Genomics Research: Prospects for Improving Livestock Productivity

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Following the "Green Revolution" that increased cereal production in some developing countries, a more holistic "doubly green revolution" has been prescribed for agriculture in the 21st Century if the global community is to both sustain the growing human population and its demand for food in a manner that conserves natural resources (Conway, 1997).

An important component of the approaches to improving productivity in the agricultural sector involves the use of biotechnology. The tools of molecular biology already contribute to the characterization of animal, plant, and microbial genetic resources. Molecular techniques have been employed to isolate genes for the diagnosis of disease and its prevention by development of safe and efficacious subunit vaccines and to capture desirable production traits in plants and animals. However, genomics research is undergoing a revolution of its own, where the emphasis is shifting from a study of single genes to a systems approach involving a study of all the genes or groups of genes that occur within an organism. This has become possible due to development of large-scale DNA sequencing strategies together with computer infrastructure and software capacity to manage the process and analyze the data produced. Bioinformatics research in combination with microarray or "DNA chip" technologies and other high throughput screening strategies herald new approaches to analyzing biological systems in the context of whole genome sequences (McKusick 1997).

The discipline of genomics offers fresh perspectives on research problems and the private sector has positioned itself to take advantage of these developments, as the return on investment is high. The international agricultural research centers of the Consultative Group on International Agricultural Research (CGIAR) must do likewise or the gap in science addressing the needs of the poor in developing countries will grow wider. In this review we highlight four project activities where the tools of biotechnology are being used at ILRI, and outline major developments occurring in genomics as these will impact on future research activities. Research partnerships are crucial for accessing some of the genomics technologies, and ILRI has formed one such linkage to address a constraint to vaccine development against a lethal disease of cattle that occurs in sub-Saharan Africa.

Constraints to Livestock Productivity in Developing Countries

There is evidence for a rapidly increasing demand for livestock products in developing countries as a result of population and income growth and urbanization leading to a shift of dietary preference away from cereal-based foods and "the next food revolution" (Delgado and others 1999). Milk and meat consumption has grown by about 3 and 5 percent per year, respectively, and is expected to increase even more by 2020. Thus, the increase in livestock production is being demand-driven although it is not evenly spread among develop-

ing countries. Livestock agriculture usually accounts for 25-30 percent of the agricultural GDP of developing countries and is thus an important component in their economies.

Most smallholder farming systems, the priority target group of the CGIAR, rear animals in a mixed crop-livestock system and livestock play an integral role in the lives and livelihood of these resource-poor farmers. Meat and milk are high-calorie foods that also provide micronutrients and are essential for improvement and maintenance of human health. Livestock are an important source of draft power and traction, activities that would otherwise be performed primarily by women and children. Livestock are able to convert otherwise indigestible crop residues into food that is fit for human consumption, and in a synergistic relationship livestock manure plays an important role in nutrient recycling that helps to sustain crop production. Manure can also provide cheap and affordable domestic fuel in certain circumstances. The sale of farm products, such as milk, can provide a daily income for the rural poor and this usually benefits women who tend to be the managers of smallholder systems. In addition, because of their high value, livestock contribute to asset building and constitute a form of social security.

There are differing constraints to increasing livestock productivity in developing countries depending on prevailing agroecological conditions. Perhaps most importantly, increase in livestock performance and productivity can be gained by changes in diet and feeding practice, from improvements in farm management strategies and disease control. Livestock with relevant productivity and disease resistance traits can also contribute to maximizing the efficiency of animal agriculture. ILRI recently re-examined the critical issues affecting livestock productivity and identified seven key areas under which most research relating to productivity enhancement and sustainability would fall:

- Improvement of livestock feeds and nutrition
- Management of natural resources as it relates to the livestock sector
- Improvement of animal health
- Characterization and utilization of livestock genetic potential
- Livestock policy analysis
- Systems analysis and impact assessment
- Strengthening livestock research capacity of the national agricultural research systems (NARS) of developing countries.

Biotechnology Research at ILRI

The tools of molecular biology are being used in five of the seven key researchable areas outlined above. Thus, there is clearly much scope for the application of biotechnology in alleviating constraints to livestock productivity. Four activities that will benefit from the developing science of genomics are outlined below.

Selection of Dual-Purpose Crops for Improved Yield

Conventional crop breeding programs tend to concentrate on selecting varieties that have high grain yield for human consumption, with less value placed on crop residues, such as leaves and stems, for animal feed. Crop residues, also called stover, tend to have poor nutritional value and efforts have been made to improve this by chemical and biological means. However, there has been little adoption of these techniques by smallholder farmers for a variety of social and economic reasons. An alternative, more environmentally friendly and practical strategy would be to increase the nutritive value of crop residues through genetic enhancement.

A collaborative project between the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), ILRI, and NARS in India aims to identify improved dual-purpose crop varieties of sorghum and millet. Crop residues play a major role in animal feed in the smallholder mixed crop-livestock farming systems in India's semi-arid tropics. Improved digestibility of crop residues as feed for ruminants will result in an increased conversion of crop material into valuable animal products such as meat, milk, manure and animal draft power. A feed simulation model estimates that as little as 1 percent increase in digestibility of crop residues would result in a 6-8 percent increase in animal products and traction capacity (Kristjanson and Zerbini 1999).

The ongoing research ranges from participatory rural appraisals of crop varieties at the farm level (Chambers 1990) to the identification of quantitative trait loci (QTLs) for residue quantity and quality (Hash and Breese 1999; Hash and

Witcombe 1994). The project addresses four major research issues:

- farmer perceptions of quality and productivity traits in crop residues
- relative importance of genetic (G) and environmental (E) variation and G x E interactions in nutritive value
- opportunities for indirect selection for stover quality in selected genotypes, based on observable morphological or agronomic characters
- application of existing and novel DNA markers to identify QTLs that contribute significantly to the observed genetic variation in digestibility traits.

Identifying the desirable heritable traits for improved stover quality and defining the existing genotypes that carry these characters will enable a final selection to be made in multilocational trials on station and on farm. These selected genotypes will then be available for the future development of new dual-purpose cultivars by conventional and marker assisted breeding techniques and ultimately through biotechnology.

Improvement of Rumen Fermentation

Molecular techniques have great potential for enhancing rumen function by allowing the introduction of new or improved fermentation activities thereby improving the utilization of poor quality feeds or expanding forage resources. Current rumen microbiology research at ILRI focuses on two major areas: detoxification of plant toxins which constitute anti-nutritional factors; and enhancement of the rate of degradation of fiber to improve the utilization of poor quality feeds.

The rumen microbes of wild ruminants are of particular interest because they survive in areas where feeds contain high concentrations of fiber and factors that are toxic for domesticated ruminants. For example, *Bison bison* have a superior ability to digest low quality forages when compared to *Bos taurus* (Varel and Dehority 1989).

Alteration of rumen function could occur via genetic manipulation of rumen organisms or by using defined microbes to supplement the rumen flora. For example, the transfer and establishment of an exotic rumen microbe in the rumen of naive animals has been described, allowing previously susceptible livestock to successfully utilize toxic *Leucaena leucocephala* as feed (Jones and Megarrity 1986). A gene from the soil bacterium *Moraxella* that allows detoxification of the plant toxin fluoroacetate has been successfully expressed in the rumen bacterium *Butyrivibrio fibrisolvens* (Gregg and others 1994) and shown to protect sheep from fluoroacetate poisoning (Gregg and others 1998). Rumen cellulolytic microbial genes have also been transferred to several noncellulolytic rumen bacteria, but it has not always been possible to alter the phenotype of genetically altered bacteria (Cheng and others 1992), indicating that there is much to be learned.

Little is known about the diversity of rumen microbes. Thus, application of molecular techniques including denaturing gradient gel electrophoresis (Muyzer and Smalla 1998), competitive PCR (Reilly and Attwood 1998), group specific hybridization probes (Zhang and others 1997) and shotgun sequence analysis of 16S ribosomal RNA genes, to characterize rumen microbial communities, will contribute much to an understanding of the ecology of rumen organisms. By linking these data with animal diets and in vitro analyses it may be possible to define key microbes and their role in the detoxification of phytotoxins and fiber degradation. Such information should lay the foundations for the manipulation of specific organisms for the benefit of domesticated ruminants.

Livestock Genetic Resources and Genetics of Disease Resistance

Over the centuries, livestock farming under different environmental conditions has resulted in breeds with traits such as heat tolerance and disease resistance, which favor their survival under these stresses. Farmers have also been breeding for a variety of attributes with a major focus on productivity traits such as increased milk and meat yields. As a result of such selective forces there exists a variety of breeds with different potential to benefit farming systems in different environments, the relative importance of one trait over another being dictated by farming conditions.

Livestock genetics research at ILRI focuses primarily on cattle, sheep, and goats, and is divided into two major activities: characterization, conservation, and use of tropical indigenous

animal genetic resources; and genetics of disease resistance.

Basic breed information and indigenous knowledge of animal husbandry is being collated to contribute to breed biodiversity information in a database, and to help guide decisions on use of breeds. Molecular characterization is being used to analyze the population genetics of African ruminant livestock. This study, the first, covering the whole of sub-Saharan Africa, has provided a basis for a comprehensive examination of the origin and classification of African cattle, and has identified a possible new center of cattle domestication in Africa (Bradley and others 1996; Hanotte and others 1999). The second area of research is currently directed at the development of DNA markers to identify the QTLs or genes that bestow disease resistance to trypanosomosis in cattle and helminthosis in sheep.

Trypanosomosis is the most important livestock disease in Africa, constituting a major constraint to livestock production, with annual losses estimated at US$1340 million without including indirect losses such as manure and traction (Kristjanson and others 1999). Conventional methods of control, such as vaccination, chemotherapy, and vector control are unavailable, expensive or difficult to sustain. The N'Dama breed of cattle, native to the tsetse-infested areas, is known to be tolerant to infection with *Trypanosoma congolense* (d'Ieteren and others 1998). Research focuses on this breed, and central to this project was the establishment of an F2 population of cattle in which the tolerance trait is segregating. Correlation of animal genotype with phenotype has lead to the identification of five chromosomal regions controlling resistance to trypanosomosis (Hanotte, O. and others, personal communication).

Helminthosis, or gastrointestinal worm infection, constitutes one of the most important animal health constraints to sheep and goat production in both tropical and temperate regions of the world (Gill and LeJambre 1996). Current control methods in industrial countries focus on anthelmintic treatment or controlled grazing. In the tropics, these control methods are limited by the high cost of anthelmintics, their uncertain availability, increasing frequency of drug resistance and limited scope for controlled grazing. There is evidence for genetic resistance among certain indigenous tropical small ruminant breeds, for example, the East African Red Maasai sheep (Baker and others 1998). Similar breeding programs and activities to that described above for trypanotolerance in cattle are under way to identify genetic markers linked to QTLs controlling resistance to helminthosis in sheep.

The ruminant research is being supported by sophisticated mouse studies where genetically resistant and susceptible strains and advanced inter-cross lines are being used as surrogate models for trypanosomosis and helminthosis research (Kemp and others 1997). Using comparative rodent and ruminant genomics, it is expected that the time to discovery of important genes that are relevant in ruminants will be considerably shortened. The products of this research will be molecular probes as markers for disease resistance. These could be used for more efficient selection in conventional breeding programs for improved performance in extant indigenous livestock breeds endowed with innate disease resistance. Alternatively marker-assisted introgression approaches could be used for development of new, productive livestock types through efficiently combining disease resistance genes with genes for enhanced productivity that already exist in many breeds in areas where the diseases of concern do not occur. By understanding the molecular basis of resistance to these diseases it may also be possible to develop novel alternative disease control strategies.

Animal Health Improvement by Vaccine Development

Vaccination offers one of the most effective and sustainable methods of disease control (Kurstak 1999; McKeever and Morrison 1998; Morrison 1999). The considerable potential of vaccine for effective disease control can be gauged by the eradication of smallpox and the global vaccines programs of the World Health Organiation. Veterinary vaccines against a number of livestock and poultry diseases have already played a critical role in increasing livestock productivity under disease challenge (Mowat and Rweyemamu 1997). ILRI's vaccine research program concentrates on two major diseases that affect ruminant livestock in Africa: trypanosomosis caused by

Trypanosoma congolense and *T. vivax*; and East Coast fever caused by *Theileria parva*.

Vaccine development against an infectious organism is more likely to succeed when there is clear evidence of acquired immunity to infection. It is then possible to define immune responses that contribute to immunity and to use screening systems based on this knowledge to identify pathogen molecules that are the targets of protective immune responses, and to incorporate these into experimental vaccines. Another effective method of disease control involves reducing the pathological effects of infection rather than parasite burden itself (Playfair, Taverne, and Bate 1991). This approach holds promise in trypanosomosis vaccine research where study of the pathogen and host-parasite interaction has so far not revealed any obvious clues for vaccine development against the organism itself. As will be described below, genomics research offers new opportunities in combating infection and disease by understanding the biology of pathogens and their hosts in greater detail.

Developments in Genomics Research

Genomics is setting new paradigms in research approaches within biological sciences, and will be a major force in enhancing the rate of progress in understanding biological systems and exploiting them for development of products. The rapid rates of progress in this field are based on high throughput technologies in the area of structural and functional genomics (McKusick 1997). Data derived from such research have the potential to significantly decrease the time frame for problem solving and to initiate novel research activities. The purpose of this section is to make the reader aware of the changes taking place in genomics. It is not intended to be an exhaustive list of the full range of methods that have been developed or those that are being developed as a consequence of the huge increase in genome sequence data. What is pertinent is that these technologies allow novel approaches to address biological problems, and because some of them currently require very specialized resources and expertise, the only way to access them is through research partnership.

A series of new platform technologies have been developed that have resulted in rapid advances in three areas that are interlinked. When taken together the area of genomics has become an incredible growth industry during 1995-99, and it is widely believed that these research areas and the immense amount of new data that they generate will fundamentally change approaches to asking and answering questions in biology. What are the changes that have occurred and what are the consequences?

First, developments in DNA sequencing have made the acquisition of whole genome sequences a reality and it is now almost routine to sequence microbial genomes. Such data, when interpreted using bioinformatics gives a complete listing of all the genes present in an organism, the genetic "blueprint" of an organism. The first genome sequence of an organism more complex than a virus was published in 1995 (Fleischmann and others 1995). Twenty-three genome sequences are now available in a public database held at the National Center for Biotechnology Information, and numerous genome sequencing projects of a wide variety of organisms, including plants and mammals, are under way

Second, a number of different types of technologies have been developed for genome analysis allowing rapid genotyping and genome expression studies using microarray technology (Lander 1999). What puts this technology into a different league is that with the growing list of whole genome sequence data available, it will be possible to scan the genomes of different organisms rapidly and to develop a systematic approach for mapping genetic traits (Brown and Botstein 1999; Chakravarti 1999).

Third, developments in computational biology or bioinformatics, which were essential in underpinning the advances in DNA sequencing and genome analysis, will increasingly allow the prediction of gene function from gene sequence (Burks 1999). Although there are currently considerable gaps in this knowledge base, it is nevertheless possible to build a theoretical framework of the biology of an organism from the listing of its genes. This forms a very powerful base for hypothesis-driven experimentation. In addition, by comparing physical and genetic maps across different organisms it is possible to significantly reduce the time frame for the identification of important genes (Bevan and Murphy 1999). The genome sequences of several microbes

are already available and soon the annotated genomes sequence of a plant (*Arabidopsis thaliania*), the fruit fly (*Drosophila melanogaster*), mouse, and humans will become available. These resources will define a new era in comparative genomics research and the biological sciences.

Relevance of *T. parva* Genome Sequence for ECF

East Coast fever (ECF) is a usually fatal disease of cattle, and approximately 24 million cattle in 11 countries in eastern, central and southern Africa are at risk (Norval, Perry, and Young 1992). ECF is characterized as a lymphoproliferative disorder and is caused by an intracellular protozoan that induces a reversible cancer-like phenotype of parasite infected white blood cells (Irvin and others 1975; ole-MoiYoi 1989). The levels of morbidity and mortality particularly in improved exotic cattle breeds are extremely high. Estimates of annual economic losses of US$168 million establish that effective and sustained control of the tick-transmitted causative agent of ECF, *Theileria parva*, would have a high impact (Mukhebi, Perry, and Kruska 1992). Conservative *ex ante* impact analysis indicates that investment in research to develop improved vaccines against ECF has a potential cost-benefit ratio of 15:1 (Kristjanson 1997). The current methods of disease control include use of acaricides to prevent tick infestation and live vaccines that rely on infection with potentially lethal parasites, followed by treatment (Radley 1981). The disadvantage of acaricide use includes cost and development of tick resistance to the treatment. Additional problems, with broader implications, include pollution of the environment and toxic residues in animal products. The disadvantages of live vaccines include strain-specific immunity, high cost, requirement for drug treatment (oxytetracycline), and a possibility of causing severe disease due to incorrect vaccine administration and requirement for a cold chain to deliver the vaccine.

It has been demonstrated that antibodies against surface components of sporozoites, the infective stage of *T. parva*, introduced into the mammalian host by feeding ticks, will inhibit their capacity to gain entry into host cells to establish infection (Musoke and others 1982). By analyzing the mechanism of immunity engendered by infection and treatment it has been demonstrated that a subset of T cells called cytotoxic T lymphocytes (CTLs) play a major role in the clearance of pathogenic, schizont-infected cells (Morrison and others 1987). Thus, antigens from two lifecycle stages of the parasite that are the targets of protective immune responses, are desirable components of a vaccine against ECF. ILRI is currently evaluating a recombinant form of p67 (Musoke, Nene, and Morizaria 1993), the major surface antigen of sporozoites, as an antisporozoite vaccine because, under laboratory conditions, p67 routinely induces immunity in about 70 percent of immunized cattle. This molecule is a promising vaccine antigen and is undergoing development to improve protective efficacy. The search for schizont vaccine antigens is complicated by the cell biology of antigen processing and CTL recognition of parasite infected cells. The specificity of CTLs is likely to be determined by interaction of a receptor on the T cell with a peptide 8 to 11 amino acid residues long that is associated with host MHC class I molecules (Collins and Frelinger 1998). Consideration of molecular mechanisms in cell biology indicates that schizont molecules must gain access to the host cell cytoplasm, and from there into the host cell MHC class I antigen processing pathway. Conventional methods to identify such peptide antigens are technically demanding, and genomics research offers an alternative approach to vaccine antigen identification.

As described previously, from the genome sequence of an organism it is possible to predict all the genes encoded within it. Schizont molecules that access the host cell cytoplasm are likely to be either secreted or shed from the cell surface. Again cell biology dictates that these types of parasite molecules will be processed by the classical secretory pathway and will contain N-terminal peptides with conserved features that can be identified from gene sequences in a high percentage of cases (Nielsen and others 1997). Thus from the complete listing of *T. parva* genes it will be possible to identify a subset of genes that contain most if not all candidate vaccine antigens, thereby overcoming the current constraint in identifying antigens recognized by cytotoxic T cells. This set of parasite genes would have to

undergo further screening to identify which ones are suitable for cattle experiments (see Nene and others 1999 for more details).

The genome sequence would also underpin other research on the parasite. It will be possible to build a hypothetical framework if the biology of the parasite and, for example, its biochemical capacity. Very little is known about the latter, and much could be inferred from the complement of parasite genes. From such information it may be possible to define novel drug targets, an approach that has already stimulated new research in chemotherapy of malaria and toxoplasmosis (Waller and others 1998; Jomaa and others 1999). It may also be possible to gain insight at the molecular level of host-parasite interaction that ultimately results in ECF. A unique aspect of the schizont, referred to earlier, is that it causes infected cells to behave like cancer cells. The schizont induces host cells to proliferate and it divides in synchrony with the host cell resulting in huge increases in schizont parasitemia (Carrington and others 1995). By understanding this phenomenon and the molecules that mediate the process, new methods of disease intervention may be developed. This research potentially has implications for human medicine, particularly leukemia research. The genome sequence would allow valuable comparative analysis of related pathogens that cause disease in livestock (*T. annulata*, *Babesia*, and *Eimeria*) or which cause debilitating diseases in humans (*Plasmodium* and *Toxoplasma*).

Partnership in Sequencing *T. parva*

To determine the genome sequence of *T. parva*, ILRI has formed a collaborative partnership with the Institute for Genomic Research (TIGR), a private but not-for-profit research institute based in the United States. TIGR is a world leader in the large-scale acquisition of DNA sequences, and pioneered the "shotgun" DNA sequencing approaches that are now being used to assemble whole genome sequences. Although this process has been used primarily with microbial genomes and purified eukaryotic chromosomes, it is believed that it can be extended to assemble the genome sequence of complex eukaryotes (Venter, Smith, and Hood 1996). TIGR also has considerable expertise in bioinformatics and microarray technology, and both will be essential in analyzing and prioritizing parasite genes for further study.

The genome sequencing project will build on the considerable data already acquired (Nene and others 1999), and has two additional collaborating institutes, which like ILRI wish to make use of the *T. parva* genome sequence data. The first is the Institute of Molecular and Cell Biology-Africa (IMCB-A), which has been recently established in Nairobi under the auspices of UNESCO. IMCB-A has a particular interest in the human-veterinary interface that *T. parva* research offers as a model system for vaccine and cancer research. The second is the Department of Infectious Diseases at the University of Hokkaido, Japan, which plans to use the *T. parva* data in comparative analysis with a related *Theileria* parasite, *T. sergentii,* that infects cattle in Southeast Asia and Japan (Uilenberg 1981).

Constraints to Delivery

Although genomics research offers new avenues leading to potential solutions of constraints to livestock agriculture, it must be recognized that the commercial implication of products resulting from such research means that intellectual property rights (IPR) must be exercised. This might seem contrary to the philosophy of the CGIAR that products developed by the CGIAR are for the public good, and to the funding sources that pledge public funds for research. It is envisaged that biotechnology product manufacture and marketing would occur through the private sector, and it is not likely that a commercial company would undertake such an activity in the absence of a framework that legally protects its investment. Thus, patents and other forms of IPR are necessary and can be used to ensure that products reach the intended client and at reasonable cost.

The issue of IPRs resulting from the genome sequence of *T. parva* has been resolved, because TIGR as a not-for-profit institute is aware of the public good that would accrue from this research. However, many problems face the commercialization of experimental vaccines. Because of the nature of the product being developed, for example, it often occupies a niche market for poor

farmers in developing countries. This is not a lucrative market for the commercial sector in the industrial countries, economic principles making it difficult to justify incurring the research and development costs. A number of the technologies in vaccine development are held by the private sector, and additional constraints may arise in a lack of freedom to operate. Licensing requirements of third party intellectual property could result in increased costs or even prohibit product development. Such constraints in the discovery to delivery pathway require novel solutions or the benefits of science will not be realized.

Conclusion

The discipline of genomics will accelerate the acquisition of fundamental knowledge about biological systems. The outputs of genomics research will change our approach to solving biological problems, and result in novel uses of biotechnology to develop and improve products for crop and livestock agriculture. The CGIAR must position itself, as has the commercial sector, to exploit the rapid advances being made and to adopt genomic technologies. This will support the strategic role of the CGIAR in global agricultural partnerships, and strengthen its ability to contribute to the principles of poverty eradication, food security, and protection of the environment.

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