APPLICATION OF BIOTECHNOLOGY IN GENETIC IMPROVEMENT,CHARACTERIZATIONAND CONSERVATION OF LIVESTOCK

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SUMMARY

Among agricultural and allied fields, animal production and health have probably benefited the most from biotechnology. Successful application of biotechnology has generally been limited to developed countries. Specifically, there are hardly any success stories of the application of biotechnology in the improvement of livestock production in Africa. This paper reviews available biotechnologies with current and/or potential application in genetic improvement, characterization and/or conservation of domestic animal genetic resources and attempts to identify those technologies which have been, or may be, applied in developing countries.

INTRODUCTION

Developing countries are faced with the challenge to rapidly increase agricultural productivity to help feed growing populations while also sustaining the natural resource base. Biotechnology is regarded as a means to meet both objectives. It is generally recognized that biotechnology has a role to play in addressing the production constraints of small scale or resource-poor farmers who contribute more than 70 percent of the food production in developing countries.

Biotechnology can be defined as any technique that uses living organisms or substances from such organisms to make or modify a product, to improve plants or animals or to develop microorganisms for specific purposes. Biotechnology is not new. Man has used these techniques for thousands of years to manufacture products such as beer, wine and bread. Conventional plant and animal breeding which involve selection and mating of phenotypically preferred individuals is a good example of age-old application of biotechnology. What is new about biotechnology has come about as a result of more recent breakthroughs such as recombinant DNA technology and associated techniques, monoclonal antibody techniques,

embryo manipulation technology, etc. These have enhanced possibilities for manipulating biological systems for the benefit of mankind.

REPRODUCTIVE PHYSIOLOGY

One of the challenges for genetic improvement is to increase reproduction rates. Several reproduction techniques are available. The commonest of these are artificial insemination (AI), embryo transfer and associated technologies. Measurement of progesterone in milk or blood which is a widely used technique for monitoring ovarian function and for pregnancy tests is also an important technology for managing the reproductive function of the animal.

Artificial insemination

No other technology in agriculture except hybrid seed and fertilizer use has been so widely adopted at a global scale as AI. Progress in semen collection, dilution and cryopreservation now enables a single bull to be used simultaneously in several countries for up to100,000 inseminations a year (Gibson and Smith, 1989). This implies that a very small number of top bulls can be used to serve a large cattle population. Additionally, each bull is able to produce a large number of daughters thus facilitating accurate progeny testing of bulls. The high intensity and accuracy of selection arising from AI can lead to a four-fold increase in the rate of genetic improvement in dairy cattle relative to that from natural mating (Van Vleck, 1981). This aspect is addressed fur ther in the next section.

In addition to its effect on intensity and accuracy of selection, Al facilitates a wider and rapid use of selected males thereby accelerating the rate of improvement. Additionally, use of AI can reduce transmission of venereal diseases in a population, reduces the need for farmers to maintain their own breeding males, facilitates more accurate recording of pedigree and is a cheaper means of introducing improved stock. However, success of AI technology depends on accurate heat detection and timely insemination. The former requires a certain level of awareness among farmers while the latter is dependent on a good infrastructure including transport network and availability of reliable means of transport.

AI is one of the most widely available biotechnologies in developing countries. Howcver, its use in these regions, particularly in Africa is far much less than in the developed countries. Although the technology is available in most African countries, it has remained generally unexploited, being only used for "exploratory" purposes mainly by research institutions. A few countries have taken the technology to the field, mostly for programmes of "upgrading" indigenous stock and as a service to a limited number of commercial farmers keeping exotic dairy cattle breeds. Countries such as Nigeria, Ethiopia, Uganda, Ghana, Botswana, Malawi, Senegal, Mali and Sudan, among others, are in this category. However, there are also countries which have used the technology more widely. Kenya and Zimbabwe, for example, have more elaborate AI systems which include national insemination services incorporating progeny testing schemes. However, even these have gone through periods of collapse or serious degeneration and have had to go through "rehabilitation" phases. The Republic of South Africa is probably the biggest user of AI technology in terms of number of inseminations. Additionally, the country has what is perhaps the best organised progeny testing scheme in the continent.

Although AI technology is available for other domestic livestock species, its use is still more generally associated with dairy cattle. The limitation of AI use in beef cattle has mainly been due to the diffculty in detecting heat in large beef herds kept on ranches and where individual cows are handled only occasionally. 1n sheep and goats there is scope for improvement of the tcchnology. The failure to develop a simple, non-surgical insemination procedure has prevented extensive exploitation of the technology in sheep (Robinson and McEvoy, 1993).

However, the technical success of laparoscopic intrauterine insemination has prompted research into less invasive transcervical procedures (Halbert et al., 1990; Buckrell et al., 1992). In Africa, research to improve the freezing-and-thawing properties of sheep semen is underway in the Republic of South Africa. AI in pigs is hampered by the inability to successfully cryopreserve boar semen.

AI is credited for providing the impetus for many other developments which have had a profound impact on reproductive biotechnology. Foote (1982) noted that studies of ocstrus detection and ovulation control which evolved out of a need to correctly time inseminations, led to the development of embryo transfer technology.

Embryo transfer

Although presently not economically feasible for commercial use on snrrll farms, embryo technology can greatly contribute to research and genetic improvement of local breeds. Advances in this area are mainly applicable in cattle. There are two procedures presently available for production of embryos from donor females. One consists of superovulation, followed by A I and then flushing of the uterus to gather the embryos. The other, called *in vitro* fertilirrtion (IVF) consists of recovery of eggs from the ovaries of the female then maturing and fertilizing them outside the body until they are ready for implantation into foster fcmales. I VF facilitate recovery of a large number of embryos from a single female animal. This reduces the cost of embryos, thus,making ET techniques economically feasible on a larger scale. Additionally, 1VF makes available embryos at the early stage suitable for such manipulations as cloning.

The principal benefit of embryo transfer is the possibility to produce several progeny from the female, just as AI produces many offspring from one male animal. For example the average lifetime production of a cow can be increased from 4 to 25 calves. Increasing the reproductive rate of selected females has the following benefits: Genetically outstanding animals can contribute more to the breeding programme, particularly if their sons are being selected for use in AI; the rate of genetic change can be enhanced if specially designed breeding schemes arc set up, which take advantage of increased intensity of female selection combined with increased generation turnover; transport of embryos is much cheaper than live animals; risk of importing diseases is avoided; facilitates rapid expansion of rare economically important genetic stocks; the stress to exotic genotypes can be avoided by having them born to dams of local breeds rather than importing them as live animals.

Embryo transfer is still not widely used despite its potential benefits. In developing countries this is mainly due to absence of the necessary facilities and infrastructure. However, even in developed countries commercial embryo transfer is still used only in specialized niches or for a small proportion of best cows in the best herds. This is mainly a cost consideration. Thus, in North America and Europe, only about one out of 500 calves born in the last decade was from ET (Seidel and Seidel, 1992). The great majority of commercial embryo transfer is with cattle. This is mainly because ET is relatively easier in cattle than the other species and also because it is more economical in cattle lie cattle are worth more). Additionally, the low reproductive rate and the long generation interval of cattle make ET much more advantageous in the species.

Embryo transfer is of particular importance in research: Production of several closely related and hence genetically similar individuals can make critical contribution to research. For example, a project at the International Livestock Research Institute (ILRI) to locate the genes responsible for tolerance of some cattle populations to trypanosomiasis required large numbers of closely related crosses of trypanotolerant and trypanosusceptible cattle. Use of ET has made it possible to generate such families thereby facilitating the search for genetic

markers of trypanotolerance. Additionally, ET could be useful in studying the extent to which a trait is influenced by the embryo (direct component) or the reproductive tract (maternal component).

Embryo sexine and cloning

Although embryo sexing may not have dramatic effects on rates of genetic gain (eg Colleau, 1991; Kinghorn et al., 1991) it can have considerable increases in efficiency. Taylor et al (1985) concluded from a study that an all-female heifer system using ET was 50% more efficient than the highest achievable in a traditional system. It has been suggested that, if multiple sexed-embryo transfer became a routine operation such as AI, beef operations based on this system could become competitive with pig and poultry production in terms of efficiency of food utilization:

Clones may be produced by embryo splitting and nuclear transfer (Macmillan and Tervit, 1990). These offer possibility for creating large clone families (Woolliams and Wilmut, 1989) from selected superior genotypes which, in turn, can be used to produce commercial clone lines (Smith, 1989). However, some studies have concluded that cloning of embryos will not increase rates of genetic progress in the nucleus, but that it offers considerable advantages in increasing rate of dissemination of superior testing genotypes in commercial populations leg Woolliams, 1989). Other potential applications of cloning include efficient evaluation of genotype x environment interactions and testing and/or dissemination of transgenics. From a research standpoint, production of identical siblings should, by eliminating variability among animals, greatly reduce the size and hence the cost of experiments.

Hormonal intervention

As has been pointed out, use of hormonal assays to *monitor* reproductive function can be a rewarding practice for both research purposes and commercial livestock operations. However, reproduction can also be *manipulated* using hormonal treatments. Although hormonal treatments have produced desirable results in some studies in Africa leg Aboul-Naga et al., 1992), lack of awareness about their use and the fact that they are not economically viable under most prevailing production circumstances limit their use. Progesterone and PMSG treatment and immunization against androstenedione increased ovulation rate in Ossimi sheep and exogenous melatonin treatment of barren Rahmam ewes resulted in increased proportion of ovulating ewes and a higher ovulation rate (Aboul-Naga et al., 1992). However, these responses did not result in increased litter size because of increased ova wastage. Thus, in addition to the impracticability arising from prohibitive prices of hormonal preparations and problems with hormonal administration at farm level, there are other technical problems with these technologies. Indeed, technologies aimed at increasing litter size in traditional small ruminant production systems should not be applied unless management, including nutrition, can be improved in concert to ensure the survival of the additional progeny.

Reproduction can also be manipulated without application of exogenous hormones. Hassan et al. (1988) have reported that exposing ewes to rams one week prior to mating ("the ram effect") increased the percentage of ewes in oestrus (and hence the percent mated) by 27%. Such management approaches offer practical options for increasing annual lamb (or kid) production in situations where other technologies are either not available or not appropriate. "Accelerated lambing" - increasing the number of lambings per year can also be used to increase annual productivity. However, in tropical and subtropical situations where animals depend on seasonally available natural pastures, this practice may not be feasible. Under such circumstances the reproductive cycle tend to be dictated by availability of feeds.

SYSTEMS OF SELECTION AND BREEDING

Genetic improvement of livestock depends on access to genetic variation and effective methods for exploiting this variation. In developed countries, breeding programmes are based upon performance recording. These programmes have led to substantial improvements in animal production. Developing countries have distinct disadvantages for setting up successful breeding programmes: Infrastructure needed for performance testing is normally lacking because herd sizes are normally small and variability between farms, farming systems and seasons are large; reproductive efficiency is low, due mainly to poor nutrition, especially in cattle; and communal grazing preclude implementation of systematic breeding and animal health programmes.

Animal evaluation technology

One of the areas in which artificial insemination has made a major contribution is in sire evaluation: Progeny testing programmes in conjunction with AI have provided exceptional opportunities to improve the accuracy of evaluation of dairy sires, especially in developed countries. AI facilitates simultaneous use of a sire in many herds (and on many dams) thereby providing reliable prediction of the genetic potential of the sire as reflected in performance of daughters.

From a genetic improvement standpoint AI has made impact both in terns of improved accuracy of predicting the genetic worth of sires and in facilitating rapid propagation of superior sires. A critical step in progeny testing is the prediction of an animal's genetic worth or breeding value from the progeny information collected in different environments. Breeding value of an animal is defined as the value of the animal as judged by the mean value of its progeny (eg Falconer, 1981). Application of this concept in selection of animals predates its formal definition and theoretical articulation of its estimation:

"The bull gives no milk, of course, yet will not a bull descended from several generations of high producing dams produce, wizen mated with a highly productive cow, calves which possess this characteristic to a still higher degree?" - Bergen, 1780.

Whether selection is based on individual's own performance or on the performance of relatives or combinations of these, the underlying principle is the same: only the best animals animals with higher breeding values should be selected. Throughout the history of animal breeding, especially diary cattle breeding, there has been a continuous evolution in the complexity of methods for animal evaluation ie breeding value estimation. The amount and type of information used in evaluation has increased from the use of physical appearance of the animal, through recording of animal performance and inclusion of records on selected traits, to inclusion of records of relatives. The substantial, but variable effects of environmental factors on tile underlying genotype has necessitated development and continual refinements of quantitative methods for accounting for (and removing) the environmental noise when estimating breeding values.

This century has witnessed significant developments and refinements in techniques for breeding value estimation. Major contributions include those by Lush (1931a, b; 1933; 1935; 1944 and 1949), Henderson (1953; 1966; 1973; 1975a, b; 1976); Henderson et al (1959); Hcndcrson and Quaas (1976), among others. Henderson's (1973) formal derivation of the mixed model equations and elucidation of the desirable properties of best linear unbiased prediction (BLUP) procedure was particularly instrumental in the development of this technology. The pace in developments in breeding value estimation procedures was accelerated in the 1980s and carly 1990s by rapid developments in computer technology. More recent developments in computer programming strategies and capacity of computers have made it possible to use better, but more computationally demanding, procedures. For example, the individual animal model is currently used as the method of choice in animal

evaluations in over 14 countries compared to only three in 1988 (INTERBULL, 1992).

The impact of selection on different livestock species has been quite variable (Table 1).The most systematic genetic improvement arising from selective breeding of animals identified through formal genetic evaluation has been witnessed in dairy cattle where annual genetic gains of1 % or more are achieved.

Table 1. *Examples of annual rates of genetic change achieved in selection experiments (E) and in practice (P)*

Species	Trait	Annual response (%)	Where achieved	Number of years	Reference
Poultry	5-9 week weight	4.1	E	10	Pym (1982)
(Broilers)	gain	6.5	P	20	Chambers et al
	7-8 week weight				(1981)
	gain				
Poultry (Layers)	Number of eggs	1.7	P	10	Flock (1979)
Pigs	Low fat depth	2.1	E	12	Hetzer and Miller
	Index of 6 traits	1.8	P	7	(1972)
	Litter size	1.5	P	4	Mitchell et al.
					(1982)
					Bichard and Seidel
					(1982)
Sheep	Weaning weight	1.5	E	10	Pattie (1965)
	Index of 7 traits	1.2	P	13	Eijke (1975)
	Litter size	2.9	P	8	Hight et al (1975)
Beef cattle	Weaning weight	0.7	E	7-26[a]	Barlow (1978)
	Yearling weight	0.6	E	9	Koch et al (1974)
	Yearling weight	0.3	P	13	Willham (1982)
Dairy cattle	Milk yield	2.2	E	13	Young and Miller
	Milk yield	2.0	E	14	(1982)
	Milk yield	1.0	P	12	Legates and Myers
	Milk yield	0.4	P	?	(1972)
	Milk yield	0.07	P	19	Van Vleck (1977)
					Szkotnicki et al.
					(1978)
					Rege(1991

[a]From 7 experiments of varying durations

Source: Adapted (with additions) from Smith (1984)

Table 2 summarizes estimates of genetic gains achieved in practice in selected countries. Although these gains are small, they are continuous and cumulative. Thus, changes of 1 to 2% could amount to 10 to 20% in ten years. Moreover, the methods are reliable and hence associated with minimal risk. Additionally, when returns are considered at a national level, the cost of genetic improvement is small (Smith, 1978).

Open nucleus breeding system and multiple ovulation embryo transfer

Multiple ovulation embryo transfer (MOET) is a composite technology which includes superovulation, fertilization, embryo recovery, short-term *in vitro* culture of embryos, embryo freezing and embryo transfer. Benefits from MOET include increasing the number of offspring produced by valuable females, increasing the population base of rare or endangered breeds or species, *ex situ* preservation of endangered populations, progeny testing of females and increasing rates of genetic improvement in breeding programmes. Genetic improvement of ruminants in developed countries has made much

progress in the last 35 or so years through the use of large scale progeny testing of males. As has been pointed out, the general failure of extensive use of AI in developing countries has implied that progeny testing schemes cannot be operated with much success. In any case the gcncrally small herds/flocks and uncontrolled breeding in communal grazing situations preclude implementation of progeny testing. Smith (1988a) has suggested that the Open Nucleus Breeding System (ONBS) may be especially valuable for developing countries where the use of AI has been a failure due to the reasons given above.

Table 2. *Examples of genetic gains (expressed as % of 1980 performance levels) obtained in practice in dairy cattle in selected countries*

Country	Breed	Period covered[a]		Average no. bulls tested per year	Trait	Annual Phenotypic gain (%)b		1980°
						Phenotypic	Genetic	average(kg)
		Phenotypic	Genetic					
Germany	German Holstein Friesian	1980-86	1975-86	1627	Milk yield	1.47	1.11	5143
					Fat yield	2.31	1.46	203
					Protein yield	1.13	1.01	174
Ireland	Holstein/Friesian	1980-86	1975-86	37	Milk yield	5.50	1.02	3210
					Fat yield	5.31	1.47	117
					Protein yield	5.34	0.97	105
Israel	Israeli Holstein	1980-87	1975-87	41	Milk yield	1.19	1.56	8178
					Fat yield	0.74	0.77	262
Italy	Black and White	1980-86	1975-86	67	Milk yield	2.24	2.13	5901
					Fat yield	1.41	1.98	216
					Protein yield	2.25	2.25	183
The Netherlands	Black and White	1978-88	1978-88	331	Milk yield	3.01	2.65	4778

					Fat yield	4.03	2.67	201
					Protein yield	3.45	2.56	159
USA	Holstein	- 1980-86	1977-86	907	Milk yield	1.98	0.99	7871
					Fat yield	1.89	0.87	283
					Protein yield	1.40	0.78	250
	Ayrshire	1980-85	1977-85	14	Milk Yield	1.77	0.70	5808
					Fat yield	1.58	0.60	226
					Protein yield	1.32	0.63	195
	Jersey	1980-85	1977-85	47	Milk yield	2.12	0.90	5267
					Fat yield	1.78	0.85	252
					Protein yield	1.62	0.71	198

[a]Period when data used in estimating phenotypic and genetic gains were collected. Year for genetic data refer to bulls' year of birth while that for phenotypic data refer to year of calving of bulls' daughters.

[b]As % of 1980 adjusted production records

[c]Number of lactations included in the evaluations and hence the averages vary: Germany - 3; Ireland - 1; Israel - 6; Italy - all; The Netherlands - 3; USA - 5. USA evaluations also require first lactation data inorder for any lactation to contribute to evaluations.

Source: Calculated by author from sire evaluation information of INTERBULL (1992)

The ONBS concept is based on a scheme with a nucleus herd or flock established under controlled conditions to facilitate selection to be carried out. The nucleus is established from the "best" animals obtained by screening the base (farmers') population for outstanding fema1es. These are then recorded individually and best individuals chosen to form the elite herd or flock of the nucleus. If ET is possible, the elite female herd is used through MOET with superior sires to produce embryos which are carried by recipient cows from the base population. The resulting offspring are reared and recorded and the males among them evaluated using, as appropriate, performance of their sibs and paternal half sibs and their own performance. From these, an elite group of males with high breeding values for the specific trait is selected and used in the base population for genetic improvement through natural service or AI. It should be noted that, while MOET improves the rate of progress substantially, it is possible to operate an ONBS without ET technology, especially in species, such as small ruminants, with high reproductive rates. Such schemes are being tried for sheep in West Asia by FAO (Jasiorowski, 1990) and in Africa (cg Yapi et al., 1994). However, availability of AI and ET, in addition to increasing rates of genetic gain, enhance the flexibility of the system. For example, germplasm from other populations can be introduced easily through semen and/or embryos. One of the advantages of a nucleus herd is that it provides opportunity to record information on more traits than is possible in a decentralized progeny testing scheme.

The ONBS can be used for the improvement of an indigenous or exotic breed. It can also be used to improve a stabilized crossbred population. The level of the genetic response depends on the size of the scheme (that is, number of participating herds or flocks and total number of animals) and the selection intensity. Additionally availability and effective use of Al will determine the impact of such a scheme, especially for dairy cattle. An ONBS can initially be developed to form a focus for national sire breeding activities. In time, and with experience, the capacity can be expanded and ET introduced to increase the rate of genetic progress.

At one time it was suggested that application of MOET in nucleus breeding schemes could increase annual genetic gains by 30-80% (eg Nicholas and Smith, 1983). More recently it has been concluded that the earlier figures were over-predictions (eg Keller et al, 1990). The over-prediction arose partly because the assumed average number of progeny (eight) per donor female was unrealistically high and partly because of wrong assumptions made about genetic parameters (Keller et al., 1990). The realistic average number of live progeny per donor flushed is in the range of 2-3 in sheep and cattle and 6-8 in goats (Macmillan and Tervit, 1990). Consideration of these figures suggest that MOET could increase annual genetic gains by 10-20% in large nucleus breeding schemes. However, costs of operating such schemes in developing countries need to be evaluated before they can be recommended.

Indicator traits

Indicator traits are characteristics which are genetically correlated to traits of economic importance and are easier to measure than the latter. Such traits are usually not the target of genetic improvements but provide an indirect means of improving a targeted trait. Blair et al (1990) have reviewed some physiological and/or metabolic characteristics which might be considered as potential indicator traits. Traits such as testicular size in rams or bulls or FSH in ewe lambs (Bodin et al., 1986) have potential as indirect predictors of fertility. Indicator traits can improve genetic response by increasing accuracy of selection and reducing generation interval. The value of an indicator trait will depend largely on magnitude of co-heritability (square-root of product of heritability of the indicator and of the target trait) and the genetic correlation between the two traits (Woolliams and Smith, 1988). Woolliams and Smith (1988) have concluded that, with high co-heritability, selection for the indicator trait alone can result in greater rates of response than is possible with progeny testing, especially when breeding values are not accurately measured by progeny testing.

Packed cell volume (PCV), an indication of the extent of anaemia, is widely used as an indicator trait for pathological conditions associated with anaemia. For example, PCV is currently used at International Livestock Research Institute (ILRI) as an indicator of the effect of trypanosomiasis and hence of trypanotolerance and as an indicator of effect of the endoparasite *Haemonchus contortus* and hence as an indirect measure of parasite resistance.

Genetic markers and marker assisted selection

A genetic marker for a trait is a DNA segment which is associated with, and hence segregates in a predictable pattern as, the trait. Genetic markers facilitate the "tagging" of individual genes or small chromosome segments containing genes which influence the trait of interest. A genetic marker need not have an effect on performance. Its value is simply that it 'marks' chromosome segments containing genes affecting performance. Markers have already been identified for some traits controlled by single genes. These include *double muscling* gene in Belgian Blue cattle, *polled* gene and *Weaver* syndrome in Brown Swiss cattle (see Archibald et al., 1994). Availability of large numbers of such markers has enhanced the likelihood of detection of major genes influencing quantitative traits. The method involves screening the genome for genes with large effect on traits of economic importance through a procedure

known as linkage analysis (eg Paterson et al., 1988). The chances of major genes existing for most traits of interest and of finding them is considered to be high (Mackinnon, 1992). Thus, Archibald et al (1994) have considered that, for mapping of QTL (where the traits are controlled by a number of loci whose individual effects may not be greater, than environmentally induced variation) the availability of suitable pedigrees; rather than inadequacy of markers, is the limitation. The process of selection for a particular trait using genetic markers is called marker assisted selection (MAS). MAS can accelerate the rate of genetic progress by increasing accuracy of selection and by reducing the generation interval (Smith and Simpson, 1986). Meuwissen and Van Arendonk (1992) have reported a selection design (granddaughter design) for dairy traits in which MAS may yield 10-20% extra genetic progress. Zhang and Smith (1992; 1993) have shown that MAS, used in combination with phenotypic information, can increase the efficiency of selection by 10-20% over and above the efficiency of selection based only on best linear unbiased prediction (BLUP) procedures. However, the benefit of MAS is greatest for traits with low heritability and when the marker explains a larger proportion of the genetic variance than does the economic trait. Lande and Thompson (1990) suggest that about 50% additional genetic gain can be obtained if the marker explains 20% of the additive genetic variance and the economic trait has a heritability of 0.2. MAS also facilitates increased rate of genetic gain by allowing measurement in young stock thereby reducing generation interval.

Genome maps of the domestic species are already beginning to become available (see Archibald et al., 1994). This should accelerate identification of markers and their use in genetic improvement. In mice, more than 20 genes conferring resistance to infectious diseases have been mapped (see Visscher and Haley, 1995). Marker identification and use in domestic livestock should enhance future prospects for breeding for such traits as tolerance or resistance to environmental stresses, including diseases. Already, identification of carriers of genes for resistance and introduction of such genes into a population seems feasible for resistance against *Trichostrongyhus colubriformis* and *Haemonchus contortus* (Gogolin-Ewens et al., 1990). It should also be possible to eliminate factors predisposing sheep to Listeriosis or Salmonellosis (Blancou, 1990). As has been alluded to, research is currently underway at ILRI (International Livestock Research Institute) to identify genetic markers for tolerance to African trypanosomiasis in N'Dama cattle and for resistance to endoparasites in Red Maasai sheep. Marker technology should provide opportunity for selecting for resistance or tolerance to these important parasites and diseases.

Transgenic animals

A transgenic animal is an animal whose hereditary DNA has been augmented by addition of DNA, through recombinant DNA techniques, from a source other than parental germplasm. Transfer of genes or gene constructs allows the manipulation of individual genes rather than entire genomes. There has been dramatic advances in gene transfer technology in the last two decades since the first successful transfer was carried out in mice in 1980 (Palmiter et al., 1982; Jaenisch, 1988). The technique has now become routine in the mouse and resulting transgenic mice are able to transmit their transgenes to the offspring thereby allowing a large number of transgenic animals to be produced. Successful production of transgenic animals has so far been reported in pigs, sheep, rabbits and cattle. The majority of gene transfer studies in livestock have been carried out in the pig. Although transgenic cattle and sheep have been successfully produced, the procedure is still inefficient in these species (Niemann et al, 1994). Niemann and Reichelt (1993) have reviewed the problems associated with transgenic technology in cattle.

Transgenesis offers considerable opportunity for advances in medicine and agriculture. In livestock, the ability to insert new genes for such economically important characteristics as fecundity, resistance or tolerance to other environmental stresses would represent a major

advance in the breeding of commercially superior stock. Indeed, disease resistance alleles are the classical example of candidates for transgenic technology (Smith et al., 1987). Another opportunity that transgenic technology could provide is in the production of medically important proteins such as insulin and clotting factors in the milk of domestic livestock. The genes coding for these proteins have been identified and the human factor IX construct has been successfully introduced into sheep and expression achieved in sheep milk (Clark et al., 1990). Moreover, the founder animal has been shown to be able to transmit the trait to its offspring (see Niemann et al., 1994). To date, the majority of genes transferred into sheep have been growth hormone encoding gene constructs. Unfortunately, in most cases the elevated growth hormone levels have resulted into a clinical diabetes situation leading to an early death of the transgenic sheep (Rexroad et al., 1990). Transgenic sheep have recently been generated which express the visna virus envelope gene (Clements et al.; 1994).

The first reports of the production of transgenic animals created a lot of excitement among biological scientists. In the field of animal breeding, there were diverse opinions on how the technology might affect livestock genetic improvement programmes. Some (eg Ward et al., 1982) believed that it would result in total reorganization of conventional animal breeding theory while others (eg Schuman and Shoffner, 1982) considered the technology as an extension of current animal breeding procedures which, by broadening the gene pool, would make new and novel genotypes available for selection. Application of the technology in animal improvement is still far from being achieved. However, consideration need to be given to its potential role in this field. Smith et al. (1987) have presented a comprehensive evaluation of strategies for developing, testing, breeding and disseminating transgenic livestock in the context of quantitative improvement of economic traits.

An important contribution of transgenic technology is in the area of basic research to study the role of genes in the control of physiological processes. The understanding of the molecular control of life process has important implications for both medicine and agriculture. For example, the generation (through mutation of an endogenous gene) of an organism which lacks a specific gene is a powerful tool to investigate the function of the gene product. This type of genetic analysis has been facilitated by the availability of *in vitro* cultures of embryonic stem cells from mice (eg Bradley, 1994).

Recent advances in *in vitro* technology *(in vitro* fertilization and maturation) will increase the number of zygotes available for gene transfer purposes. This, plus utilization of embryonic stem cell (Stice et al., 1994) and primordial germ cell (Stokes et al., 1994) technologies should enhance the efficiency of gene transfer in cattle and sheep considerably.

Maior genes in livestock

For the application of marker technology, and indeed, for transgenesis, it is necessary to be able to find genes or chromosome segments which explain a significant proportion of the variation in the trait of interest. Virtually all major genes currently known in livestock were first detected by "eyeballing", ie examination of recorded data. Methods for searching for major genes are increasingly getting more sophisticated. For example, statistical methods now exist for screening populations for major genes without the use of genetic markers (Hoeschele, 1988; LeRoy et al., 1989; LeRoy and Elsen, 1992; Knott et al., 1992). However, use of marker technology (see earlier) is perhaps the most rigorous method for searching for major genes. This involves evaluating large numbers of animals for the (quantitative) trait of interest and genotyping them for a number of markers (eg Soller et al., 1979). Table 3 presents some examples of major genes in livestock. Identification of genes with large effects (using modern gene mapping methods) will allow us to unravel the molecular basis of production differences in livestock. This should have important implications for genetic improvement. It is considered that the more recent technologies such as MAS will not replace existing animal breeding

techniques; rather, they will complement them: That is, genetic evaluation methods which combine both known and unknown quantitative trait loci are likely to be the most effective.

Table 3. *Examples of major genes in livestock*

Species	Major gene	Main advantage	Reference
Pigs	Halothane gene	increased lean meat yield	Fujii et al. (199 1)
	Moishan gene	Increased litter size	Rothschild et al. (1994)
Sheep	Booroola, hwerdale and Javanese genes	higher ovulation rate	Montgomery et al. (19)3); Piper w id Bindon (1982); Bradford (1980,); Davis et al. (1991)
	Callipyge muscling gelle	Higher lean meat yield	Cockett et al. (1994)
Cattle	Double muscling gene	Higher lean meat yield	Hanset and Mlcllallx (1985a,b)

MOLECULAR CHARACTERIZATION OF ANIMAL GENETIC RESOURCES

Developing countries are endowed with the majority of the global domestic animal diversity in the form of landraces, strains or breeds. Some livestock breeds in these countries are in immediate danger of loss through indiscriminate crossbreeding with exotic breeds. The importance of indigenous livestock breeds lies in their adaptation to local biotic and abiotic stresses and to traditional husbandry systems. The indigenous genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production on a range of environments. However, most of these animal genetic resources are still not characterized and boundaries between distinct populations are unclear. In such cases breeds are defined on the basis of subjective data and information obtained from local communities. Reliance on these criteria as the basis for classification or development of improvement and/or conservation strategies may be misleading. Additionally, historical evidence is not always accurate, relying as it often does on subjective judgements. Archival research can reveal much about the original type of a breed or strain but it is molecular genetic evidence which is factual and precise. It is in this sphere that biotechnology has an important role.

Genetic uniqueness of populations is measured by the relative *genetic distances* of such populations from each other. Polymorphism in gene products such as enzymes, blood group systems and leukocyte antigens which have traditionally been used for measuring genetic distances are rapidly being replaced by polymorphism at the level of DNA, both nuclear (eg Jeffreys and Morton, 1987) and mitochondrial (eg Loftus et al., 1994) as a source of information for the estimation of genetic distances. The first DNA polymorphism to be used widely for genome characterization and analysis were the restriction fragment length polymorphisms (RFLP) (Southern, 1975) which detect variations ranging from gross

rearrangements to single base changes. Minisatellites sequences of 60 or so bases repeated many hundreds or thousands of times at one unique locus within the genome have been used to generate DNA fingerprints typical of individuals within species (Jeffreys and Morton, 1987). Microsatellites (Weber and May, 1989) repeats of simple sequences, the commonest being dinucleotide repeats - are abundant in genomes of all higher organisms, including livestock. Polymorphism of microsatellites takes the form of variation in the number of repeats at any given locus and is generally revealed as fragment length variation in the products of PCR amplification of genomic DNA using primers flanking the chosen repeat sequence and specific for a given locus (Kemp and Teale, 1991). Ease of identification and of sequence determination (Moore et al., 1992) and need for only small amounts of DNA, are some of the advantages of microsatellites. Additionally, because microsatellite polymorphism can be described numerically, they lend themselves to computerized data handling and analyses (Teale etal., 1994). Microsatellites can be used in non-PCR systems in a similar way to numsatellite probes (Haberfeld et al., 1991).

Randomly amplificd polymorphic DNA (RAPD) (Williams et al., 1990) has been extensively used for genetic characterization of a wide range of organisms. The technique uses short (up to 10 base) primers to amplify nuclear DNA in the PCR. The procedure does not require knowledge of the sequence of DNA under study: primers are designed randomly. The basis of the polymorphism detected by this method is that products are either generated in PCR or not. Complete sequencing of the genome is the ultimate form of genetic characterization. Sequencing has traditionally been expensive and laborious, but with the advent of automated sequencing this is changing rapidly. However, sequencing is unlikely to be used as a technique of choice for genetic characterization.

Regardless of which method is used, the ultimate goal in genetic characterization for conservation is to obtain a measure of available diversity. Nei and Takezaki (1994) have reviewed statistical methods for estimating genetic distances and for constructing phylogenetic trees from DNA sequence data and have concluded that different analytical methods may produce different results. Tcale et al. (1994) have commented on what considerations should be made in using DNA polymorphism data for genetic distance estimation and have cautioned that great care his to be taken in selecting characterization methods and in interpreting the resulting data. While recognizing the importance of the uniparental mode of inheritance of mitochondrial DNA in detecting underlying population structure not discernible from analyses of nuclear DNA, Loftus et al. (1994) have concluded that mitochondrial DNA analysis may not be sufficient to resolve breed differences within Africa. MacHugh et al. (1994) have suggested that microsatcllite polymorphism may be more suitable when trying to discriminate between closely related populations.

CONSERVATION OF ANIMAL GENETIC RESOURCES

The terms conservation, preservation, *ex-situ* and *in-.situ* are used here according to the definition given by FAO (1992). There arc several ways, differing in efficiency, technical feasibility and costs, to conserve animal genetic resources. Developing and utilizing a genetic resource is considered the most rational conservation strategy. However, there are cases where ex *situ* approaches are the only alternatives. *Ex-situ* approaches include: Maintenance of small populations in domestic animal zoos; cryoprescrvation of semen (and ova); cryopreservation of embryos; and some combinations of these. Brem et al. (1989) have reviewed biotechnologies for ex situ conservation.

Cryopreservation of gametes, embryos or DNA segments can be quite effective and safe approach for breeds or strains whose populations are too small to be conserved by any other means. The safety of these methods has been demonstrated by background irradiation studies. For example, studies based on irradiation of mouse embryos exposed to the

equivalent of hundreds of years of background mutation showed no detectable damage (Whittington et al., 1977).

Regeneration of offspring following transfer of frozen-thawed cmbryos has been successful for all ma ior domestic species, except buffalo (Tcale ct al., 1994). In cattle, the transfer of frozen-thawcd embryos is now a commercial practice and embryo survival rate after thawing can be as high as 80% with a pregnancy rate of about 50%. Cryopreservation of oocyfies followed by successful fertilization and live births have been achieved in the mouse. Cryopreserved bovine oocytes have been successfully matured and fertilized *in vitro* and zygotes developed to blastocyst stage (Lira et al.. 1991). These trends strongly suggest that long-term cryoprcservation of mammalian oocytes will be possible (Tcale et al.. 1994). Respective pregnancy rates of 58 and 50% for fresh and frozen-thawed *in vitro* produced embryos have been reported (Lu et al.\\, 1990) and calves have been produced from transfer of both split and frozen-thawed *in vitro* produced cmbryos.

Economic aspects of genetic conservation in farm animals has been assessed by Bren et al (19 84). The study concluded that costs of *ex,situ* live animal conservation wits moderate to high while costs of long term cryopreservation of gametes were low. Developments in genetic engineering, cryobiology, cell biology and embryology will provide techniques that may enhance our ability to preserve germplasm *in vitro*. Techniques such as transfer of DNA within and between species and the production of viable transgenic animals are far from practical application. However, biotechnology will certainly contribute newer and cheaper methods for preservation such as storage of catalogued DNA. At present, other than live animal and embryo preservation, the other techniques do not allow preservation of genomes in a form which can be reactivated *in tolo* at a later stage. but they permit the preservation of individual genes or gene combinations for possible future use.

Conservation of indigenous animal genetic resources should be one of the priority livestock development activities for developing countries. The critical importance of these resources to their owners in developing countries need not be emphasized. Their importance to developed countries is also becoming evident: There is an increasing importation of tropical germplasm by these countries. It is highly likely that these resources will become of increasing importance to the industrialized countries either as sources of unique genes or when environmental concerns necessitate change in production systems. Developed countries should, thus, assist in the conservation and development of these resources. Technology for cryopreservation of semen and embryo is sufficiently developed to be applied in developing countries. What is missing is financial support to implement conservation programmes. Such support has been provided for world-wide conservation activities for plant germplasm. There is also a strong case for support of animal genetic resources conservation.

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