</td>
<td>CD</td>
</tr>
</tbody>
</table>

Where the probability that one gamete of offspring and other gamete(Y) in the population inherit allele of same origin at locus of recombination ratio c with the marker, r(Xi,Y), can be calculated from the probability between parental gamete and Y, r(Vj,Y) and r(Wk,Y). Subscript denote the type of marker allele that the gamete have. This table covers all the possible cases, since allele type A, B, C, D and the parents V ,W denote only the pattern.

Simulation

A simulation study was conducted for this method of evaluating correspondence probability (Takeda et al., 1996). It was assumed that a gamete have 10 linkage groups and each has a length of 100 cM. It was simulated whether the gamete chosen from X...
Table 2. Mean, standard deviation of evaluated probability and correlation between evaluated probability and realized proportion with different number of available marker loci

<table>
<thead>
<tr>
<th>case</th>
<th>number of marker loci</th>
<th>mean</th>
<th>s.d.</th>
<th>correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.1251</td>
<td>0.017</td>
<td>0.474</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.1250</td>
<td>0.026</td>
<td>0.599</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.1251</td>
<td>0.030</td>
<td>0.668</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.1251</td>
<td>0.030</td>
<td>0.745</td>
</tr>
</tbody>
</table>

The distribution of evaluated probability becomes wider as the area covered with markers becomes larger. The correlation becomes higher as the number of available marker becomes larger.

Table 3. Mean, standard deviation of evaluated probability and correlation between evaluated probability and realized proportion with different number of allele types on each marker locus

<table>
<thead>
<tr>
<th>number of marker loci</th>
<th>heterozygosity</th>
<th>mean</th>
<th>s.d.</th>
<th>correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.50</td>
<td>0.1250</td>
<td>0.018</td>
<td>0.584</td>
</tr>
<tr>
<td>3</td>
<td>0.67</td>
<td>0.1251</td>
<td>0.029</td>
<td>0.745</td>
</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>0.1251</td>
<td>0.036</td>
<td>0.815</td>
</tr>
<tr>
<td>5</td>
<td>0.80</td>
<td>0.1250</td>
<td>0.040</td>
<td>0.850</td>
</tr>
</tbody>
</table>

The probabilities were calculated for different numbers of allele types of each marker in case 4. As the number of allele type loci becomes larger, the marker becomes more informative because of the higher heterozygosity.

and Y in Figure 2 inherited the same grandparental allele on a loci at every 1 cM (total 1000 loci). The proportion of loci where inherit the same allele, realized proportion, was compared with the evaluated probability by this method.

The evaluated probabilities were calculated for 4 cases having different numbers of available marker loci (Figure 3). Case 1: 1 marker located at 50 cM from one end of each linkage group (total 10 markers) was used to evaluate the area from 30 to 70 cM. They covered 40% of whole gamete and coancestry 0.125 was used to other area. Case 2: 2 markers located at 30 and 70 cM of each linkage group (total 20 markers) were used to evaluate the area from 10 to 90 cM. They covered 80% of the gamete. Case 3: 3 markers located at 20, 50 and 80 cM of each linkage group (total 30 markers) covered the whole gamete. Case 4: 5 markers located at 10, 30, 50, 70 and 90 cM of each linkage group (total 50 markers) covered the whole gamete.

Three types were randomly put on the grandparental allele of each marker locus. Then, we change the number of allele types put on each marker locus from 2 to 5 in Case 4. These evaluations were done for 100,000 simulated families.
Figure 1. The simulated distribution of the proportion that the gametes of X and Y of family in Fig. 2 inherited the same grand parental allele. The proportion has mean 0.125 as coancestry indicate, but it is distributed from 0 to 0.50 and has a deviation of 0.067.

Figure 2. The family of the simulation. X and Y are cousins each other both paternal and maternal. It is simulated whether a pair of gametes from X and Y, one from each, inherit the same grandparental allele on loci throughout the gamete.
Case 1: 1 marker for each linkage group
position of the marker
0 30 50 70 100 (cM)
marker used for evaluation coancestry 1 2 coancestry

Case 2: 2 markers for each linkage group
position of the markers
0 10 30 50 70 90 100 (cM)
marker used for evaluation coancestry 1 1 2 2 coancestry

Case 3: 3 markers for each linkage group
position of the markers
0 20 35 50 65 80 100 (cM)
marker used for evaluation 1 1 2 2 3 3

Case 4: 5 markers for each linkage group
position of the marker
0 10 20 30 40 50 60 70 80 90 100 (cM)
marker used for evaluation 1 1 2 2 3 3 4 4 5 5

Figure 3. Position of the markers and the area where the probability is calculated using markers for each case of the simulation. Each of 10 linkage groups has markers at the same position. Case 1: 1 marker located at 50 cM from one end of each linkage group was used to evaluate the area from 30 to 70 cM. The coancestry 0.125 was used for the other area. Case 2: 2 markers located at 30 and 70 cm of each linkage group were used to evaluate the area from 10 to 50 cm and from 50 to 90 cm respectively. Case 3: 3 markers located at 20, 50 and 80 cm of each linkage group were used to evaluate the area from 0 to 35, 35 to 65 and 65 to 100 cm respectively. Case 4: 5 markers located at 10, 30, 50, 70 and 90 cm of each linkage group were used to evaluate the area from 0 to 20, 20 to 40, 40 to 60, 60 to 80 and 80 to 100 cm respectively.

Result and Discussion
A distribution of realized values simulated has already shown in Figure 1. The mean was 0.125 as coancestry indicate, but it ranges from 0 to 0.50 with standard deviation of 0.067. Figure 4 shows a simulated distribution of the evaluated probability of case 4. Figure 5 shows the scattered diagram of the evaluated value of case 4 and the realized value of the first 1000 simulations. Table 2 shows the mean and standard deviation of evaluated probability and the correlation with realized proportion.

The distribution of evaluated probability becomes wider as the area covered with markers becomes larger. The correlation becomes higher up to 0.75 in case 4 as the number of available marker becomes larger. These correlations are high even 0.47 of
Figure 4. The simulated distribution of the evaluated probability. The probability calculated for case 4. The distribution ranges 0.03 to 0.30 and has mean 0.125, standard deviation 0.03.

Figure 5. Scattered diagram of the evaluated probability and the realized proportion. The realized proportion and the evaluated probability of the first 1000 simulations in case 4 are plotted.
case 1, since the ordinary coancestry have only one value of evaluated probability, 0.125.

Table 3 shows the mean and standard deviation of evaluated probability and the correlation between evaluated probability and realized proportion in case 4 when we change the number of allele types on each marker locus. The marker becomes more informative because of the higher heterozygosity as the number of allele types becomes larger. The correlation reached 0.85 with 5 types of alleles on each marker loci.

Although the situations in this simulation, the number and length of linkage group or the position of marker loci, are different from that in real animals, the value of correlation would not be so different, if the heterozygosity of markers and the ratio of area covered are similar.

This simulation indicated that use of marker information gives us more precise evaluation for the relationship between gametes than the ordinary methods. It enables us to select the mating pair effectively to keep genetic variability high in a small population.

Reference
New Endocrinological Methods for Production of Germ Cells in Birds

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Introduction

Despite recent advances in biological techniques, little has been incorporated into conservation biology. We have been attempting to apply modern endocrinological methods to conservation biology, especially in helping to breed endangered avian species including techniques for captive breeding programs (Ishii, et al., 1995). To select individuals for efficient breeding, from a captive colony of an endangered avian species, it is necessary to know the gametogenic and endocrine conditions of the ovary and testis of birds in the colony. To estimate these conditions, measurement of plasma levels of hormones which are directly related to the gonadal conditions is necessary, since direct observation of the gonad by laparotomy may not be preferred. However, it is also not recommended to catch such precious birds for collection of blood samples. To solve these problems, we have developed a method to measure sex steroid hormone levels in avian feces which reflect sex steroid hormone levels in blood. We first present some of our data showing how accurately fecal sex steroid levels reflect plasma sex steroid levels in Japanese quail which were used as a model species. We also present an example of the application of this method to an endangered species, the "Toki" or Japanese crested ibis (Nipponia nippon).

According to the estimated gonadal condition by fecal sex steroid analysis method, we can select individuals for breeding. If none of the examined individuals shows an active gonadal condition, we have to stimulate the testis or ovary of some individuals in order to promote breeding. For breeding mammals, including man with regressed gonads, administration of gonadotropic hormone is commonly used. However, the situation in birds had been different until recently. Even in domestic hens with regressed ovaries, researchers have not been able to induce ovulation, the final step of the ovarian gametogenic activity, by hormone administration. Accordingly, we developed a method to induce ovarian growth and ovulation in female birds by administration of a hormone (Wakabayashi et al., 1994). The method for hormonal induction of the ovarian processes - the oocyte development, ovulation and oviposition - is described.
Fecal sex steroid analysis in birds

*Extraction methods:*

We employed three different methods, depending on the species, to extract sex steroids from fecal samples of birds. The simplest method was extraction using water as the extraction medium followed by radio-immunoassays of the steroids. This method was used in the Japanese quail. In the Japanese crested ibis, we had to use an organic solvent for extraction and LH20 column chromatography for removing substances which disturbed measurement of steroid hormone by radioimmunoassay. A more complicated method was developed to measure sex steroids in feces of the rock ptarmigan (*Lagopus mutus*). Feces of this bird contained much lipid-like substances which had to be separated from the steroids before radioimmunoassay. For this purpose, we used extraction with an organic solvent followed by a high performance liquid chromatography.

*Fecal sex steroid study in the Japanese quail:*

To demonstrate the usefulness of fecal sex steroid analysis method in estimating gonadal activities, some of our results with the Japanese quail are presented.

In one experiment, we used three groups of male Japanese quail:

1. normal intact adults kept under a long-day photoperiodic regimen,
2. normal intact adults kept under a short-day photoperiodic regimen and
3. castrated adults kept under the long-day photoperiodic regimen.

Mean concentrations of androgen (testosterone plus dihydrotestosterone) in blood plasma and feces were compared between the three groups and also between plasma and feces. The mean concentrations in feces changed in close association with those in plasma (Figure 1). In the next experiment using females, feces were collected from two groups of Japanese quail hens, one laying at a certain period of the day and the other non-laying. It is well known, that progesterone in plasma shows a transient rise around the time of the ovulation in laying hens. We collected fecal samples from laying hens and progesterone in the samples was measured. The mean concentration of progesterone in feces had a clear peak around the time of ovulation that was estimated from the oviposition time in the laying group but no significant peak in the non-laying group (Figure 2). Thus, we could show that the gonadal activity can be estimated by monitoring fecal sex steroid hormone. Unlike mammals, birds excrete both urine and feces together.
Figure 1. Mean concentrations of testosterone in blood plasma (pg/ml) and feces ($10^{-1}$ pg/dropping) in normal adult males kept in a long-day photoperiodic regimen, normal adult males kept in a short-day photoperiodic regimen and castrated males kept in the long-day photoperiodic regimen.

Figure 2. Mean concentrations of estradiol-17 beta in blood plasma ($10^{-2}$ pg/g) and feces (pg/dropping) in normal adult females kept in a long-day photoperiodic regimen, normal adult females kept in a short-day photoperiodic regimen and immature females.
Sex steroids are metabolized to glucronide or sulfate forms before they are excreted. However, sufficient quantities of the free form (circulating form) of the steroids were detected in feces in our experiments. We suppose that they moved from the circulatory system into the contents of the digestive duct by diffusion, because they can easily pass through cell membranes.

**Fecal sex steroid study in the Japanese crested ibis:**

We applied the fecal sex steroid analysis method to assess the reproductive condition of a male, Japanese crested ibis. The mean concentrations of androgen for several days in three summer months in 1994 and a period for several days around New Year's day of 1995 are shown (Figure 4). Compared to the summer months, the concentration was significantly higher in the period around New Year's day when nuptial plumage coloration appeared. It is clear that the fecal androgen concentration reflects well the gonadal activity.

**Hormonal induction of the oocyte growth, ovulation and oviposition in the Japanese quail**

Repeated injections of gonadotropin preparations do not induce ovulation in hens of female domestic birds (Wakabayashi et al., 1994). We studied changes in the luteinizing hormone (LH) concentration in blood plasma in Japanese quail. The LH concentration showed a transient increase and dropped to subnormal levels after several hours. Then, we decided to use the osmotic pump (ALZET Co.,) for the chronic treatment of female Japanese quail with gonadotropin. These birds were kept under a short-days for a certain period before and during the experiment. Their ovaries were considered to be in the completely regressed condition at the start of the experiment. The pump was loaded with chicken pituitary glycoprotein fraction which is rich in gonadotropins, and implanted into the abdominal cavity. The flow rate of glycoprotein was 12.5 μg/hr. This quantity was equivalent to 1.5 μg chicken LH/hr. High gonadotropin levels in plasma were maintained over a period of two weeks, when plasma gonadotropin levels were determined by radioimmunoassay. The ovary of the females treated with pituitary glycoprotein for about two weeks, by means of the osmotic pump, was well developed and contained a number of oocytes containing the yolk (Figure 5). However, no ovulation was induced in these females. To mimic the LH surge which
Figure 3. Change in the mean concentration of progesterone in feces in laying females of the Japanese quail. The time of ovulation, 18:00-20:00, was estimated from the oviposition time.

Figure 4. Comparison of fecal testosterone levels between different seasons in the male Japanese-crested ibis named "Midori" or "Green". Values are means and their standard errors of two to three successive days in each season.
induces ovulation in normal hens, we injected chicken pituitary glycoprotein daily for the last five days of the osmotic pump treatment in female Japanese quail kept under the short-day regimen. Four of the 7 treated females laid 7 eggs during the injection period. One of them laid four eggs and each of the remaining three one egg. All the eggs were incubated artificially and two of them hatched. The chicks were males and one of them was confirmed to be fertile by mating with an adult virgin female. We have been successful in stimulating the testis of male Japanese quail kept under a short-day regimen by this treatment.

**Conclusion and Discussion**

The fecal sex steroid analysis method is a useful tool to estimate the reproductive condition of birds non-invasively. We may apply this method to monitor the reproductive condition of birds in feral populations. Furthermore, in the case of captive breeding of
birds, we may select individuals for breeding from a captive colony of birds using this method. However, selection of an appropriate extraction method is important to obtain a satisfactory result.

Our hormone administration study was the first successful case of hormonal induction of a series of ovarian events, i.e. oocyte development, ovulation and oviposition, in birds. Combination of the two different means of hormone administration, the osmotic pump and injection, is considered to be the cause of our success. However, for the actual application of this method for captive breeding of endangered birds, problems still remain to be solved. First, the success rate of about 40% needs to be improved. It is also important to clarify the problem of the immunological response to hormones from heterologous species. We are now conducting studies to improve the success rate and also examining the possibility of using commercial mammalian gonadotropin preparations such as pregnant mare serum gonadotropin and human chorionic gonadotropin. Preliminary data indicate that these preparations are effective.

References


Use of Chimeric Animals to Preserve Animal Genetic Resources

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1. Production of chimeric animals

Chimeric animals are composite animals containing genetically different cells derived from more than two fertilized eggs. There are two major techniques for making animal chimeras: aggregation and injection methods.

1.1 Aggregation method

8-cell stage embryos are generally used for the aggregation method. After removing the zona pellucida using 0.5% pronase or acidic Tyrode solution, the embryos are stuck together with a medium containing phytohemagglutinin. The aggregated embryos form blastocysts which are transferred into the uterus of pseudopregnant female to obtain chimeric offsprings (Figure 1). The aggregation method is simple and efficient for murine embryos, but it is not suitable for some livestock animals, because of the difficulty of embryo culture when the zona pellucida is removed.

1.2 Injection method

Blastocyst stage embryos are widely used for the injection method. The trophoderm cells of blastocysts are broken by immunosurgery using antibodies and complement for isolation of inner cell mass (ICM) cells. The isolated ICM cells are injected into blastocoel using a micromanipulator, and the injected blastocysts are transferred in the same way as in the aggregation method (Figure 2). Although the injection method requires skill, a shorter time in embryo-culture and the non-removal of zona from embryo makes this method appropriate for many livestock animals.

1.3 Production of chimeric pigs

In contrast to the numerous experiments involving chimeric mice, there have been relatively few reports of chimeras produced from livestock such as sheep (Fehilly et al. 1984; Butler et al. 1987), cows (Brem et al. 1984; Williams et al. 1990; Picard et al. 1990), and pigs (Kashiwazaki et al. 1992).

We examined two injection methods for making chimeras between Chinese
Table 1. The production of piglets following blastomere or ICM cells transplantation (Onishi et al., 1994)

<table>
<thead>
<tr>
<th>Recipient embryos breed* stage</th>
<th>Donor cells breed* stage</th>
<th>No. of embryos transferred</th>
<th>black</th>
<th>white</th>
<th>chimera</th>
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<tbody>
<tr>
<td>M 4-8 cell</td>
<td>LW 8 cell</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Exp. I M 8-16 cell</td>
<td>L 8-16 cell</td>
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<td>4</td>
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<tr>
<td>L 8 cell</td>
<td>M 8 cell</td>
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<td>41</td>
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<tr>
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<td>6</td>
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</tr>
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<td>M blastocyst</td>
<td>L blastocyst</td>
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</tr>
<tr>
<td>M blastocyst</td>
<td>L blastocyst</td>
<td>7</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>21</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*M=Meishan; L=Landrace; LW=Landrace x Large White

Meishan and European Landrace or Landrace x Large White pigs (Table 1). The Meishan breed has a black coat color whereas the other breeds have a white coat. In the first experiment, blastomeres were transplanted into embryos at the 4-16 cell stage. Of 41 transplanted embryos transferred into 3 females, 12 were single-colored, but no overt chimeras were obtained. 2 piglets in 2 litters were derived from injected blastomeres, and 10 piglets in 3 litters were derived from recipient blastomeres. In the second experiment, ICM cells of Day 6 Landrace embryos were injected into the blastocoel of Day 6 Meishan embryos. Of 35 injected embryos transferred into 3 females, 2 overt chimeras of each sex were obtained in a single litter (Figure 3) (Onishi et al. 1994).

2. Analysis of chimerism

The ideal cell markers for analysis of chimeras should be cell-localized, cell-autonomous, stable, and present in detectable quantities in all cells (McLaren 1976). In the mouse, the most widely used markers are strain-dependent enzyme isoforms that are detectable by electrophoresis. Although many antigenic and biochemical markers have been reported in pigs, very few markers are available for characterization and distinction of each breed. Mitochondria are present in all cells, and mitochondria have their own DNA. Mitochondrial DNA (mtDNA) polymorphism in domestic animals has been reported in many species. In pigs, restriction fragment length polymorphism of mtDNA of Chinese breeds is quite different from European breeds (Watanabe et al. 1985, 1986; Mikami et al. 1988; Takeda et al. 1995). Therefore, we examined the efficiency of mtDNA polymorphism as a cell marker in the analysis of chimerism.
The cloned 3 kb EcoRI fragment of Landrace mtDNA was used as a probe for detection of BglII-digested mtDNA in the chimeric pigs. The BglII fragment of Meishan (16.5 kb) and Landrace (4.4kb) mtDNA are clearly identified in all organs of the overt male chimera (Figure 4). The same chimerism was detected in the blood of overt female chimera. No chimerism was detected in the single-colored piglets derived from the experiment. These results clearly showed that mtDNA polymorphism can be used as a cell marker in chimeras (Onishi et al. 1994).
3. Use of chimeras; Chimeras derived from ES cells

Chimeras have provided valuable material for studies of embryology and developmental biology (McLaren 1976). Recently, chimeras also have played an important role in genetic modification of the germ line of animals through use of embryonic stem (ES) cells (Robertson 1987; Joyner 1993). ES cells are cultured cells which are derived directly from the ICMs of blastocysts. ES cells have normal karyotype and resemble ICM cells especially in their ability to contribute to all tissues including the
gametes in chimera. Great care should be taken in the culture of ES cells for the maintenance of high differentiation ability. The use of feeder layer, together with high quality medium, and the accurate time of cell-passage are important factors for maintenance of ES cells. Following introduction of DNA into ES cells, the targeted ES cells can be selected and injected into blastocysts for making chimeras. The targeted ES cells are transmitted through the germ line of chimeras (Figure 5). Although ES cells are a powerful tool for genetic alteration of animals, at present, the establishment of ES cells seems to be limited to the mouse. We have not been successful in making chimeras derived from cultured ICM cells of porcine blastocysts. Wheeler et al. (1994) reported the live chimeric pigs following injection of long term cultured ICM cells, but they did not mention the germ line transmission in the chimeras. The study of isolation of ES cells from livestock animals should be continued.

4. Chimeric pigs for conservation of genetic resources

Obtaining live offspring from embryos preserved at -196 °C is important in animal breeding and conservation of genetic resources. Recently, live piglets were obtained from frozen embryos after removing cytoplasmic lipid (Nagashima et al., 1995). However, porcine embryos are still difficult to maintain in long term storage at low temperatures. In contrast to the troublesome procedure of embryo freezing, ES cells are easy to cryopreserve like other tissue culture cells. In the mouse, the production of offspring from cryopreserved ES cells is widely carried out through the germ line of chimeras. From this point of view, if porcine ES cells are established, it

![Figure 5. Diagram to show the production of offspring from targeted ES cells. (a) establishment of ES cells from ICM cells. (b) introduction of DNA into ES cells. (c) selection of targeted ES cells. (d) production of chimeras. (e) breeding to maintain targeted alleles.](image-url)
will be possible to obtain piglets derived from frozen ES cells by making chimeras. Nuclear transfer techniques may be the another way to produce complete ES cell-derived pigs.

References
Questions and answers in Session 4

Question to Dr. Ishii.
Q. Dr. Ishii, how do you get individuals from testicular cells of Japanese Crested Ibis (*Nipponia nippon*)? (Naito)
A. We plan to use nuclei of cryopreserved spermatogonia of a male *Nipponia nippon* of Japanese origin and exchange nuclei with the nuclei of the Chinese male. Then, we may obtain germ cells of the Japanese origin in Chinese individuals. However, unfortunately, we have not been able to find germ cells in cryopreserved testicular cells of the Japanese male which died recently. (Ishii)

Questions to Dr. Takeda.
Q. Dr. Takeda, how many animals are necessary for maintaining the heterozygosity of small populations of animals? (Sekikawa)
A. This depends on how many generations we are talking about and the level of heterozygosity, and how many markers we have available. We have to consider each case separately. (Takeda)
C. The more heterozygous the loci, or polymorphic genes are present in a population the larger the population size is needed for maintaining the genetic structure of the population. For example, the Chillingham cattle of the United Kingdom has survived with about 30 females for many centuries because it is highly homogeneous. (Bodo)
Q. Are you using gene-markers to study qualitative and quantitative traits? (Astuti)
A. The genotype of the markers are used to estimate the variability of the neighbour loci which could be either quantitative or qualitative. However the marker loci should be qualitative so that we can distinguish the genotype clearly. (Takeda)

Questions to Dr. Onishi.
Q. Do you have further plans to make transgenic pigs? (Kagami)
A. At present I have no plans. (Onishi)
Q. Did you observe any heterotic effects in the germline of chimeric pig? (Kagami)
A. Chimeric heterosis for reproductive and growth performance was obvious in the chimeras derived from two inbred strains of mouse. At present, we plan to examine relationships between the degree of chimerism and reproductive traits in chimeric
pigs. (Onishi)

Q. How did you prove the germline transmission from your chimeras? Could you explain how you established the ES cell? (Sekikawa)

A. Meishan has a black coat, while the Landrace has a white coat. In pigs white is dominant to black. Therefore, the progeny test by mating with Meishan bore established that the white coloured piglets were derived from Landrace oocytes and black coloured piglets were from Meishan oocytes.

There is one paper about the establishment of ES cells derived from pigs (porcine) blastocysts. The authors succeeded in making chimeras using the established porcine ES cells. However the authors did not mention the transmission of ES cells through a chimera. (Onishi)
TECHNICAL REPORTS

Session 5

Use and information management

Chairpersons
H. Hayakawa
J. M. Matias
Conservation and Use of the Kacang Goat in Indonesia

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Abstract
Kacang goats are the native goats of Indonesia. Based on the population size of the breeding females, the present status of this breed is normal from a conservation perspective. Not much have been done to conserved this breed, if any. Conservation is mostly due to geographic isolation, historical and cultural value of the breed. Demand of improving production and the social changes in the rural life to some extent influence and may threaten the genetic resources of this breed. Therefore a conservation program is necessary to prevent genetic loss and dilution. This paper discuss the reasons and benefits of conserving this breed, the value and potential uses along with information on performance and management.

Introduction
Three major goat breeds are known in Indonesia, the Kacang goat, the Local goat, and the Etawah Grade goat. Nearly all goat populations are raised by smallholder rural farmers. Each farmer raises only a small number.

The largest goat populations are in Java, followed by Sumatra and Sulawesi. Most of the goat breed populations are dominated by the Local goat which is a crossbred between Kacang and Etawah Grade goat. The genetic background and development of local goat is unknown. People often do not differentiate the local goat and the Kacang goat and just call them the Kacang goat. In some places they call the local goat other names. The local goat can be distinguished easily from the pure Kacang goat (Figure 1 and 2) as they show characteristics between the Kacang and the Etawah Grade goat.

The Kacang goats have low productivity and crossbreeding is undertaken to improve production. However, this breed has not been properly evaluated and fully exploited, though they support the economy of the smallholder rural farmers especially in marginal lands. There is no doubt that this breed plays an important role in the economy of farmer households. The value of this breed such as biological, cultural and scientific uses are many. Some factors have been identified which threaten the genetic resource of this breed. There are reasons for and benefits from a conservation program for the Kacang goat.
Goat Populations and Distribution

More than 90% of the world's nearly half billion goats (*Capra hircus*) are found in developing countries. Half in Asia and one third in Africa. These goats are called microgoats. They weigh less than 35 kg fully grown. They are notable for their high reproductive rates, rapid growth, early maturity, tasty meat, ease of handling, and tolerance of climatic stress and poor feeds (National Research Council, 1991).

Kacang goat is the one that can be classified as microgoat. Besides the Kacang goat in Indonesia there are two other goat breeds, the Local goat and the Etawah Grade goat. The total population for these three breeds in 1992 was over 12 million head, with an average rate of increase of 3.8% per annum from 1989 to 1992 (DGLS, 1994).

Goats are distributed throughout Indonesia, however, they are concentrated mostly in Java. The distribution of goats in Indonesia is shown (Table 1).

The Local goat is the dominant goat in Indonesia and can be found throughout Indonesia. This breed is a crossbreed between the Kacang goat and the Etawah Grade goat. The genetic background of the crossbreed is unknown and varies due to uncontrolled mating.

The distribution of purebreed Kacang goat is limited to certain areas especially in the marginal lands and in the isolated hilly and mountainous areas without easy road access.

Separate population data for each goat breed is not available yet, but it is estimated that in 1992 the purebred Kacang goats numbered approximately 2 million head, and the Etawah Grade goat about 0.5 million head, and about 9 million Local goats. To obtain an exact number of each breed is not easy because many reports do not differentiate the Local goat from the Kacang goat. In some places the Local goat has a local name such as Jawa Randu in Central Java, Bligon in Yogyakarta region, Krikil in Sragen, Marica in South Sulawesi, and Gembrong in Bali.

In Central Java the population of Kacang goats is concentrated in the north of Bengawan Solo river, on the mountain range of Kendeng and Kapur Utara in the district of Sragen, Grobogan, Purwodadi, Blora and Rembang. Other areas can be mentioned like Lebak and Serang districts in West Java, district of Jambi, Palembang, and Lampung in Sumatra.
Table 1. Distribution of goats in Indonesia in 1989 to 1992 (million head)

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sumatra</td>
<td>1.124</td>
<td>2.256</td>
<td>2.353</td>
<td>2.470</td>
</tr>
<tr>
<td>Java</td>
<td>6.541</td>
<td>6.669</td>
<td>6.695</td>
<td>7.035</td>
</tr>
<tr>
<td>Kalimantan</td>
<td>0.167</td>
<td>0.171</td>
<td>0.178</td>
<td>0.195</td>
</tr>
<tr>
<td>Sulawesi</td>
<td>1.028</td>
<td>1.075</td>
<td>1.106</td>
<td>1.110</td>
</tr>
<tr>
<td>West Nusa Tenggara</td>
<td>0.380</td>
<td>0.366</td>
<td>0.386</td>
<td>0.359</td>
</tr>
<tr>
<td>East Nusa Tenggara</td>
<td>0.457</td>
<td>0.447</td>
<td>0.434</td>
<td>0.528</td>
</tr>
<tr>
<td>Maluku and Irian Jaya</td>
<td>0.206</td>
<td>0.214</td>
<td>0.230</td>
<td>0.246</td>
</tr>
<tr>
<td>East Timor</td>
<td>0.091</td>
<td>0.097</td>
<td>0.100</td>
<td>0.116</td>
</tr>
<tr>
<td>Indonesia</td>
<td>10,994</td>
<td>11,295</td>
<td>11,483</td>
<td>12,059</td>
</tr>
</tbody>
</table>


Kacang Goat Native Breed to Indonesia

Kacang goat is a native breed to Indonesia. Devendra (1983) reported that this breed are also found in Malaysia and Philippines. Before the 1920's goat's in Indonesia were the Kacang goat. In 1923, during Dutch colonization, the Etawah goat (Jamnapari) was introduced from India (Soepardjo, 1980).

Etawah goats have a larger body frame, long hanging ears, convex face, large horns, the udder are well developed and they are used for milk production. The Etawah breed was imported with the intention of improving the meat and milk production of the native Kacang goat. These imported goats were then bred and developed in the breeding center of Pangarasan (Madura), and from this center the Etawah goats were distributed to other animal husbandry centres such as Cirebon, Pekalongan, Purworedjo, Blitar, Nganjuk, Nusa Tenggara, North and South Sulawesi, and North Sumatra (Soedjai, 1952 and Aldjufri, 1971). From that time the Etawah grade goats were known as descendants from crosses between the native Kacang goats and the Etawah bucks. Then during the development of the goat population, Etawah grade goats were used for crossing with Kacang goats. Local goats are the results of uncontrolled crossing with Etawah grade goats. Due to the early history of the distribution of Etawah goats, in the regions where Etawah goats were distributed and in the surrounding regions now only Local goats can be found. The population of Local goats has increased dramatically and is widely distributed because of the government support for crossing the Kacang goats with the Etawah grade goats.

Kacang goats are well adapted to the agroclimatic conditions of Indonesia and any selection that has taken place over the years has been entirely by natural selection.
Crossing the Kacang goat with other breeds might cause a dilution and loss of the genes related to adaptation and other valuable genes as yet unidentified.

Purebred Kacang goat has a certain characteristics which is manifested in the small and compact body frame, erect ears, flat nose, short neck, short horns, and the back rises slightly higher than the shoulders. Mature males and females weigh approximately 25 kg and 20 kg respectively with the average height at wither of 60 - 65 cm in males and 56 cm in females (Devendra and Burns, 1970). Kacang goats are known as a prolific goat and they produce kids all year around (Devendra, 1983).

Management and Performance

Goats are raised by smallholder rural farmers. One in five farmers in Indonesia

<table>
<thead>
<tr>
<th>Traits</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>At one year old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>12 - 17</td>
<td>10 - 15</td>
</tr>
<tr>
<td>Height at wither, cm</td>
<td>45 - 53</td>
<td>43 - 50</td>
</tr>
<tr>
<td>Body length, cm</td>
<td>44 - 51</td>
<td>42 - 48</td>
</tr>
<tr>
<td>Heart girth, cm</td>
<td>50 - 5</td>
<td>48 - 56</td>
</tr>
<tr>
<td>Ear length, cm</td>
<td>10 - 12</td>
<td>10 - 12</td>
</tr>
<tr>
<td>Ear width, cm</td>
<td>6 - 7</td>
<td>6 - 9</td>
</tr>
<tr>
<td>Birth weight (average) kg</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Weaning weight (average) kg</td>
<td>7.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Body weight at 15 mo</td>
<td>17 - 20</td>
<td>-</td>
</tr>
<tr>
<td>Carcass, %</td>
<td>45.5</td>
<td>-</td>
</tr>
<tr>
<td>Preweaning ADG, gr</td>
<td>57.6</td>
<td>47.1</td>
</tr>
<tr>
<td>Postweaning ADG, gr</td>
<td>31.2</td>
<td>24.1</td>
</tr>
<tr>
<td>Age at puberty, mo</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Body weight at puberty, kg</td>
<td>8 - 10</td>
<td>9 - 10</td>
</tr>
<tr>
<td>Service/conception(days)</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>Gestation length (days)</td>
<td>-</td>
<td>145</td>
</tr>
<tr>
<td>Body weight a first parity, kg</td>
<td>-</td>
<td>12 - 24</td>
</tr>
<tr>
<td>Litter size at first parity</td>
<td>-</td>
<td>1.2</td>
</tr>
<tr>
<td>Sex ratio at birth</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Kidding Interval(days)</td>
<td>-</td>
<td>207 - 391</td>
</tr>
<tr>
<td>Survivability (single) %</td>
<td>87</td>
<td>85</td>
</tr>
<tr>
<td>Survival (twins) %</td>
<td>61</td>
<td>50</td>
</tr>
</tbody>
</table>

raises goats or sheep (Soedjana, 1993). The herd size is usually 2 to 7 head and 2 to 3 head is most common. Farmers raise the goats under traditional management systems, and goat rearing is only a secondary farming activity. Goats are reared under grazing and browsing systems, cut and carry systems and farmers in urban areas rear goats under scavenging systems. Goats are usually grazing and browsing along the road sides, banks, wastelands and uncultivated rice fields in the morning and penned at night. Under cut and carry system farmers give cut-grass, legume tree and other tree leaves and sometimes the goats graze. In urban areas goats scavenge in the market, along the road and in garbage dumps. At night goats are tethered in the barn or the farmer’s house. Only few farmers use a raised floor pen.

Not all farmers own their own buck. There is uncontrolled mating, goats are free to mate indiscriminantly. Generally does nurse their kids until weaning which is after three months.

Little has been done in disease prevention and control. Loses of kids are more common than loses of adults. The most common diseases are scabies, diarrhoea, pink eye, orf, bloat and gastro intestinal parasite investation. Rural farmers use traditional medicine to cure disease. The use of traditional medicine varies from region to region. Near cities, farmers use traditional medicine less frequently due to the availability of veterinary services that help farmers by monitoring diseases (Sutama and Djajanegara, 1992). In traditional medicine, diarrhoea is treated by giving bamboo leaves or ground
guava leaves mixed with boiled water. Bloat is cured with ground ginger rhizome mixed with black coffee and boiled water. To overcome the internal parasites farmers use ground black tumeric (Temum hitam) rhizome mixed with water for drenching. Scabies usually is treated by rubbing with a solution of lubrication oil and sulphur.

The performance of Kacang goats has been summarized in Table 2. Data in Table 2 indicates that the productivity of Kacang goat is low in terms of weight and gain.

The Reasons and Benefits of Conservation

Bodó (1990) suggested two reasons why a population should be conserved, these are:

1. The endangered status.
2. Genetic value.

Further, Diwyanto and Handayani (1995) stated that the conservation of native livestock in Indonesia could be justified for economic, biological and socio-cultural reasons. All these reasons can be used to justify the conservation of Kacang goats. Kacang goats are a valuable genetic resources especially in relation to genetics of adaptation. Kacang goats are adapted to the local climatic conditions, the poor quality of feed resources, the management system under which they are kept, and resistance to some infectious and parasitic diseases.

The status of populations, according to Bodó (1990), can be categorized into five groups based on the number of the breeding females i.e. normal, insecure, vulnerable, endangered, and critical. The present status of Kacang goat population can be categorized as normal.

Although the status is normal this breed as a purebred is influenced and threatened by several factors. These factors are:

1. Improving productivity of crossbreeds.

This has been implemented through crossbreeding Kacang with Etawah grade bucks, and has been carried out over decades with the objectives of increasing the size of the animal and improving milk productivity. Crossbreeding with uncontrolled mating will cause a dilution and loss of the genes related to adaptation and other genes that might by valuable in the future. The Kacang goats cannot maintain their original type, size, conformation and performance due to crossbreeding. Efforts should be made to improve
the production of Kacang goats while still maintaining the purity of the breed. On the other hand, crossbreeding with other breeds can be used as a tool to produce commercial goats to meet the market demand.

2. Socio-economic changes in the rural areas.

More people especially young people are leaving rural areas, looking for a better life and job in the nearby cities. Farmer's interests have changed due to modernization and they are becoming reluctant to raise goats. Rapid changes in transportation infrastructure make it easier to purchase and bring other goat breeds to rural areas.


Disease and parasite control are not commonly practiced. Mortality is quite high in young kids. Though the Kacang goats are believed to be resistance to disease and parasites long exposure to disease and parasite will cause mortality.

The benefit of conservation can be viewed from 1. The economic uses, 2. The biological uses, 3. The cultural uses, and 4. The scientific uses.

1. The economic uses

The economic uses of a given stock are determined by the performance, the adaptation, and disease resistance (FAO, 1990). The Kacang goats are believed to have a potential for adaptation and disease resistance. The performance of Kacang goats in term of production is low but this goat has some economic advantages for the smallholder rural farmers. Like other microbreeds they are less expensive to buy, less of a financial risk to maintain, give a faster return for investment, use space efficiently, are easy and cheaper to manage (National Research Council, 1991). These goats support the economy of the farmers. In Java, goats contribute 10 to 30% of the farmers income (ISRN, 1992). Further goats are used to buffer investment risk and provide capital and cash. To attain greater economic benefits farmers should be informed of how to be good at animal husbandry including the raising of goats. Further, the advantages of the genetics of adaptation and disease resistance should be exploited.

2. The biological uses

Kacang goats as the native breed of Indonesia, enrich the biological diversity of
the country. Kacang goat might have some valuable genes related to some biological traits that have not been used by farmers such as the gene for prolificacy. Some biological traits might not be found in exotic animals and they will be needed to develop new crossbred animals.

Kacang goats are particularly useful for meat production. Other uses are for producing organic fertilizers (manure) and for producing hides. As a source of animal protein, goat meat is an appropriate source of animal protein to support the national program of improving per capita protein consumption in rural areas. It is a relatively inexpensive meat that rural households can afford and there is increasing meat consumption as household expenditure increase (Soedjana, 1993).

At present the national goat meat production is 76,590 tons, which is 16% of the total meat supply from ruminant livestocks (DGLS, 1994).

3. The cultural uses

Kacang goat are used in religious ceremonies. People sacrifices the Kacang goats at funerals, to celebrate the birth of a child, and as a brideprice in marriage. All of these are rooted to the Indonesian cultural heritage especially important in rural life.

4. The scientific uses

Kacang goats are used as experimental animals in production research, genetic research and nutrition research. Recently, researchers are using fistulated goats to study *in sacco* and *in vitro* digestibilities. Compared to fistulated large ruminants the use of fistulated goat is cheaper and needs less space, so goats are becoming more popular for such experiments. In the future with the application of biotechnology the genetic potential can be exploited for the benefit of mankind.

**Conservation of Kacang Goat**

Livestock conservation must be conducted within the livestock's own environment. A sufficient population size for each genotype is necessary. It's known that the preserved genotype will always be the result of combined effects of genetical and environmental variables, the latter being peculiar to the breeding environment. The breeding environment where the livestock has evolved or environments that are thought to be similar to the original one will enable them to live and reproduce (Matassino *et al.* 1993). One way to conserve the genetic resources of livestock is by conservation of
individuals either in situ or ex situ.

In situ conservation or on-farm conservation might be the appropriate way for Kacang goats to be conserved whereby populations are raised by the farmers in their own environment.

The most effective on-farm conservation is that which is nationally promoted and combines elements of existing national programs (Diwyanto and Handayani, 1995). A government-sponsored plant and animal conservation effort exist in Indonesia such as the National Genetic Resource Committee (Komisi National Plasma Nutfah/KNPN). The national program should pursue the genetic improvement of Kacang goats while on the other hand maintaining the genetic diversity of the breed. KNPN as a single entity should initiate the efforts and give suggestion and recommendations concerning the breeding policy of government.

At the local level where the pure Kacang goats are found, a group of farmers with the same interest should be organized. Local government help to such groups with knowledge of animal husbandry practices and means to prevent the use of other goat breeds for breeding. Commonly farmers buy and sell their goats in the nearby livestock markets which are located within the radius 10 to 15 km from their home. In the livestock market farmers purchase the bucks or does. To prevent crossing with other breeds, the local livestock services should established a buffer zone and make a rule that only Kacang goats are allowed to be sold inside this buffer zone.

Implementation of on-farm conservation includes the national policy to conserve the Kacang goats, evaluation at the phenotypic and genetic level to know more about biological characteristics of the Kacang goats, to establish an organization for on-farm conservation, and training of technical personal to maintain the program.

Conclusions

The population of the Kacang goat is decreasing due to crossing with Etawah grade buck with the intention of improving productivity. Kacang goats have a genetic value for adaptation and prolificacy. Continuous and uncontrolled crossing will cause the loss and dilution of these valuable genetic resources.

These reasons and the benefits justify conserving Kacang goats. One way to conserve this breed is by on-farm conservation. A breeding policy for the Kacang goat should be established to support the efforts of conservation.
The genetic potential of Kacang goat should be properly evaluated and exploited in the existing conditions where they are exposed.

References


Introduction

Animal experiments occupy an important position in the broad field of life science. In experiments we can use any animals, but they can be classified into 3 categories, that is, laboratory animals, domestic animals and captive wild animals. The laboratory animals are developed as sophisticated tools for use in research. They have been bred under genetic, microbiological and environmental control. Domestic animals are easy to use in experiments, but most of them have been bred without considering their genetic or microbiological background. The term "experimental animals" is used in a broad sense for animals used in experiments, including laboratory animals and animals in the process of development for laboratory use. Generally experimental animals have been developed for use in the field of medical and pharmaceutical science as a substitute for human being. On the other hand, in the field of veterinary medicine and animal science, domestic animals are targets for study and can be used for experiments. However, large domestic animals are disadvantageous for use in the laboratory. In the present paper the survey and development of experimental animals for veterinary medicine and animal science of use as substitutes for large domestic animals is described.

Worldwide, there are about 4,500 species of mammals, 8,600 species of birds, 6,000 species of reptile, and 3,000 species of amphibia. However, there are only 10 commonly used experimental animals. The most popular experimental animals are laboratory rodents like mice and rats. They are genetically and microbiologically sophisticated. There are more than 400 inbred strains of mice (Festing, 1993). Microbiologically controlled rodents, such as germ free and SPF (specific pathogen free) mice, have been established. Therefore laboratory mice have been called "living test tubes", and they have contributed very much to progress in life science.

The advantage of using domestic animals in veterinary medicine and animal science research, is that the results of experiments can be directly applied to the field. While, the disadvantages of domestic animals for use in research is their large body size which affects:-
1. Handling and management requires experts;
2. These animals are costly to purchase and maintain;
3. For chemical and irradiation experiments large doses are required which is dangerous for experimenters;
4. Facilities and equipment have to be prepared especially for each animal.

Small laboratory rodents have been used even in the field of veterinary medicine and animal science as well as domestic animals. However, they are omnivorous animals having a single stomach. Their physiological characteristics are quite different from those of domestic ruminants. For these reasons, new experimental animals to act as substitutes for domestic ruminants are needed in the field of veterinary and animal science (Goto, 1986a).

**Survey and development of experimental animals**

As candidates for experimental animals as substitutes for domestic animals, particularly for ruminants, we can classify them to 4 categories:-

1. Small herbivorous rodents with a complex stomach harboring cellulytic bacteria;
2. Small- or microbreeds of livestock;
3. Small wild relatives of the domestic ruminants;
4. Transgenic mice added with characteristics of ruminants introduced;

In the following discussion the first three approaches will be discussed.

The steps in the development of experimental animals are shown (Figure 1). First of all, we develop a list of as many animal species as possible as candidates to be developed, from bibliographical sources. In the next step, the listed animals are examined in detail by bibliographical survey and by
### Table 1. List of microlivestock

<table>
<thead>
<tr>
<th>Microcattle</th>
<th>Microsheep</th>
<th>Microgoat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini-Brahman</td>
<td>Navajo-Churro</td>
<td>Black Bengal (Teddy)</td>
</tr>
<tr>
<td>NDama</td>
<td>Entre Douro e Minho</td>
<td>Sudanese Dwarf</td>
</tr>
<tr>
<td>Muturu</td>
<td>Galician</td>
<td>American Pygmy</td>
</tr>
<tr>
<td>Small Zebu</td>
<td>Magra (Chokha)</td>
<td>Tersai</td>
</tr>
<tr>
<td>Dwarf Zebu (Mongalla)</td>
<td>Southern Sudan Dwarf</td>
<td>Katjang</td>
</tr>
<tr>
<td>Ovambo</td>
<td>Mandya (Bandur)</td>
<td>Hejazi</td>
</tr>
<tr>
<td>Nuba Dwarf</td>
<td>Corsican</td>
<td>Creole</td>
</tr>
<tr>
<td>Kedah-Kelantan</td>
<td>Churra do Campo</td>
<td>Small East African</td>
</tr>
<tr>
<td>Sinhala (Dwarf Zebu)</td>
<td>Sitia</td>
<td>Chapper</td>
</tr>
<tr>
<td>Mashona</td>
<td>Common Albanian</td>
<td>Changthang (Ladakh)</td>
</tr>
<tr>
<td>Florida Scrub</td>
<td>Soay</td>
<td>West African Dwarf</td>
</tr>
<tr>
<td>Rodope</td>
<td>Landim (Small East African)</td>
<td>Chinese Dwarf</td>
</tr>
<tr>
<td>Abyssinian Shorthorn Zebu</td>
<td>West African Dwarf</td>
<td>Sinai (Black Bedouin)</td>
</tr>
<tr>
<td>Taiwan Black</td>
<td>Javanese Thin-Tail</td>
<td>Barbari</td>
</tr>
<tr>
<td>Chinampo</td>
<td>Berber</td>
<td>Gaddi</td>
</tr>
<tr>
<td>Tibetan Dwarf</td>
<td>Greek Zackel</td>
<td>Sudanese Nubian</td>
</tr>
<tr>
<td>Hill cattle</td>
<td>Zel (Iranian Thin-Tailed)</td>
<td>Mauritian</td>
</tr>
<tr>
<td>Nilotic</td>
<td>Pag</td>
<td>Heuk Yumso</td>
</tr>
<tr>
<td>Bavenda</td>
<td>Zeta Yellow</td>
<td>Crioulo</td>
</tr>
<tr>
<td></td>
<td>Florida Native</td>
<td>Southern Hill Goat</td>
</tr>
<tr>
<td></td>
<td>Hejazi</td>
<td>Nubian Dwarf</td>
</tr>
<tr>
<td></td>
<td>Hu (Huyang)</td>
<td>Shiba</td>
</tr>
<tr>
<td></td>
<td>Arab</td>
<td>Tokara</td>
</tr>
<tr>
<td></td>
<td>Marwari</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virgin Island White Hair</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roccia (Steinschaf)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>North Ronaldsay</td>
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### Table 2. Small wild ruminants

<table>
<thead>
<tr>
<th>Small ruminants</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse-deer (Tragulus spp. and Hyemoschus aquaticus)</td>
<td></td>
</tr>
<tr>
<td>Musk deer (Moschus spp.)</td>
<td></td>
</tr>
<tr>
<td>Asian microdeer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muntjac or Barking deer (Muntiacus spp.)</td>
</tr>
<tr>
<td></td>
<td>Water deer (Hydropotes inermis)</td>
</tr>
<tr>
<td>South American microdeer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pudu (Pudo spp.)</td>
</tr>
<tr>
<td></td>
<td>Brocket (Mazama spp.)</td>
</tr>
<tr>
<td></td>
<td>Huemul (Hippocamelus spp.)</td>
</tr>
<tr>
<td>Duiker (Cephalopus spp. and Sylvicapra grimmia)</td>
<td></td>
</tr>
<tr>
<td>Small African antelopes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Royal antelope (Neotragus pygmaeus)</td>
</tr>
<tr>
<td></td>
<td>Pygmy antelope (Neotragus batesi)</td>
</tr>
<tr>
<td></td>
<td>Suni (Neotragus moschatus)</td>
</tr>
<tr>
<td></td>
<td>Dikdik (Madoqua spp.)</td>
</tr>
<tr>
<td></td>
<td>Klipspringer (Oreotragus oreotragus)</td>
</tr>
</tbody>
</table>
Table 3. Body size of small wild ruminants

<table>
<thead>
<tr>
<th>Animal</th>
<th>Head &amp; body length (cm)</th>
<th>Shoulder height (cm)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pudu</td>
<td>80 - 90</td>
<td>38</td>
<td>7 - 9</td>
</tr>
<tr>
<td>Blue duiker</td>
<td>55 - 72</td>
<td>45 - 50</td>
<td>4 - 6</td>
</tr>
<tr>
<td>Kirk's dikdik</td>
<td>55 - 65</td>
<td>37 - 45</td>
<td>3 - 4.5</td>
</tr>
<tr>
<td>Royal antelope</td>
<td>45 - 55</td>
<td>20 - 28</td>
<td>1.5 - 2</td>
</tr>
<tr>
<td>Chinese muntjac</td>
<td>65</td>
<td>40 - 50</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4. Body size and weight of mouse-deer (Family: Tragulidae)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Head &amp; body length (cm)</th>
<th>Shoulder height (cm)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African water chevrotain</td>
<td>75 - 85</td>
<td>35 - 40</td>
<td>7 - 15</td>
</tr>
<tr>
<td>Indian chevrotain</td>
<td>45 - 55</td>
<td>25 - 30</td>
<td>2.2 - 2.7</td>
</tr>
<tr>
<td>Larger mouse-deer</td>
<td>50 - 75</td>
<td>30 - 35</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Lesser mouse-deer</td>
<td>30 - 47</td>
<td>20 - 25</td>
<td>0.7 - 2</td>
</tr>
</tbody>
</table>

Table 5. Reproduction traits of lesser mouse-deer

<table>
<thead>
<tr>
<th>Trait</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeding season</td>
<td>No, continuous breeder</td>
</tr>
<tr>
<td>Estrous cycle</td>
<td>14 - 16 days</td>
</tr>
<tr>
<td>Mating period</td>
<td>2 days</td>
</tr>
<tr>
<td>The earliest age of copulation (male)</td>
<td>166 days</td>
</tr>
<tr>
<td>The earliest age of copulation (female)</td>
<td>258 days</td>
</tr>
<tr>
<td>The shortest gestation period</td>
<td>132 days</td>
</tr>
<tr>
<td>Actual gestation period</td>
<td>134 ± 2 days</td>
</tr>
<tr>
<td>Post-partum estrous</td>
<td>Yes, 30 minutes after parturition</td>
</tr>
<tr>
<td>Litter size</td>
<td>one, rarely two</td>
</tr>
</tbody>
</table>

field survey in their actual habitats, as occasion demands. Judging the probability and value to develop the animals, we make a decision on a few animal species as candidates as experimental animals. In the case of overseas animals, it is necessary to check the status of the animals in their native countries, because the animals of interest to be developed are often rare and protected by law. For introduced animals export and import permission is necessary. Obtaining export permission of animals is often the most difficult step in the development of animals. Recently, some governments have a tendency to refuse export of animals to foreign countries, whether the animals are rare
Import permission is also difficult to obtain. For even-towed ungulates or artiodactyls, Japan rarely gives import permission, since protection of livestock from serious infectious diseases is a priority. Foot-and-mouth disease is most feared due to its high infectivity. Consequently, animals from Southeast Asia, Africa and South America are prohibited from import into Japan in principle. However, in the case of academic research or display at zoos, import permission may be issued after rigorous quarantine inspection. Before applying for import permission, we must gather information about the past and present hygienic state in relation to animals of the exporting country, then obtain an official certificate of the animals' health. With both the export and import permission, animals can be transferred to Japan, and they are inspected at a quarantine station for 14 or 35 days depending on animal species and hygienic state of the exporting country. After that they can be introduced into facilities for the animals maintenance. During the first 3 months after the introduction, if the animals' health alters the quarantine office should be informed.

On rearing of the introduced animals, we must improve food, cage and others to become more adequate for them. We should not only rear the animals successfully, but also proliferate them effectively. For the successful breeding, we studied their reproductive traits and behavior. Pairing for reproduction is very difficult to wild animals, because they are often solitary and struggle each other at the time of pairing. Some species permit mating partner at only the breeding season in the wild.

While establishing a breeding colony, the animals must be tamed to enable easily handling and their physiological characteristics must be studied. Finally we should distribute the animals for use in experiments.

**Experimental animal candidates**

1. Small herbivorous rodents with complex stomach

Small rodents are useful animals for experiments, because of their small body size, short life span, early sexual maturity and large litter size. They are very convenient for researchers in the field of veterinary medicine and animal science, if small rodents with complex stomach similar to rumen are developed as replacement for ruminants. The vole, *Microtus*, is a herbivorous rodent having a stomach which consists of two compartments, forestomach and pyloric stomach. The forestomach is covered with
stratified epithelium and cellulolytic bacteria are harbored there (Kudo & Oki, 1984). More than 50 species of Microtus spp. distributed worldwide. Among them, Microtus oeconomus, M. montebelli, M. arvaris, M. agrestis, M. ochrogaster and M. pensylvanicus have been bred in laboratories. In NIAH we have maintained Japanese field vole, Microtus montebelli (Figure 2), for more than 15 years (Goto, 1986b). During their maintenance, some mutations occur in the colony and are maintained as mutant strains. They are a good model substituting for large domestic ruminants to use in nutritional physiology (Kudo & Oki, 1984). However, the limitation of the vole is that it is susceptible to domestic animal diseases.

(2) Microbreeds of livestock

There are many kinds of small livestock in the world (National Research Council, 1991). Most of them have been maintained as native breeds in isolated rural areas. With advances in communication and trade, these traditional breeds are declining. A few microbreeds have been developed by selective breeding. Table 1 shows a list of microbreeds of cattle, sheep and goat. Among microcattle, Mini-Brahman (Bonsai cattle) is the smallest breed which has been developed in Mexico by selective breeding by Professor Berruecos and his colleagues. However this breed is more than 130kg and inconvenient for experiments.

Small sheep and goat are about 15kg in adult body weight. Actually the West African dwarf goat and Shiba goat were bred for use in experiments. In Japan the Shiba goat has been examined for many physiological traits. Katjang is a small goat distributing through Southeast Asia, China and Pacific islands. The Katjang goat of Indonesia (Figure 3) is also a candidate for an experimental animal, because of their small body size, easy handling and good reproductive performance (Fukuta, 1993). The advantage of the microgoat as an experimental animal is that maintenance and handling is already established. The size is adequate for training medical students surgery. In future microgoat and microsheep will prevail as experimental animals in veterinary medicine, animal science and also in medicine.

(3) Small wild ruminants

Many kinds of small ruminants, order Artiodactyls, suborder Ruminantia, are distributed through different families and genera (Table 2). These animals inhabit Africa,
South America and Southeast Asia. Table 3 and 4 shows their body size and weight. The smallest is lesser mouse-deer of Southeast Asia and royal antelope of Africa. We have little information about the royal antelope. The mouse-deer consists of one family of 2 genera and 4 species. Mouse-deer inhabit Asia except one species, the African water chevrotain (Dubost, 1986).

Among the small wild ruminants, blue duikers have been bred at the Pennsylvania State University (Goto, 1986a). The lesser mouse-deer is maintained at the Institute for Medical Research (IMR), Malaysia; Universiti Pertanian Malaysia (UPM), Malaysia; and National Institute of Animal Health (NIAH), Japan (Fukuta, 1996).

Breeding of lesser mouse-deer at NIAH

Lesser mouse-deer, *Tragulus javanicus*, is the smallest ruminant in the world. Adult body weight is about 1.5 kg and rarely over 2 kg. The lesser mouse-deer has neither horn nor antler (Figure 4). The male has long upper canines as tusks. We are interested in this animal and plan to develop it as an experimental animal as a substitute for domestic ruminants. Fortunately we have been able to import lesser mouse-deer twice from Malaysia. The first time 2 pairs were introduced from IMR in 1989, and second time 15 heads were introduced from UPM in 1995.

To maintain lesser mouse-deer, we use stainless steel rabbit cages as do IMR and UPM. The cages are two cages (57x57x47 cm) joined together, and a partition is able to separate them into 2 compartments (Figure 5). At UPM half of the connected-cages are used to keep a pair and good reproductive performance was achieved (Kudo et al., 1996). The animals are fed on rabbit pellet feed, banana, carrots, long beans and broken cubed hay for the first few years, then the array of food was reduced to rabbit pellet feed, banana and cubed hay. Nowadays we give rabbit pellet feed, sweet potatoes and broken cubed hay and supplemented with carrots.

The lesser mouse-deer rests in the daytime and moves actively around sunrise and sunset, but they are not nocturnal which is described in many books (Fukuta, 1996). The stomach is large and occupies most of the abdominal cavity. It consists of 3 compartments; rumen, reticulum and abomasum (Figure 6 and 7). A small vestigial omasum is recognized between the reticulum and the abomasum. The reticulo-rumen volume is larger than that of goat and cattle. Protozoa and large bacteria are found in the rumen. Cellulolysis ability of the rumen bacteria in the lesser mouse-deer is high
Figure 2. A Japanese field vole, *Microtus montebelli*.

Figure 3. Katjang goat in Indonesia.

Figure 4. Lesser mouse-deer, *Tragulus javanicus*, maintained at NIAH. Male (front right) has long upper canine tooth acts like a tusk.
Figure 5. Stainless steel cages for lesser mouse-deer. Two original rabbit cages are connected.

Figure 6. Rumen of lesser mouse-deer.

Figure 7. Schematic drawing of stomach of lesser mouse-deer.

Figure 8. Young lesser mouse-deer of 2 months old. The animal is tamed by hand feeding.
compared with those of cattle and buffalo (Kudo et al., 1996). The reproductive traits of the lesser mouse-deer are summarized (Table 5).

The disadvantage of lesser mouse-deer as an experimental animal is its long gestation period and only one individual per litter. However, they can become pregnant continuously by post-partum estrous and deliver young twice or three times a year. Therefore, the animals proliferate more effectively than large domestic animals, if a breeding colony is established. Although the lesser mouse-deer is said to be delicate and shy, we can tame them by hand feeding (Figure 7).

Conservation of animal species and the development of experimental animals

Animal welfare groups target animal experiments. For these groups the development of experimental animals might be considered exhaustion of animal resources. To develop new experimental animals, however, it is necessary to establish methods to maintain animals safely and in conditions where they can proliferate effectively under human control. Rare animals in the wild are threatened with extinction. Experimental animal research can give insights into how best to proliferate rare species.

In the past, the procedure of rearing and breeding experimental animals has helped rescued rare animals from extinction. For example, in the case of the volcano rabbit (Romerolagus diazi) which inhabits restricted areas of mountains around Mexico city they were threatened with extinction. Several pairs of the rabbits were introduced into Japan and proliferated by researchers who major in experimental animals (Matsuzaki et al., 1982). Young volcano rabbits were exported back to Mexico from Japan. For rare animals in the wild, the procedure of rearing and breeding of experimental animals may contribute to their conservation.

Acknowledgments

The author wish thank Dr. N. Goto, former Professor of Animal Breeding, Kobe University, and Dr. H. Kudo, National Institute of Animal Husbandry, for their helpful suggestions and encouragement. Lesser mouse-deer were obtained from Dr. N. H. Fuzina, Institute for Medical Research, Malaysia, and Dr. S. Jalaludin, vice-chancellor, Universiti Pertanian Malaysia. Their help is gratefully acknowledged.

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University of Leicester, Leicester, U.K.


Information System for Animal Genetic Resources in the Asian Region

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Summary
The Food and Agriculture Organization of the United Nations (FAO) recently developed a Special Action Programme for Global Management of Animal Genetic Resources. The establishment of Domestic Animal Diversity Information System (DAD-IS) is one of very important components of the Programme. In addition, the FAO has started a regional project, supported by the Japanese government, entitled "Conservation and Use of Animal Genetic Resources in Asia and the Pacific". This project covers 12 Asian countries and is for 4 years. One of the major activities is to make an inventory of domestic animals and to obtain better population data in the region. The Regional Databank will be established at the project office in Bangkok. Furthermore, an Asian Network will be established. E-mail system will be set up at specific coordinating institutions in each of the twelve participating countries. These institutions will act as national centers, the project office in Bangkok is expected to be regional center and this Network will be linked to the DAD-IS (Global Center) in Rome.

1. Introduction
It is no exaggeration to say that the Asia-Pacific region is a treasure house of animal genetic resources. The percentage of world's animal species and breeds in this region is 32% for cattle, 96% for buffaloes, 56% for pigs, 40% for sheep, 57% for goats, 43% for chicken, 83% for ducks (Sasaki, 1995). Furthermore, according to the Global Data Bank for Animal Genetic Resources of the Food and Agriculture Organization of the United Nations (FAO), the total number of farm animal breeds which were registered is 2,719 in the whole world and 746 breeds (about 27%) are located in the Asia-Pacific region (FAO, 1993b).

The global environment has altered as a result of human activities, development of social and economic system and rural development. As a result, the number of the animal species on earth, including wild life and domestic animals, is decreasing rapidly. This trend is marked in the developing countries including those of the Asian region. The population of many pure indigenous breeds is likely to diminish as a result of crossbreed-
ing, replacement or neglect. It is supposed that many indigenous species are at risk of extinction.

FAO started a new regional project entitled "Conservation and Use of Animal Genetic Resources in Asia and the Pacific". This project is supported by the Japanese government. The author was sent to the FAO by the Japanese government and participated in the activities of this project for 18 months. Based on the experience of that period, the author introduces the activities of the project, with special focus on the information system for the animal genetic resources in the Asian region.

2. The recent international movement on animal genetic resources

Before discussing the main issues, it is useful to understand the recent international movement on animal genetic resources.

Since its establishment, FAO has been involved in the conservation of the animal genetic resources. During the 1980's, FAO implemented many activities in cooperation with the UNEP (United Nations Environment Programme). In 1989, the Committee of Agriculture of FAO, which was held in Rome, Italy, discussed Animal Genetic Resources. The Committee reviewed the past, present situation and the future prospects for animal genetic resources activities. The Committee recommended orientation and the problems for FAO and for the national governments. Afterwards, the FAO held two Expert Consultations on Animal Genetic Resources: (a) A global program for sustainable development and (b) Management of Global Animal Genetic Resources in 1989 and 1992, respectively. The programmes and activities were designed and implemented based on the recommendations of these consultations. During the same year, the decision of the United Nations Congress on the Environment and Development (the "Earth Summit"), which was held on Rio de Janeiro in Brazil in June 1992, and the Convention on Biological Diversity, which was ratified in December 1993, gave recognition to the necessity of global scale activities on conserving biodiversity. At the same time, a new perspective was added that of sustainable agriculture. Based on these developments, the FAO developed plans for the conservation of domestic animal diversity and is in the first stage of these activities. Global Management of Animal Genetic Resources was discussed at the Committee of Agriculture of the FAO held in March 1995 in Rome, Italy. Based on the decision of this Committee, FAO recently developed a Special Action Programme for the conservation of animal genetic resources. The Programme intends to
establish as quickly as possible a sound primary infrastructure for conserving animal genetic resources which will involve many countries and organizations.

3. Special Action Programme for the Animal Genetic Resources

FAO designated a number of imperative actions for the successful management of animal genetic resources (Hammond, 1994). These actions include:

1) identify and understand unique resources
2) develop and properly use these resources
3) monitor the status of the resources at risk
4) preserve those unique resources which are not currently in demand
5) train and involve national scientists
6) communicate to world opinion the importance of the sustainable management of animal genetic resources.

Furthermore, the FAO developed a Global Strategy for the Management of Animal Genetic Resources. This Strategy includes the following components:

* An intergovernmental mechanism
* A geographically distributed global structure
* Activities, grouped into seven work elements
* Expert panels ensuring sound advice for the Strategy’s implementation

The specific activities to execute this programme are composed of some key features as follows:

1) Execution of regional project identification missions to promote effectively

<table>
<thead>
<tr>
<th></th>
<th>Buffalo Cattle</th>
<th>Yak Horse</th>
<th>Ass</th>
<th>Sheep</th>
<th>Chicken</th>
<th>Geese</th>
<th>Camel</th>
<th>Deer</th>
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<tbody>
<tr>
<td>China</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>10</td>
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<td>4</td>
<td>21</td>
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<tr>
<td>Laos</td>
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<td>6</td>
<td>13</td>
<td>2</td>
<td>16</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 1. Number of Animal Breeds collected by the Project
many conservation activities

2) Establishment of geographically distributed structure
   To establish this structure, it is necessary to build the components as follows:
   (1) umbrella projects in each region of the world in order to involve and
       assist governments in designing and implementing national action plans
   (2) a coordinating institution within each country with an active country
       coordinator
   (3) a global focus in FAO Headquarters in Rome

3) Formation of a Domestic Animal Diversity Information System (DAD-IS)
   To form DAD-IS, it is necessary:
   - to develop rapidly the Global Data Bank on Animal Genetic
     Resources
   - to expand rapidly the World Watch List for Domestic Animal
     Diversity

4) Fostering an in-situ conservation strategy

5) Introduction of an ex-situ conservation strategy
   - Establishment of national and international gene banks

6) Monitoring of populations of breeds at risk

7) Implementation of a specific global research activity
   - To establish the amount of diversity in each domestic species, and
     the contribution of breeds to diversity
   - To study a genetic distance
   - To make genetic maps

8) Implementation of the activity for the communication of the information and
    the training of the experts

9) Development of the guidelines to establish a plan for the international
    activities and the national activities

4. The project of "Conservation and Use of Animal Genetic Resources in Asia
   and the Pacific"

This project is one of FAO's trust fund projects supported by the Japanese
government. The Japanese government provides US$1.82 million to FAO and FAO
carries out the project activities. This project is coded "GCP/RAS/144/JPN". The
duration of the project is for 4 years from December 1993 to December 1997 and covers 12 Asian countries. The participating countries are: Bhutan, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Thailand and Vietnam. These countries covers over 85% of the animal genetic resources in the region and therefore over 25% of the global resources.

The main activities are:

1) To identify and monitor the animal genetic resources of the 12 countries
   - To make a regional inventory of all known breeds, breeds varieties and populations for all livestock species (buffalo, cattle, horses, pigs, sheep, goats, poultry, ducks, deer, yak) with information on population sizes, as well as on evolution of populations and also with some performance data. For two countries, it is a first time to do an actual breed survey.
2) To preserve and simultaneously enhance the productivity of those indigenous breeds at risk of disappearing.
3) To train national experts on techniques for description of breeds, data handling and in-situ and/or ex-situ preservation.
4) To analyze and publish all information related with Animal Genetic Resources collected or created during the project.
5) To establish the Asian Network on Conservation of Animal Genetic Resources.

The initial activities are concerned with collecting the latest population data on each breed in each country. The project office is located in the building of FAO's Regional office for Asia and the Pacific (RAPA) in Bangkok, Thailand. The staff of the project are a Chief Technical Adviser sent by FAO Headquarters, a Senior Animal Resources Officer sent by Japanese government (the author) and a secretary employed in Bangkok.

National Coordinators were nominated and decided in each of the 12 countries. They are responsible for the project activities in each country and the institution at which each works is the Coordinating Institution for that country and will be the center for the within country network.

5. Establishment of the Network on Animal Genetic Resources in Asian region

As mentioned above, the establishment of the Network is one of main objectives
To understand the concept of the Network, it is useful to understand the Global Data Bank for Animal Genetic Resources at FAO Headquarters in Rome and World Watch List for Domestic Animal Diversity.

1) The Global Data Bank for Animal Genetic Resources

With the help of many organizations and individuals throughout the world, FAO initiated the global breed survey of main domestic animals in 1991 and established a Global Data Bank for Animal Genetic Resources at FAO Headquarters in Rome in 1992. This Global Data Bank will have a core role in the Global Network. The Data Bank now includes initial data on about 2,800 breeds (and varieties of breeds) of 7 species (ass, buffalos, cattle, goats, horses, pigs and sheep) and population size data for about half of these breeds. It is planned to include camels and poultry and the survey of these two species is progressing now.

The list of breeds is still in the development phase and it is certain to change as information accumulates. Population data are currently entered into the Global Data Bank.

For each breed, the following information are entered -- its origin, population size, physical and morphological features, uses and special attributes, production records and management conditions -- in addition to information which is less commonly found of the project "GCP/RAS/144/JPN". This is the first stage in the global programme of FAO.
such as genetic distances and genetic conservation programmes for the breed. The world dictionary of livestock breeds by Mason (Mason, 1988) was used as the primary source of information on breeds and breed varieties for the data bank. This book provides breed names and synonyms, the geographical location of the population as well as giving a basic description of the origin, physical appearance and main uses of each breed.

2) The World Watch List for Domestic Animal Diversity

Together with UNEP, FAO published the first edition of the World Watch List for Domestic Animal Diversity in 1993 (FAO, 1993b). This list will act as another component of the global early warning system for domestic animals and was called for under the Convention on Biological Diversity. The early results suggest that at least 30% and possibly as high as 40% of all animal genetic resources are currently at high risk of extinction. The simple criteria used to determine the risk of extinction was, less than 1,000 breeding females and less than 20 breeding males remaining. The majority of the breeds at risk are in developing regions. Adequate records do not exist to enable us to obtain a reliable estimate of either loss rates of these breeding resources or of domestic animal diversity itself. The second edition will be published soon.

![Figure 2. The model structure of each participating country.](image)
3) The Network on Animal Genetic Resources in the Asian region

The regional center for the Network is located in the office of the project "GCP/RAS/144/JPN" which is in the RAPA. The Asian databank is also located in the project office. This regional databank has a core role in the Network. The institutes, in which the National Coordinator works in each of 12 countries, will be a national center and the national databank will be developed there. The national databank is also essential for the national Network and it will also have an important role in the Asian Network. In these institutes, E-mail systems are being set up and these institutes will be linked to the regional center in Bangkok. The model structure of the Asian Network is shown (Figure 1) and the model structure of each country is shown (Figure 2). The regional center is linked to the global center in Rome. These structures are a component of Domestic Animal Diversity Information System (DAD-IS). Namely, DAD-IS is composed of a Global center, Regional centers and National centers. DAD-IS will be operated through Internet based on a Personal Computer. DAD-IS will serve as an information axis for the FAO's Special Action Programme and as a global early warning system. DAD-IS will also provide easily available databank, and have research design and analysis, training and communication capabilities. The basic model structure for DAD-IS is shown (Figure 3).

Each country should organize its own databank according to its circumstances.
Figure 4. White Lamphun Cattle in Thailand.

Figure 5. Ac Chicken in Vietnam.

Figure 6. Tre Chicken in Vietnam.

Figure 7. A yak in Nepal.
Once a national databank is established, in principle, each country should take care of the conservation of its own animal genetic resources. The countries participating in the project will be responsible for decisions concerning the breeds/populations to be conserved. They will be influenced in these decisions by some guidelines common to all countries involved in this activity. Conservation of animal genetic resources must be a national initiative. Given the substantial differences in needs and appropriate approaches in individual countries, the organization of national conservation will vary widely. It is also important that private and public interests within in each country are incorporated into the strategy for conserving animal genetic resources to ensure an effectively, coordinated national program. The support of strong national programs is essential to the success of regional and international conservation efforts. Ultimately, livestock production and improvement programs will benefit from international support and assistance from enhanced international movement of animal genetic resources.

It is necessary to create an appropriate infrastructures in each country. New institutional facilities to support field activities will be needed. They need not be large, but they must be suitable for liaison with national governments, international bodies both governmental and NGO's (Non Governmental Organizations's), and with bilateral organizations working on animal genetic resources. They should include Cryogenic Animal Gene Bank(s).

6. Some information on the animal genetic resources in the Asian region

The animal breeds collected by the project by the end of June 1995 is shown (Table 1). The number of the animal breeds collected is 181. This number includes only 7 countries, and for 3 countries this does not include all of the breeds because the breed survey is not yet complete. These data were sent to FAO Headquarters and were entered to the Global Data Bank for Animal Genetic Resources.

The project collected information on the breeds at a serious risk in each country. And the project decided to provide budgetary support to some of the breeds which needs urgent action for conservation. Some of the breeds are as follows:

1) South China Pig in Malaysia
2) Shwe Ni Gyi Cattle in Myanmar
3) White Lamphun Cattle in Thailand (Figure 4)
4) Ac Chicken in Vietnam (Figure 5)
5) Tre Chicken in Vietnam (Figure 6)

The yak in Nepal, is not endangered but it is one of very particular breed in Asia, is shown (Figure 7).

7. Conclusion

This project is a first umbrella project for animal genetic resources in the world and it is conducted as a Special Action Programme of FAO. This project is also being used as a pilot scheme for the global programme. It is significant that this project was started in Asia. The author wishes the project success in attaining its objectives.

References


Questions and answers in Session 5

Comment from Dr. Mikami
Q. In Japan where are pcr primers available? (Astuti)
C. For cattle, the Shirakawa Institute of the Livestock Technological Association provides more than 200 pcr primer sets for MS markers. In pigs, the National Institute of Animal Industry and STAFF Institute also provide about 200 primers sets. We are studying linkage analysis between these markers and economic traits using large resources (reference) family produced by crossing between genetically divergent breeds, for example between improved breeds and native breeds. (Mikami)

Question to Dr. Yamada
Q. When you mention collected breeds from Asian countries do you mean data or live animals? Why are Indonesia and the Philippines not on the list? (Matias)
A. Collected data on breeds. By the end of June we had not received the questionnaire sheet from those two countries. (Yamada)
Nepalese Animal Genetic Resources
(Paper presented at the MAFF Seminar)

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Summary
Nepal is a small mountainous Himalayan country with an area of 147,181 km² and 18.5 million people. The livestock population in Nepal comprises of 6.234 million cattle, 3.073 million buffaloes, 0.911 million sheep, 5.452 million goats, 0.605 million pigs, 13.601 million poultry, and 0.392 million ducks.

Nepal has diverse ecosystems and diverse genetic resources of plant, animals and other species. Nepal has different types of wild animal genetic resources such as Gauri Gai, Arnii buffalo, wild pig, musk deer, Jungle fowl, tiger, elephant, rhinoceros, deer, bear, wolves. Nepal has varieties of plants that are found in natural habitat that are common to subtropical to temperate and alpine type of climate and Himalayan ranges.

Nepal is rich in domesticated animal genetic diversity also. The domesticated animal genetic resources contribute about one/third of the National Agricultural Domestic Gross Product. The livestock supply milk, meat, draft power, manure, hides and skin and wool as the product sources of livelihood of many poor Nepalese farmers. The contribution of livestock in the high hill and mountain is higher than in Terai Region where crops dominate the socioeconomic condition of the farmers compared to the hills and mountains where the land available for crops is scarce.

Distribution and availability of different breeds of buffaloes, cattle, sheep, goat, pig, ducks, poultry is described. Their present status in term of evaluation of the production performances and other characteristics of the available breeds is discussed. The Lulu cattle, Achhami Cattle, Lime Buffalo, Yak/Nak, Pate breed of pig and Kage sheep may become extinct soon if suitable measures are not taken. They need conservation as the number is decreasing rapidly as a result of crossbreeding and replacement by exotic breeds. The economic performance of these native breeds of domesticated animals is low compared to some imported breeds.

Natural selection has been operating for many generations on the livestock population in different parts of the country. Physical barriers to movement of animals isolated for many generations has resulted in considerable population diversity in Nepal. Many breeds and strains exist within each species and they are still not well identified, characterized and evaluated for exploitation particularly for traits like resistance to hardy condition and diseases.

Buffalo is the main livestock species contributing about 52% of the livestock GDP. The Swamp type called the Lime and the riverine type called the Parkote are the two types of local buffalo found in Nepal. In addition, Terai and other uncharacterised types are also found in the country. Murrah are an imported breed which is spreading all over the country for both crossbreeding purposes and to keep as a pure breed for milk and meat production. The Swamp type of buffalo needs conservation as this type may be lost if they
are not protected. The wild buffalo Arna is being conserved in the Koshi Tappu National Park.

Lulu, Siri, Achhami, and Hill cattle and Terai Cattle are the five types of cattle found in Nepal. The Lulu, Achhami and Siri cattle need immediate attention to conserve them as their numbers are declining rapidly. The Gauri Gai is been conserved in Chitawan National Park.

The Yak/Nak, Chauries, Jhyopkyos are reared in the high mountains and Himalayas. They are multi purpose animals for milk, meat, draft power for plowing, transportation of goods in high mountain on small tracks. Their numbers are decreasing rapidly and they are in need of characterization, evaluation, and genetic improvement by adopting a farmers group breeding approach.

The goat meat is accepted by all the Nepalese people. Goat meat is in always in high demand. The goat population is increasing. Chyangra in the high Himalayas, Sinhal in the high mountains, Khari at mid hills, and Terai goats are the four types of goat based on the ecological system of the country. The goat has been subjected to cross breeding with the imported goats from India particularly Jamunapari and Barberi.

Bhyanglung, Baruwal, Kage, and Lamptuchire are four breeds of sheep occur in Nepal. The Kage breed has been decreasing at fast rate and soon it may extinct if proper measures are not taken.

Sakini and Naked Neck are the poultry type breed found in Nepal. They are kept as backyard scavengers. They have been cross breed with New Hampshire and Australorp. Some Nepalese Red Jungle Fowl is found in forest areas.

Hurrah and Chwanche are the two types of pig found in the Terai and Hills of Nepal. Wild pigs are also found in the Terai and High Mountain areas. The wild pig needs conservation. The Chwanche and Hurrah breed could be exploited for their small size and early maturity.

During the last three decades the Government of Nepal has been giving emphasis to production and propagation of imported animal breeds of different domesticated species. Native breeds were not given due consideration compared to economically competitive imported breeds. Some awareness has been created by the organization of a Workshop on animal genetic resources conservation in Nepal' by joint collaboration between Nepal Agricultural Research Council, National Animal Science Research Institute, Animal Breeding Division, Department of Agricultural Development and United Nation Department Programme, 11-13 April 1994.

The Nepal Agricultural Research Council is the coordinating national institute for Agricultural Research including bio-diversity. The crop sector of biodiversity is at a more advanced stage than the animal biodiversity. The lack of manpower and laboratory facilities are the main constraints and there needs to be a focus on increasing the research capability in relation to animal biodiversity research. Genome analysis is not done in Nepal either for plants or animals. Foreign Research Institutions support both financial and technical will be needed to carry out such research work.

**Introduction**

Nepal is a small Himalayan country with a population of 18.5 million and area of 147,181 km². Nepal is blessed with the worlds highest mountains, deep gorges, fast flowing rivers and gorgeous valleys. Despite the small size of the country, Nepal has a
diversity of ecosystems, animals and plants. The tremendous differences in ecology and environment within a short distance are mainly due to big changes in mountain topography, isolated valleys and niches and varying hill slopes and aspect. Such topography has generated tremendous bio-diversity in plant and animal genetic resources. Basically the whole country can be divided into five geographical regions. Trans-Himal /High Himal, High mountain, middle mountain, siwalik/ lower hills and the Terai (Figure 1).

Animal Genetic Resources Across Different Ecological Region

*Trans-Himal/ High Himal Range* (Figure 1)

This region is characterized by the area situated at higher than 2500m and adjacent to the Tibetan border behind the Himalayan mountain ranges. This area is characterized by high peaks, steep slope, narrow valleys and gorges. The climate is arid, dry, cold and windy. The rainfall ranges from 200-800 mm/annum with intense solar radiation, high winds and high evaporation rate.

The people living in the high Himalayan areas are Sherpas in Solukhumbu, Thakalis in the Dhaulagiri, Bhotias in Humla, Dolpa and other areas bordering Tibet. They are involved in the barter system of agricultural products with Tibetan neighbours. The barter system has been disturbed recently due to easy access with India. For the Serpas their lifestyle has changed, many are tourist guides or camp helpers.

The major vegetation of this region consist of dwarf shrubs with some perennial pastures and weeds.

The major domesticated animals raised in these areas are Chyangra goat, Lulu cattle, Yak Chauries, Kirko, and Bhyanglung sheep, Tibetan horse and donkey. About 3% of the total Nepalese population live in this region. Potato and buckwheat are the major food crops.

The animals are kept in a nomadic migratory system herds are taken to higher altitudes in summer and lower altitudes in winter. Previously farmers were taking animals across the Tibetan border.

*High Mountain Range* (Figure 1)

This range lies at an attitude of 2200-4000m lying south of the Himalayan ranges. The climate and vegetation varies from temperate to alpine. High peaks are
covered with snow in the winter and when the snow melts these change to alpine pasture. The major feed resources for animals are from alpine meadows and shrub forest cover, and some from the crop land. The major animals raised in this region are Yak, Chaureis, Kirko Cattle, local hill cattle Baruwal/Dhorel Sheep, Sinhal Goat and hill buffalo. About 8% of the Nepalese population live in this region.

Animals are reared in a nomadic migratory management system taking the animals to alpine grazing areas in summer and to lower altitudes in winter. Animals are used to transport produce from Tibet and from the Terai during summer and winter respectively. The sheep and goats in the mid western region are also used for carrying food grain from the Terai and salt from Tibet.

The people residing in this area are Tamang, Limbu, Gurung, Thakali, Bhotias, Magar. They live in a poor environment by using the natural resources of the region. Their main livelihood is from livestock supported by some crops such as potatoes, buckwheat, wheat, millet that are grown in limited areas.

**Mid Mountain Ranges** (Figure 1)

This Zone represent the areas ranging from 800-2400m lying north of Siwalik ranges. The climate and vegetation is mainly sub temperate and temperate with a considerable variation in micro environment.

This region is the mostly densely populated with about 46% of the people living in this region. Brahman, Chhetri, Gurung, Magar, Newar are the main dwellers of this region.

Common practices of rearing animals in the region include a semi nomadic migratory system, stall feeding and taking the animals out to graze in the day time and keeping in sheds at night. Some intensive animal production systems are common in areas surrounding city areas.

The major feed resources come from grazing on high meadows, shrubs, communal grazing lands, waste lands, and forests and a limited amount of crop by products. Different type of fodder trees are common in this areas for feeding animals during the lean period.

These major animals raised in this region are Hill cattle, Achhame cattle, Siri Cattle, Baruwal and Kage sheep Sinhal Goat, Lime and Parkote buffalo, local black pig (Chwanche) Sungur, Pundi and Sakini and Naked Neck chicken.
Figure 1. Map of Nepal. 1) Trans-Himal/High Himal Range (2,500 - 8,848 m), 2) High Mountain Range (2,000 - 2,500 m), 3) Mid Mountain Range (700 - 2,000 m), 4) Siwalik hill (300 - 700 m) and 5) Terai Region (below 300 m). Bar = 200 km.

Siwalik hills (Figure 1)

This region includes mainly low land and upland forest and agricultural areas ranging form 200 m to 1500 meter elevation. The climate is sub-tropical to sub-temperate. The major feed supply comes from forest grazing, crop lands and waste lands. Most of the animals are raised under sedentary management system based on the grazing in the surrounding forest, fodder trees, agricultural lands/fallow, waste land, terraces and rivers.

Animals are kept in sheds during night and are taken to grazing areas during the day. Stall feeding the milking animals is also common. Paddy straw is the most common feed for stall feeding, as well as, additional feeding during lean period of available feeds form grazing land.

The Tharu and the migrants from India and hills and mountain areas are the people living in the regions.

The genetic resources available in this region are Kage sheep, Khari goat, Chwanche and Pundi pig, lime buffalo, Pahadi hill cattle. Some exotic breeds and their cross breed with buffalo, sheep, cattle and pig are also raised in this region.
**Terai Region** (Figure 1)

This region is the plain situated below 300m and is the main crop growing area of the country.

The Tharu and migrants from India and different regions of Nepal live in this region. The region is the main grain production center for the whole country.

The major feed supply comes from cropland residues and grazing fallow land, waste land and roadside verges. Growing of fodder crops is increasing in the region.

The animals are kept in sheds during night and are taken out for grazing during the day. Feeding animals with paddy straws is quite common. Intensive stall feeding of milking animals is common around markets and cities.

The major animal resources in the region are Terai Cattle, Terai Buffalo Terai Goat, Hurrah pig and Lampuchhre sheep. The cross breeding of Terai cattle with Jersey, Holsteins Friesians, and Terai buffalo with Murrahs is increasing.

**Brief Description of Different Breeds of Different Animal Types**

**Buffalo Genetic Resources**

Wild buffalo or Arna (*Bubalus arnee*) are seriously endangered and have are conserved in Kosi Hill Tappu riverine terraces at 600-2000ml. Their morphological characteristics are rounded for head with large horn and dark black body color. They measure 3.2 m long from mouth to root of the tail and 1.8 m high at the shoulder. Little is known about production and reproduction performances of these buffalo. Genome analysis, electrophoresis and other studies will be areas to conduct future research.

Domesticated buffalo provides about 53% of the livestock contribution to the national GDP and about 71% of the total milk (87,700 tons) and 64.4% of total meat. About 12% of the farmers use buffalo as draft animals for ploughing and for transporting agricultural products. Two type of buffalo are found on Nepal, the riverine and the swamp type. The swamp type buffalo differs from riverine genetically in color and body appearance.

**Lime:** Nepalese swamp-type buffalo (*Bubalus bubalis*) are believe to have been introduced to Nepal long time ago from China. They are found in the mid hill and high hill region of Nepal. The animal is characterized by chevrons of gray or white hair below the Jaws and under the chest. Its Sickle shaped horn curved towards the neck.
population of lime is decreasing very fast through indiscriminate crossbreeding with riverain type. Pure males of this type are difficult to find these days. This breed has poor milk production and has been replaced by riverain buffalo.

**Parkote:** The native riverain type buffalo are mainly in the lower hills and Terai. They have not been characterized. They are used to cross with Murrah breeds for milk production. Parkote are medium size breed slightly bigger than lime and smaller than terai buffalo.

**Non-descript buffalo:** There are many non-descript buffalo in Nepal some of these have been introduced from India. They have not been studied well. They have not been evaluated for the production, reproduction and other parameters.

**Imported buffalo:** The main imported breeds are Murrah. Some NiliRavi types can be seen in some places.

**Cattle Genetic Resources**

The wild cattle, Gauri Gai (*Bos gaurus*) are found in Chitawan National Park and they have not been studied for their production, reproduction and other parameters.

About 7.3 million domestic cattle are reared by 2 million Nepalese farmers. The cattle population comprises 3.2 million adult male mainly for ploughing and 2.3 million adult females which provide 30% of the total milk supply (876,000 tons).

**Terai white zebu cattle:** Most Terai cattle have white hair and black skin. Crossbreeding with Haryana Cattle has been practiced for draft purpose. The height at wither is about 141 cm for bulls and 131 cm for cows. Body weight of bull is about 360-500kg and of cows about 350kg. For production and reproduction parameters this breed needs to be further studied.

**Black hill Cattle:** Diversified types of black hill cattle exists in Nepal. They have been kept isolated for long time due to topographical barriers.

**Siri Cattle:** According to Epstein (1977) Siri Cattle were introduced to Nepal through Darjelling from Bhutan. The few Pure Siri Cattle that exist in Illam may become extinct soon. However some crossbreed Cattle are found in Illam district. The Siri cattle are black and white in color with well developed udder. They are larger than the black hill cattle. The neck is fairly short and dewlap is moderate in size.

**Lulu Cattle:** The humpless cattle are reared in Mustang district. Similar type of cattle are also found in Solukhumbu area brought from Tibet. Their number is decreasing...
Achhami Cattle: This cattle being small in size are known as "Sano Gai" meaning dwarf cattle. They are few in numbers and are not well characterized. The pure form of Achhami Cattle is difficult to find and they are decreasing in number because of crossbreeding with other breeds. They need to be characterized and identified for physical, production and reproduction performances.

Imported breeds of Cattle: The Jersey, Friesian, Brown Swiss and Ayreshire are the milk breeds that were introduced to Nepal. They have been used for their semen. The Haryana Breeds have been used for cross breeding with the terai cattle for draft purpose.

Yak Genetic Resources

Yak (Bos Grunniens) are kept in the high Himalayas where they are well adapted to severe cold and can withstand lack of food for a number of days. Yaks are found in Solukhumbu, Jomsom, Dolpa, and Mugu but have not been well studied because of remoteness and difficulty of access to the rugged terrain. They descend only to 4000m above sea level in winter and graze at 6000-7000m during the summer months. They are reared as a transhimalayan animals. These multipurpose animals have been used for meat, milk, ploughing, and for transporting goods over small tracks in the high mountain. The number of Yak/Nak is decreasing very fast. Both Yak and Nak has been used for crossbreeding purposes to produce Urang Chauris (Jhoopkyos) and Dimzo Chauris (Jhyopkyos). The gene flow from these crosses stopped because no further generation are produced after the first and second crosses as they are sterile and are selective to breed the female with either Lang or hill cattle.

Milk protein polymorphism in Yak/Nak cattle and their hybrids were examined electrophoretically by Yoshi Kayoto and others from the University of Tokyo in 1988. The result suggest that gene flow from high mountain to low areas of Nepal is low. The genetic constitution of Yak was estimated to be much differentiated from breeds of European and Indian cattle.

Goat Genetic Resources

The goat represent the largest population of small ruminants in the country. They are distributed all over the country. Different breeds exist in different ecological areas. The management system also varies with the elevation. The various types of goat
raised in the country are as follows:

**Chyangra:** This type of goat is reared in the Himalayan ranges of the country. It is well adapted to cool, dry, semidesert snowy type of climate. The Chyangra found in Nepal are smaller than those of Tibet. This type of goat is known as Kasmiri in some areas and Chyapu in other areas. The male weighs about 45-60kg and the female weighs about 35-50 kg. Each she goat produces one kid per year and about 125-200gm at Pasmina. The have not undergone any artificial selection and have been reared in the yak rearing ranges. They have not been studied well because of remoteness and difficulty of getting to areas where they are reared. They are mainly reared by the Humli, Dolpali and Sherpa people. They are decreasing in number because of restrictions in the grazing areas of Tibet.

**Sinhal:** This high hill goat is kept along with flocks of Baruwal sheep in the ranges of 500-1500m above sea level. Male buck weighs about 40-50kg and the female weighs 30-40 kg. Both male and female have horns and medium sized ears. The goats are generally of mixed color black and white or pure white or pure black. This breed is being indiscriminatly crossbreed with Chyangra or Khari breeds. Their number are decreasing rapidly.

**Khari breed:** This breed is reared in the lower mountain areas. These are smaller than other breeds of goat. Males weigh 30-40kg and female weigh 25-30kg. Both sexes have horns and the coat is multicolored. They are a prolific breed and twinning is a common trait. The frequency of breeding is also high and three pregnancies in two years is possible. Sometimes 4 kids per kidding can be observed in farmers flocks.

**Terai goat:** They are long eared and are of mixed type. They resemble Jamunapari. They are bigger in body size with body weight of male 40-50kg and of female between 30-40 kg. Other imported breeds of goat are Jamunapari and Barberi from India. A few crossbreeds with Sanen are also available. They are a good milk yielder. The crossbred with Sanen have a higher milk yield than other breeds in Nepal.

**Sheep Genetic Resources**

Four different type of sheep are found in Nepal. They have undergone natural selection only. Their genetic variation seems to be very high.

**Bhyanglung:** This breed originated in Tibet. They are reared in the border areas with Tibet. They are kept at altitudes in excess of 3000m. They are adapted to the high cold
desert type of ecosystem where they live. They cannot survive on lower hills. Adult males weigh between 30-45 kg and females weigh 25-35 kg. Their wool is very good for Nepalese carpet making blended with New Zealand carpet wool. Their wool is finer than that of other breeds. They have not been studied for their production and reproduction characteristics.

**Baruwal Sheep:** This breed is a hardy mountain breed. Males weigh between 35-45 kg and females weigh between 30-35 kg. Their legs are long and strong and are suitable for nomadic sheep rearing. They can run fast in the poor hill and mountain environment. They are also used for transportation of goods from the Terai to mountains and from Tibet to lower hills. The heads are large with small ears. The wool is generally white but some have black and brown patches on the coat. Rams have curled horns and ewes are hornless or with smaller horns. They are kept mainly for meat and wool in the western region and also for draft purposes in the mid-western region.

**Kage Sheep:** Kage sheep are a valley breed found in the mid mountain and hilly regions ranging in elevation from 600m to 1500m. This is the smallest breed of subtropical type and are generally polled females. Adult males weigh between 20-25 kg and females 15-20 kg. The wool yield is about 300-500 g per year per sheep. They are non-seasonal breeders. Twinning is common in the Kathmandu valley while twinning is rare in the Pokara valley. It is now difficult to find pure male kage sheep because of indiscriminate crossbreeding.

**Lampuchhre Sheep:** This breed resembles kage type but has mixed color on the neck and head and is much bigger than Kage with long tail and is raised in the western Terai. The body is generally covered with coarse white wool.

**Imported sheep breed:** Polwarth, Merino, Romney Marsh, Border Leicester, and Rambouillet have been introduced for crossbreeding.

**Pig Genetic Resources**

Most of the 496,000 pigs in Nepal are the native type. They are reared by the Tharu, Magar, Limbu, Damain, Sharkee people of the country. Many breeds have not been characterized and identified for their phenotypic, production and reproduction performances. Chwanche pigs are found in the western region in the Magar community, Sungur is found in in the mid western and western region, Pundi is found in the eastern region, Hurrah or Pate is found in the Terai region. These breeds are raised on a small
scale in a backyard scavenging system with a small amount of kitchen waste, grain by-product, and other type of waste as feed. They are raised with almost no veterinary care and are supposed to be disease resistant to many diseases and parasites. They become sexually mature when they are about six months of age.

Some wild pigs are also available in the forest ranging from Terai to Jumla areas to an elevation of about 3000m. They have not been studied thoroughly.

Some of the imported breeds of pigs in Nepal are Yorkshire, Landrace, Saddleback Hampshire, Tameworth and Duroc. Their production performances characteristics in Nepal are being evaluated.

**Horses, Mules and Asses**

There are about 19,000 horses, mules and asses in Nepal. Mules are more common in Western Nepal where they are mostly used for transportation of goods in the high mountain areas. The different type of horses found in different parts of Nepal are named after the respective districts. Namely Mustangi in Mustang, Jumli and Humli in Jumla and Humla districts respectively. They are believed to have originated in Tibet. However these horses are smaller than the Tibetan type. The number of asses are not known.

Blood protein polymorphisms of Tibetan asses in Nepal were examined and substantial genetical differences between asses and horses were reported by Kawamoto et al. (1988).

**Poultry Genetic Resources**

The Sakini type and Naked neck type of poultry are available in different parts of the country. They are small, their eggs are small, and characterized by broodiness of females. They are given no health care and are raised in a scavenging system with household waste and free grazing around the houses. They are resistant to many parasites and many diseases and can withstand poor environmental conditions. Their meat is said to be tastier than the broiler commercial type. Naked neck are said to be meatier than the full feather type. They are said to be more suitable to warmer climates.

**Duck Genetic Resources**

Muscovy ducks and species are found in Nepal.
Genomic Analysis

Electrophoretic study on blood protein and milk protein of yak, cattle and their hybrids and blood protein of asses, mules and horses of Nepal and of native goats were studied by Takao Nishida and other investigators in 1988. The same group made a study of different breeds of sheep for the genetic distances and similarity by taking blood sample from these breeds. Karyotype of sheep, native pigs and of some native chicken were also studied by the groups.

Genomic analysis has not been done for any species of domesticated livestocks. Nepal is a good place for future genomic study of these domesticated animals.

Institutional Structures

The Animal Breeding Division, Nepal Agricultural Research Council is undertaking the leading role for studying the genetic resources and has the mandate for research has been given to NARC as an apex body. The Animal Breeding Division has been working with Animal Breeding and Artificial Insemination Section of the Department of Livestock Services. The Institute of Agriculture and Animal Science teaches agriculture, veterinary and animal sciences.

Acknowledgments

The author would like to offer his sincere thank to Dr. Tsutomu Furukawa, Department of Animal Genetic Resources, Dr. H. Seko, Genetic Resources Coordinator; and to Dr. Masahiro Nakagahra, Director General; National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Japan; for their invitation, encouragement and providing the financial support and for organizing the seminar to present this paper. The author is happy to acknowledge Dr. Upendra Mishra, Director, Livestock and Fisheries; Mr. B.K. Baniya Director, Planning and Coordination and to Mr. Jagadish Chandra Gautam the Executive Director, Nepal Agricultural Research Council for providing me the opportunity to visit Japan to present this paper.

References


Buckley J.T. Presented at Rare Breed International 1-5 August Queen's University, Kingston, Ontario, Canada.


GENERAL DISCUSSION

Chairpersons
Y. Tanabe
I. Bodó
Questions and answers in General Discussion

Comments by Dr. Tanabe
C. I want to mention three topics that were not discussed in depth during the workshop.
1. More attention should be paid to the quality of products in animal production. Native animal products are usually superior than those of modern breeds.
2. Adoption of modern engineering techniques to analyse meat quality without killing the animal would be useful. For example, magnetic resonance imaging with computer tomography are useful techniques to detect the marbeling pattern of beef cattle.
3. The mechanism of heterosis should be elucidated. In maize, a high (0.8%) coefficient of correlation was observed between the yield (an expression of heterosis) and the genetic distance detected by DNA polymorphism (RFLP). No research has been conducted on this point in animals. Genetic distance among strains or lines is easily detected by randomly amplified polymorphic DNA. This technique will be very useful to predict the expression of heterosis is chickens. (Tanabe)

Comments by Dr. Bodó
C. Heterosis which can occur with preserved animal breeds should be mentioned. Heterosis occurs when well adapted females (from ancient breeds) are crossed with top males. (Bodó)
C. Quality is a very difficult topic and it is difficult to agree on a definition because different markets have different quality requirements. Thus, high quality is only that which commands a high market price. (Bodó)

Comment by Dr. Sekikawa
C. Disease resistance is important. In the next stage of conservation of animal genetic resources the linkages between the phenotype and genotype (DNA polymorphic markers) is necessary because both phenotypic and genotypic diversity are important. (Sekikawa)

Comment by Dr. Ishii
C. Diversity is the key to conservation of animal genetic resources. I think selection of
genetic resources to conserve on the basis of commercial quality is unwise, because the standards for quality varies around the world as pointed out by Dr. Bodo. Peoples perception of good quality also changes over time. So conservation of broad diversity is most important. For preservation, making genomic libraries is most economic. (Ishii)

Comment by Dr Li Ning

C. We can consider three levels of conserving animal genetic resources - animal level, cell level and gene level. The methods and financial requirements for each differs. There are also advantages and disadvantages to each level. Of these three levels, genomic libraries represent a powerful and efficient opportunity for conserving animal genetic resources. The genomic library can also be readily distributed for many uses. (Li Ning)
CLOSING REMARKS
Closing remarks

HIDEFUMI SEKO
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Thank you very much Dr. Tanabe and Dr. Bodó for your excellent chairmanship of the general discussion.

We have now reached the end of the indoor part of this workshop. I am sure you are looking forward to seeing sunlight tomorrow during the outdoor part of the workshop.

On behalf of the organizing committee of the workshop, I should deliver a few words.

Dr. Bodó reminded us that world wide conservation of native breeds and landraces of animals related to agriculture is at a relatively early stage of development compared with plants and microorganisms. But during this workshop a large amount of data and impressive results from many years of research have been presented. It appears, however, that in many countries much more effort needs to be directed towards animal genetic resources conservation. We are all too well aware of the extinction and imminent extinction of many animals. We cannot be reminded too often that "extinction is forever".

To effectively and efficiently conserve animal genetic resources many questions have to be answered. As the discussion yesterday on the cryopreservation of chicken semen indicated, we need to understand more about effective cryogenic preservation of delicate reproductive tissues.

I am very conscious that in Asia meat consumption is increasing rapidly as people across the region have greater spending power. As Dr. Matias discussed, in the Philippines, rapid economic development is resulting in a rapid increase in demand for meat. We are likely to be challenged in the future, to supply the demand for meat, much more than for the supply of agricultural plant products for human consumption. It will require concerted international efforts to conserve animal genetic resources that will provide the raw materials for improvement of farm animals. A vital task. However, meat production must be done in an environmentally sound way that does not threaten other elements of the environment.
The MAFF International Workshop on Genetic Resources aims at promoting research exchange and collaboration on the development of technologies and global strategies for conservation and use of genetic resources in national programs and international research institutions. This workshop has been of a small scale, but I anticipate that by continuing these efforts in the future, we will contribute to the promotion of research and collaboration in conserving and evaluating animal genetic resources.

As a plant breeder I have learned much over the last two days of intensive discussions. I thank everyone for their wholehearted participation.

I thank my colleagues here in Japan for their hard work in preparing papers for this meeting. I would like thank all the cooperating institutes in Japan and the Agriculture, Forestry and Fisheries Research Council for their support.

To friends who have come here from different parts of Asia and Europe it has been marvelous to have you with us. We look forward to collaboration and interaction in the years to come. We wish you a safe journey home when you leave at the end of the week.

It is now my honor to close the indoor part of this workshop.
To all Happy Christmas and Happy New Year.
Thank you.
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