BOVINE GENOME

MAPPING AND TRYPANOTOLERANCE

PROCEEDINGS OF A WORKSHOP HELD AT ILRAD

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The International Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 with a mandate to develop effective control measures for livestock diseases that seriously limit world food production. ILRAD's research program focuses on African animal trypanosomiasis and East Coast fever, a form of theileriosis.

ILRAD is one of 17 centres in a worldwide agricultural research network sponsored by the Consultative Group on International Agricultural Research. In 1991 ILRAD received funding from the African Development Bank, the European Economic Community, the Rockefeller Foundation, the United Nations Development Programme, the World Bank and the governments of Australia, Belgium, Canada, Denmark, Finland, France, Germany, India, Italy, Japan, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom and the United States of America.

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Introduction

A Workshop entitled *Bovine Gene Mapping: Application in Research Directed to Understanding and Utilization of Trypanotolerance in Cattle* took place at the International Laboratory for Research on Animal Diseases in Nairobi on 9–11 April, 1991.

The Workshop was held as a result of recognition at ILRAD of two significant facts. First, trypanotolerance in certain livestock types offers a potentially important means of controlling the effects of trypanosomiasis, particularly as a component of integrated control systems, and, second, research directed towards development of a linkage map of the bovine genome is gathering pace in several laboratories worldwide.

The identification of markers of genes controlling trypanotolerance would considerably facilitate the exploitation of the trait. A set of highly polymorphic and mapped markers distributed across the genome is clearly an important step towards this goal. The Workshop was therefore held to bring together researchers in the field of trypanosomiasis and trypanotolerance and researchers in genome mapping in cattle.

The Workshop attempted to perform two functions. First, to act as a forum for alerting the bovine gene mapping community to trypanotolerance as an important target trait for linkage map application and to update the gene mappers on trypanotolerance research and resource populations segregating the trait. Second, to inform the Laboratory of the status of bovine gene mapping worldwide with a view to facilitating strategic planning of trypanotolerance gene mapping.

Herein, following an introduction to the subject of the Workshop, are collected the reports of the gene mapping laboratories and of those laboratories and groups concerned directly with trypanotolerance research. Also presented here is a summary of the final roundtable discussion, and the Workshop's recommendations concerning genome mapping development and the production and use of trypanotolerance resource material.

Workshop objectives

- Inform participants of the activities of all the groups represented and their plans for future research in the area of gene mapping in cattle.
- Discuss resource sharing between laboratories working towards a linkage map in cattle and make arrangements for the extension of existing collaborations.
- Give participants the opportunity to learn about livestock trypanosomiasis and trypanotolerance.
- Give participants an update on the program at ILRAD for the generation and phenotyping of a trypanotolerance resource population.
- Determine the level of interest in the various laboratories in the ILRAD resource families.
- Discuss the practicalities of developing an International Bovine Trypanotolerance Mapping Group.
- Obtain an indication of likely commitment, on the part of various laboratories, to bovine map development and to the trypanotolerance mapping experiment at ILRAD.
- Obtain critical comment on the design of the trypanotolerance resource population being generated at ILRAD.

Mapping and trypanotolerance

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Trypanosomiasis is unquestionably a major constraint on livestock production in many parts of the world and most especially in sub-Saharan Africa where those parasites transmitted by tsetse flies are prevalent. It has been estimated that an area of 50 million km^2 of the African continent is affected by livestock trypanosomiasis and that 150 million cattle are at risk in the 37 countries infested by the vector.

There are no effective vaccines against trypanosomiasis and, indeed, natural infection generally fails to result in protective immunity. In such a situation, control has hitherto relied upon the use of a limited number of trypanocidal drugs and various methods of reducing vector densities, such as trapping of flies, spraying of insecticides etc. This situation is unsatisfactory for a number of reasons. Drugs are expensive, sometimes difficult to obtain, and, most importantly, the parasites they are intended to control often respond by developing resistance. Vector control can be costly and laborious, and may involve negative environmental impacts, especially when insecticide application and bush clearance are involved. The advent of the newer, simple insecticide-impregnated `targets' has represented a significant advance in that, with relatively modest outlays, large reductions in vector density are possible. However, the requirement to maintain targets is a continuing one because of the constant threat of reinvasion in `cleared' areas.

ILRAD is currently mandated to work towards improved control of trypanosomiasis. The research program is driven by prospects of advances in four main areas. These are the areas of diagnosis and epidemiology, vaccination, chemotherapy and natural host resistance.

Studies of the basic biology of trypanosomes are being undertaken with a view to identification of parasite components which could be made the targets of immune or chemotherapeutic attack. The processes of disease are being studied with the prospect that if they can be understood, interventions can be designed, whether drugs or vaccines, to limit pathogenesis even in the face of continuing parasite infection. Finally, the mechanisms by which a minority of cattle in the tsetse-infested areas of West Africa are able to withstand levels of challenge which prove fatal to other cattle types are under study. This natural resistance to infection and its effects, which is widely known as trypanotolerance, is a feature of both West African longhorn and shorthorn *Bos taurus* types, represented by the N'Dama and Baoule breeds respectively.

Important features of trypanotolerance are:

- it involves a significant degree of genetic control;
- it is probably highly heritable;
- it is probably controlled by two or more genes;
- it is most obviously characterized by improved parasite control, the ability to maintain normal blood parameters, and the ability to remain productive in the face of challenge levels which prove fatal to susceptible cattle types;

- it is not dependent on previous challenge; and
- it is not parasite specific.

As a means of control of trypanosomiasis, the breeding and dissemination of trypano-tolerant cattle has many attractions. It would reduce reliance on chemotherapeutic and insecticidal agents with consequent reduction in the negative environmental effects of widely practised current control methods. Most importantly, perhaps, the farming of genetically-resistant livestock is a robust means of disease control because, once established in a system, the maintenance of such livestock is largely independent of continued sophisticated and expensive inputs. Realistically, however, trypanotolerance is likely to make maximum impact as part of an integrated package of control measures in which minimal, and possibly strategic, use of trypanocides is practised and in which some degree of vector control can be achieved. With genetic resistance established in the livestock population, the most serious shortcomings of other control methods can be avoided; these being the development of drug resistance in the parasites concerned and the difficulties involved in totally removing the tsetse fly from its habitat.

The mechanisms of trypanotolerance are not known and two approaches are being adopted to achieve an understanding of the phenomenon. First, parameters of immune response and pathogenesis are being compared in experimental challenge situations in N'Dama and Boran cattle. Such an approach may reveal where significant differences lie between these extreme cattle types. Hitherto, this has met with a degree of success and it appears that there are differences in the generation of both antibody and cell-mediated immune responses and in the generation of cytokines between the breeds. Work continues on the basic mechanisms underlying these differences.

The second approach to trypanotolerance was suggested by a developing interest in a number of laboratories around the world in generating a linkage map of the bovine genome. The possibilities for the application of a putative map, with respect to providing genetic markers of the trypanotolerance trait, were first raised by Soller and Beckmann several years ago.

Subsequently there have been two major developments which can be expected to contribute greatly to the success of a `trypanotolerance mapping experiment'. First, new types of marker loci have been discovered offering great advantages over RFLPs and even over the minisatellite fingerprinting loci. These new microsatellite markers are relatively easy to identify and apply. The second development has been the initiation of crossbreeding programs at ITC and ILRAD directed towards the generation of N'Dama $\rangle$Zebu cattle in which the segregation of trypanotolerance can be expected to occur, and in which coinheritance of the trait and marker loci can be studied.

The ultimate objectives of the `marker approach' to trypanotolerance are:

- provision of markers for marker-assisted selection.
- provision of markers to guide initial steps in the identification of genes controlling the trait.

With trypanotolerance genes identified, it will become possible to consider transgenic approaches to cattle design which would incorporate resistance to trypanosomiasis. The identification of the genes will also contribute to an understanding of how resistance to an important infectious disease, caused by a parasite capable of evading immune response, operates.

It is important to realize that the benefits of such knowledge may not be restricted to the area of bovine trypanosomiasis. First, it is quite possible that similar genes and

mechanisms controlling trypanotolerance operate in small ruminants. With the degree of conservation which is increasingly apparent at the genome level between small ruminants and cattle, knowledge obtained in N'Dama animals may be applicable to, and readily applied in, other trypanotolerant breeds and species. Further, the basic mechanisms of trypanotolerance may operate in control of other infections in both ruminants and non-ruminants.

At the present time, several laboratories are beginning to develop microsatellite markers and have initiated limited collaborations in order to speed their mapping and thus facilitate the generation of a useful linkage map of the bovine genome. It is generally accepted that between 150 and 300 marker loci, with relatively even distribution across the genome, is a reasonable target for a primary linkage map. The number of mapped genes in cattle currently places the species behind only man, mouse and rat in the mapping table of species, and well ahead of other ruminants. However, a universally accepted collaboration based on agreed reference material for linkage map development has not been achieved to date. Such collaboration in the placement of new loci on a single map, similar to that adopted for map development in man, would greatly enhance progress.

With an N'Dama-Boran crossbreeding program now well established at ILRAD, and with the initial responses to trypanosome challenge of the first F1 N'Dama × Boran cattle now becoming available, the convening of a Workshop on the bovine genome and application of genetic maps to the trypanotolerance question is timely. A linkage map offers the possibility to identify trypanotolerance genes and thus contribute greatly to the broader problem of trypanosomiasis control. Conversely, the opportunity to contribute to an understanding of trypanotolerance could serve as a stimulus to international collaboration on the bovine genome.

LABORATORY REPORTS

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It was at the Human Genetics Congress in Chicago in 1966 that I first encountered a serious discussion of the use of genetic markers for mapping of quantitative trait loci (QTL). This was at a plenary session where Thoday reported on his work in mapping polygenes in Drosophila, and suggested that similar methods could be used for man. His methods seemed to require the use of specially constructed tester chromosomes, however, and, if anything, implied that application to agricultural species would be complex.

In 1972, I came across a paper by Spickett and Thoday (1966) describing a QTL mapping study in Drosophila in which a simple experiment based on a single marker per chromosome gave results essentially similar to those obtained using a much more complex genetic system. This sort of experiment seemed to be one which could be implemented in agricultural species. As a result I began to consider just what the design and power of such experiments could be and how the information obtained could be used in genetic improvement programs. Exploration and development of these themes has since become the major research activity in my laboratory, and has included four components.

- Development of statistical designs and analyses for mapping of loci (QTL) affecting polygenic quantitative traits of economic or biological importance in plant and animal populations.
- Development and evaluation of marker-assisted breeding methods.
- Development of DNA level genetic markers for use in genetic analysis and marker-assisted genetic improvement.
- Experimental studies aimed at mapping of QTL affecting traits in specific populations.

I now present a brief review of our research in these areas, with particular reference to bovine populations, where applicable.

STATISTICAL DESIGNS AND ANALYSES FOR QTL MAPPING

The simplest system for analysis, and the one which we first considered in order to get some initial notion of the size of experiment that might be required for marker-QTL linkage studies, is a cross between inbred lines homozygous for alternative alleles at both marker and QTL loci (Soller *et al.,* 1976). It turned out that experiments of manageable size (from a few hundred to 2000) backcross (BC) or F2 individuals would have high power for detection of linkage between QTL and marker loci. Power would drop rapidly, however, with increased proportion of recombination between marker and QTL, and with lack of fixation for alternative marker and QTL alleles in the two lines crossed.

Since animal populations are generally not at fixation for alternative alleles at either marker loci or QTL, it seemed clear that experiments based on crossing populations would not be a generally effective means of mapping such species. Instead we explored the power of an alternative design, based on within-sire analyses, in which the marker effect was expressed as a marker component of variance in a hierarchical analysis of variance, with markers nested within sires (Soller and Genizi, 1978). Power, in this case, turned out to be markedly less than for a cross between inbred lines. In part this was due to the fact that for diallelic markers, many sires and offspring were uninformative with respect to marker-QTL linkage.

Our involvement in the trypanotolerance mapping program led us to yet a third situation. Initially we had hoped that the N'Dama × Zebu cross would turn out to be similar to a cross between inbred lines, i.e., at or close to fixation for alternative alleles at loci affecting trypanotolerance, and also at a large number of marker loci. Although it still seems plausible that the two breeds are reasonably close to fixation for alternative alleles at the loci conferring trypanotolerance, for all markers that we have examined, the same alleles appear to be present in both populations. This led us to consider appropriate modes of analysis for a cross between interbreeding populations which are at fixation for alternative alleles at the QTL, but overlap with respect to marker alleles (Beckmann and Soller, 1988). It turns out that the analysis can still be carried out as for a cross between inbred lines, but must be individually applied to each F2 family and each marker system, taking account of marker information on parental and F1 parent individuals. Power was relatively low for diallelic markers, but increased markedly for polyallelic markers, with most of the gains obtained by four alleles. Similar analyses and conclusions also hold with respect to analyses based on backcross populations (Mackinnon and Soller, 1991). Backcross populations have the advantage that they may be more useful than an F2 as a starting point for marker-assisted introgression. Backcross populations may also be more powerful than F2 populations for mapping traits showing much dominance.

In addition to these three main designs we have also considered designs aimed at more accurate mapping of QTL with respect to markers using maximum likelihood and other approaches (Weller, 1986; 1987) and methods for reducing number of individuals that have to be genotyped in marker/QTL linkage studies by genotyping only those animals falling into the selected upper and lower tails of the phenotypic distribution (Lebowitz et al., 1987). Our analysis showed that use of such selected populations would result in significant savings only when selection was rather intense. Our approach involved consideration of marker allele frequencies in the selected tails. Later studies (Lander and Botstein, 1989) showed that power could be increased by basing the analysis on the quantitative trait values of the selected individuals. Further analyses showed that for experiments intended for genetic analysis of a single trait, it will almost never be worthwhile to genotype more than the upper and lower 25% of the population (that is 50% of the total population). The contribution of the middle 50% to statistical power is essentially nil (Darvasi and Soller, 1991a). We have also considered the use of replicated progenies for QTL mapping in crosses between inbred lines (Soller and Beckmann, 1990) and of progeny tests in animal populations (Weller et al., 1990), showing that these designs can be very useful in increasing power of marker/QTL mapping experiments for low heritability traits.

Studies recently completed in our laboratory show that marginal costs of marker genotyping as compared to costs of rearing and evaluating individuals with respect to

the quantitative traits under analysis will determine the optimum spacing of marker loci for a marker/QTL linkage analysis (Darvasi and Soller, 1991b). In many cases, even when highly saturated marker maps are available, the most cost-effective use of experimental resources will involve relatively wide marker spacing for initial gross mapping of QTL, even though this may require raising and evaluating larger numbers of experimental individuals. In another study we considered application of sequential analysis to marker/QTL mapping and showed that, for mapping aimed at a single trait, sequential methods can reduce by half the number of animals genotyped (Motro and Soller, 1991).

MARKER-ASSISTED BREEDING

My initial interest in marker-assisted breeding was as a means of increasing the effectiveness of dairy cattle sire selection. In particular, it seemed to me that marker-assisted selection could prove useful for preliminary selection of candidate bulls for progeny testing (Soller, 1974). The basic notion was that elite sires would be evaluated for marker-QTL linkage phase and the information used to select among their sons those individuals carrying marker alleles in coupling to favourable QTL alleles. However, initial studies showed that a single diallelic marker, even if known to be linked to a QTL having a relatively powerful effect on milk production, could make only a minor contribution to overall genetic progress (Soller, 1978). Among the many problems were the fact that for diallelic markers, many sires would be homozygous at the marker locus and hence not amenable for analysis. Also, for diallelic markers accuracy of marker/QTL phase determinations in the elite sires would be low, since many daughters would be uninformative.

1980 was the advent of RFLP markers as a means of providing total genome coverage in marker QTL linkage studies, and in dairy cattle in particular (Beckmann and Soller, 1983). Using such markers, even though the contribution to genetic progress of any single marker would remain low, it could be shown that when many markers were utilized, marker-assisted selection of candidate bulls could make an appreciable contribution to genetic progress (Soller and Beckmann, 1982; 1983). With the advent of polyallelic markers, effectiveness can be shown to increase even more (Kashi *et al.,* 1990), since a greater proportion of sires will be heterozygous at the marker locus, and a greater proportion of daughters will be informative of marker/QTL linkage phase.

The relative weakness of marker-assisted selection for within population genetic improvement stimulated consideration of marker-assisted introgression of favourable alleles from one population to another. We found that utilization of genetic markers can provide a manyfold increase in the effectiveness of QTL introgression, especially when introgression is based on the use of a pair of markers bracketing the QTL of interest and selection is simultaneously carried for the desired donor alleles and against the remainder of the donor genome (Soller and Plotkin-Hazan, 1978; Soller and Beckmann, 1983; 1988). This approach would appear to be particularly useful in animal genetic improvement where marker-assisted introgression can provide a powerful means of introducing useful alleles from one population to another (Beckmann and Soller, 1987; Soller and Beckmann, 1989).

Recent studies in mapping of genetic diseases in man show that markers in close linkage to a locus responsible for a genetic disease often show a marked degree of

linkage disequilibrium with respect to the disease. These and other results led us to carry out simulation studies aimed at evaluating the possible role of linkage disequilibrium between marker and QTL for genetic analysis and marker-assisted selection (Marko and Soller, 1991). The results showed that for markers in close linkage to QTL, linkage disequilibrium could be expected to develop rather rapidly in closed populations with relatively small effective numbers, and might provide a useful means for selection.

DEVELOPMENT OF DNA LEVEL MARKERS

Until 1980 markers available for studies in animal populations included primarily blood group and biochemical polymorphisms. Although relatively numerous, these provided only very partial genome coverage. Consequently, prior to 1980, designs and evaluations for marker/QTL mapping and marker-assisted selection were more in the realm of theoretical exercises than intended for real world application. Matters changed radically with the advent of DNA level markers. I was introduced to these in 1980 by my colleague Jacques S. Beckmann, who independently had come to the concept of RFLP markers and their potential for breeding purposes, and was then engaged in a search for such polymorphisms in tomato. Indeed, shortly after our initial conversations, he demonstrated the existence of an RFLP distinguishing tomato species using a heterologous probe. Together we began a search for RFLPs in dairy cattle and were successful in demonstrating our first RFLPs in 1986 (Beckman *et al.,* 1986); others soon followed (Hallerman *et al.,* 1987; 1988a; 1988b; reviewed in Fries *et al.,* 1989).

The further advent of DNA fingerprints and locus specific VNTR markers led us to search for these markers in cattle as well. Although we never succeeded in uncovering a locus-specific VNTR marker in cattle, our efforts did lead us to a new `Jeffreys-type' minisatellite marker giving useful fingerprints in cattle and other species (Kashi *et al.,* 1990a). We also were among the first to demonstrate that simple repeated motifs such as poly(TG), poly(GATA) and others (termed `microsatellites') could also uncover highly informative DNA fingerprint patterns (Kashi *et al.,* 1990b).

With the discovery that microsatellite sequences themselves exhibited a great deal of variation in tandem repeat number (Tautz, 1989; Weber and May, 1989) and that this variation could be uncovered by use of PCR methods to provide a rich source of locus specific polyallelic markers, our research has focused on these sequences. The great advantage of microsatellite markers, as compared to other classes of DNA-level marker, is that it is not necessary to send probes from one laboratory to another—all that is required is to share the sequences of oligonucleotide primers flanking the microsatellite tracts (Beckmann and Soller, 1990). Our current program involves screening a bovine genomic library for clones containing microsatellite sequences, isolating and sequencing the inserts to locate the microsatellite, choosing and synthesizing appropriate flanking oligonucleotide primers for PCR, determining optimum conditions for PCR amplification, and examining the products for polymorphism on sequencing gels. Results are promising and our goal is to develop some 30 bovine polymorphisms of this sort. We intend to make these polymorphisms public as they become available and hope that other laboratories will do this as well. A reasonable goal, worldwide, would seem to be 200 public microsatellite polymorphisms available by 1993, and a complete 20 cM public bovine gene map based on these markers by 1995.

EXPERIMENTAL STUDIES

Even before the advent of DNA level polymorphisms, we carried out a large-scale QTL mapping experiment in tomato, using ten markers (morphological and isozyme) and examining 18 traits in a 2000 plant F2 derived from an interspecies cross in tomato (Weller *et al.,* 1988). The purpose of the experiment was to test whether the theoretical notions we had been developing could actually map genes. They did! and in 1987 we were able to publish the first QTL map in a species other than Drosophila (Weller, 1987).

We are now engaged in a variety of marker-QTL mapping programs based on DNA level markers. These include two studies in chickens: one on the effects of endogenous viruses and chicken homeobox genes on traits of economic importance (Iraqi *et al.,* 1991); the other a large-scale program aimed at mapping loci affecting growth rate and egg production in a cross between layer and broiler strains. We also have a program under way in dairy cattle based on the candidate gene approach, in which we are using DNA level polymorphisms and protein percent.

A major event for applied studies in our laboratory was my participation in a conference on animal biotechnology held at Armidale, Australia, in 1985 (Soller and Beckmann, 1985). I gave a talk on RFLP markers in genetic analysis and improvement. Peter Brumby, then director of the International Livestock Centre for Africa (ILCA), was also present at the conference and, although I left before meeting him, J.S.F. Barker forwarded to me an inquiry of his as to the possibility of using RFLP markers to characterize trypanotolerant cattle in Africa. This was my first exposure to animal trypanosomiasis (although human sleeping sickness and the tsetse fly were a part of the mythology of my boyhood). Dr. Brumby's inquiry was tremendously exciting and I replied that if there were a trypanotolerant breed available, we could do better than characterize it—we could go ahead and try to map the loci leading to trypanotolerance and use this as a basis for marker-assisted introgression of trypanotolerance to other breeds, and introgression of useful economic traits from other breeds to trypanotolerant cattle. Eventually, through the good offices of John Hodges of FAO, this led to a FAO consultantship on the genetics of trypanotolerance at the International Trypanotolerance Center (ITC) at Banjul. Together with my colleague Jacques Beckmann, and Ian MacIntyre, Bakary Touray, Derek Clifford and others at ITC, a trypanotolerance program based on a cross between the trypanotolerant N'Dama and trypanosensitive Zebu was worked out in detail (Beckmann and Soller, 1987) and presented at an international genetics seminar held at the formal opening of the ITC in 1987.

We feel honoured and extremely fortunate to have been invited to participate in the trypanotolerance mapping program at ILRAD and look at this as the central focus of our laboratory and statistical efforts at this time.

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SWISS FEDERAL INSTITUTE OF TECHNOLOGY

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Most researchers attending this Workshop are devoted to mapping genes in domestic animal species, particularly in cattle. Knowing the chromosomal map position of a gene has many benefits, a fact that will certainly not be disputed during this Workshop. What should be discussed, however, are the strategies involved in developing the maps, and I would like to combine the presentation of the activities in our laboratory with an outline of our general mapping strategy.

The ultimate mapping goal is the chromosomal location of any gene of interest. The availability of a sufficient number of marker loci is a *conditio sine qua non* to achieve this goal. Therefore, the intermediate goal of all mapping activities should be the saturation of the chromosomes with highly polymorphic marker loci. The functions of these loci can be summarized as follows: (1) They serve as genetic markers in linkage studies involving economic trait loci. They are the highly polymorphic species-specific marker loci designated `type II anchor loci' by O'Brien (1991). (2) They allow correlation of the map of the agriculturally relevant genes with the map consisting of `type I anchor loci' representing evolutionarily conserved coding genes (O'Brien, 1991).

DIFFERENT TYPES OF VNTRs SERVE AS MARKER LOCI

The type II anchor loci should be highly polymorphic to allow and facilitate linkage studies in any given pedigree. A PICz0.60 is considered reasonable. O'Brien (1991) has listed as an additional criterion the free availability of the respective molecular probes as clones or as sequence data for PCR purposes. For complete coverage of the cattle chromosomes we need about 100 appropriately spaced marker loci. Everything points now to different types of VNTRs becoming the basis of any genome covering marker system. We have been involved in the mapping of bovine minisatellite VNTRs (Georges *et al.,* 1991). The respective probes were developed by Genmark. The regional mapping of some of these VNTR loci by radioactive *in situ* hybridization gave no indication of a telomeric clustering as was observed for human minisatellite VNTRs (Figure 1). Some of the probes were also mapped by panel mapping in the laboratory of J. Womack (Texas A and M University). The combined *in situ* and panel mapping allowed the assignment of several syntenic groups to chromosomes. Although the minisatellite VNTR markers may be evenly distributed throughout the bovine genome, there are disadvantages associated with this type of marker. The free availability of the majority of the probes developed thus far is uncertain. Moreover, several laboratories (including ours) found it difficult both to develop and to apply minisatellite probes.
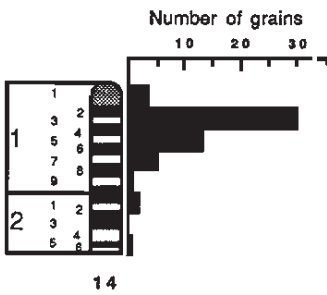
Fortunately, another type of marker has been recently detected and found to be ubiquitous in mammalian genomes, so called microsatellite VNTRs. We decided to concentrate our activities on these markers. Since the visualization of the microsatellite
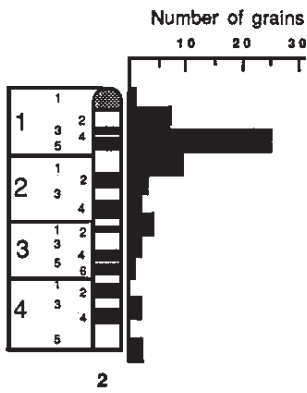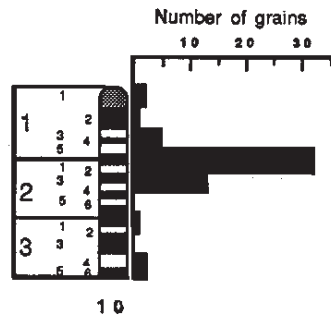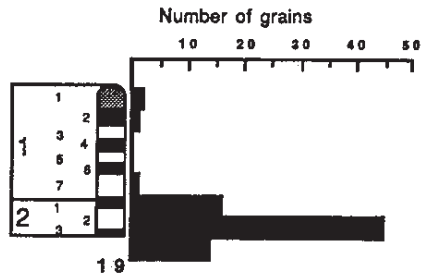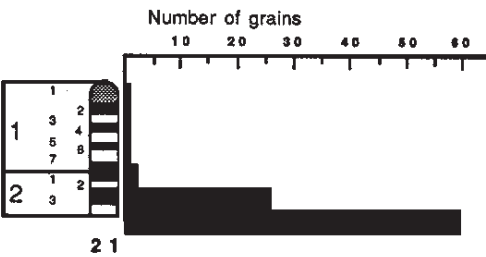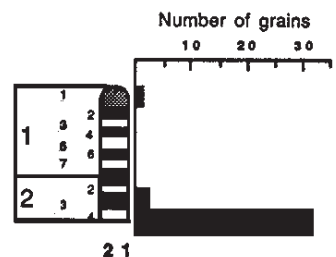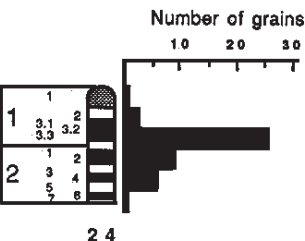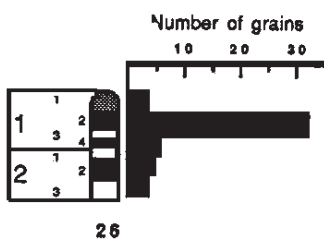
FIGURE 1. *In situ* mapping of some minsatellite VNTRs (for details see Georges *et al.,* 1991).

polymorphism is based on PCR, these markers are immediately available in the STS (sequence tagged site) format (Olson *et al.,* 1989). Beckmann and Soller (1990) introduced the acronym STMS (sequence tagged microsatellite site). We have searched the bovine sequence entries of GenBank and EMBL for repeated short nucleotide motifs constituting microsatellites. Genes that were found to contain microsatellites and to be polymorphic are listed in Table 1.

ISOLATION OF MICROSATELLITES FROM A PLASMID LIBRARY

We also physically isolated microsatellites using PCR. The procedure is described below.

- Screen partial plasmid library with poly(dC-dA)-poly(dG-dT) (500bp inserts in pBluescript).
- Pick positive colonies with toothpick. Inoculate fresh LB medium. Freeze cells.
- Pick few of the remaining cells of each positive colony. Lyse cells by boiling in water and use as substrate for PCR with primers complementary to the T3 and T7 promoter sequences of pBluescript.
- Electrophorese, blot and hybridize again with poly(dC-dA)-poly(dG-dT) to confirm the presence of the microsatellite.
- Of confirmed positive colonies pick a few cells directly from the frozen culture. Lyse and PCR as described in step 3.
- Electrophorese in low temperature gelling agarose. Cut out.
- Sequence both strands directly in the low temperature gelling agarose using a modified Sequenase protocol. The sequencing primers are the same as the PCR primers.
- Design primers.
- Use DNAs from a panel of unrelated animals as substrate for PCR. Electrophorese through 3% NuSieve agarose to obtain preliminary information about the degree of polymorphism.

Using this approach we were able to isolate the 8 microsatellite loci listed in Table 2. The majority of them was polymorphic when analyzed on 3% NuSieve agarose. DNA samples from N'Dama and Boran animals at ILRAD are part of our routine polymorphism screening panel. Some of the microsatellites have been mapped in the laboratory of J. Womack by applying the PCR to a panel of hybrid lines.

ISOLATION OF MICROSATELLITES FROM A COSMID LIBRARY

A serious drawback of the above described marker searching approach is that the probes for the microsatellite loci are available as relatively short plasmid inserts. Regional mapping by *in situ* hybridization is not straightforward. That is why we are now applying a different approach to isolate STMS markers. This approach involves screening a partial cosmid library with poly(dC-dA)-poly(dG-dT). About every second cosmid contains at least one microsatellite. Positive cosmids are isolated and cut with frequently cutting enzymes, electrophoresed, blotted and hybridized again with poly(dC-dA)-poly(dG-dT). Positive bands are subcloned in pBluescript. The colonies of bacteria containing recombinant pBluescript are then treated as described above.

TABLE 1. Polymorphic microsatellites detected by database query.

Gene	Locus	Map position	Repeat motif	PCR primers (5'–3')									Number of alleles
Steroid 21-hydroxylase	CYP21	23	CA	GGA	GGG	TTA	CAG	TCC	ATG	AGT	TTG		13
				TCG	CGA	TCC	AAC	TCC	TCC	TGA	AG		
Cytokeratin	KRT10	19	T	TGT	CTA	AAA	TGC	TGT	AGC	TTT	GGT	G	3
				GTT	GGG	TCC	TTA	CTA	AAT	AAC	GAG	C	
GTPase activating protein	GAP,RASA	7	TG	CCC	TTC	CGC	TTT	AGT	GCA	GCC	AG		4
				GGG	CCA	CAG	CCC	AGG	ATC	GGG	AGC		
Beta-subunit of follicle stimulating hormone	FSHB	15	AT, GT	CTT	GGG	ATA	TAG	ACT	TAG	TGG	CAT	G	9
				GCA	CAA	GTC	ACA	GTT	TCT	AAG	GCT	A	
Retinol binding protein 3, interstitial	RPP3	U29	CA, TA	GAC	CTT	CTA	TGC	TTC	CAC	TCT	AG		4
				GCT	TTA	GGT	AAT	CAT	CAG	ATA	GC		
Major histocompatibility complex, class II, DR beta *pseudogene 2*	BOLADRBP2	23	GT, GA	AGG	CAG	CGC	CGA	GGT	GAG	CGA			2
				TCC	AAC	ACT	CAC	CTG	GAC	GTA	GC		

TABLE 2. Microsatellites isolated from plasmid library.

Laboratory designation	Locus	Map position	Number of CA repeats*	PCR primers (5'–3')								Number of alleles[†]
ETH131	D21S4	21	19	GTG	GAC	TAT	AGA	CCA	TAA	GGT	C	4
				GCT	GTG	ATG	GTC	TAC	GAA	TGA		
ETH 225	DU2S1	U2	18	GAT	CAC	CTT	GCC	ACT	ATT	TCC	T	3
				ACA	TGA	CAG	CCA	GCT	GCT	ACT		
ETH152	D5S1	5	17	TAC	TCG	TAG	GGC	AGG	CTG	CCT	G	4
				GAG	ACC	TCA	GGG	TTG	GTG	ATC	AG	
ETH121	D8S2	8	23	CCA	ACT	CCT	TAC	AGG	AAA	TGT	C	5
				ATT	TAG	AGC	TGG	CTG	GTA	AGT	G	
ETH1112	DU6S1	U6	17	AGT	GGA	TCC	TGC	ATG	TTA	TGC	CG	2
				CCA	GAC	GGA	CCT	TTG	TGG	GCA	A	
ETH185	N.A.	N.A.	22	TGC	ATG	GAC	AGA	GCA	GCC	TGG	C	5
				GCA	CCC	CAA	CGA	AAG	CTC	CCA	G	
ETH153	N.A.	N.A.	11	GAT	GAC	TGA	GCA	ACT	GAG	CAT		N.P.
				CCT	GAG	GCT	GAT	ATA	TTC	TCT	C	
ETH123	N.A.	N.A.	13	GTC	AGA	CAT	GAC	TGA	GTG	ACT		N.P.
				CTG	CTA	TAG	AGG	TAT	AGG	AGG		

* Determined by sequencing of plasmid insert
[†] Determined in a panel of 15 unrelated animals of different breeds by electrophoresis through 3% NuSieve agarose
  N.A.—not assigned
  N.P.—not polymorphic

MAPPING OF COSMID-DERIVED MICROSATELLITE LOCI BY FLUORESCENT *IN SITU* HYBRIDIZATION

The regional mapping of the cosmids containing polymorphic microsatellites is achieved by competitive *in situ* suppression (CISS) and non-radioactive (fluorescent) *in situ* hybridization (NISH) as described by Lichter and Ward (1990) and Lichter *et al.* (1990). Radioactive *in situ* hybridization is basically a statistical approach requiring the analysis of up to one hundred metaphase spreads for the location of a gene. This is, particularly in cattle with 30 difficult chromosome pairs, a tremendous task. Non-radioactive hybridization using biotin labelling and signal detection after incubation with avidin-FITC is very efficient, provided that the sensitivity is sufficient. Cosmids usually contain sufficient unique sequences to yield a strong signal. The characteristic double dots representing signals from each chromatid are indicative of specific hybridization. These double dots can be easily distinguished from the few scattered background dots. The analysis of five to ten metaphase spreads is usually sufficient for mapping a gene.

COSMID-DERIVED STMS (CSTMS) AS PHYSICAL AND GENETIC REFERENCE LOCI ON ALL BOVINE CHROMOSOMES

We believe that it should be possible to routinely map a large number of cosmid-derived STMS by this approach. Several reports demonstrate the feasibility of large scale *in situ* mapping of cosmids to human chromosomes (e.g. Lichter *et al.,* 1990; Yamakawa *et al.,* 1991). The mapping of about one hundred cosmids would most likely place a marker

on each bovine chromosome. These STMS could be easily assigned to syntenic groups by PCR analysis of a hybrid panel. The result of this combined physical mapping approach would be the assignment of the remaining unassigned syntenic groups to chromosomes. (Only 13 of the 29 autosomal syntenic groups have been chromosomally assigned to date.) These cosmid derived STMS would also serve as genetic reference loci, since they are selected based on a high degree of polymorphism. This Workshop might be a good forum to discuss to what extent cosmid-derived STMS and their assignment by *in situ* hybridization should become part of a general mapping strategy.

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CSIRO, DIVISION OF TROPICAL ANIMAL PRODUCTION

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Our Program was initiated just over three years ago and is directed largely at cattle with secondary emphasis on sheep. Because of the high degree of homology at the DNA level between these two species, there is a natural interchange of information and reagents. Our mission is to identify and map genes of economic importance in animal production. Because this field of livestock research is still in its infancy, there is need for a substantial amount of basic research in order to develop efficient tools and strategies for gene mapping. Our current activities include construction of a primary bovine linkage map, DNA marker isolation and the mapping of a number of simply inherited traits. Future activities will centre around the genetic analysis of polygenic traits and the identification of markers linked to major genes. Because of the scale of the task in livestock gene mapping, we believe international cooperation to be not only desirable but essential for the rapid development of the technology and its application in the livestock industries. For this reason we support efforts to establish links between national programs and between research groups.

CURRENT ACTIVITIES

Construction of a primary linkage map

A genetic or linkage map is an essential tool for gene mapping where the reverse genetics approach is to be used. We are contributing to the development of a bovine genetic map in a number of ways.

Reference families

We have collected and stored both DNA and viable lymphocytes from 16 full sib families for use in linkage mapping studies. The reference families comprise around 170 animals in two and three generation pedigrees where parents are *Bos taurus* \ *Bos indicus* or *Bos indicus* strain cross animals (Hetzel, 1991). The DNA is currently being shared with four other groups around the world and linkage data is being collated in a central database. Our families, together with those being collected by Jim Womack (Texas A & M University) and Alan Teale (ILRAD), will be a valuable ongoing resource in bovine gene mapping.

Linkage mapping

We are initially targeting particular chromosomes on which to build linkage maps. Genes previously assigned to syntenic groups have been mapped using RFLP markers

defined by coding sequences. Up till now, more than 30 genes have been mapped in the reference families. Partial maps of chromosome 5 (Barendse *et al.,* 1991) and chromosomes 14 and 19 (W.J. Barendse, unpublished) have been produced. As highly polymorphic single locus markers become available, they are being mapped within our families. Together with the efforts of other groups, a public primary linkage map with an average spacing of 20 cM between markers should be available within several years.

In situ *mapping*

The *in situ* hybridization technique has been used to assign the growth hormone (Hediger *et al.,* 1990) and follicle stimulating hormone B chain (Hediger *et al.,* 1991) gene loci in cattle and sheep. Several single locus minisatellite sequences isolated by us have been assigned to syntenic groups by Jim Womack and then localized to chromosomes by *in situ* hybridization (R.D. Drinkwater *et al.,* unpublished). One of these localizations has assigned syntenic group U10 to chromosome 1. We intend to continue to use *in situ* hybridization studies to assign unassigned syntenic groups to chromosomes in order to increase the resolution of the bovine physical map.

Marker isolation

There is a requirement for a large number of highly polymorphic DNA markers for use in reverse genetics studies. We chose to isolate variable number tandem repeat (VNTR) sequences of the minisatellite and microsatellite types. We initially evaluated heterologous minisatellite sequences in cattle and sheep as a source of multiple genetic markers for linkage analysis (Drinkwater *et al.,* 1990). Levels of polymorphism were lower than expected and clustering of loci was evident. The isolation of minisatellite sequences from cattle has proceeded but has not been especially productive. Only a small number of variable single locus probes have been produced (R.D. Drinkwater, unpublished). These probes also produce multilocus patterns when used at low stringency.

Our major effort is now on microsatellite markers since they utilize the elegant PCR technology and are easier to isolate. Microsatellites associated with coding sequences in cattle have been found to be variable and are also conserved between cattle, sheep and humans (Moore *et al.,* 1991). Microsatellites based on $(CA)_n$ repeats are both numerous and apparently randomly distributed. We now have around ten systems working in cattle and others are at various stages of development (S.S. Moore, unpublished).

Genetic Markers

We are currently seeking genetic markers for the following three single gene traits.

Booroola gene

This gene trebles ovulation rate in Merino sheep through a reduction in inhibin level. Close linkage to several candidate genes has been excluded. Using multilocus minisatellite probes, over 85 markers have also been screened for close linkage without success (Drinkwater *et al.,* 1991). The efficiency of this approach has been limited by the size of the available half sib families. Large families will become available through collab-

oration with INRA, France, greatly increasing the probability of detecting linkage. The use of single locus probes is also being planned. Other groups in New Zealand, Germany and Israel are also pursuing this gene.

Polled gene

The gene, which controls the presence of horns in *Bos taurus* cattle, is dominant. We have collected half sib families in which the polled gene is segregating. Using multilocus probes, we have excluded close linkage to 25 minisatellite markers. In collaboration with Genmark, USA, we are carrying out a linkage study using their highly polymorphic markers.

Pompe's disease

There is a genetic mutation in cattle which results in a metabolic disorder known as Pompe's disease. The gene is recessive and lethal. We have defined an RFLP marker using the candidate gene approach (Hetzel *et al.,* 1989). The mutation in Brahman cattle has been localized to a 3' fragment of the gene but development of a PCR based diagnostic test has progressed only slowly.

FUTURE ACTIVITIES

As the tools for livestock gene mapping are developed in the near future, our activities will focus on the genetic analysis of polygenic traits and the identification of markers closely linked to major genes. Most of the economically important traits in livestock are controlled by multiple genes though the number of genes and size of effects are largely unknown. We will use the mapping technologies to search for major gene effects on meat quality and parasite resistance in cattle. In order to carry out this research, we are identifying, generating and collecting appropriately documented family groups. For instance, we are producing two large progeny groups from F1 Charolais × Brahman bulls on the assumption that major genes will be segregating in such pedigrees. Other *Bos indicus* × *Bos taurus* families are also being used.

There are two other areas where advances are required in order to address polygenic traits. Firstly, marker analysis systems need to be improved so that the genotyping of large numbers of animals is efficient both in terms of time and money. We will contribute to the development of suitable systems for genotype measurement, data capture and processing. Secondly, there is scope to refine the statistical approaches to linkage mapping in animal populations. In particular, the use of maximum likelihood methods to estimate genetic parameters (e.g. gene effect, frequency, recombination distance, mode of action) in half-sib family designs needs to be optimized since the screening of such families could become common-place in livestock.

INTERNATIONAL COOPERATION

Gene mapping in livestock is in its infancy and a lot of the basic research remains to be done. On the other hand, the international human genome research programs will

generate much information about mammalian genomes as well as generate mapping technologies which can be immediately transferred to livestock. There is tremendous scope for international cooperation in livestock mapping research and we stand ready to participate in such programs.

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INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES

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ILRAD's gene mapping effort has two main thrusts; generation and phenotyping of a trypanotolerance resource population and the generation of genetic markers contributing to an internationally developing bovine gene map. A third, although lower priority, interest concerns the relationship of African cattle types one to another. This is currently being approached through the analysis of mitochondrial DNA.

A TRYPANOTOLERANCE RESOURCE POPULATION

Some years ago, ILRAD acquired cryopreserved N'Dama embryos (West African longhorn cattle *Bos taurus)* from The Gambia. From 10 successful embryos the population of N'Dama cattle at ILRAD has now grown to more than 40, which is primarily being used in comparative studies, with Boran *(Bos indicus)* cattle, of the immunology and pathology of trypanosomiasis. The N'Dama is a trypanotolerant breed whereas the Boran is relatively trypanosusceptible.

Four of the five original N'Dama bulls obtained from the 10 embryos are forming the source of trypanotolerance genes in a crossbreeding exercise with Boran cows. The objective, as far as the F1 generation is concerned, is to produce one full-sib family of 30 crossbred calves from each bull-cow pair using artificial insemination and embryo transfer into surrogate Boran females. This exercise is now well advanced with two of the F1 families complete (either born or *in utero,* with most now born).

Calves are born at the ILRAD ranch (Kapiti Plains Estate), which is free of trypanosomiasis. After weaning at 8 months of age and a 2-month post-weaning period on the ranch, they are moved to the Laboratory. After a further 2-month-period of acclimatization, during which baseline physiological data and data on their immune status is collected, they are challenged at 1 year of age with *Trypanosoma congolense* IL1180 by the bites of 5 infected tsetse flies *(Glossina morsitans centralis).*

Cattle are monitored for up to 100 days post-challenge and data are collected on a regular basis on:

- packed cell volume percent and blood haemoglobin concentration (as measures of anaemia);
- parasite concentrations in peripheral blood;
- parasite antigen levels in peripheral blood;
- leukocyte levels in peripheral blood (total and differential counts);
- serological responses to invariant trypanosome antigens; and
- body weight.

Animals in which the packed cell volume percent falls to 15 or less at any stage are treated and are effectively removed from the experiment (as far as further observations are concerned). Animals remaining under observation at 100 days post-challenge are treated to effect cure. Plasma and sera are stored against future needs to examine other parameters of response as they may be identified. It is planned to produce an F2 generation, work on which will begin before the end of 1991. At the present time it is intended to produce and phenotype approximately 150 F2 animals. DNA is prepared from the peripheral blood of all animals and stored appropriately.

GENERATION OF GENETIC MARKERS

ILRAD, like most other laboratories involved in the bovine gene map, is currently placing the bulk of its effort in the area of microsatellites. We conducted a search of the GenBank database and found four sequences which contained dinucleotide repeats. PCR primers were made to amplify these regions and three of them (IRBP, FSHB and CYP21) yielded polymorphic PCR products. We demonstrated Mendelian inheritance with all of these.

This initial study led us to the conclusion that polymorphic microsatellites were likely to be valuable sources of markers. They have a number of advantages over other systems. They are easy to exploit and they are highly polymorphic. Perhaps their most important advantage over minisatellites or RFLPs is that they are easily transferred between laboratories; simply exchanging two short primer sequences amounts to exchanging a marker. Microsatellites also appear to be frequent; if three of the three hundred bovine sequences searched in GenBank contain useful microsatellites then this suggests that there are more than enough such regions in the genome to construct a high resolution map. We have thus begun a program to locate and sequence short stretches of genomic DNA containing dinucleotide repeats. This exercise is likely to occupy most of the group's efforts in the near future.

ILRAD also has expertise in the study of the major histocompatibility complex (MHC). This highly polymorphic genetic region located on chromosome 23 controls the expression of cell surface antigens which are responsible for presentation of peptides to T cells and which, therefore, are pivotal in immune responses. We are able to detect a large number of MHC alleles in N'Dama and Boran cattle by serological methods and will use these as genetic markers in the N'Dama/Boran crossbreeding experiment. We have also employed class I serology and microsatellite amplification to confirm linkage between CYP21 and the MHC.

BREED RELATIONSHIPS

The third area of interest is bovine breed relationships; a particular interest being the relationship of N'Dama cattle to other breeds. In this respect, mitochondrial DNA, which appears to be rapidly evolving, may provide useful insights. To this end we are comparing mtDNA from different populations of N'Dama cattle, from East African Zebu types and from European breeds. We have designed primers based on mitochondrial DNA sequence for the purpose of amplification and restriction digest analysis. Initial results are informative and therefore encouraging.

TEXAS A & M UNIVERSITY

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Utilizing panels of hybrid somatic cells, our laboratory has assigned over 200 loci to bovine synthetic groups. Eleven of the 29 autosomal groups have now been assigned to chromosomes, primarily by *in situ* mapping in the laboratory of R. Fries. Most of the 200 loci selected for mapping were comparative anchor loci, i.e., their homologs were previously mapped in humans and mice. We have used both the techniques of Southern blotting with homologous and heterologous probes and PCR of hybrid cell DNA primed with published bovine sequences. A comparative map is presented with the human physical map as a reference standard. These 200 loci identify 34 evolutionary break-points on the bovine-human comparative map. The same loci identify 49 disruptions in a human-mouse comparison. These data support the hypothesis that the bovine genome is more highly conserved relative to human than is the mouse. This comparative map will facilitate the extrapolation of genomic data between three mammalian species.

Recent unpublished bovine assignments include:

	Gene	*Bovine*	*HSA*
OT	Oxytocin	U11	20
ARVP	Arginine-vasopressin	U11	20
INHBA	Innihin-BA	U13	7
FIO	Coagulation factor X	U27	13
CHGA	Chromogranin A	U4	14
LDLR	Low density lipoprotein receptor	U22	19
CGA	Glycoprotein alpha	U2	6
FUCA1P	Fucosidase, alpha L-1 pseudogene	U17	2
PGY3	P glycoprotein 3	U13	7
IGF2	Insulin like growth factor 2	U7	11
IGF1R	Insulin like growth factor 1 receptor	U4	15
HCK	Hemopoietic cell kinase	U11	20
ESR	Estrogen receptor	U2	6
INSR	Insulin receptor	U22	19
HP	Haptoglobin	U9	16
LHB	Luteinizing hormone-beta	U9	19
YES1	Yamaguchi sarcomal viral oncogene homolog	U28	18
PCKB	Protein kinase C, beta	U8	16
ADRB2R	Adrenergic, beta-2 receptor	U22	5
POMC	Proopiomelanocortin	U16	2
PRIP	Prion protein	U11	20
ACO2	Aconitase 2, mitochordrial	U3	22
IFNO	Interferon omega	U18	—
TP	Trophoblast protein	U18	—

CGPRA	CGMP Phosphodiesterase-alpha	U22	—
CGPRB	cGMP Phosphodiesterase-beta	U15	—
CGPRG	cGMP Phosphodiesterase-gamma	U21	—
CGGC	cGMP gated channel protein	U15	—
CGPCA	cGMP phosphodiesterase cone protein-alpha	U26	—
TCRA	T cell receptor, alpha	U5	14
TCRB	T cell receptor, beta	U13	7
TCRG	T cell receptor, gamma	U13	7
CD3E	Antigen CD3 epsilon	U19	11
CD3D	Antigen CD3 delta	U19	11
CD8A	Antigen CD8A	U16	2
D4S43	Human anonomous sequence	U15	4
D4S10	Human anonomous sequence	U15	4
QDPR	Quinoid dihydropteridine reductase	U15	4
KIT	Hardy-Zukerman 4 feline sarcomal viral onogene	U15	4
IGJ	Immunoglobulin J. polypeptide linker protein	U15	4
ADH2	Alcohol dehydrogenase (class 1), beta	U15	4
IF	Complement component I	U15	4
FGB	Fibrinogen, beta	U23	4
FGG	Fibrinogen, gamma	U23	4
F11	Coagulation factor XI	U23	4
CSF2	Colony stimulating factor 2	U22	5
RPS14	Ribosomal protein S14	U22	5
PDGFRB	Platelet derived growth factor receptor, beta	U22	5
FGFA	Fibroblast growth factor, acidic	U22	5
CSF1R	Colony stimulating factor 1 receptor	U22	5
C9	Complement component 9	U10	5
HGMCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase	U5	5

We have also been studying the inheritance of natural resistance to *Brucella abortus* in cattle. To date we have shown that natural resistance (R) to brucellosis can be increased from 18% (19/105) to 58.6% (17/29) by mass selection in one generation of selective breeding of RXR. The genetic analysis of the mating results are consistent with two or more genes controlling the resistant phenotypes. We have observed that the IgG2a-A1 and A2 allotype response to *B. abortus* LPS, postchallenge with *B. abortus* is significantly different between R and S cattle. The ability of macrophages from R and S cows to control replication of *Salmonella dublin* and *S. typhimurium in vitro* correlates with resistance to *B. abortus in vivo* and *in vitro.* From these studies it appears that both arms of the immune system are important in resistance to bovine brucellosis, but the relative importance of each arm is unknown. We are unable to determine if gene markers that are linked to natural resistance to intracellular pathogens *(Salmonella, Mycobacteria* and *Leishmania)* in mice are linked to a conserved bovine homolog of the *Bcg* gene complex in these cattle, because of the low polymorphism information content (PIC) of these small sibships. We are currently producing five embryo transfer sibships (all are paternal half-sibs) that are genetically segregating natural resistance/susceptibility to brucellosis for genetic analysis of challenge phenotypes and marker genes to determine if there is a bovine homolog of the murine *Bcg* gene in these cattle.

INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH

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GENERAL BACKGROUND

The Institute of Animal Physiology and Genetics Research (IAPGR) has traditionally been associated with the improvement of livestock and the implementation of new technologies in animal production. The Edinburgh Research Station is organized into five departments—Molecular Genetics, Molecular Endocrinology, Immunogenetics, Biometrical Genetics and Ethology. As a broad theme the research is directed to understanding physiological processes, in particular growth, reproduction and immunology. Molecular genetic approaches are used to identify genes affecting specific traits and a well established molecular biology base enables the cloning, manipulation and reintroduction of genes of interest. In addition IAPGR has a strong Biometrical Genetics group who have developed powerful breeding strategies and which provides statistical back-up across the Institute.

We have identified the need for genetic maps of livestock species to facilitate marker-assisted selection (MAS) and reverse genetics. Markers flanking genes controlling quantitative traits will enable genes to be moved from one breed to another resulting in a `compound' genotype comprising best genes from several breeds. A complete genetic map will also allow the detection of genes affecting quantitative traits, and enable their effects to be estimated. Ultimately the genes themselves could be identified and cloned enabling direct manipulation and reintroduction.

Currently there are two gene mapping programs at IAPGR, an EEC-sponsored PigMaP project and the Bovine Project, currently funded through the Ministry of Agriculture, Food and Fisheries. Both projects are presently focusing on the identification of polymorphic probes.

CATTLE GENETIC MAPPING

Objective

Our initial objective is the construction of a low resolution genetic linkage map of the bovine genome by studying segregation of markers in full-sib families selected from a multiple ovulation embryo transfer (MOET) herd. A 20 cM map should be sufficient to detect markers linked to quantitative trait loci in families in which the trait segregates. The long term objective is to identify markers for milk production and quality and growth and disease susceptibility, particularly BSE. The construction of a linkage map requires an animal resource, genetic markers, a database and appropriate statistical methodology.

Animal Resource

In order to achieve our initial goal of a low resolution map we will use selected full sib families from a MOET herd. This herd is derived from 4 elite sires and 20 donor dams, and the objective is to generate families with 8–16 progeny. We calculate that in order to create a linkage map at 20 cM resolution, 5 families with 16 progeny or 10 families of 8 progeny will be sufficient.

In order to identify markers for genes affecting production traits it will be necessary to identify populations in which the trait of interest segregates. Improved breeds still show variation in the selected trait and although fixed for the major genes they still segregate minor `fine tuning' genes. Families segregating such genes are available within the national cattle population and within cattle available at the Institute. We also have two herds of Friesian cattle selected for high and low milk production whose ICC diverge by about 20%. To identify major genes controlling production traits, inter-specific crosses between divergent breeds will be required.

Markers

The development of a linkage map using a herd derived from a restricted panel of highly selected founders, from within one breed, will require probes showing the maximum degree of polymorphism. The advantage of selecting markers that are polymorphic in this herd is that the majority should also be polymorphic in other populations.

Most of the markers used will be anonymous DNA sequences (RFLP, mini- and micro-satellite). We are presently screening heterologous RFLP probes and attempting to identify homologous mini- and micro-satellite probes from a bovine library. Putative markers will be selected from those showing polymorphism on a panel of 5–10 unrelated individuals from the herd. The objective of approximately 200 markers evenly spaced throughout the genome will require co-operation between as many laboratories as possible.

Database and statistics

The Institute is currently installing computing facilities to handle the PiGMap database. The database will run on software, now under development, which is compatible with the existing human and mouse databases. The methodology for the identification of linkage between large numbers of markers, and ultimately QTL, is also an area receiving attention. Hardware and software will be used for both porcine and bovine mapping programs.

DISEASE RESISTANCE

Bovine spongyform encephalopathy (BSE)

A `slow virus' infection similar to one known in sheep, Scrapie, has appeared in the cattle population of the UK. As of now 25,826 cases have been confirmed. The infectious agent is unknown, but infection results in the modified expression of a cellular glyco-protein—PrP. This modified expression causes vacuoles to appear in the brains of infected animals, which progressively lose co-ordination.

In mice and sheep, alleles at a Scrapie incubation period locus (sinc) affect the incubation period prior to an infected animal displaying symptoms. This locus is linked to the PrP locus. In cattle a constant incubation period of about 3 years is observed, but a substantially longer incubation period allele may also exist. We are using the markers which are being developed for the linkage map to attempt to identify other loci affecting incubation period and resistance to infection.

One problem we have encountered is that the challenge is unknown and that there may be a long incubation genotype that has yet to be observed, thus apparently healthy animals may either have not been challenged, or may yet display symptoms. However, markers which are not associated with loci affecting BSE infection should be evenly distributed between affected and unaffected animals, while markers linked to relevant loci will show a skewed distribution. Using a sample size of 400 animals we would expect to identify markers tightly linked to loci affecting BSE infection even with only 25% challenge.

COLLABORATION

Markers

The major effort in the initial stages will be in the generation of markers. We would hope that a large number of markers that we will use can be obtained from other laboratories. Likewise as we develop our own markers we will make every effort to make these freely available.

Animal resources

DNA from the full-sib families that we have access to should be freely available, although the quantities will be limited. DNA from our BSE panel, and ultimately animals selected for QTL studies, will also be available. In order to address major genes affecting QTL, crosses between divergent breeds may be required. This will be an expensive and lengthy process and may well be best addressed collectively rather than as individuals.

Databases

A central repository for information is desirable. Our database and information will ultimately be available to all laboratories with which we collaborate.

CONCLUSION

In conclusion, although we will be making a substantial effort to generate a linkage map at the IAPGR, the project is of a scale that requires free and open collaboration between as many laboratories as possible internationally.

TRINITY COLLEGE

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Cattle are animals of enormous economic significance, both in the developed and developing regions of the world. They have existed in a synergistic relationship with humanity for some 10,000 years and their biogeography and evolution have been profoundly influenced by this association. Populations around the globe differ in physical and physiological characteristics, particularly in the existence of two sub-species, *Bos taurus* and *Bos indicus*. We seek to apply the techniques of DNA analysis to measure genetic distances between six European, three Indian and four African breeds of cattle.

A sampling strategy was adopted with a view to intensive genome analysis (examination of many loci, especially mitochondrial sequences) of ten or more representative individuals of each breed. The European breeds chosen were Aberdeen Angus, Charolais, Friesian, Jersey, Hereford and Simmental. Irish artificial insemination stations were the chosen source for samples due to the facilities suitable for obtaining the large amounts of blood (500 ml) necessary for mitochondrial DNA extraction. In addition, pedigree records were available to ensure minimum relatedness between the animals in each breed.

Sampling from the developing world has also been completed. Indian cattle selected were the Sahiwal, Tharparkar and Hariana. Sahiwal originate from Pakistan and are one of the most widely used dairy breeds of Zebu or *Bos indicus* type. They are small in number but have been used in the upgrading of local cattle in many countries. Tharparkar also originate from Pakistan, are slightly larger than Sahiwal but have similar dairy qualities. The Hariana are the predominant breed of northern India and have lesser milk yields. They show vigour and persistency as draught animals. Ten mitochondrial and nuclear DNA samples were extracted from unrelated representatives of each of the above on site at the National Institute for Animal Genetics, Karnal, Haryana, India. The Ongole cattle of southern India were also intended for sampling but this seems unlikely at this stage in our project.

East African breeds selected were the Butana from central Sudan, east and north of Khartoum, and the Kenana, originating from Sudan, south of Khartoum. These two are representatives of *Bos indicus* and are regarded as the `best' African milk breeds. Ten mitochondrial DNA samples and 24 and 38 nuclear DNA samples, respectively, were obtained at the Shukaba Research Station, Wad Medani, Sudan.

Sampling in West Africa was conducted at the University of Ibadan, Ibadan, Nigeria. Twelve mitochondrial and 19 and 29 nuclear DNA samples, respectively, were extracted from the N'Dama and White Fulani breeds. The latter, of Zebu type from northern Nigeria, represent half of the cattle in the country and typically inhabit the savannah belt, moving south in the dry season. They are triple purpose—milk, meat and draught—and thrive under a variety of conditions. The N'Dama are the best known of several breeds of small humpless cattle found in West Africa. These are considered to

be *Bos taurus,* descendants of Hamitic Longhorns of North-East Africa, which have survived in Guinea and surrounding countries due to a tolerance with respect to trypanosomiasis (Maule, 1990).

Various crossbreeding programs are in place in tropical countries aiming toward improvement of local cattle. Depending on factors to which genetic distance between subjects is a contributor, appropriate strategies may be breed replacement, some form of synthetic, rotational crossing, or grading up to half or three-quarter exotic. As pursuit of inappropriate strategies may lead to costly and widespread disappointment, especially with the acceleration afforded by artificial insemination and other techniques, additional theoretical underpinning to cross design is of value (Cunningham and Syrstal, 1987).

An additional contribution made by a knowledge of the genetic origins of breeds is as a guide to the allocation of resources for breed conservation. Whether achieved by maintenance of herds or cryogenic techniques this involves considerable expense and in cases where a subset of a given number of breeds may be selected it may be more expedient to concentrate on a selection of populations which are most distantly related to each other.

These studies would also be expected to help in the historical reconstruction of migrations and contribute to the general knowledge of the evolution of DNA sequences.

It is hoped to engage in an extensive survey of the bovine genetic material. The rate of change of DNA is known to depend on factors such as whether a region is coding or not, the level of repetitiveness (simplicity) present and whether it is mitochondrial, autosomal and possibly sexlinked. The different modes of transmission of mitochondrial (maternal), autosomal (sexual) and Y-chromosomal DNA (paternal) are an additional reason for examining each in turn.

The advent of polymerase chain reaction (PCR) technology has revolutionized the determination of short DNA sequences. This procedure amplifies regions of DNA, delineated by primer sequences, and enables sequencing of these regions without lengthy cloning procedures. This technique lends itself readily to sequence comparisons between individuals in a population or closely related populations. Once the sequence of an area of interest is known, primers can be designed for PCR amplification and sequencing. These oligonucleotides are suitable for all individuals examined, provided that the individuals are not too genetically distant.

Bovine mtDNA is a highly conserved molecule comprising 22 tRNA genes, 13 protein coding genes and 2 rRNA genes (Anderson *et al.,* 1982). The organization of these genes is very compact with some genes actually overlapping. Apart from small numbers of interspersed bases the only non-coding region is the D-loop. This is a region of about 910 base pairs and is involved in the regulation of DNA replication and transcription.

Previous studies (Hausworth *et al.,* 1984) have indicated that the D-loop tends to evolve at a higher rate than the rest of the mtDNA. Therefore a sequence comparison of this region should be most efficient at detecting any differences present between individuals at the mtDNA level. Wrischnik *et al.* (1987) carried out such a study on humans which helped resolve some discrepancies from an earlier RFLP study (Cann *et al.,* 1987). It was therefore decided to examine the D-loop in further detail to determine whether discrimination between breeds from a particular geographical location is possible.

Evident from initial sequencing are a number of sequence divergences, especially between *Bos indicus* and *Bos taurus* animals. Approximately 50% of these are shared

by two Hariana subjects and one divergence is shared by Indian and African Zebus. These results are encouraging given only approximately one third of the D-loop has been examined but are insufficient for the construction of a robust phylogeny.

Both Indian and European populations have been examined for mtDNA restriction fragment length polymorphisms using whole mitochondrial restrictions visualized using agarose gels and silver staining. Most restriction enzymes could readily distinguish between breeds from the different continents. However, the majority of polymorphic sites observed in any particular continental population showed more variation within a given breed than between breeds. These high levels of intra-breed polymorphism tended to obscure the much lower levels of intracontinental, inter-breed polymorphism, thus making breed discrimination difficult. Many more enzymes and individuals would need to be examined in order to determine accurate genetic distances.

The most efficient nuclear DNA markers have proved to be those incorporating copy number variation of simple sequence repeat units. In 1989 three groups published a PCR-based technique for rapid detection and screening of simple sequence length polymorphisms (SSLPs) (Litt and Luty 1989; Weber and May 1989; Tautz 1989). We have applied this technique to most of the samples collected to-date.

The computer facilities of the Irish National Centre for BioInformatics were used to search the GenBank/EMBL DNA sequence database for bovine sequence entries containing simple sequence regions. The database was searched for sequences displaying loose homology to 15 unit dinucleotide repeat sequences, i.e. $(CA)_{15}$, $(AT)_{15}$ etc.

Twelve dinucleotide simple sequence regions emerged and two of these microsatellites have exhibited length polymorphism. The microsatellites analysed are as follows:

- *Bovu* 1: (CA)n repeat in the 7th intron of the bovine steroid 21-hydroxylase gene.
- *Bovu* 2: (CA)n(AT)n repeat in the 3' untranslated region of the 4th exon of the bovine interphotoreceptor retinoid-binding protein gene.

An interesting result has been the clear differences in allele frequencies between Indian and European breeds in the latter and a similar, if less marked, result in the former. It is hoped to extend the autosomal DNA analysis to include PCR and Southern blot detected RFLPs, minisatellites and possibly direct sequencing and single strand conformational polymorphism examination of chosen regions. The Y-chromosome may be accessible for analysis through the knowledge of an unusual repeated sequence (Perret *et al.,* 1990).

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LABORATOIRE DE GENETIQUE BIOCHIMIQUE, INRA

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The Laboratory of Biochemical Genetics is part of the Animal Breeding and Genetics Department of INRA (National Institute for Agricultural Research) in France. Originally, the Laboratory was a farm animals blood group laboratory but, little by little, research was developed in other fields, mainly on milk protein polymorphisms and the cattle major histocompatibility system. Because of the dramatic increase of routine blood typing activities, it was decided in 1985 to establish an independent blood typing service, while the Laboratory of Biochemical Genetics remained a purely research-oriented group. Three years ago, research on the bovine MHC was stopped to concentrate mainly on milk protein genes and on DNA polymorphisms. In 1990 it was decided to start developing systematic studies on the genetic map of cattle. Two cytogeneticists were recruited and joined the group.

Present activities of the Laboratory are thus focused mainly on structure and function of milk protein genes and the bovine genetic map.

STRUCTURE AND FUNCTION OF MILK PROTEIN GENES

Activities in this field include gene sequencing (ovine alpha-La and beta-Cn genes, c-d caprine alpha $_{s1}$-Cn gene), analysis of polymorphism at the level of both proteins and DNA, and use of transgenic animals (mice) as tools for understanding regulation of gene function. In the field of milk protein polymorphisms, efforts are now being concentrated on caprine alpha$_{s1}$-casein. In this casein, mutant alleles with decreased synthesis activity exist at high frequencies in European dairy breeds, which partly explains the low protein content of milk. Typing of alpha$_{s1}$-casein alleles using DNA methodologies will start in the near future. This is one of the very first examples of the use of molecular genetics in selection. This case also shows that, even for a trait considered as typically polygenic, situations exist where alleles with major effects may be found.

THE BOVINE GENETIC MAP

Although systematic research on the bovine genetic map is quite a recent project in our Laboratory, several contributions have been published in the past. The close linkage of the genes coding for the four casein species, alpha$_{s1}$, B, K, alpha$_{s2}$, was first established by our group. We also made contributions to the genetic fine structure of the complex cattle blood group systems. The bovine genome group includes at present 6 scientists, 2 students and 5 technicians. The main orientations are as follows:

- assignments of genes using somatic cell hybrids. The cell lines are those produced by M.C. Hors-Cayla and S. Heurtz in Paris.

- chromosomal location of genes by *in situ* hybridization, with attempts to use non-radioactive probes.
- RFLP studies using as far as possible probes for additional genes.
- microsatellite markers: in addition to systematic screening, special attention has been paid to a microsatellite site within the K casein gene.
- minisatellites: we have developed cooperation with the group of G. Vergnaud, in Paris, who introduced the use of polymerized synthetic random sequences (STR) as probes to detect DNA polymorphism in man. Their probes were first successfully used by G. Guerin in the horse. They are now going to be tested in cattle where they detect polymorphisms.
- to carry out genetic studies, DNAs from several families are being collected, until now within a breed.

Such a project cannot be successful without collaborations. The genome project (bovine in Jouay-en-Josas, but also pig in Toulouse) was accepted in October 1990 as a priority project by INRA. Additional posts are thus expected. In addition, the Ministry of Research has decided to establish a genome project over a period of five years.

As far as cooperations are concerned, the Laboratory is included, in INRA, in a more general group including quantitative geneticists and bio-mathematicians. In the EEC, an international network is to be organized by the International Society for Animal Genetics (ISAG). The Laboratory is ready to discuss any other possibility of cooperation.

THE UNIVERSITY OF GUELPH

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Disease prevention by vaccination, vector control, antibiotics or chemotherapy is effective in many instances but control of complex diseases, such as trypanosomiasis, remains elusive. Host resistance to pathogens and to disease induced by them requires a complex response to variable virulence gene products. Disease resistance is therefore polygenic and the genes and gene products involved are largely unknown. Livestock may have been selected to some extent for disease resistance when bred under pressure of enzootic disease. However, attempts to optimize disease resistance by genetic and breeding methods have generally not been undertaken.

Optimal resistance to infection should be a function of optimal host immune response and non-specific resistance-mediating attributes. Resistance to disease may additionally involve an optimal balance between host responses that are potentially resistance-mediating but simultaneously induce inflammation, fever, cachexia or other disease signs. Since populations are at risk from several potential pathogens varying in virulence attributes and in host response required for resistance, there is an inherent risk in breeding animals to resist any single disease. Inverse relationships between resistance/susceptibility to pairs of diseases are known (Simonsen, 1987) and in experiments involving selection of mice for antibody production, resistance based upon cellular activity was compromised (Mouton *et al.,* 1979). A strategy of selection based upon optimal antibody, cell-mediated immunity and innate resistance-mediating traits may provide overall enhancement of disease resistance in populations of livestock.

Indirect selection may be based upon measured resistance-related traits and correlated markers, such as the major histocompatibility gene (MHC) products, in known linkage with resistance-mediating mechanisms. In this way natural or artificial infection is avoided together with related requirements for standardized infection methods, objective quantitative criteria of disease, infection containment and control. Indirect selection criteria may be chosen which offer low risk to potentially valuable breeding animals and which may be utilized without confounding effects from variable individual infection and acquired resistance status. Resistant populations could therefore be derived in remote locations in which it may be impractical to work with exotic pathogens.

The question of whether or not indirect selection may be used to enhance disease resistance has been addressed in a series of experiments using pigs. Pigs were chosen for their fecundity and availability of MHC (SLA)-defined miniature pigs (Sachs). Methods were evaluated which may have broad relevance in livestock breeding. These experiments have been reported and also briefly summarized (Wilkie *et al.,* 1990). Two general approaches were taken. The first involved evaluation of immune and innate-resistance related traits in pigs of four SLA haplotypes (SLA[a,c,d, and g]) in all homozyg-

ous and heterozygous genotypes. The g haplotype is a recombinant (Thisthelwaite *et al.,* 1983) having SLA class II genes of d and class I genes of c allowing analysis of the relative effects of these SLA genes. Haplotype, sire, dam, litter and other effects were determined by linear general model and least squares analysis while heritability was calculated by the SAS VARCOMP procedure (Helwig and Council, 1982). In another study, Yorkshire pigs were selected using Estimated Breeding Values (EBV) calculated using restricted maximum likelihood under an individual animal model (Henderson, 1984).

To assess immune and innate resistance-related functions standard protocols were used to induce and measure primary and secondary antibody to hen egg white lysozyme (HEWL), sheep erythrocytes (SRBC) and (T,G)-A,L; serum IgG and IgM concentration (by radial immunodiffusion, RID), serum lytic complement (CH50), cutaneous delayed hypersensitivity (DTH) to PPD of *Mycobacterium tuberculosis* (BCG), cutaneous contact allergy (CH) to dinitrochlorohenzene (DNCB), *in vitro* blood lymphocyte blastogenesis to concanavalin A (con-A) and PPD and cultured blood monocyte function in uptake and killing of *Salmonella typhimurium* and *Staphylococcus aureus*. Antibody and cell-mediated immunity (CMI) to an *S. typhimurium* aromatic mutant were also determined. The purified o-polysaccharide of the parent *S. typhimurium* was used to determine lymphocyte blastogenic and passive haemagglutinating antibody. Antibody avidity was measured by a chaotropic ion (KSCN) elution system in enzyme immunoassay for antibody to HEWL.

RESULTS OF EXPERIMENTS IN SLA-DEFINED MINIATURE PIGS

Antibody and Cell-Mediated Immune Response (Mallard *et al.,* 1989a). Dam, sire and litter influenced immune response traits ($p \leq$ 0.12–0.001, 0.88–0.001 and 0.69–0.001 respectively) while SLA and sex had lesser effects ($p \leq$ 0.72–0.01 and 0.95–0.02). SLA dg, gg and dd formed a response group by rank but rank position varied by time after immunization. This group was superior in response to HEWL, (T,G)-A,L, SRBC and DH to PPD but low in ranked CH response to DNCB. Heritability was estimated for HEWL (0.81–1.17) and (T,G))-A,L (0.11–0.44) but not for SRBC, CH and DNCB due to negative sire variance. Heritability of DH response to PPD was 0.03 and 0.13 for 24- and 48-hour post-challenge measurements.

Immune Response to S. typhimurium (Lumsden, 1989). Primary antibody response was influenced by litter ($p \leq$ 0.026–0.0001) at various times after immunization but none of the variables included in the statistical model influenced secondary antibody response. Litter also influenced antibody response after challenge infection ($p \leq$ 0.0052). There were no significant differences in antibody response by SLA haplotype although dd, cd and dg were usually highly ranked. For CMI, litter ($p \leq$ 0.011) and sire ($p \leq$ 0.019) influenced primary response at days 6 and 13 post immunization (pi) respectively while SLA ($p \leq$ 0.004) and litter ($p \leq$ 0.026) influenced secondary response on day 20 pi. Only litter influenced response after challenge ($p \leq$ 0.0001). The effect of time by haplotype was significant ($p \leq$ 0.0002) with dd, dg and gg being either higher or lower in ranked response than aa ac, cc and cd at various times pi.

Serum Immunoglobulin Concentration (Mallard *et al.,* 1989b). IgG concentration was influenced by sire ($p \leq$ 0.001), dam ($p \leq$ 0.002 and 0.02 for 24 hr and 48hr RID),

SLA (p≤ 0.01) and litter (p≤ 0.02 and 0.06 for 24 and 48hr RID). Haplotypes dd, dg and gg had very significantly more serum IgG than others and heritability estimates were 0.13 and 0.27 for 24 and 48hr RID. IgM concentration were influenced only by litter (p≤ 0.001 and 0.009 for 24 and 48hr RID) and heritability is assumed to be zero because of negative sire variance.

Serum Lytic Complement (Mallard *et al.,* 1989c). Activity (CH50) was determined before and after immunization. Prior to immunization only litter and dam (p≤ 0.03 and 0.06) influenced the trait while none of the variables influenced post-immunization CH50. The average CH50 differed significantly (p≤ 0.001) pre- and post-immunization with a tendency for haplotypes other than dd, dg and gg to have elevated CH50. Differences between haplotypes were not significant. Heritability for CH50 was not estimated because of negative sire variance.

Uptake and killing of S. typhimurium *and* S. aureus (Lacey *et al.,* 1989). Litter and SLA had significant effects on uptake and killing as measured at 4 and 8 weeks of age (p≤ 0.0001 for litter and p ≤0.0769–0.001 for SLA in various assays), while sire did not contribute significantly. SLA behaved in group response fashion with dd, dg, gg and cc high or low in the rank order. An apparent effect of age was significant but subsequent studies suggest a cyclical effect with low response at 8 weeks, possibly due to weaning-related stress.

Antibody avidity and maturation of avidity between primary and secondary immune responses were not significantly influenced by SLA but were by litter (p≤ 0.01–0.05). Pigs of the dd haplotype had significantly greater avidity maturation than the others as did the dg and gg haplotypes although not significantly so (Appleyard *et al.,* 1991a).

RESULTS OF SELECTION EXPERIMENTS WITH YORKSHIRE PIGS (Mallard *et al.,* 1989d)

In the F0 generation, sire and gender influenced primary response to HEWL (p≤ 0.05) but not to (T,G)-A,L while sire significantly influenced secondary response to both antigens.

Heritability and genetic correlations were used to determine traits for inclusion in the selection index. These were HEWL antibody (day 21 pi), IgG concentration (day 0), DH to PPD (24hr post-stimulation), lymphocyte blastogenesis to con-A and killing of *S. typhimurium* by cultured blood monocytes. F1 progeny of the H and L groups differed significantly for HEWL, con-A and PPD responses (p≤ 0.03, 0.03 and 0.007) as well as for the correlated traits SRBC and CH to DNCB (p≤ 0.10 and 0.01). IgG concentration and monocyte functions did not differ by selection group. Heritability values for combined F0 and F1 generations were 0.25, 0.23, 0.08, 0.08 and 0.00. Avidity of antibody to HEWL was not correlated with antibody quantity, however the H group had significantly (p≤ 0.005) higher average avidity than the L group (Appleyard *et al.,* 1991b). At the third generation of selection the lines are significantly separated by EBV for HEWL, con-A and PPD. Production characteristics also favoured the high response line.

The results suggest that resistance-related traits can be influenced by selection using EBV and that MHC genotype has a variable influence which is generally less than other terms such as litter. It remains to be determined if there are differences in disease resistance between SLA haplotypes or between selected lines.

Trypanotolerance may be obtained by selective breeding based upon the relatively resistant N'Dama breed using resistance-related traits, identified in comparison of N'Dama and the relatively susceptible Boran cattle, as indirect markers. Difficulties inherent in the low fecundity of cattle may be overcome by use of advanced breeding methods including *in vitro* fertilization of prepubertal oocytes and embryo cloning (Georges and Massey, 1991). If low and high response groups were selected simultaneously the progeny would facilitate mapping gene loci which may contribute to trypanotolerance and the availability of divergent response groups would aid ongoing investigations of the mechanisms of resistance as well as attempts to develop alternative control systems such as vaccines or chemotherapeutics. Efficacy in the low response group would be the best predictor of efficacy in random populations. Since trypanosomiasis is not the only indigenous disease in areas of interest the potential of the selection scheme to enhance overall disease resistance would minimize risk. Also, observed production efficiency benefits in similarly selected pigs may indicate that productivity could also be improved in cattle simultaneously with improved disease resistance.

An integrated program of selective breeding based upon EBVs, advanced embryo transfer and artificial insemination technology, vaccine and chemotherapy development and gene mapping may pragmatically improve disease resistance in African cattle and establish infrastructure for an indigenous cattle improvement and breeding industry.

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INTERNATIONAL TRYPANOTOLERANCE CENTRE

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Following the successful transfer of N'Dama embryos from The Gambia to ILRAD in 1983 and the subsequent demonstration by ILRAD scientists that trypanotolerance is undoubtedly genetically based, more interest has been given towards understanding the genetics of trypanotolerance.

In 1987, ITC organized a consultation, with FAO funding, which was carried out by Soller and Beckman with a view to preparing a project document and funding proposal for a project entitled `Towards an understanding of the genetic basis for trypanotolerance in the N'Dama cattle of West Africa'. ITC also organized a workshop in London in January 1988 which resulted in a project document and funding proposal entitled `Mapping trypanotolerance loci of the N'Dama cattle of West Africa'. Unfortunately these projects were not funded. However, ITC undertook to produce Zebu × N'Dama animals and the first batch of F1 calves was born in December 89/January 90. ITC has collaborated with various institutes by providing DNA and specimens for BoLA typing from Zebu, N'Dama and Zebu × N'Dama animals.

ITC is placed in an ideal position as a source of genetic material with regard to trypanotolerant cattle. ITC owns a total of approximately 2000 N'Dama cattle at 3 stations including 600 females of breeding or near breeding age. N'Dama bulls bred at ITC have been tested for trypanotolerance following artificial infection by monitoring PCV levels and weight changes during infection. A small breed herd of Zebu (Senegalese Gobra) is also kept to provide comparison between trypanosensitive and trypanotolerant cattle. The males of this breed have been used to provide the sires for the F1 animals (Zebu × N'Dama). At present there are 20 F1 animals aged 16 months.

The EEC-funded (ILRAD/ILCA) component of ITC has been working in Gambian village herds for the past 5 years. Data on health and productivity of N'Dama cattle in different tsetse challenge situations has been collected. Thus cohorts of high producing animals living under various levels of natural tsetse challenge can be identified. Gambian herd owners are co-operative and ITC staff now have considerable experience in working in village situations.

ITC has a good relationship with the Department of Livestock Services of the Ministry of Agriculture of The Gambia. At present, as a result of the Livestock Department Project, ITC has access to approximately 50,000 village animals, i.e. one sixth of the Gambian national herd.

In addition ITC has signed, or is in the process of signing, approved Memoranda of Agreement with members of OMVG states (Gambia, Senegal, Guinea Bissau and Guinea Conakry) and the MRU (Mano River Union) states, which are Guinea Conakry, Liberia and Sierra Leone. These are institutional arrangements with the National Agricultural Research Systems of these states. The importance of these agreements is emphasized by the fact that almost 80% of all N'Dama cattle are found in these six countries. ITC has also recently signed a Memorandum of Agreement with CRTA which

is situated in Burkina Faso. This agreement will foster closer links between ITC and CRTA and is important as CRTA carries out work on the other major trypanotolerant breed, the West African Shorthorn.

In summary ITC has access to large numbers of trypanotolerant cattle both on station and at village level in The Gambia. Trypanosensitive Zebu cattle originally from Senegal are available and these have been used to produce a first group of Zebu × N'Dama animals. ITC has developed links with major research institutes in the sub-region which could allow access to a very large number of trypanotolerant animals in the future.

Thus ITC is in a strong position to collaborate on a formal basis with other institutes in research directed at the understanding and utilization of trypanotolerance in cattle.

INTERNATIONAL LIVESTOCK CENTRE FOR AFRICA

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This paper focuses on some aspects of, and relationships between, trypanosome infection, anaemia control and animal performance that have been recently evaluated in sites of the African Trypanotolerant Livestock Network in Gabon and Zaire, using N'Dama cattle. The results are discussed in the time sequence of the steps actually followed. Some results are published and are available, some in press and some recently submitted.

RELATIONSHIPS BETWEEN CRITERIA OF TRYPANOTOLERANCE AND LIVESTOCK PERFORMANCE

At Mushie Ranch, Zaire, calving interval records were built up from N'Dama cows maintained for 3.5 years under a high natural tsetse-trypanosomiasis challenge. Monthly blood samples were examined by the buffy coat method to detect the presence of trypanosomes. The species of trypanosome was identified and the intensity of infection quantified as a parasitaemia score. The degree of anaemia was quantified by measuring packed red cell volume percent (PCV). Attempts were made to control other possible causes of anaemia; ticks by weekly dipping and internal parasites through a pasture management system involving extensive grazing conditions, no night paddocks, and regular burning of pastures.

Trypanotolerance criteria and cow productivity

The comparative influences of time detected parasitaemic, parasitaemia intensity representing control of development of parasitaemia, and PCV value representing control of development of anaemia were measured on calving interval, calf weaning weight and cow productivity (weight of weaner calf per cow per annum) using least squares mixed model procedures.

Results are summarized in Table 1 and significant findings included cows detected parasitaemic for a low length of time having a 14% shorter calving interval and a 15% higher productivity than their contemporaries that were parasitaemic for a high length of time. The effects of parasitaemia intensity were not significant. In contrast, animals maintaining a high PCV value had an 11% shorter calving interval, a 9% heavier calf weaning weight and a 24% superior cow productivity over those maintaining a low PCV value. So, control of development of anaemia, as measured by average PCV value, appeared to be the criterion of trypanotolerance most closely linked to overall cow productivity, in this production system where attempts had been made to control systematically other possible causes of anaemia.

Repeatabilities of trypanotolerance and performance traits

Repeatabilities, between calving intervals, for the three trypanotolerance criteria and the three performance measures, all expressed as traits of the cow, were computed. Traits

TABLE 1. Comparative sizes of influences of trypanotolerance criteria on productivity.

Criteria	Calving interval		Calf weaning weight		Cow productivity	
	days	%	kg	%	kg	%
Low versus high time detected parasitaemic	−68	−14.2	2.8	2.1	17.1	15.5
Low versus high parasitaemia score (within high time detected parasitaemic)	−20	−4.1	3.2	2.0	5.6	5.2
High versus low PCV (within low time detected parasitaemic)	−27	−6.3	7.8	5.8	12.6	10.4
High versus low PCV (within high time detected parasitaemic)	−59	−11.5	12.2	9.4	23.7	24.1

with significant repeatabilities were calf weaning weight (0.35), average PCV over the calving interval (0.33) and time detected parasitaemic during the calving interval (0.23). The repeatability of PCV value was reasonably high and almost equal to that of calf weaning weight. Thus the ability to control development of anaemia, as indicated by PCV value, might well be a useful criterion of trypanotolerance with which to identify more trypanotolerant individual animals.

Criteria assessment in young animals

Simultaneous evaluation of the relative effects of criteria of trypanotolerance, in both the preweaner calf and its dam, on calf performance (weaning weight) showed that calf PCV values were at least as important as dam PCV values. Thus evaluation of criteria of trypanotolerance in an animal might be feasible before it reached maturity, but would need to be sufficiently long after weaning for the preweaning influence of the dam to have disappeared.

A FIELD TEST FOR TRYPANOTOLERANCE IN YOUNG N'DAMA CATTLE

The Zaire study suggested that investigations into practical field tests for trypanotolerance should focus on the use of post-weaners, maintained for varying lengths of time in as high natural tsetse-trypanosomiasis challenge situations as possible. Three such tests were carried out in which a total of 436 one-year-old N'Dama cattle were maintained for 12, 18 and 24 weeks under a medium tsetse-trypanosomiasis challenge at the Government Ranch (OGAPROV) in Gabon.
 Every 4 weeks in the first test, and every 2 weeks in the second and third tests, blood samples were examined by the buffy coat method to measure infection criteria. The degree of anaemia was estimated by measuring the PCV. Attempts were made to control other possible causes of anaemia. On the last day of the first and second tests all animals were treated with Samorin (isometamidium chloride) at the rate of 1.0 mg/kg bodyweight by intramuscular injection to evaluate the recovery of PCV values.

Trypanotolerance criteria and animal growth

The comparative sizes of the influence of parasitaemia control and anaemia control on N'Dama daily liveweight gain are summarized in Table 2. Ability to control the

TABLE 2. Comparative sizes of influences of parasitaemia control and anaemia control on growth. (Constructed from least squares means for classes tested within all periods when detected as parasitaemic.)

Test number	Statistic	Parasitaemia control		Anaemia control	
		High score	Low score	High PCV	Low PCV
1	Animal numbers	57	57	58	56
	Growth (g/day)	248	273	283	238
	S.E.	23.5	23.9	21.0	20.6
2	Animal numbers	51	51	46	56
	Growth (g/day)	97	106	135	68
	S.E.	14.4	11.0	27.2	26.3
3	Animal numbers	27	49	40	36
	Growth g/day)	81	89	111	59
	S.E.	15.6	19.5	21.1	32.0
Overall	Animal numbers	135	157	144	148
	Growth (g/day)	142	156	176	122
	S.E.	19.0	19.1	23.2	25.9
Mean difference in growth (%)			+10	+44	

development of anaemia had a major effect on daily weight gain, four times that of the ability to control parasitaemia. Above-average PCV values, as a measure of anaemia control, resulted in a 44% superior daily weight gain over below-average PCV values.

Post-test recovery of PCV values after trypanocidal drug treatment

At the completion of the first test (Table 3), trypanosome prevalence was 30%, and the PCV values of groups that had been detected parasitaemic for varying percentages of the 12 week test period ranged from 33.1% for those never detected as parasitaemic, to 18.8% for those parasitaemic for 80% of the period. Coefficients of variation, indicating the amount of variation in PCV values within each group, ranged from 15.9% in animals never detected as parasitaemic to 26.5% in animals parasitaemic for 80% of the test period. On the last day of the test all animals were treated with Samorin at the rate of 1 mg/kg. When PCVs were measured 30 days later, major recovery of PCV had taken place, even those parasitaemic for 80% of the test period had a PCV value of 34%, and the coefficients of variation within all groups were reduced to 8.7%.

In the second test (Table 4), 73% of the recovery achieved one month after the trypanocidal drug treatment had been reached in 9 days.

GENETIC ASPECTS OF CONTROL OF ANAEMIA DEVELOPMENT

Blood typing for parentage determination allowed genetic parameters of measures of control of anaemia to be evaluated.

The heritabilities of, and genetic and phenotypic correlations between growth, average PCV and lowest PCV reached on test are shown in Table 5. The heritability of bodyweight at the start of the test when animals averaged 50 weeks of age was 0.49 ± 0.32. This is within the normally reported range for this trait, while the large standard error could be a reflection of the small number of five progeny available per sire. When all environmental and parasitaemia information was taken into account, the heritability

TABLE 3. PCV response to trypanocidal drug treatment after 1987 test.

Percentage of time detected parasitaemic	PCV at treatment (%)		PCV 30 days after treatment (%)	
	Mean	c.v.[*]	Mean	c.v.[*]
0	33.1	15.9	36.5	8.7
25	26.7	18.7	34.8	8.7
50	22.1	22.8	33.0	8.8
80	18.8	26.5	34.0	8.7

[*] Coefficient of variation

TABLE 4. PCV responses to trypanocidal drug treatment after 1988 test.

Percentage of time detected parasitaemic	PCV at treatment (%)		PCV 9 days after treatment (%)		PCV 28 days after treatmen (%)	
	Mean	c.v.[*]	Mean	c.v.[*]	Mean	c.v.[*]
0	33.2	12.2	36.9	8.0	37.7	8.5
10	33.6	12.0	37.9	7.8	38.8	8.2
20	31.9	12.8	36.1	8.3	38.3	8.4
35	29.1	13.9	34.0	8.7	34.9	9.1
60	27.2	14.9	32.3	9.2	35.5	9.0

[*] Coefficient of variation

of growth over the test period was 0.39 ± 0.32, again within the expected range for growth over a 3-month period. The heritabilities of both PCV measures were higher than the corresponding heritability of growth in all analyses and were 0.64 ± 0.33 and 0.50 ± 0.32 when all environmental and parasitaemia information was utilized.

When all environmental and parasitaemia information was taken into account, the genetic correlation between average PCV and growth was 0.70 ± 0.42 and between lowest PCV reached and growth, 0.28 ± 0.55. These values, coupled with the higher heritabilities of the PCV measures, indicate some possibility of selection on PCV values for control of anaemia development.

So at this stage it seemed possible to achieve something with the anaemia control component as measured by PCV when infected, but not with actual infection data as measured by the buffy coat technique.

ANTIGEN-DETECTION TEST FOR MORE ACCURATE MEASUREMENT OF TRYPANOSOME INFECTION

In an attempt to measure infection more precisely, antigen-detection enzyme immunoassays developed by Dr. V. Nantulya and colleagues at ILRAD were used for the diagnosis of *Trypanosoma vivax, T. congolense* and *T. brucei* in the same group of N'Dama cattle in Gabon. The assays are based on monoclonal antibodies which recognize trypanosome antigens specific for each of the three species.

TABLE 5. Heritabilities of, and genetic and phenotypic correlations between, growth, average PCV and lowest PCV reached on test.

	Growth	Average PCV	Lowest PCV reached
(a) Parasitaemia detection and parasitaemia score not included in analysis			
Growth	0.22±0.28	0.41±0.73	−0.13±0.74
Average PCV	0.35	0.35±0.30	0.96±0.20
Lowest PCV reached	0.29	0.72	0.48±0.31
(b) Parasitaemia detection included in analysis			
Growth	0.38±0.30	0.71±0.42	0.28±0.55
Average PCV	0.32	0.63±0.33	0.99±0.17
Lowest PCV reached	0.25	0.66	0.51±0.32
(c) Parasitaemia detection and parasitaemia score included in analysis			
Growth	0.39±0.31	0.70±0.42	0.28±0.55
Average PCV	0.32	0.64±0.33	1.00±0.17
Lowest PCV reached	0.25	0.67	0.50±0.32

Buffy coat and antigen test results

An average of 6 assays per animal were carried out over a 92-day period. Of the animals detected parasitaemic by the buffy coat technique, 90% were positive to the antigen test. More importantly, 40% of the animals with negative parasitological findings were also found to be antigen positive.

Genetic aspects of parasite control

When antigen positive, parasite negative animals were classified as having more ability to control parasite growth than parasitaemic animals, a significant sire effect suggested some possibility of a degree of genetic control being involved. Thus the ELISA might offer a practical possibility for selection of trypanotolerant animals based on infection criteria.

ANTIGEN-ELISA AND EFFICIENCY OF SELECTION OF N'DAMA CATTLE

Further work was then set up to more accurately evaluate relationships between trypanosome infection as measured by antigen detection enzyme immunoassays (antigen ELISA), anaemia as determined by average packed red cell volume (PCV) and animal performance as assessed by daily weight gain. Ninety-nine N'Dama cattle in Gabon were exposed to natural tsetse challenge at 11.5 months of age and recorded 14 times over a 13-week period.

Prevalence of mixed species infections

Approximately half the animals were found to be infected for an average of 5 of the 14 times that they were examined: 38% with *Trypanosoma congolense,* 13% with *T. vivax* and 49% with a mixed infection.

TABLE 6. Parasitaemia and antigenaemia relationships.

Parasitaemia status	Antigenaemia status	Animals Number	%
Positive	Positive	38	90
Positive	Negative	4	10
		42	
Negative	Positive	43	40
Negative	Negative	63	60
		106	

TABLE 7. Heritability estimates of daily weight gain and trypanotolerance criteria using only parasitaemic and antigenaemic animals.[*]

	$h^2 \pm$ S.E.
Daily weight gain	0.33 ± 0.47
Lowest PCV reached	0.45 ± 0.48
Average PCV	0.57 ± 0.49
Parasite control	1.08 ± 0.50

[*] 79 progeny of 21 sires

Effects of *T. vivax* and *T. congolense* on animal growth

T. congolense infections had significant deleterious effects on animal growth, while *T. vivax* infections did not. Animals found, on several repeated occasions, to be infected with *T. congolense* had significantly lower PCV values than those demonstrated to be infected on fewer occasions. Animals capable of maintaining PCV values, even when antigen ELISA-positive on a high number of occasions, grew at the same rate as uninfected animals. Animals that could not maintain PCV values when infected had poorer growth.

Antigen ELISA has the potential to increase the efficiency of selection of trypano-tolerant N'Dama cattle under tsetse challenge in the field in three main ways. Accurate identification of trypanosome species, especially in mixed species infections, clarifies linkages between infection, anaemia and animal performance. Detection of animals antigenaemic without patent parasitaemia could allow individuals with superior ability to control trypanosome infection to be identified. More accurate measurement of the proportion of time an animal is infected allows more accurate evaluation of its anaemia control capability.

INTERNATIONAL LIVESTOCK CENTRE FOR AFRICA

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Any comprehensive livestock productivity and disease research program for Africa must aspire to a better understanding and exploitation of trypanotolerant livestock. This research is part of ILCA priorities and continuing support (ILCA, 1987).

ILCA is coordinating international research on the better understanding of factors affecting the performance of trypanotolerant animals and effectiveness of trypanosomiasis control measures in collaboration with its partners in sub-Saharan Africa (Network participants), overseas and with ILRAD.

Research programs were established in order to improve livestock production in tsetse-affected areas of Africa by achieving a better understanding of genetic resistance, acquired resistance, environmental factors which affect susceptibility and the efficacy of control measures, and by ensuring optimal application of both existing knowledge and recent research findings. A network for trypanotolerance research was developed because it was impossible to cover the many aspects involved in the problem of trypanosomiasis and trypanotolerance in one or very few research situations.

The African Trypanotolerant Livestock Network was established in 1983 in different trypanosomiasis risk situations throughout tropical Africa. A very large body of data has now been built up on various breeds and their crosses under different levels of trypanosomiasis risk in different ecological and management situations. A comprehensive description of all the Network sites and the standard research methodologies used has been given in the publication entitled *ILCA/ILRAD Trypanotolerance Network: Situation Report, December 1985.* There were 13 collaborative research situations in 10 countries—Senegal, The Gambia, Cote d'Ivoire, Togo, Nigeria, Gabon, Zaire, Ethiopia, Kenya and Tanzania (ILCA, 1986a; 1986b).

Standard protocols were defined for the collection and recording of data, usually monthly, and a system established for its entry, valuation, storage, analysis and return. For the past four or so years, all this has been carried out using microcomputers both at individual sites and at the Nairobi coordinating office of the Network. This has proved to be very effective, and descriptions of the approach and results of the first 3 years of research were published by ILCA and ILRAD in 1988 as the proceedings of a Network meeting (ILCA/ILRAD, 1988).

The objectives in the first years were to establish baseline values for assessing levels of animal health and productivity in the different situations. This having been achieved, internationally recognized specialists reviewed, at the same Network meeting, their fields of interest and contributed to Network development through evaluation of the results presented by Network scientists, and they indicated subject areas where, in order to maximize subsequent progress in the Network, it would have been advantageous to redeploy some resources, modify or redesign some protocols and design further in-depth studies. Aspects considered included the representativeness of Network sites, the modifications of the standardized techniques used, studies to quantify genetic variation

for trypanotolerance and the design of intervention experiments. As a result, the research program is now more focused on fewer sites with research being undertaken in greater depth under the following themes:

- Trypanosomiasis epidemiology, including estimates of tsetse challenge to evaluate the possible contribution it can make in planning for increased livestock production; diagnosis of trypanosomiasis; factors affecting susceptibility; interactions with other diseases.

- Effects of trypanosomiasis on animal performance, reproductive cycle and milk extraction.

- Identification of trypanotolerance traits and their linkages with animal performance.

- Definition of selection criteria for trypanotolerance and to estimate phenotypic and genetic variances of, and covariance between, trypanotolerance and production traits. Our aims under this theme are firmly anchored in conventional quantitative approaches to the genetic improvement of disease resistance, which provide information of direct practical value for animal breeding.

- The possibility of marker gene identification as an additional potential tool for the identification and use of more trypanotolerant individuals (with ILRAD).

- Biological and economic evaluation of productivity responses to intervention: the aim in this theme is to develop and evaluate control aspects to the point that they are incorporated into packages to be delivered as soon as practical to farmers. It is through this theme that research results are integrated into strategies to improve livestock productivity in tsetse-affected areas of Africa.
Work areas include:

- the use of insecticide-impregnated traps and screens for tsetse control;

- the effect of trypanocidal drugs on cattle health and production;

- the efficacy of nutritional supplementation to reduce the deleterious effects of environmental and physiological stresses on stability of trypanotolerance and productivity under trypanosomiasis risk;

- the introduction of N'Dama cattle in village farming systems in tsetse-affected areas of central Africa where cattle husbandry was not practised in the past; and

- development and evaluation of pilot selection programs based on criteria of trypanotolerance.

Network research stations are tackling components of trypanotolerance research they are best equipped to handle in a cost-effective manner. They are grouped to complement each other in terms of disease level, tsetse challenge, livestock breed and specific production systems: Sites in The Gambia and Senegal at the International Trypanotolerance Centre (ITC) and at the Institut Senegalais de Recherches Agronomiques Kolda (ISRA) concentrate on research in nutrition, reproduction and milk extraction under various levels of trypanosomiasis risk; sites at the Societe de Developpement des Productions Animales (SODEPRA) in Boundiali, Cote d'Ivoire, on research involving interaction between trypanosomiasis and other diseases, tsetse control aspects, and on comparative returns from the use of trypanotolerant cattle and sheep; sites in Gabon and Zaire on genetic and animal breeding aspects under high natural trypanosomiasis challenge; and associated sites in Ethiopia and Kenya on susceptible livestock (ILCA, 1990).

There are currently 6 countries where collaborative research activities are coordinated by ILCA from west to east: Senegal, The Gambia, Cote d'Ivoire, Gabon, Zaire and Ethiopia. Activities in Burkina-Faso at the Centre de Recherche sur les Trypanosomoses Animales (CRTA) and in Kenya are also conducted in liaison with the Network but ILCA inputs are at the planning and exchange of results levels.

ILCA's ability to link international, regional and national organizations into collaborative research programs providing extensive access to on-farm experiments throughout the ecological zones concerned by trypanosomiasis greatly contributes to its successful achievements in trypanosomiasis research.

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ROUNDTABLE DISCUSSION

Roundtable discussion—a summary

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Although the Workshop was primarily concerned with the application of a linkage map consisting of 150–300 highly polymorphic markers in families segregating trypanotolerance, it became clear from the outset in discussion that although various groups were now in the process of developing markers, the mapping community had not developed a formal means of coordination of effort leading to an `international map', such as that built on the CEPH material for human mapping. It was agreed that in view of the fact that resource families segregating traits of interest (including trypanotolerance) were now in the process of construction and that the means to fund bovine mapping programs in Europe and North America were under active discussion (but with no firm decisions yet made), the present meeting was timely.

All participants were agreed that as far as the Workshop discussion was concerned, there were two basic areas for consideration, these being, first, coordination of development of a map and, second, its application to trypanotolerance.

The group felt strongly that in the development of maps, common reference families for placing marker loci and genes were essential to efficient progress. It was pointed out (Womack, Hetzel) that the Texan and Australian groups had already agreed to share their reference family material and put their respective markers across each other's families. Womack suggested that the 2 largest ILRAD families (N'Dama × Boran), added to the families in Texas and Australia, would produce good family material to be shared by all. There was some concern (Fries) that not all laboratories with an interest in bovine gene mapping were represented (although all of the most active laboratories were) and that others may have, or be in the process of developing, useful families. However some participants (Hetzel, Womack) pointed out that they were already funded to obtain linkage information and wished to proceed with the material now known to be available. It was agreed that international collaboration beyond the rather informal links which currently existed between some of the groups represented was unlikely to be furthered to any significant degree before the 1992 meeting of the ISAG at Interlaken.

Following discussion of views previously expressed concerning focusing on the immediately available ILRAD/Texan/Australian families, the group agreed to a suggestion from Dr. Hetzel to draw together information on these families which Dr. Williams would take to an imminent meeting of potential European mappers (Edinburgh, 13–14 April). Following a suggestion (Wilkie) that the journal of the ISAG *(Animal Genetics)* should be used to build consensus around the available families, Drs. Hetzel, Womack and Teale agreed to seek to place the collated information on the families in the journal and invite their use. It was agreed that in this way it might be possible to achieve the degree of coordination in bovine linkage map development which so far has failed to materialize. The consensus view was that collaborative mapping should therefore proceed using the 3 family sets with the possibility of including others which may

become available on the basis of discussions in Interlaken in 1992, involving a wider group.

On the second subject of discussion, development and use of trypanotolerance resource material, all of the active mapping groups represented expressed their willingness to contribute to the development of markers of trypanotolerance in the ILRAD resource families. There was general recognition of the importance of the trait and of the usefulness of markers once developed for marker-assisted selection and ultimately for identification of trypanotolerance genes. Dr. Teale recognized that as the current experiment proceeded, it might become apparent that the original design of the F2 population may not be optimal and pointed out that the ILRAD group would welcome input from those present on the best way to construct the third generation. Dr. Soller agreed to give consideration to family development based on the latest approaches to linkage identification and to communicate results to ILRAD in July.

There was agreement that the approach to F1 development and phenotyping for trypanotolerance currently being taken at ILRAD was appropriate. Dr. Soller made the point that in the case of the Israel group, the ability to map trypanotolerance simultaneously with map development made acquisition of funding considerably easier. Indeed, at the present time, it was only in this way that the Israel Laboratory was able to proceed.

It was suggested (Hetzel) that, although not a requirement of the trypanotolerance mapping exercise, it would be valuable to include purebred N'Damas raised alongside F1 and Boran animals in comparative challenge experiments. Dr. Teale agreed to give consideration to breeding of a limited number of N'Dama at Kapiti Plains Estates (where F1s and Borans are reared prior to challenge) for this purpose. This would involve transferring N'Dama embryos from ILRAD to Kapiti Plains Estates for implantation into Boran surrogates.

The Workshop discussion briefly touched on the management of mapping data as it accumulates. Dr. Fries indicated his willingness to continue recording data until such time as an agreed computerized database could be established. Drs. Hetzel and Williams both indicated that there were possibilities of establishing computerization of a bovine linkage map in their respective laboratories. Discussion was not taken further as it was felt that decisions on databases and their management could be deferred for discussion in a broader forum at Interlaken.

Dr. Teale raised the question of funding, pointing out that this could be considered in the separate contexts of linkage map development and trypanotolerance. The general indications from the group were that funding was available to them for the foreseeable future for linkage map development. This was the case for the Edinburgh, Zurich, College Station, Rockhampton, Jouay-en-Josas and Nairobi groups. As previously indicated, this was tied to trypanotolerance mapping in the case of the Jerusalem Laboratory. It was, however, emphasized by the group that rate of progress with development of maps and with their application was almost directly proportional to the available funds. Additional funding could therefore speed the process of development and mapping of markers.

It was suggested (Teale, ole-MoiYoi) that ILRAD might be persuaded to seek funding on behalf of an international collaborative group. They felt that this particular research area and the approaches being taken were in tune with current CGIAR thinking and strategies, which are to some extent based on identification, conservation and utilization of genetic resources for sustainable and environmentally sound improvement in food productivity.

Dr. Gray (Director General, ILRAD) made the point that ILCA could make a significant contribution in this area, particularly as the research and application of results fitted within the broad outlines of ILCA's mandate. Dr. Trail agreed and suggested that the value of a linkage map as far as ILCA is concerned would not be restricted to its use in mapping trypanotolerance genes. Dr. Gray suggested that Dr. d'Ieteren bring this to the attention of ILCA management and assess response which could then be communicated to his ILRAD colleagues.

Dr. Gray also suggested that a `cover document' outlining the requirements for, and application of, a bovine linkage map, naming collaborators and specifying roles would be a valuable first step in seeking funding from international funding agencies. Dr. Teale undertook to stimulate efforts as far as possible to obtain funding through the Centres for trypanotolerance gene mapping, which would serve to finance an International Bovine Trypanotolerance Gene Mapping Group. Funds would be used for purchase of consumables, support of additional staff in collaborating laboratories, communications, database management and analysis, and for travel expenses.

The Chairman (Dr. ole-MoiYoi) then requested the participants to state what contributions they were in the process of making, and those which they would like to make, both in the area of map development and in application to trypanotolerance. Responses were as follows:

Soller (Jerusalem). The Jerusalem Laboratory would clone 40–60 new markers over the next two years and score 20–30 of them in the trypanotolerance families at ILRAD. The markers could be made available to other laboratories for mapping in the other reference families (Texan and Australian).

The Jerusalem Laboratory would also concentrate effort on development of statistical techniques for mapping of trypanotolerance genes and, in the process, reassess the number of F2 animals required in the current ILRAD experiment.

Womack (College Station, Texas). The Texas Laboratory would undertake to make their somatic cell hybrid panel generally available and would be prepared to both distribute DNA and apply markers from other laboratories to the panel in Texas.

The Laboratory has 6 large full-sib families from which two would be selected for reference material. Texas would undertake to collect and disseminate DNA from their families and also distribute DNA from the other reference families.

The intention in Texas was also to develop 20–30 markers over the next two years which would be run over the reference and trypanotolerance resource material.

Hetzel (Rockhampton). The Australian Laboratory intends, with outside help, to map 160 markers in their families over the next two years, approximately 50 of which were expected to be generated in-house.

Dr. Hetzel was prepared to help in distribution of DNA from all reference families. He also informed the group that his laboratory had established the basis of a database and had acquired the CEPH software. He was prepared to use this database for information on the reference family set.

The Laboratory would attempt chromosome-specific libraries, whilst recognizing the difficulties involved. It would also undertake to assign syntenic groups to chromosomes using *in situ* hybridization technology.

Clifford (ITC, Banjul). ITC, as described in Dr. Clifford's presentation to the Workshop, is currently engaged in breeding N'Dama × Zebu animals and in assessing their response to challenge. Materials from, and information relating to, these animals will be made available.

Fries (Zurich). The Zurich Laboratory will map the 14 markers they are currently working with in the reference families decided by the Workshop. The Laboratory will also work up the system of cosmid cloning of microsatellites which may have particular value for *in situ* hybridization and thus for screening for physical location.

Dr. Fries will continue to record and make available map information obtained from the literature and mapping community until such time as this is taken over by the database.

Wilkie (Guelph). The Guelph Laboratory, being primarily interested in genetics of immune response, will extend its current activities in mini pigs to dairy cattle. By selective breeding, immune response (high and low) resource families will be constructed.

Grosclaude (Jouay-en-Josas). The French Laboratory will isolate microsatellite markers and also continue to produce and map `synthetic' tandem repeats. The Laboratory will put its markers across the trypanotolerance families.

Williams (Edinburgh). The Edinburgh Laboratory is about to begin marker generation. Developed markers will be mapped in the agreed reference families. The Laboratory is also committed to establishing a database. There are plans to identify resource populations segregating dairy traits.

Kemp (ILRAD). A major thrust of the Nairobi Laboratory will continue to be the generation of N'Dama × Boran families and their phenotyping for trypanotolerance. DNA from 3 generations of two of the large crossbred families will be made available as reference material for marker/gene mapping. DNA from all of the animals will be made available to those laboratories collaborating on trypanotolerance mapping.

The Laboratory also plans to produce 25–30 microsatellite markers and map these in the reference families over the course of the next two years.

Recommendations

- For the purpose of developing a linkage map of the bovine genome, every effort should be made to focus the attention of all laboratories in the field on a common set of reference families. Drs. Hetzel, Teale and Womack should collate information on the best families in Australia, Kenya and the USA and then, through Drs. Williams and Grosclaude, seek other nominations from the European group meeting in Edinburgh from 13–14 April. A selected family set should then be detailed in *Animal Genetics,* inviting their widespread use as bovine reference families.

- The recommended reference family set should be reviewed at the next meeting of the International Society for Animal Genetics (ISAG) in Interlaken in 1992.

- An International Bovine Trypanotolerance Mapping Group (IBTMG) should be formed to further collaborative efforts to identify markers of trypanotolerance in N'Dama cattle.

- ILRAD, possibly together with ILCA, should attempt to obtain funding for an IBTMG at a level which would support the manpower and consumables requirements to run markers across trypanotolerance families in the collaborating laboratories. Other expenses which would need to be covered are those relating to database management, data analysis, communications, travel and secretarial expenses.

- The projected size of the F1 N'Dama $\times$ Boran families at ILRAD, and the construction of the third generation, should be reviewed in the light of developments in marker identification. Such a review might make a reduction in numbers possible.

APPENDIX:
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