Application of Molecular Genetic Technologies in Livestock Production: Potentials for Developing Countries

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Application of Molecular Genetic Technologies in Livestock Production: Potentials for Developing Countries

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Abstract: Application of Molecular Genetics in livestock production has been reviewed indicating that why molecular genetics is important in the presence of conventional livestock improvement programs. Molecular genetic technologies like marker assisted selection, PCR, DNA sequencing, tran genesis and cloning have been discussed. The paper includes the applications of Molecular biotechnologies in animal improvement like animal health, nutrition, growth and in animal breeding and genetics. Scope of molecular genetic technologies in developing countries has also been reviewed.

Key words: Molecular genetics · marker assisted selection · PCR · trangenesis · cloning · livestock production · developing countries

INTRODUCTION

MOLECULAR GENETICS

Molecular Genetics is the study of the genetic make up of individuals at the DNA level. It is the identification and mapping of genes and genetic polymorphisms. There are opportunities for using molecular genetics to identify genes that are involved in variety of traits. Armed with this information it would be possible to select improved livestock on the basis of their genetic makeup. If applied with care, the use of molecular information in selection programmes has the potential to increase productivity, enhance environmental adaptation and maintain genetic diversity. The first task is to understand the genetic control of the trait of interest and then to identify the genes involved.

CONVENTIONAL LIVESTOCK IMPROVEMENT PROGRAMMES

Conventional animals breeding programmes depend on selection programmes based on phenotypic selection where traits are measured directly and animals with superior performance in the traits are used as breeding stock where the trait is limited, such as milk production, progeny test schemes have allowed the genetic merit of the sex not displaying the trait to be estimated. There are several problems associated with phenotypic selection:

1. Narrowing the genetic base of a population.
2. The approach can only be applied to traits that are easily measured.
3. High costs.

In traits that are displayed only in adults, which include most of the production traits, it is necessary to raise a large number of individuals for which the trait is recorded, so that a few can be chosen for breeding. In the case of progeny testing for milk production, the costs are very high, as the test sires have to be raised and then the daughters themselves raised and bred before the trait can be measured and the elite sires selected.

WH Y MOLECULAR GENETICS?

The use of molecular genetic technologies potentially offer a way to select breeding animal at an early age (even embryos); to select for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual. The broad categories of existing gene-based options include;

- Molecular analysis of genetic diversity
- Animal identification and traceability
- Reproductive enhancement;
- Transgenic livestock;
- Germ line manipulation and;
- Gene based trait selection;

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- Animal health: diagnosis, protection and treatment;
- Ruminant and non-ruminant nutrition and metabolism;

To date, most genetic progress for quantitative traits in livestock has been made by selection on phenotype or on Estimated Breeding Values (EBV) derived from phenotype, without knowledge of the number of genes that affect the trait or the effects of each gene. In this quantitative genetic approach to genetic improvement, the genetic architecture of traits has essentially been treated as a 'black box'. Genetic progress may be enhanced if we could gain insight into the black box of quantitative traits. Molecular genetics allows to study the genetic make-up of individuals at the DNA level.

The main reasons why molecular genetic information can result in greater genetic gain than phenotypic information are:

1) Assuming no genotyping errors, molecular genetic information is not affected by environmental effects and, therefore, has heritability equal to 1
2) Molecular genetic information can be available at an early age, in principle at the embryo stage, thereby allowing early selection and reduction of generation intervals
3) Molecular genetic information can be obtained on all selection candidates, which is especially beneficial for sex-limited traits, traits that are expensive or difficult to record, or traits that require slaughter of the animal (carcass traits).

MOLECULAR GENETIC TECHNOLOGIES

Molecular genetic technologies are summarized in Fig. 1.

1. Marker assisted selection technology: Animal scientists are currently hanging their hopes on genetic markers. These markers have no function of their own they simply identify a particular region of genetic instructions. This region will contain hundreds of genes that do have a function, but we do not generally know which genes they are or what their function is. We can follow the inheritance of many different markers in families of animals and see whether inheritance of any of these markers is associated with improved performance. If they are, we then know that one or more genes in the region of the marker are having a beneficial effect. We do not need to discover which genes are involved, but can go to use the information on the genetic markers to make future selection decisions, since animals that inherit the marker will also inherit the useful effects associated with it. This is known as marker assisted selection, the use of genetic technology and MAS in animal production has moved from a theoretical concept to the beginnings of practical application during the 1990s. The low and medium density linkage maps that have been constructed generally consist of several hundred to over a thousand micro satellite markers distributed throughout the genome. Using well established statistical techniques and specially constructed three generation families, linkage between these markers and production traits can now be established. While this provides encouragement that there are potentially useful genes to be found, it is of little immediate value in selection, because the regions of the genome involved tend to be large. Further cycles of work are therefore required to locate the gene or genes involved more precisely. The methodology for creating these higher density maps is still evolving and a number of different approaches are being used [20].

- Positional cloning of markers from defined regions by chromosome micro-dissection, or use of yeast (YAC) or bacterial (BAC) artificial chromosomes
- By cross-reference to the much better documented human and mouse genomes, it may be possible to infer the approximate location of a functional gene and also something about the nature of its action [21]

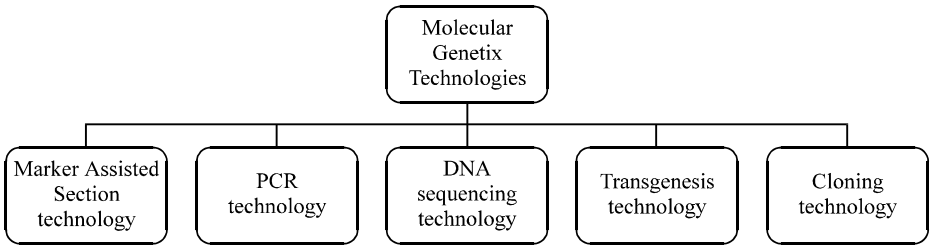

Fig. 1: Molecular genetic technologies

During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or genomic regions that affect quantitative traits. This has provided opportunities to enhance response to selection, in particular for traits that are difficult to improve by conventional selection (low heritability or traits for which measurement of phenotype is difficult, expensive, only possible late in life, or not possible on selection candidates). Examples of genetic tests that are available to or used in industry programs are documented and classified into causative mutations (direct markers), linked markers in population-wide linkage disequilibrium with the QTL (LD markers) and linked markers in population-wide equilibrium with the QTL (LE markers) [9].

Some examples of MAI in livestock are available. Hanset *et al*. [18] reported on the successful introgression of the halothane normal allele into a Piétrain line that had a high frequency of the halothane-positive allele. They used LD foreground selection on markers linked to the RYR locus. Yancovich *et al*. [45] used marker-assisted background selection to speed up the recovery of the broiler genome when introgressing the naked-neck gene from a rural low-BW breed into a commercial broiler line. Gootwine *et al*. [13] reported on MAI of the Booroola gene (FecB) into dairy sheep breeds using LD markers for foreground selection. In developing countries, programs for the introgression of disease resistance or tolerance genes are being considered for cattle.

Genetic markers can also be used to control inbreeding, parental verification and product tracing. Pedigree verification is an important aspect of the use of molecular markers in several breeding programs [37].

Applications of molecular markers: Molecular markers can play an important role for livestock improvement through conventional breeding strategies. The various possible applications of molecular markers are short-range applications or immediate and long-range applications [1].

Short-range or immediate applications: Molecular markers have several immediate applications like parentage determination, genetic distance estimation, determination of twin zygosity and freemartinism, sexing of pre-implantation embryos and identification of disease carrier. In the following subsections, each of these applications have been discussed briefly.

(I) Parentage determination: Since the breeding value of an animal is generally estimated using the information available from its relatives, the knowledge of correct parentage is therefore a prerequisite. Parentage testing using molecular markers yields much higher exclusion probability (>90%) than the testing with blood groups (70-90%) or other biochemical markers (40-60%) Highly [14] polymorphic DNA fingerprinting markers [19]. are quite useful for this purpose. Recently, DNA fingerprinting with oligoprobes (OAT18 and ONS1) has been successfully used for determining the parentage of IVF buffalo calf [29]. With the advent of PCR-based microsatellite assays, a large number of microsatellite panels have been reported that are useful for parentage testing in different livestock species. For example in cattle, Glowatzki-Mullis *et al*. [15] demonstrated that using two triplex microsatellite co-amplification systems, wrong parentage can be excluded with almost 99% accuracy. In addition, molecular markers also serve as a useful tool for animal identification, particularly for verification of the semen used for artificial insemination.

(ii) Genetic distance estimation: Genetic distance, a measure of overall evolutionary divergence, i.e. genetic similarities and dissimilarities between two populations (such as between species, breeds, strains), serves as an useful tool for authentication of the pedigree, for characterization of different breeds or strains within a species and for evaluation of the change in variation in species over time. In principle, genetic distance can be measured on the basis of polymorphic characters occurring at the different levels, viz. morphological, biochemical, cellular and DNA level. Allelic frequencies of blood groups [23] as well as those of other biochemical loci, e.g. serum and milk proteins [3] have been used extensively for the estimation of genetic divergence of different livestock species. However, a great amount of genetic variations at protein loci remain undetected, since changes in the underlying nucleotide sequences may not necessarily lead to corresponding change in the amino acid sequences owing to degeneracy in the genetic code. Molecular markers capable of generating individual specific DFP patterns, useful for establishing familial relationships [20, 21] can serve as an alternative. The similarities between the DFP patterns that are expressed by band-sharing values, provide a reliable method for evaluating genetic distance amongst populations [7, 24].

Presently, the PCR-based RAPD fingerprinting assays are being used for characterization of zebu cattle breeds [16], for detection of genetic variations in cattle

and sheep [25] and characterization of highly inbred chicken lines in poultry [33].

(iii) Determination of twin zygosity and freemartinism: Correct knowledge of zygosity of twins, particularly in monotocus animal, is very important. Monozygotic twins provide means for epidemiological as well as for genetical studies and also help in transplant matching. Individual specific DNA fingerprinting techniques have potential applications in determination of twin zygosity [17] and demonstration of spontaneous XX/XY chimaerism [10]. Demonstration of XX/XY chimerism in heterosexual bovine twins, by PCR-RFLP assay using sex-chromosome-specific primers, has enabled the identification of freemartin animal [26, 43].

(iv) Sex determination: Sexing of pre-implantation embryos can serve as an important tool for improving herd for a desired purpose. A large number of invasive and noninvasive methods for sexing embryos are available. However, ideally the technique to be applied should not have any adverse effect on embryo survivability, its conception rate and subsequent development. Besides, the technique should be simple and easy to carry out, repeatable and accurate and time saving. Though embryos can be sexed by cytogenetical method [44], this method is quite accurate but invasive and needs a large piece of embryo. The molecular markers on the other hand, have potential application in determination of sex of pre-implantation embryos, since the embryos can be sexed using male-specific or Y-chromosome-specific DNA sequence as probes[43]. However, this method is time consuming as well as tedious. Sexing of embryo using PCR-based approach [30, 41], involves amplification of male-specific DNA fragment and its visualization in ethidium-bromide-stained agarose gel following electrophoresis. The PCR-based method of sex determination offers several advantages over all the other methods: (i) It can be carried out in less than five hours with almost 100 per cent accuracy [34]. (ii) It is less invasive and requires very small quantities (in nanograms) of DNA for PCR assay, which can be isolated from two to eight cells biopsied from the embryo [41] (iii) It can be done at an early stage of embryo e.g. blastocyst stage (6 to 8 days) or even earlier at the 16-32 cell stage [30] (iv) The use of multiplex PCR allows simultaneous genotyping for important loci like milk proteins, diseases carrier, etc.

(v) Identification of disease carrier: Many of the most serious incurable diseases result not from infections with bacteria or viruses but defects in genomes of the hosts. Certain allelic variations in the host genome lead to susceptibility or resistance to a particular disease. Kingsbury [22] reported that a particular RFLP in the Prion protein (Prn P) gene was responsible for the variation in host's response to the causative agent and the incubation time of Bovine Spongiform Encephalopathy (BSE). DNA polymorphism occurring within a gene helps to understand the molecular mechanism and genetic control of several genetic and metabolic disorders and allows the identification of heterozygous carrier animals which are otherwise phenotypically indistinguishable from normal individuals. In case of genetic disorders caused by a single point mutation, for example citrulinaemia [8], Bovine Leukocyte Adhesion Deficiency (BLAD) [38] and deficiency of uridine monophosphate synthatase (DUMPS) [39] in cattle; hyperkalemic periodic paralysis in horses and malignant hyperthermia in pigs [11], carrier animal possessing the defective recessive allele can be identified easily using PCR-RFLP assay. Using micro satellite (TGLA116) marker, Georges *et al*. [12] demonstrated the identification of carrier animals of weaver disease in cattle.

Long-range applications: The foremost long-range application of molecular markers in conventional breeding includes mapping of the QTL by linkage. Such mapping information, if available, particularly, for those loci which affect the performance traits or disease resistance/susceptibility, can be used in breeding programmes by either within-breed manipulations, like marker-assisted selection of young sires, or between-breeds introgression programmes.

(I) Gene mapping: Molecular markers have three-fold applications in gene mapping: (i) A marker allows the direct identification of the gene of interest instead of the gene product and consequently, it serve as a useful tool for screening somatic cell hybrids. (ii) Use of several DNA probes and easy-to-screen techniques, a marker also helps in physical mapping of the genes using in situ hybridization. (iii) The molecular markers provide sufficient markers for construction of genetic maps using linkage analysis. Genetic maps are constructed on the basis of two classes of molecular markers [31]: Type I markers, that represent the evolutionary conserved coding sequences (e.g. classical RFLPs and SSLPs), are

useful in comparative mapping strategies where polymorphism is not an essential prerequisite. However, these are mostly single locus and di-allelic (SLDA) and thus are not useful for linkage analysis. On the other hand, the type II markers (like microsatellites markers) have higher polymorphism information content than conventional RFLPs and can be generated very easily and rapidly. Therefore, major efforts are being made to produce gene maps based on the type II markers. Further utilization of molecular markers developed from DNA sequences information, namely ASO and STMS polymorphic markers are also helpful in rapid progress of gene mapping.

(ii) Marker assisted selection: The concept of Marker Assisted Selection (MAS), utilizingthe information of polymorphic loci as an aid to selection, was introduced as early as in 1900s [40]. However, its application in genetic improvement of livestock species has been limited due to lack of suitable genetic markers. The discovery of DNA-level polymorphism in eighties and their subsequent use as molecular markers has renewed interest in the use of genetic markers in selection of breeding stocks. Implementation of MAS essentially involves two steps: Identification of the marker loci that is linked to QTL of economic importance, followed by the utilization of linkage association in genetic improvement programme. Once linkage between a QTL and a marker locus is established, it is possible to recognize the alternative QTL allele inherited by the individual. Such information can then be used for the selection of the breeding stock.

Pongpisantham [32] found that the inclusion of markers could increase up to 15% the genetic response to selection for growth rate in a population of chickens, compared with selection based on family selection. Ruane and Colleau [35] found an increase of 6 to 15% from MAS in the selection response for milk production in cattle nucleus that used multiple ovulation and embryo transfer (MOET) in the first six generations of selection.

2. PCR technology: The PCR is a method that efficiently increases the number of DNA molecules in a logarithmic and controlled fashion. PCR is a major scientific development and tag polymerase the enzyme essential to PCR's success. The chemistry involved in the PCR depends on the complimentarity (matching) of the nucleotide bases in the double stranded DNA helix. When a molecule of DNA is sufficiently heated, the hydrogen bands holding together the double helix are disrupted and the molecule separates or denature into single strand.

PCR analysis involves the use of DNA polymerase and synthetic primers to replicate DNA *in vitro*. Starting with just 1 copy of the target sequence, billions of copies can be generated within an hour. The process involves temperature cycle. At high temperature the DNA melts, the temperature is then lowered to one where the primers can base pair (anneal) with the target and the DNA polymerase can synthesize the DNA molecule. PCR principles are denaturizing, annealing and elongation.

Denaturation: When a double stranded DNA molecule is heated to 94°C, the paired strands will separate (denature. This allows the primers access to the single stranded DNA templates.

Annealing: The reaction mixture is cooled (about 50°C) to allow primers to select and bind (hyberidize) to their complementary positions on the ssDNA template molecules.

Elongation: The single stranded DNA/primer solution is heated to 72°C. in the presence of the heat stable polymerase, PCR buffer'dNTP's and magnesium (Mg^{2+}) molecules, the replication procedure begins. By the repetition of the cycle, the target DNA template becomes double. And after 30 cycles the reaction procedure one million copies of the target DNA fragment.

Use of PCR: PCR can be used very effectively to modify DNA. Modification means that addition of restriction enzyme sites and generation of desired site directed mutations or deletions.

PCR has a profound effect on all molecular studies including those in diagnostic area. The PCR has dramatically impacted on diagnosis of genetic and infectious disease. The PCR today plays a central role in genetic typing of organisms or individuals and molecular epidemiology.

3. DNA sequencing technology: DNA sequencing is the process of determining the exact order of the billions of chemical building blocks (called bases and abbreviated A, T, C and G) that make up the DNA.Genomics, the science of identifying the entire set of genes of living organisms is revolutionizing biological sciences and has become a driving force in mergers and divestments involving many of the world's largest corporations. DNA sequencing technology is now being used by public and private researchers to decipher the genetic blueprint of humans, plants, animals and micro-organisms. As the efficiency of

DNA sequencing technology accelerates, genomics milestones are being reached far ahead of schedule.

Highest resolution of DNA variation can be obtained using sequence analysis. Sequence analysis provides the fundamental structure of gene systems. DNA sequencing is generally not practical to identify variation between animals for the whole genome, but is a vital tool in the analysis of gene structure and expression [6].

An aid in the development of livestock genome maps, has been the high level of conservation of gene sequences between humans, cattle, sheep, goat, pig and mice. By such reason, once loci of particular DNA sequences has been mapped in one species, the information is frequently of help in the genome mapping in another. Livestock species mapping had been greatly facilitated by the increasing availability of human and murine sequences [4, 36].

Examples of current genome mapping information available in the internet for several mammal species [27] can bee seen on the following websites.

http://sol.marc.usda.gov/genome/cattle/cattle.html
http://sol.marc.usda.gov/genome/swine/swine.html
http://ws4.niai.affrc.go.jp/dbsearch2/mmap/

4. Cloning technology: Cloning technology allows us to generate a population of genetically identical molecules, cells, plants or animals. Because cloning technology can be used to produce molecules, cells, plants and some animals, its applications are extraordinarily broad. Any legislative or regulatory action directed at "cloning" must take great care in defining the term precisely so that the intended activities and products are covered while others are not inadvertently captured.

Molecular or gene cloning: Molecular, or gene, cloning, the process of creating genetically identical DNA molecules, provides the foundation of the molecular biology revolution and is a fundamental and essential tool of biotechnology research, development and commercialization. Virtually all applications of recombinant DNA technology, from the Human Genome Project to pharmaceutical manufacturing to the production of transgenic crops, depend on molecular cloning.

The research findings made possible through molecular cloning include identifying, localizing and characterizing genes; creating genetic maps and sequencing entire genomes; associating genes with traits and determining the molecular basis of the trait.

Animal cloning: Animal cloning has helped us rapidly incorporate improvements into livestock herds for more than two decades and has been an important tool for scientific researchers since the 1950s. Although the 1997 debut of Dolly, the cloned sheep, brought animal cloning into the public consciousness, the production of an animal clone was not a new development. Dolly was considered a scientific breakthrough not because she was a clone, but because the source of the genetic material that was used to produce Dolly was an adult cell, not an embryonic one.

Recombinant DNA technologies, in conjunction with animal cloning, are providing us with excellent animal models for studying genetic diseases, aging and cancer and, in the future, will help us discover drugs and evaluate other forms of therapy, such as gene and cell therapy. Animal cloning also provides zoo researchers with a tool for helping to save endangered species. Cloning may also be used commercially for animals.

5. Trangenesis: Gene transfer (or Tran genesis) means the stable incorporation of a gene from another species in such a way that it functions in the receiving species and is passed on from one generation to the next. In mammalian species, the transfer is mostly done by direct injection of the foreign DNA into the nucleus at the early embryonic stage. Gene transfer has been achieved in all the major livestock species and since the first success in 1985, more than 50 different transgenes have been inserted into farm animals. Because so many separate steps are involved, the success rates are often low usually one or two per cent. This imposes an enormous cost in the case of cattle; so most work has been done in mice, pigs and sheep [5]. As a broad generalization, it can be said that in farm animal species about one in ten injected and transferred embryos survives and about one in ten of these carries the transgene, or transferred genetic construct [42]. Among these transgenic animals, further wastage can be expected. Normally about half express the transgene. In those, which do show expression, the gene may be activated in unintended tissues or at abnormal times in the animal's development. This unpredictability of gene expression is perhaps not surprising, given that it is currently not possible to control the site of integration into the host genome, nor the number of copies integrated. Furthermore, transgene transmission to the next generation is sometimes abnormal. The expectation would be that 50 % of offspring would inherit the transgene. However, this is true in only 70 % of the cases in mouse studies. The most widely accepted

explanation is mosaicism, where the transgene is present only in some cells of the developing parental embryo. It is clear that a great deal more detailed research will be required to overcome these difficulties. A particularly promising approach is to develop methods of verifying that an injected embryo actually carries the transgene before it is implanted. In principle, this could increase success rates tenfold. It is difficult to see production of transgenic livestock contributing to improved efficiency of animal production until the efficiency of the process of producing transgenics is dramatically improved. Predictability of the timing and location of expression will also need to be improved. One consequence of variable expression has been to produce unacceptable side effects on the health and welfare of animals. Consumer concern from lack of convincing information on transgenics and antipathy to transgenesis is very strong in many countries and both producers and consumers would reject a technology which had negative effects on animal welfare. Furthermore, transgenesis for enhancing livestock production must compete with other technologies. Genes promoting productivity (meat, milk, wool) or reducing costs (disease resistance) are most likely to be found within the species concerned. Normal selection programs can be quite efficient in utilizing them where there measurement and recording in herds/flocks is feasible. The use of markers could add to the efficiency of the process. If a gene is sufficiently well characterized to permit its use in transgeesis, then it will also be possible to genetically characterize individuals carrying the gene and to make direct selection and propagation highly efficient.

The objective of gene transfer is to produce in the animal a protein which it does not normally produce. This can be done for two kinds of proteins. The first group would be expected to improve the normal functioning of the animal. In dairy animals, most consideration has been given to genes which modify fat or protein synthesis in the mammary gland. Transgenic animals have one or more copies of one or various foreign gene(s) incorporated in their genome or, alternatively, selected genes have been 'knocked out'. The fact that it is possible to introduce or to delete genes, offers considerable opportunities in the areas of increasing productivity, product quality and perhaps even adaptive fitness. In initial experiments, genes responsible for growth have been inserted. The technology is currently very costly and inefficient and applications in the near future seem to be limited to the production of transgenic animals as bio-reactors. What is the potential significance of these advanced technologies for developing countries and what are the technical, societal, political and ethical determinants of their application?.

The pivotal technological advances discussed above provide for the first time realistic prospects for directed genetic alteration of the livestock genome. While the key molecular and reproductive techniques require expertise, the skills are not prohibitively specialized and widespread adoption of transgenic approaches can be anticipated. In many cases, transgenesis can be expected to augment breeding programs with traditional objectives, allowing rapid and predictable introduction of genetic modifications affecting size, growth rates, food conversion efficiencies, product quality and waste management into animals of high value. Compared with introduction of genetic modifications by interbreeding, this approach would reduce the requirement for phenotypic screening.

APPLICATIONS OF MOLECULAR BIOTECHNOLOGIES IN ANIMAL IMPROVEMENT

Various molecular biotechnology applications in animal improvement are summarized in Fig. 2.

a) Applications of molecular biotechnologies in animal health: Animal diseases are a major and increasingly important factor reducing livestock productivity in developing countries. Use of DNA biotechnology in animal health may contribute significantly to improved animal disease control, thereby stimulating both food production and livestock trade.

I) Diagnostics and epidemiology: Advanced biotechnology-based diagnostic tests make it possible to identify the disease-causing agent(s) and to monitor the impact of disease control programmes, to a degree of diagnostic precision (sub-species, strain, bio-type level) not previously possible. For example, DNA analysis of Bovine Viral Diarrhoea Virus (BVDV) has been shown to be composed of two genotypes, BVDV1 and BVDV2. Only the latter was found to produce haemorrhagic and acute fatal disease and diagnostic tests to distinguish between the two are under development. Enzyme-immunoassay tests, which have the advantage of being relatively easily automated, have been developed for a wide range of parasites and microbes. Relevance and accessibility of these diagnostic tests to the livestock industry in developing countries are suggested for debate.

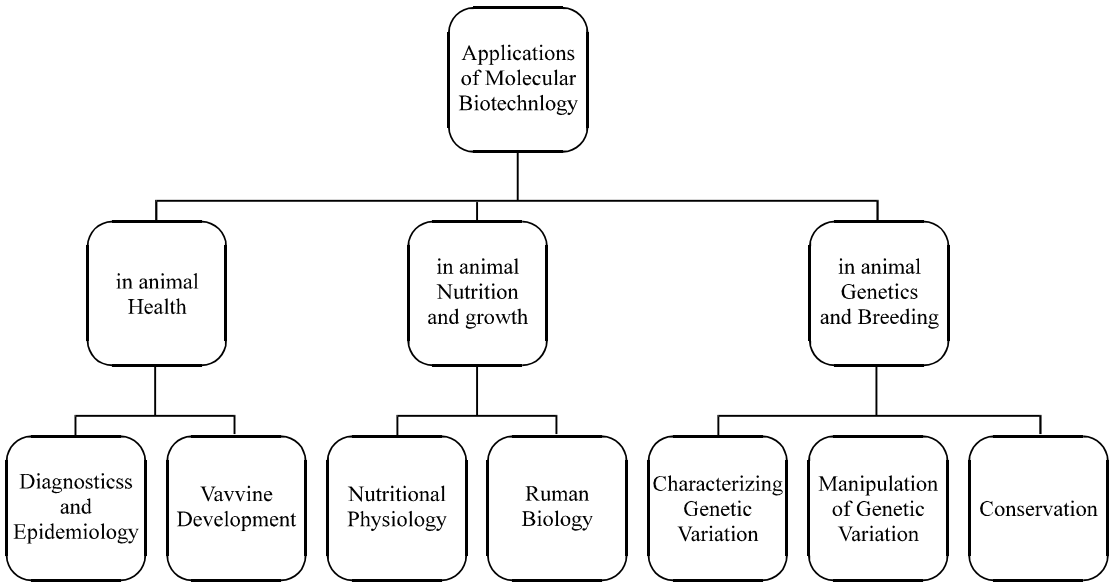

Fig. 2: Applications of molecular biotechnologies in animal health, nutrition and breeding

Molecular epidemiology is a fast growing discipline that enables characterization of pathogen isolates (virus, bacteria, parasites) by nucleotide sequencing for the tracing of their origin. This is particularly important for epidemic diseases, where the possibility of pinpointing the source of infection can significantly contribute to improved disease control. Furthermore, the development of genetic probes, which allow the detection of pathogen DNA/RNA (rather than antibodies) in livestock and the advances in accurate, pen-side diagnostic kits, considerably enhance animal health programmes.

ii) Vaccine development: Although vaccines developed using traditional approaches have had a major impact on the control of foot-and-mouth disease, rinderpest and other epidemic and endemic viral, mycoplasmal and bacterial diseases affecting livestock, recombinant vaccines offer various advantages over conventional vaccines. These are safety (no risk of reversion to virulent form, reduced potential for contamination with other pathogens, etc.) and specificity, better stability and importantly, such vaccines, coupled with the appropriate diagnostic test, allow the distinction between vaccinated and naturally infected animals. The latter characteristic is important in disease control programmes as it enables continued vaccination even when the shift from the control to the eradication stage is contemplated. Recombinant DNA technology also provides new opportunities for the development of vaccines against parasites (e.g. ticks, helminthes, etc.) where conventional approaches have failed. What is the status and potential for the use of these technologies in developing countries?.

b) Applications of molecular biotechnologies in animal nutrition and growth

i) Nutritional physiology: Applications are being developed to improve the performance of animals through better nutrition. Enzymes can improve the nutrient availability from feedstuffs, lower feed costs and reduce output of waste into the environment. Prebiotics and probiotics or immune supplements can inhibit pathogenic gut micro-organisms or make the animal more resistant to them. Administration of recombinant somatotropin results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows. Immuno-modulation can be used for enhancing the activity of endogenous anabolic hormones.

In poultry nutrition, possibilities include the use of feed enzymes, probiotics, single cell protein and antibiotic feed additives. The production of tailor-made plant products for use as feeds and free from antinutritional factors through recombinant DNA technology is also a possibility. Plant biotechnology may produce forages with improved nutritional value or incorporate vaccines or antibodies into feeds that may protect the animals against diseases.

ii) Rumen biology: Rumen biotechnology has the potential to improve the nutritive value of ruminant

feedstuffs that are fibrous, low in nitrogen and of limited nutritional value for other animal species. Biotechnology can alter the amount and availability of carbohydrate and protein in plants as well as the rate and extent of fermentation and metabolism of these nutrients in the rumen. The potential applications of biotechnology to rumen micro-organisms are many but technical difficulties limit its progress. Current limitations include: isolation and taxonomic identification of strains for inoculation and DNA recombination; isolation and characterization of candidate enzymes; level of production, localization and efficiency of secretion of the recombinant enzyme; stability of the introduced gene; fitness, survival and functional contribution of introduced new strains.

Methods for improving rumen digestion in ruminants include the use of probiotics, supplementation with chelated minerals and the transfer of rumen microorganisms from other species.

c) Applications of molecular biotechnologies in animal genetics and breeding: The eventual application of molecular genetics in breeding programs depends on developments in the following four key areas:

1) Molecular genetics: identification and mapping of genes and genetic polymorphisms
2) QTL detection: detection and estimation of associations of identified genes and genetic markers with economic traits
3) Genetic evaluation: integration of phenotypic and genotypic data in statistical methods to estimate breeding values of individual animals in a breeding population
4) Marker-assisted selection: development of breeding strategies and programs for the use of molecular genetic information in selection and mating programs.

Most animal characteristics of interest to food and agriculture are determined by the combined interaction of many genes with the environment. The genetic improvement of locally adapted breeds will be important to realizing sustainable production systems.

The molecular genetic technologies provide a major opportunity to advance sustainable animal production systems of higher productivity, through: characterizing and better understanding animal genetic variation; manipulating the variation within and between breeds to realize more rapid and better-targeted gains in breeding value and in conserving genetic material.

I) Characterizing and better understanding of animal genetic variation: The use of micro satellites in genetic distancing of breeds is gaining momentum. The increasing knowledge of mammalian genetic structure and the development of convenient ways of measuring that structure, has opened up a range of new possibilities in the areas of animal and product identification. Parentage verification by livestock breed and registry associations has for many years been based on blood typing. This is now being replaced by typing based on micro satellite characterization. Within a few years, the conversion to the DNA methodology will be complete. The advantages of the new system are substantial. Better precision in identification should be possible, because the number of independent loci typed can be increased at will. The value of any particular locus depends on the number and relative frequencies of the alleles present in the population, as well as on the ease at which it can be amplified and read in the laboratory. The International Society of Animal Genetics (ISAG) is well advanced in the standardization of panels of micro satellite loci in each of the main species. A further advantage should be reduced cost, as automated methods replace conventional gel reading and as DNA chip technology or mass spectrometry eventually makes gel based methods obsolete. Further economies are possible through the use of hair rather than blood in the provision of samples. Producers of animal products, particularly meat, face increasing and legitimate demands from consumers for the greater guarantees of the integrity of the food production chain. This includes certification of production systems as well as guarantees related to the origin of the product. In the wake of the BSE crisis in Europe, this is of particular concern to beef producers. Methods for using the new DNA technology to provide traceability in meat production have been developed.[28] The basis of the methods is the taking of a sample from the animal or carcass and its characterization by a unique DNA profile. Any product derived from that animal can then be unequivocally linked to the animal by a matching DNA analysis. Current work is concentrated on refining these techniques and reducing costs so that they can be used to provide widespread consumer guarantees of traceability of product.

ii) Increasing the speed of genetic improvement of locally adapted breeds: There are many links in the chain to realizing rapid genetic progress in the desired goals, with the objective being to rapidly transmit from selected breeding parents to offspring those alleles which

contribute to enhanced expression of the traits of interest. In developing countries, generation intervals are generally longer for all animal species of interest than in developed countries. How can DNA technologies be used to reliably realize intense and accurate selection and short generation intervals and to enable genetic improvement of these many locally adapted breeds to contribute to the required livestock development?

There is rapid progress in the preparation of sufficiently dense micro satellite linkage maps to assist in the search for genetic traits of economic importance. Can these linkage maps be used to develop strategies of MAS and marker-assisted introgression to meet developing country breeding goals? How should this be approached? Given the limited financial resources, how might work for the developing country breeding programmes strategically utilize the rapidly accumulating functional genomic information of humans, mice and drosophila?

iii) Molecular conservation: The first step in considering the sustainable management or conservation of a particular population of animals is genetic characterization. How unique is it in genetic terms? How different is it from other populations? How wide or narrow and therefore how endangered, are its internal genetic resources? In the past, these questions could only be answered in very indirect ways. The development of efficient methods of reading the molecular structure of populations has added a totally new range of instruments which can be used for the development of rational and balanced genetic management strategies.

The most widely used of these techniques is the characterization of a population at a range of micro satellite loci [28].

Why to conserve the genetic diversity of livestock?: Extinction is not a concern for the major domestic species, which benefit from society's protective custody. Large total population sizes are maintained to ensure adequate product supplies and the species' basic needs are usually met. Total population sizes are generally in the hundreds of millions, even though the effective population size may be much smaller.

The compelling need for conserving domestic species is to prevent the loss of the many differentiated populations that, because of geographic or reproductive isolation, have evolved distinct characteristics and now occupy different environmental niches.

Methods of preserving animal germplasm: Three basic approaches can be identified for preserving genetic diversity: maintaining living herds or flocks, cryo-preserving gametes or embryos and establishing genomic libraries. The vast majority of livestock genetic resources will continue to be maintained in living herds and flocks, many of which are privately owned. If the size of the breeding population is sufficiently large and the population is not decreasing in number, directed conservation efforts will not be required. Periodic inventories of animal populations can provide early warnings of any changing patterns in production or use that may alter the diversity within breeds. A major advantage of preserving live herds and flocks is the opportunity for selection, thereby allowing the breed to adapt to shifting environmental conditions. Frozen storage of gametes and embryos offers a cost-effective method to preserve the genetic material of a breed for an indefinite period of time. Collection and freezing of semen is relatively simple and, if samples are collected from a sufficient number of males, allows the preservation of essentially all the genetic variation in a stock. The costs of collection are not excessive, although in remote areas where accessibility to equipment and facilities may be problematic, they can be higher. The cost of maintaining the samples in frozen storage is low. Methods for collecting, handling and freezing embryos, although, more complex than those for semen, also offer efficient means of preservation. Embryo storage has not been used for conservation, in part because of the cost of sampling, but research on collecting and handling oocytes and embryos is advancing rapidly. Cryo-preservation can complement efforts to preserve live populations and it should be used as a safeguard when population numbers are dangerously low or when certain breeds or lines are likely to be replaced with other populations.

As noted earlier, genomic libraries are of little use for breed preservation. As technologies develop, however, they may provide an important mechanism not only for conserving diversity, but also for accessing particular genes. Their value will be enhanced with continuing advances in molecular genetics research.

POTENTIALS FOR DEVELOPING COUNTRIES

Capacity-building: There is a serious need for capacity-building, in developing countries, if new biotechnologies are to be successfully applied to improve the management of animal genetic resources, for the benefit of farmers and consumers. This capacity building needs to be at all

levels. There is a need to strengthen competence in the areas of science and technology, but also for regulatory issues and policy analysis. With the rapid changes occurring through the livestock revolution, it is equally important to address the needs of the smallholder livestock owners, particularly women, who bear ultimate responsibility for the management of most of the world's animal genetic resources and to improve their literacy, education and access to technology, services and capital [2].

Communication and public awareness: There is a need for Governments to inform the public as to the benefits and risks of new biotechnologies and their potential role in the management of animal genetic resources. In building national development strategies and the Global Strategy in general, it therefore appears crucial to address from the beginning the question of public awareness, education and information.

The role of the public and private sectors: The new biotechnologies have targeted improvement in livestock production in industrial countries and have increasingly been developed in the private sector. Such research will necessarily concentrate on species and breeds that can generate a near-term profit, to the exclusion of research on less profitable species or traits. At the same time, there has been limited public investment in animal biotechnology in most developing countries and only modest support for more conventional livestock research and development to improve productivity, nutrition and the health of farm animals. Few livestock breeding programmes exist in developing countries capable of applying molecular breeding and genomics, through marker-assisted selection and gene-assisted selection, as an aid in selection of improved livestock breeds. This situation is unlikely to change without significant investments in the public and private sectors. It should be noted that such programmes will need linkages to strong conventional animal breeding programs, since the interpretation of the genomics data requires information on observed production traits.

These factors point to the need for continued and additional public sector investments, in developing countries, in developing and applying biotechnologies in the characterization, sustainable use and conservation of animal genetic resources, where local capital is unavailable and where private sector investment is unlikely to be commercially attractive in the short and medium term. Other key public sector research targets include improved diagnostics and therapeutics, particularly vaccines against the major livestock diseases, where information coming from the study of pathogen genomes can help develop more effective disease control. Governments need to consider how best to support private-public sector collaborations in animal biotechnology research [2].

Regulatory systems and food safety: A key role for governments is to ensure that an open, transparent and effective regulatory system in place, that permits the harmonious development of animal production, particularly in the light of the on-going livestock revolution, so as to maximize production while minimizing ecological risks.

Intellectual property management: The use of new and frequently proprietary, biotechnologies in the management of animal genetic resources will require developing countries more systematically to consider their relevant intellectual property policies and legislation, in order to provide enabling environments for the conservation and utilization of animal genetic resources and for associated public and private sector research and product development. This may be an important issue for member Governments of the WTO, in the review of the TRIPS Agreement. The following issues will be of importance for the future use of new biotechnologies in the management of animal genetic resources:

1) harmonization of IP regimes;
2) access by the public sector and by the emerging private sector in developing countries to the enabling technologies required for biotechnological research and development;
3) the nature of research exemption under patent regimes, especially for public goods orientated research;
4) the possible use of licensing and/or patent exemptions, for example, to develop generic veterinary products [2].

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