QTL MAPPING AND GENE MARKERS FOR RESISTANCE TO INFECTIOUS DISEASES IN SHEEP AND CATTLE

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INTRODUCTION

The impacts of infectious diseases are of economic significance in both intensive and extensive livestock production systems. Currently, a combination of control strategies is utilized, relying on vaccination, sanitation, therapeutic/pharmaceutical applications, quarantine, and eradication. The selection of genetically resistant, or tolerant hosts as a further strategy has been encouraged by the widespread evidence for host genetic variation in resistance for most diseases of interest (Axford *et al.* 2000). However, conventional genetic improvement programmes aimed at increasing profitability have found it difficult to incorporate disease resistance in multi-trait breeding objectives. Hurdles have been difficulties in accurately and cheaply identifying resistant genotypes for breeding replacements, selection for resistance to multiple diseases simultaneously, co-evolutionary pressures on pathogens forcing the evolution of parasite resistance and the potentially negative impact of selection on production traits. It is also important to assess the potential effectiveness of marker based selection at the level of an entire population rather than at level of the individual (Gibson and Bishop 2005).

STATE OF QTL MAPPING FOR DISEASES OF ECONOMIC SIGNIFICANCE

The last 10 years has seen a myriad of so called Quantitative Trait Loci (QTL) mapping experiments in livestock species (Brenig *et al.* 2004). Some studies have recently emerged which have concentrated on the identification of QTL and/or genes which confer resistance to disease. Marker assisted selection (MAS) for disease resistance is particularly attractive since disease traits are generally difficult to measure, and in most cases have low heritability. Within the limited scope of this review, we restrict our interest to such studies in cattle and sheep. Within these species, infectious diseases having the greatest economic impact include parasites (nematodes and trematodes, trypanosomes, cutaneous myiasis,), bacterial diseases (brucellosis, mastitis, paratuberculosis or Johne's disease, dermatophilosis and salmonellosis) and viral diseases (foot and mouth, bovine leukaemia and blue tongue). However not all have attracted equal investment in QTL searches, with the major investigations described below. Typically the studies have fallen within two broad types. Many aim to identify novel regions within the genome that contain genes of moderate to large effect by employing full genome-wide screens, with subsequent investigation of positional candidate-genes located within QTL regions. The alternative and complimentary tactic is to examine candidate-genes directly based on prior information gained from functional studies, or comparative studies in other species. The synergistic information is starting to support genetic selection applications in the field.

Mastitis. Mastitis is a ubiquitous disease caused by a complex bacterial infection affecting the mammary tissue of cows with production losses of $3billion *per annum* in US dairy industry alone. Somatic cell score (SCS) is a trait measured in most commercial cattle herds, as an indicator for susceptibility to mastitis, and has been utilized in numerous full or partial genome QTL studies. Findings from twelve such studies were summarized by Khatkar *et al.* (2004) but ongoing updating of the combined QTL table may be found at www.vetsci.usyd.edu.au/reprogen/QTL_Map/. Figure 1 depicts all QTL discussed in this paper, in a chromosomal representation. BTA7, BTA18, BTA21 and BTA23 have five or more reports for QTL, although this may well reflect the greater proportion of times these chromosomes have been investigated. Only three QTL studies report on mastitis as a

phenotype (Figure 1), but with the exception of QTL on BTA11 and BTA14, little agreement between studies is evident. It is also hard to find any obvious alignment of QTL for SCS and mastitis, given that every chromosome barring BTA6, BTA17 and BTA25 now holds QTL for SCS. Prospective large scale QTL studies that are already underway in sheep include the Genesheepsafety E.U.-funded project involving collaborators from France, U.K., Italy and Spain which promises mastitis as a phenotype (Barillet *et al.* 2003), while for dairy cattle there is a large QTL project initiating in Germany (Schwerin *et al.* 2004). Rupp and Boichard (2003) review associations that have been made between genes (Blad, lactoferrin, lysozyme and the MHC) and mastitis resistance and conclude few offer scope for MAS without further work.

GI Nematodes. Gastro-intestinal nematodes (GIN) are responsible for loss of productivity and death in ruminants, through inefficiency of feed utilization and anaemia. Their management is increasingly difficult with the development of resistance to multiple classes of drenches, making alternative strategies of control more attractive. Results of 6 QTL studies with genome wide or partial scans in sheep have been reported (Figure 1), most recently reviewed by Dominik (2005). At least five other studies in sheep remain unreported ("Golden Ram" Merino project : Marshall *et al.* 2005, Indonesian Thin Tail Project : Raadsma *et al.* 2002, Maasai x Dorper at ILRI : Baker *et al.* 2003, Falkiner Merino Flock and SheepGenomics Australia Merino flock : Frank Nicholas, pers. comm.). The consensus alignment of QTL reported to date suggests that OAR1, OAR3, OAR6 and OAR20 may harbour gene(s) of interest (Figure 1). OAR3 in particular is almost always reported, which suggests that a highly influential gene controlling resistance is located here. Comparative genome analysis may also inform on chromosomal regions of importance to GIN resistance. Of relevance are the QTL and microarray studies being conducted on a 5 generation Angus cattle herd which has reported QTL following infection by *Ostertagia ostertagi* and *Cooperia oncophora* (Gasbarre *et al.* 2004). Targeted candidate gene association studies have been made with interferon gamma (OAR3), IgE, and Il-4, Il-5 and Il-10 (OAR5) (Sayers *et al.* 2005, Benavides *et al.* 2002, Clarke *et al.* 2001), and the MHC region (OAR20) (reviewed by Dominik 2005). However, failure to find an association has also been reported for the MHC and IFNG genes, which suggests caution in using these markers without prior evaluation (Crawford *et al.* 1997).

Johne's disease, Bovine tuberculosis, Salmonellosis and Brucellosis. Infection by *Mycobacterium avium* subsp. *paratuberculosis* leads to Johne's disease, a chronic enteritis of ruminants and humans with losses to the cattle industry estimated at $1.5 billion *per annum* in the US (Chacon *et al.* 2004). Association between disease resistance and genotypic variation has been found with the solute carrier family II member A1 gene (formerly NRAMP1), while IL-10, SOCS3, TNF-alpha and IL-12 expression levels varied between infected and potentially resistant uninfected cattle (Weiss *et al.* 2005). NRAMP1 was also found to be associated with natural resistance to intracellular bacteria in mice (from Barthel *et al.* 2001) and men, and maps to BTA2 in cattle (Zanotti *et al.* 2002). In cattle, SSCP analysis has revealed a microsatellite polymorphism in the 3` end of this gene to be associated with Salmonellosis and Brucellosis resistance (Adams and Templeton 1998) and examination of 135 Holstein Fresian cattle has associated NRAMP1 microsatellite alleles with *Mycobacterium bovis* resistance (Zanotti *et al.* 2002) although this association was not confirmed by Barthel *et al*. (2000).

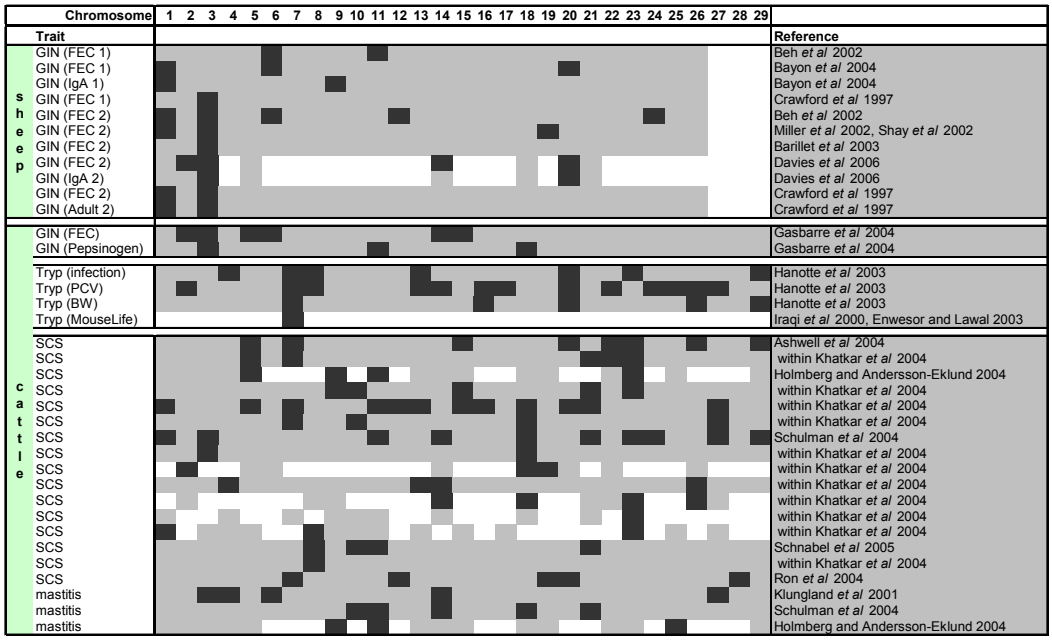

Figure 1. QTL studies in sheep and cattle. Dark grey=significant or suggestive. Light grey=non-significant. White=unexamined or non-defined. FEC=faecal egg count, 1=naïve infection, 2 = 2nd infection, Adult = adult worms, Pepsinogen = serum pepsinogen, Tryp = trypanosome, PCV = packed cell volume, BW = body weight, mouse life = putative position of the *Tir1* locus of mouse in the cattle genome.

Resistance to tick infestations and tick borne diseases. Ticks and tick borne diseases may affect up to 80% of the world's cattle population (Minjauw and de Castro 2000) with associated impacts of $13-19 billion *per annum*. Natural resistance to the tick *Boophilus microplus* is being seen as an alternative to the use of acaricides, as they are increasingly expensive and ineffective due to acaricide-resistance. Segregation analysis has suggested that a recessive QTL for tick resistance may be segregating in tropical beef cattle populations, independent of QTL for worm resistance (Henshall *et al.* 2001). Significant association studies have been carried out between tick resistance and MHC class I alleles (Stear *et al.* 1984) and class II alleles (Acosta-Rodrigiez *et al.* 2005).

Trypanosomiasis. Trypanosomiasis is a parasitic disease carried by the Tsetse fly in tropical Africa, resulting in anaemia, a lowering of immune responsiveness, appetence and often death if left untreated. Estimated costs are in the order of $1.34 billion annually in Africa (Kristjanson *et al.* 1999). The estimated potential benefit of introducing an effective form of trypanosome control is $700 million per year (Kristjanson *et al.* 1999), which highlights the potential importance of employing host trypanotolerance in breeding. Hanotte and co-workers (2003) have reported on the only QTL study of trypanotolerance in N'Dama and Boran cattle. An autosomal screen was carried out with phenotyping of packed cell volume, level of parasitaemia, and body weight following infection with *T. congolense* (QTL results in Fig. 1).

Ovine Footrot, Flystrike and Body Strike (cutaneous myiasis). These three cutaneous diseases are considered to be the greatest economic impact in sheep production after internal parasitism. Association has been made with footrot resistance and the ovine MHC (Litchfield

et al. 1993, Escayg *et al.* 1997). Associations described in Escayg *et al.* (1997) underpin the development of one of the world's first genetic tests for footrot resistance, as discussed later.

Dermatophilosis. Infection with *Dermatophilus congolensis,* can result in a bacterial skin disease in both sheep and cattle causing loss of productivity and a 15% mortality rate in extreme cases. Maillard *et al.* (2002) report on a very strong association between a BoLa Class II haplotype and relative resistance/susceptibility among two groups of unrelated zebu Brahman cattle, classified according to their widely differing reactions to infection. The association has been confirmed in five other populations, including other breeds. They selected solely against the susceptibility haplotype in a field trial, and successfully reduced the incidence of disease from 76% to less than 2%, while maintaining MHC variability.

Fasciolosis in sheep and cattle. Both temperate and tropical infection with *Fasciola hepatica* and *Fasciola gigantica* respectively, have an estimated impact on production of $US 3 billion *per annum* (Piedrafita *et al*. 2004). Relatively few investigations have been carried out on genetic sources of variation in these diseases with some anecdotal evidence for breed variation in both sheep and cattle (Piedrafita *et al*. 2004). Raadsma *et al*. (2002) report on a medium size study to identify QTL for fasciolosis in sheep and through comparative genomics identify equivalent regions in cattle. Reports to date suggest significant QTL for fluke number on OAR 10, 11, 17 and 18, with simultaneous immunological studies identifying functional candidates.

FROM QTL TO QTN

Most primary QTL screens have confidence intervals that are typically in the order of 10's of centimorgans, thus encompassing potentially 100's of positional candidate genes. Without strong evidence for a positional candidate, further refinement in map position is required to target functional mutations (Quantitative Trait Nucleotide or QTN) in causal gene(s) underlying the QTL. Fine mapping may refine the QTL location to no less than the smallest haplotype structures, in the order of 500-50kb in sheep and cattle. While the temptation may be to saturate the primary QTL mapping resources with additional markers in target regions, this is unlikely to be of benefit in linkage analyses (LA), since typically no additional recombination is observed. A solution is to utilize historical recombination events within the population from which the mapping family is drawn. Such linkage disequilibrium (LD) mapping in combination with LA adds significant power to fine mapping. However even with LA/LD analysis, it is unlikely that mapping alone will lead to definitive causative genes given the mean within-species short range linkage disequilibrium 'wall' of about 0.5cM or 10-50 genes. Nevertheless fine mapping strategies remain valid if markers in close linkage disequilibrium to the QTN can be defined and applied for MAS. Two examples are given below.

Fine mapping the interferon gamma gene for GIN resistance in sheep. Following a genome wide QTL study (Crawford and McEwan 1998), fine mapping of OAR3 in sheep narrowed down the QTL confidence interval to a 5cM region, encompassing the interferon gamma (IFNG) gene as the lead positional candidate (Paterson *et al.* 2001). Interestingly, while many studies reported significant association between markers predominantly near to the 5` region of the gene and resistance, the phase of the resistant haplotype was not consistent (Crawford and McEwan 1998. Paterson *et al.* 2001, Coltman *et al.* 2001, Sayers *et al.* 2005) therefore excluding this region as causative for this important QTL. Furthermore, at least two studies have reported a lack of association between IFGN haplotypes and resistance to GIN. The first was in Romney lines selected for traits independent of parasite resistance (Dukkipati *et al.* 2005) where a founder effect or correlated selection were raised as possible explanations. The second was in the Suffolk breed (Sayers *et al.* 2005). The latter is interesting since the Suffolk population appeared to have four segregating IFNG haplotypes, (instead of the two present in other breeds), representing a potentially highly informative recombination between two closely

linked markers. While it is still possible that the QTN may be located within IFNG, other possibilities within 1Mb 5` of the bovine IFNG gene include Loc507778 (Similar to IL22), MADP-1, loc532695 (similar to perihilin1 isoform 2), FLJ20436 (similar to hypothetical protein), LOC407194 (cyclin T1) and intriguingly, BSM (bovine submaxillary mucin2). To distinguish between several candidates, contributions from other fields are increasingly important, as described under 'Positional Functional Genes'.

Fine mapping Trypanosome resistance. ILRI researchers have made use of parallel studies in mice as a resource to assist the fine mapping of putative QTL, taking advantage of a comparative genomic approach. Three major QTL were revealed on MMU17 (*Tir1*), MMU1 (*Tir3*) and MMU5 (*Tir2*) (Kemp *et al*. 1997, Iraqi *et al.* 2000). Comparative studies of map data between cattle and mice revealed a region of homology of about 300,000 bp between BTA7 and MMU17, lying within the principal QTL in both species (Figure 1). It is therefore possible that the underlying genes in these two QTL could be the same, leading to their eventual identification (Enwezor and Lawal 2003). The *Tir1* QTL falls over the MHC region, TNFa and Heat Shock Protein70.3 and 70t, but HSP70.1 is excluded (Nakamura *et al.* 2003). It is worth noting that mice and cattle immune systems behave differently when infected by trypanosomes, and interpreting such results warrants caution (Naessens *et al.* 2002).

POSITIONAL FUNCTIONAL GENES

Full transcriptome analyses (such as microarrays, SAGE, MPSS), together with QTL studies, have been conducted for mastitis (Schwerin *et al.* 2003), for trypanosomiasis (Hill *et al.* 2005, Berthier *et al.* 2003), for GIN and innate immune response in cattle (Gasbarre *et al.* 2004, Donaldson *et al.* 2005) and GIN in sheep (Diez-Tascon *et al.* 2005, Keane *et al.* 2006). Joint results provide a significant avenue to identify so called positional functional candidate genes. All derived genes may typically be screened *in vitro* and *in vivo*, and sequenced to identify putative QTN for use in mapping as direct markers. However unequivocal proof that a specific mutation is causative of a QTL or a phenotype change remains elusive in livestock, with limited scope for gene substitution and targeted mutation to obtain such proof. NRAMP1 may provide the best example of an available loss/gain studies in a functional system. For this gene, there appears to be both conservation of protein sequence and function between cattle and mice. This has allowed an expression study of specific alleles of the bovine NRAMP gene under the control of the bovine NRAMP1 promoter, in NRAMP1-susceptible mice macrophage cell lines (Barthel *et al.* 2001), without the interfering effects of *in-vivo* genetic background.

MARKER APPLICATIONS FOR IMPROVEMENT OF DISEASE RESISTANCE

Despite the intensive search for QTL for disease resistance, few have been applied in commercial applications in livestock. The only known application of MAS using QTL in livestock is found in a collaboration between INRA, LABOGENA and 8 breeding companies. Here, SCS is selected utilizing 2-4 microsatellite markers in regions on BTA 10, 15, and 21, in French commercial cattle breeds (Boichard *et al.* 2002). In New Zealand, the so-called Footrot Gene Marker Test (FGMT) makes use of an association between alleles of the MHC-DQA and resistance to footrot, and has been implemented for large scale testing among ram breeders (Hickford *et al.* 2005, Bates and Hickford 2005). A different test for markers within the IFNG gene has been patented in New Zealand (renewal date 15 April 2009) (Crawford and McEwan 1998), and offers resistance to intestinal nematodes. MAS for the PrP allele for scrapie resistance in sheep is now compulsory in Europe and demonstrates a widespread application of MAS when genes of major effect can be identified.

CONCLUSIONS

Infectious diseases continue to have significant impact on livestock production world wide. A plethora of QTL mapping studies in sheep and cattle have primarily focussed on production traits, with relatively few screens targeting disease resistance. The information to date on

SCC/mastitis in dairy cattle, GIN in sheep and trypanosomiasis in cattle suggests that many putative QTL maybe be present. Slowly a picture is emerging on which chromosomes consistently harbour QTL for key diseases. Significant investment in QTL confirmation and fine mapping to near direct markers is required before commercial applications for MAS will be seen at a large scale. Augmenting QTL and candidate gene mapping studies with linked functional genomics approaches will inform positional functional candidates to be screened for possible QTN. Until specific QTL for disease resistance have been identified, it is difficult to ascertain if 'broad based' disease resistance genes are likely to operate with resistance to multiple diseases. In the absence of such information, each QTL application will need to be considered on its own merit, with a comprehensive evaluation for impact on other production traits and other diseases not identified as the primary target for MAS.

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