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Review Article

Molecular Approaches for Disease Resistant Breeding in Animals

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ABSTRACT

Improved animal health is a major objective in current animal breeding strategies, but is difficult to achieve by traditional\ breeding methods. Disease resistance traits are thus among the most difficult to include in classical biometrical farm animal genetic improvement programmes. Disease resistant breeding is one of the strategies that can be adapted for selective breeding of livestock to perk up animal health and ultimately the productivity. In genomic approaches to improve of disease resistance, the criterion for selection is shifted from phenotypically expressed disease status to allele status at the de–oxy ribonucleic acid (DNA) level. This mode of selection is termed marker assisted selection (MAS). Chromosome design mapping within population along with genomic analysis and association studies have found important place in the context of disease resistant breeding. Vivid studies have been conducted from time to time on linkage mapping of disease resistance loci within populations. Marker–assisted introgression (MAI) are employed when resistance alleles at one or more disease resistance loci (DRL) is identified in a donor population, mapped with respect to a marker locus or marker haplotype, and the marker phase is established. Use of molecular markers to enhance resistance of livestock to disease is an advanced biotechnological tool. They have been used in conservation decision; decision on utilisation to confirm the hypothesis of different mechanisms of genetic control and to confirm the hypothesis of different mechanisms of genetic control. This mini review shed lights on different molecular tools and techniques adapted so far for disease resistant breeding in farm animals.

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INTRODUCTION

Improved animal health is a major objective in current animal breeding strategies, but is difficult to achieve by traditional\ breeding methods. There are many documented instances of breed and individual differences in genetic disease resistance among farm animals. Furthermore, historical evidence documents the presence of genetic resistance to many infectious diseases in man, and current studies in humans are providing increasing evidence of genetic predisposing factors to many degenerative diseases. Thus, selection for genetic disease resistance provides a potential avenue for improving the health status of farm animals, increasing productivity and reducing the need for pharmaceutical intervention, in this way reducing costs and delaying the appearance of resistant pathogens (Whitelaw and Sang, 2005; Zhao et al., 2012). During the last fifteen years after the development of the first transgenic animal the genetic engineering rationale is production of animals that are having altered traits. The objective in most of the instances has been either alteration of traits for improvement of efficiency of production or alteration of the properties of the animal products. From a variety of mammals cloning of the milk protein genes have been done (Maga and Murray, 1995).

Disease resistance traits are among the most difficult to include in classical biometrical farm animal genetic improvement programmes. This is primarily due to the fact that biometrical programmes require good field measurement of the disease status on the animals under selection or their relatives. The incidence of infectious diseases is apparently strongly influenced by environmental factors, such as exposure to the pathogen. Thus, if one depends on field exposure, levels of exposure will necessarily be greatly dissimilar among individuals, so that much of the phenotypic variation will be due to differences in degree of challenge. Also, in years of low challenge there

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will be little variation to speak of, which again does not permit effective selection. If one depends on artificial challenge, the costs of setting up a suitable system and the direct costs of productivity loss due to development of disease are high. Moreover, even when challenge is adequate, development of the disease is determined by numerous factors in addition to genetic resistance status so that the heritability of resistance is generally low. A further complication is that disease resistance is most often an 'all–or–none' trait. Although there may well be quantitative polygenic variation in resistance status, the observations are often limited to 'sick or healthy'. This, too, reduces heritability compared with other polygenic traits, such as milk production. Furthermore, in the framework of an overall commercial breeding programme aimed at productivity traits as well at disease resistance, disease exposure and disease development will affect phenotypic expression of productivity traits so that selection for disease resistance occurs at the expense of effective selection for productivity (Michelmore, 1995; Nash and Freeman, 2004).

In principle, when resistance alleles are segregating within a breeding population, many of these difficulties can be overcome by utilising family selection or progeny testing methods. In dairy cattle, where very large half–sib sire families are routinely produced by artificial insemination and where individual daughters are scattered over many farms and environments, there is sufficient information in the daughter population to allow effective selection for disease resistance. Accessing this information, however, is very difficult. In most instances, uniform veterinary records are unobtainable from commercial herds. In poultry, where large half–sib families can also be produced, some breeders have set up specific testing facilities (*e.g.* for Marek's disease) in which part of a family is exposed to uniform disease challenge, providing information for family selection, but this is an expensive programme. Thus, with the outstanding exception of Marek's disease, biometrical approaches have been ineffective in obtaining genetic improvement in disease resistance traits (Vallejo et al., 1998).

MOLECULAR METHODOLOGIES FOR DISEASE RESISTANCE BREEDING

In genomic approaches to the improvement of disease resistance, the criterion for selection is shifted from phenotypically expressed disease status to allele status at the DNA level. This mode of selection is termed 'marker–assisted selection' or MAS. In principle, MAS can enable selection for disease resistance without exposure to disease challenge and allows highly accurate selection which is unaffected by environmental factors (Fadiel et al., 2005). Thus, MAS provides an ostensibly feasible approach to selection for genetic disease resistance in farm animals. The realisation of this potential in practice requires the following:

a) Comprehensive genome maps of the various farm animal species
b) Genomic analysis of the resistance trait, leading to mapping and identification of DRL
c) Incorporation of genomic information on disease resistance loci (DRL) which are identified and mapped in this way within the framework of an MAS genetic improvement programme. These three components are treated in detail below:

Construction of Comprehensive Farm Animal Genome Maps

A genetic map can be divided into three components: a linkage map, a physical map and a comparative map. A prerequisite for the development of a good linkage map is the availability of a large number of genetic markers. A genetic marker is a character showing genetic polymorphism with a simple Mendelian inheritance that can be scored easily. The most frequently used genetic markers in current genome research are so–called microsatellites. Microsatellites are short tandem repeats of a 1–5 base pairs (bp) motif; the genetic polymorphism is due to variable numbers of the tandem repeat. Microsatellites are analysed by polymerase chain reaction (PCR) amplification and the polymorphism is revealed as length differences by gel electrophoresis (51). A mammalian genome contains on the order of 100,000 microsatellites which are more or less randomly distributed in the genome. Microsatellites may be located within or near a gene, but are most often isolated as anonymous DNA segments. The next generation of genetic markers will most probably be single nucleotide polymorphisms (SNPs), i.e. simple base pair substitutions. SNPs are even more numerous in the genome than microsatellites, but the major reason why SNPs will be the future marker type is the ease of typing. There is currently intensive technology development in this field and it is very likely that large–scale screenings of SNPs can be carried out at low cost in the near future. A linkage map is constructed by family segregation analyses, which means that the inheritance of genetic markers is traced from heterozygous parents to their progeny. Linkage mapping is based on the observation of genetic recombination events which occur between the two chromosome homologues during meioses. Genetic markers which are located on different chromosomes or are far apart on the same chromosome show independent segregation, whereas genetically linked markers show co–segregation. The closer the markers are, the lower the frequency of recombination. A linkage map contains information on the relative order of markers along the chromosome as well as the map distance between markers. Map distances are measured in centiMorgans (cM) and 1cM corresponds to a recombination frequency of 1%. A comprehensive description of the principles and statistical methods used for linkage mapping includes informative linkage maps comprising 500–1,500 markers that have been established for all major farm animals (Clark and Whitelaw, 2003).

A physical map includes information on the physical location of genes and genetic markers. The traditional way to perform physical mapping has been to utilise somatic cell hybrid (SCH) panels which are constructed by fusing cells from two species, e.g., pig and hamster. Such hybrid cells tend to lose chromosomes, and clones which contain different subsets of chromosomes from the species of interest can be isolated. Genomic DNA is isolated from each clone and any DNA marker can be analysed for its distribution in the panel of clones. The screening of an informative SCH panel containing on the order of 30 clones will reveal the chromosome on which a certain gene or genetic marker is located. Genes which have been assigned to the same chromosome are said to be syntenic. More

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recently, a more advanced form of SCH mapping, termed radiation hybrid (RH) mapping, has been developed. The difference in this form of mapping is that the cells from the species of interest are irradiated before fusion to the recipient cell line. As a consequence, the genome is fragmented to segments of 1 to 10 million bp, and a larger number of clones (about 100) are needed to obtain complete genome coverage. The advantage is that a much better resolution is obtained. RH mapping is now widely used to develop ordered, high–resolution physical maps. A more direct method for physical mapping is fluorescence in situ hybridisation (FISH). With this method, DNA probes are labelled with a fluorescent reporter molecule and allowed to hybridise with a fixed chromosomal spread. The hybridisation signal is detected and the cytogenetic location determined. Large insert genomic libraries, constructed using yeast artificial chromosome (YAC) vectors which harbour inserts in the range 150–1,000 kilobases (kb) or bacterial artificial chromosome (BAC) vectors which harbour inserts in the range 100–200 kb, are invaluable resources for genome analysis. For example, large insert libraries are used to produce sets of overlapping clones (termed 'contigs') flanking a gene of interest before genome sequencing (Kadarmideen et al., 2006).

Comparative gene mapping is of central importance in all gene mapping programmes in farm animals. Comparative gene mapping is possible because vertebrate species share the same basic set of genes, and most genes are so well conserved that homologous genes can be identified even between distantly related species, such as humans and chicken. Moreover, genome organisation is well conserved, which enables the identification of large, homologous chromosome segments shared between species. The major impetus for comparative mapping is that the genetic maps of humans and mice are far more advanced than the farm animal maps. For instance, a human genetic map containing approximately 16,000 coding sequences has been reported. Thus, the comparative map provides a link between a farm animal map and the advanced human and mouse maps. The comparative maps are constructed by linkage or physical mapping of coding sequences previously mapped in other species. A major contribution to this field has been Zoo–FISH analysis which has been carried out in pigs, cattle, horses and cats. In this method, human chromosome–specific probes are labelled with fluorescence and used to paint metaphase spreads of other genomes. This allows a direct visualisation of homologous regions (Niemann et al., 2003; Rothschild, 2004). The method has not yet been used successfully in the chicken. The linkage, physical and comparative maps are well integrated since many loci have been mapped by more than one method and hundreds of coding sequences are included in the map. The genetic maps of the farm animals are now powerful tools for genome analysis which can be exploited to map any gene controlling a phenotypic trait, provided adequate family material with accurate phenotypic records is available. The procedure is straightforward for traits with a monogenic inheritance and has already been used successfully for the mapping of coat colour genes, morphological traits such as polledness in cattle and some inherited disorders. However, most traits of economic importance in farm animals show a multifactorial inheritance, which means that they are controlled by an unknown number of genes and influenced by environmental factors. Genome mapping can still be used to identify so–called quantitative trait loci (QTL), but fairly large amounts of family materials are needed and the statistical treatment of data is more demanding. Convincing examples for the detection of QTL in farm animals have been reported (Manly et al., 2005; Price, 2006; Miles and Wayne, 2008).

Genomic Analysis; Mapping and Identification of Disease Resistance Loci

There are two major approaches for identifying genes controlling a phenotypic trait: association analysis and linkage analysis using genome maps. An association analysis operates in long–standing random mating populations that are in a long–range linkage equilibrium situation, and tests for population–wide short–range linkage disequilibrium existing over a chromosomal region of less than 1 cM. Linkage analysis is based on the co–inheritance of random markers and linked trait genes in situations of long–range linkage disequilibrium (up to 20 cM), as is found within families, in F2 or backcross (BC) populations derived from crosses between widely diverged breeds or in inbred lines. Once performed, however, a linkage analysis will naturally lead to an association test for fine mapping of the uncovered DRL. The availability of high quality records with respect to disease status or physiological traits correlated with disease resistance is an absolute prerequisite for successful DRL identification, whether based on association testing or linkage analysis (Orr, 2001; Bazer and Spencer, 2005).

Association Studies; the Candidate Gene Approach

Association analysis is appropriate as the first line of investigation, when a plausible candidate gene for a DRL has been identified. An association analysis is also appropriate when a chromosomal region containing the DRL has been identified through prior linkage analysis. An association will be found if there is strong, population–wide linkage disequilibrium between the DRL and a genetic polymorphism in the candidate gene or in the chromosomal region containing the DRL. Association tests will now be discussed in detail in the context of candidate genes, and the topic will be returned to briefly in the context of 'fine mapping' of DRL uncovered by linkage analysis.

Candidate genes for a trait are genes that are directly involved in the physiology or development of a given trait. Thus, candidate genes are identified on the basis of their known function. For example, the genes of the major histocompatibility complex (MHC) are important candidate genes for resistance to infectious diseases. A candidate gene may also be one that controls a similar phenotype in another species. A major weakness of the candidate gene approach at present is that the large majority of genes have not yet been identified, nor have their functions been clarified. Furthermore, it is likely that in at least some instances, the genes responsible for resistance will not have an obviously related function. Nevertheless, with the rapidly growing knowledge concerning vertebrate genes and their function, the candidate gene approach becomes more and more powerful. The first step in performing an association test of a candidate gene with trait variation is to detect one or more genetic polymorphisms in the gene. This requires that sequence information is available for the gene at the genomic or complementary DNA (cDNA) level. Fragments of the gene are then amplified and examined for sequence variation by direct sequencing or by any of the less laborious methods for

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detecting sequence polymorphisms, such as PCR restriction fragment length polymorphism (PCR–RFLP) or single–stranded conformational polymorphism (ssCP). Although an association test can be carried out on the basis of a single polymorphic site, it will be more powerful to consider a number of sites so as to identify the various haplotypes present at the candidate gene in the population (or populations) of interest. This is so because a given allele at any single polymorphic site may be common to a number of allelic variants, which have different functional attributes. Haplotype identification in farm animal populations is simplified by the small number of alleles expected in such populations due to their small effective numbers (Perez–Iratxeta et al., 2002).

A possible association between a candidate gene and disease resistance may be revealed as an allele frequency difference between a resistant and susceptible population. However, excluding the possibility that the allele frequency difference has another cause, such as random drift or selection for some trait unrelated to disease resistance, is always difficult. Therefore, an association test will generally be based on an association between alleles at individual polymorphic sites or a combination of marker sites (haplotypes) at the candidate gene locus and resistance/susceptibility. An association test can be carried out either within a random mating population or in the F2 progeny of a cross between resistant and susceptible populations. In both cases, it should be noted that an observed allele frequency difference between resistant and susceptible individuals may be due to a locus in linkage disequilibrium with the polymorphic site (or haplotype) at the candidate gene. In the case of a within–population study, the extent of linkage disequilibrium will be rather small (< 1 cM), so this is not a very serious concern. In the case of a population derived from a recent cross between resistant and susceptible populations, however, significant linkage disequilibrium will be found extending 20 cM or more to both sides of the candidate gene. In this case, the possibility that the candidate gene is simply serving as a 'marker' locus is not negligible. In both instances, therefore, candidate gene/resistance association within additional independent random mating populations must be confirmed (Tabor et al., 2002; Zhu and Zhao, 2007).

An association analysis is normally carried out by a statistical evaluation of the incidence of disease among individuals with different genotypes at the candidate locus. Alternatively, the association is expressed as a difference in allele frequency between resistant and susceptible individuals. In both cases, a false positive result may be obtained in within–population studies if the population is stratified. For instance, if sire families are non–randomly distributed among affected and non–affected individuals, this will lead to allele frequency differences at many loci in the genome. Therefore, an association test must be carried out with carefully matched controls, and family relationships must be included in the statistical model. Any single polymorphic site in an association analysis will usually not be the causative mutation affecting resistance. Consequently, false negative results may be obtained in cross–family population–wide studies if the genetic polymorphism used in the analysis is in linkage equilibrium with the causative mutation. This can be avoided if the analysis is based on multi–site intragenic haplotypes at the candidate gene, since at least one of the intragenic haplotypes will necessarily be co–extensive with the causative mutation. Alternatively, the polymorphic site at the candidate gene can be used as a marker in a half–sib or full–sib family–design linkage analysis (see below). This will uncover linkage, even in the event of complete linkage equilibrium between the marker site and the causative mutation, but statistical power in this case will be only one–sixth to one–tenth of that obtained in an association test with complete linkage disequilibrium between marker site and causative mutation (Wayne and McIntyre, 2002).

The candidate gene approach has been applied successfully to a number of inherited disorders in farm animals. The identification of the causative mutations for citrullinaemia and bovine leucocyte adhesion deficiency (BLAD) in cattle, as well as hyperkaelemic periodic paralysis (HYPP) and severe combined immune deficiency (SCID) in horses, were all guided by previous molecular characterisation of corresponding inherited disorders in humans and/or mouse. Another interesting case is the Nramp (natural resistance–associated macrophage protein) gene which was first identified as a major locus controlling natural resistance to Salmonella infection in mice, and more recently in chicken as well. Once a putative association of candidate gene with resistance has been revealed by association test or linkage studies, the next obvious step is to characterise further the candidate gene at the molecular level to identify the causative mutation. This can be accomplished by sequence analysis of cDNA or genomic DNA obtained from disease–resistant and susceptible animals. Structural mutations affect protein structure and occur in the coding sequence or at exon/intron borders affecting the splicing of messenger RNA (mRNA). Regulatory mutations change gene expression and are to be found in the promoter region, in the 3'–untranslated region and also in regulatory elements located at a considerable distance from the coding sequence. It is often necessary to study the expression pattern of the gene to reveal the presence of a regulatory mutation. When a putative mutation has been identified, it is important to provide genetic or functional evidence that the mutation is really causing the phenotypic effect and is not a linked polymorphism. However, except for simple monogenic disorders where the mutation leads to an obvious defect in the protein product, providing formal evidence for a causal relationship is often difficult and transgenic experiments may be required. For polygenic traits, such as disease resistance, the causative mutations are unlikely to represent obvious deleterious mutations but rather more subtle changes in the structure or expression of proteins.

Therefore, distinguishing causative mutations from linked polymorphisms and proving causal relationships will be difficult. A major challenge for future genome research will be to develop strategies to overcome this problem. However, this does not detract from the ability to use identified candidate genes for purposes of marker–assisted selection.

Experimental Designs for Linkage Mapping Disease Resistance Loci Using Complete Genome Marker Maps

The strength of linkage mapping is that the entire genome can be scanned for the presence of DRL by using anonymous genetic markers spread right across the genome. Prior information on gene function or DRL location is not

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required. In principle, linkage mapping can potentially identify all DRL of appreciable effect that distinguish between populations or that are segregating within a population. In practice, limitation in population size and genotyping capacity often render this unfeasible. Experimental designs for mapping QTL using complete genome marker maps are divided into the following two groups:

i. Designs based on crosses between resistant and susceptible populations, which primarily include F2 and backcross (BC) mapping populations.
ii. Designs for implementation within segregating populations, which primarily include full–sib and half–sib designs; useful variants include the granddaughter and chromosome designs.

Following initial mapping of DRL, congenic lines containing a single DRL can be constructed to provide a basis for physiological studies of gene action. Simulation studies show that even very large–scale BC or F 2 experiments can locate a QTL of moderate effect (d = 0.2 or 0.3 standardised units) only to within a confidence interval (CI) of 10 to 20 cM or more. CIs are much greater for full–sib and half–sib designs. Tightly linked markers are required, however, for effective manipulation of DRL in MAS or introgression programmes, and as a stepping–off point for positional cloning. A variety of approaches have been proposed for fine mapping of DRL after the initial mapping. Whole genome scans for DRL require large amounts of genotyping. In appropriate instances, however, selective genotyping and selective DNA pooling can be used to reduce greatly the genotyping requirements of the mapping project.

Designs Based on Crosses between Populations that Differ Widely in Disease Resistance

When populations are available which differ significantly in resistance to the disease of interest, a powerful approach for mapping DRL is to generate a mapping population by crossing the populations to produce an Fx generation. Resistance status of the parents and F1 progeny can be usefully compared to estimate the degree and direction of dominance of resistance/susceptibility. In cases where the F1 exhibits intermediate resistance, statistical power will be greatest if the F1 animals are intercrossed to produce an F2 generation. If dominance is present, the F1 animals should be crossed back to the population exhibiting the recessive phenotype to produce a BC generation. For mapping DRL, the F2 or BC progeny are scored individually for disease resistance and genotyped individually with respect to a panel of markers that span the genome. In the initial scan for DRL, marker spacing should be no more than 4 0 cM, but little is gained by a marker spacing that is less than 10 cM. A DRL in linkage to a marker is detected as a statistically significant difference in mean resistance score between alternative marker genotypes in the BC or F2 progeny, i.e., between MM and Mm marker genotypes in the BC design and between MM and mm marker genotypes in the F2 design. The number of informative meioses needed to detect a DRL will depend on the size of the effect and on the degree and direction of dominance (Soller and Andersson, 1998).

When the two crossed populations differ strongly in allele frequency at markers alleles and DRL, BC and F2 designs have good efficiency and experiments of moderate size (N = 1,000) can uncover most QTL of moderate effect with good power. When the populations crossed differ at the DRL but share many alleles at the marker loci, more complex analyses must be employed, but power remains high. When many markers are available, markers that show a maximum frequency difference between the populations can be searched for and the linkage analysis can be based on these. When evidence of linkage between a genetic marker and the DRL has been obtained, further markers from the particular region are genotyped to verify the linkage. In this case, various sophisticated analyses, termed 'interval mapping', based on maximum likelihood or regression methods, are available to determine QTL location more exactly with respect to the marker map (Mackinnon and Weller, 1995; Pasyukova et al., 2000; Chen, 2003).

Fine Mapping of Disease Resistance Loci Uncovered in Crosses between Populations

Advanced intercross lines (AIL) have been proposed as a means for fine mapping of DRL. An AIL is produced by randomly and sequentially intercrossing a population which initially originated from a cross between two widely differing populations. This provides increasing probability of recombination between any two loci. Consequently, the genetic length of the entire genome is stretched by a factor of approximately t/2, where t is the usual generation number (*i.e.* for an F 10 generation, t = 10). In an AIL, the many recombination events required for fine mapping of DRL are accumulated in a single, relatively small population over the course of many generations, rather than by producing and examining many progeny in a single large F2 or BC generation. In this way, an approximately 95% CI of DRL map location of 20 cM in the F2 is reduced to about 4 cM after 8 additional generations (F 10), analysing the same population size for the same DRL.

An alternative approach to fine mapping is based on the 'genetic chromosome dissection' method, originally developed by Drosophila (fruit fly) geneticists. In genetic chromosome dissection, a DRL is first assigned to a relatively broad CI by linkage mapping. The DRL allele status of the chromosomal region is then determined in the two chromosomes of one or more individuals which are heterozygous for the DRL and also for a series of ordered markers spanning the region containing the DRL. Recombinant chromosomes derived from the heterozygous individual are then examined for DRL status by progeny testing, or by developing recombinant congenic strains. By choosing the starting individuals appropriately and combining information on DRL status and recombinant points of the recombinant chromosomes, the DRL can be located within the boundaries of a very small sub region. In the best case, the width of the sub region will be proportional to CI/2n, where CI is the original confidence interval of DRL location, and n is the number of recombinant chromosomes examined in this way.

Experimental Studies of Disease Resistance Loci in Crosses between Populations

An elegant demonstration of the power of linkage mapping for detecting DRL in a population cross design was recently provided by Cheng and colleagues, who were studying Marek's disease in chicken. They developed an F2 mapping population consisting of over 300 animals by crossing two inbred lines which differed in resistance to Marek's disease. Several symptoms of the disease were recorded after an

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experimental challenge with a defined strain of the virus. A genome scan revealed eight chromosomal regions with statistically significant effects. Recombinant congenie lines are currently being developed to characterise further these putative DRL.

A major project aimed at mapping loci conferring resistance to trypanosomiosis in cattle is currently underway at the International Livestock Research Institute at Nairobi. This project is based on crosses between the trypanotolerant N'Dama cattle of West Africa and the trypanosensitive Boran cattle of East Africa. Over 200 F 2 animals have been produced and are being phenotyped with respect to trypanotolerance and genotyped with respect to a battery of markers covering the bovine genome. Following the experimental mapping programme, fine mapping of the trypanotolerance loci will be carried out in field populations derived from hybridisation of resistant and sensitive stocks. These will be based on short–range linkage disequilibrium, admixture and identical–by–descent approaches. Parallel to this effort, a search for candidate genes controlling trypanotolerance is being performed in mice. Genome–wide scans for genes controlling resistance to trypanosomiosis in crosses between inbred lines of mice have uncovered three putative resistance loci. These are now being subject to very fine resolution mapping utilising advanced intercross lines and interval specific congenie strains (Blakesley, 2004; Aulchenko et al., 2007).

Linkage Mapping of Disease Resistance Loci within Populations

In many instances, resource populations showing high resistance with respect to the disease of interest are not available, or their utilisation for DRL mapping by crossing is inconvenient; yet biometrical studies provide evidence for segregating DRL in the population under selection. In this case, linkage mapping of DRL is carried out on a within population basis, by means of half–sib or full–sib family designs. In a half–sib design, a number of families are produced, each by mating a single male to a number of females, to produce progeny that are related to one another as half–sibs. Such families are produced in cattle, for example, where a single sire can have many hundreds or thousands of daughters by artificial insemination. Large half–sib families can also be produced in poultry and pigs, by mating a single sire with numerous females. In a full–sib design, a number of families are produced, each by passing a single male and a single female, to produce progeny that are related to one another as full–sibs. Large full–sib families can be produced in pigs and fish.

The statistical power of half–sib and full–sib family designs is much less than that of BC or F 2 designs. There are two reasons for this, as follows:

a) Marker–DRL linkage is detected when in some families the difference in mean disease resistance between marker genotypes is greater than expected on the basis of sample variation alone. This will be found only in those families where the parents are heterozygous at the DRL. For half–sib designs, this must be the single sire that is the common parent of each family. For full–sib designs, this must be one or both of the parents of the full–sib family. On Hardy–Weinberg considerations, however, at most half of the parents of the full–sib or half–sib families can be expected to be heterozygous for a DRL, and only these parents will be informative for marker/DRL linkage.
b) In both of the family designs, since the population from which the parents are chosen is generally in linkage equilibrium, the same marker genotype will be associated with resistant DRL alleles in some families but with susceptible DRL alleles in other families, depending on the specific 'marker allele'–'DRL allele' linkage phase in the parents of the given family. For this reason, marker–DRL linkage does not turn up as an overall difference in mean disease status between marker genotypes averaged across all families but rather as a significant between–marker–genotype effect, in a hierarchical analysis of variance, with markers nested within families. Taken together, these two factors reduce the statistical power of half–sib and full–sib family designs to the point where they have only about one–sixth to one–tenth the power of an F 2 design. As a result, in outcrossing species very large populations must be analysed to map a reasonable proportion of the segregating DRL of interest (Womack, 2005; de Koning et al., 2008).

Granddaughter and Chromosome Designs

In a granddaughter design, DRL mapping is based on mean resistance status of the progeny of an individual, rather than on resistance status of the individual itself. This increases the heritability of the resistance measure and also converts it from a categorical trait ('healthy or sick') to a continuous quantitative trait. Both of these factors will increase the power and precision of DRL mapping. Granddaughter designs can be applied in current selection programmes in dairy cattle and layer chickens, where males are routinely progeny–tested for sex–limited traits (milk and egg production) that come to expression only in their daughters. The chromosome design is an attempt to achieve, for within–population linkage mapping, statistical efficiency comparable to that of BC and F2 designs. In this design, a large mapping population is produced by recurrent intercrossing and expansion of the progeny of a single parental pair over a number of generations. By the third generation, population numbers for species with high reproductive capacity (poultry, pigs) will be sufficient for mapping purposes. Each generation, from and including the second generation, can serve as a mapping population. In the two founding parents of a chromosome design population, each chromosome is represented by only four exemplars: two derived from the male founder parent and two from the female founder parent. This introduces a very high degree of long–range linkage disequilibrium in the parental and following generations. As a result, the statistical power of a chromosome design can approach that of an F2 design. In addition, the population can be continued for additional generations to accumulate data and meiotic recombinations, thus providing the benefits of an AIL for fine mapping purposes. As a result of the initial inbreeding step, which can be accompanied by severe inbreeding depression, however, a chromosome design population must be initiated with a number of independent parental pairs, continuing with which ever family shows the best reproductive capacity (Wang et al., 1998; Womack, 2005).

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Fine Mapping of Disease Resistance Loci Uncovered in Linkage Studies within Populations

The CI of DRL map location derived from within–population linkage studies will generally be even wider than those obtained for F2 or BC populations, so that follow–up studies aimed at fine mapping of uncovered DRL are essential for implementation of MAS or for positional cloning. In some cases, this can be achieved by simple accumulation of data. The CI of DRL map location will be inversely linear to population size. Thus, in dairy cattle or poultry where very large total numbers of progeny can be accessed across many sires in half–sib designs, the reduction of CI of map location to useful proportions may be possible by this means. This implies, however, that it is possible to phenotype very large numbers of individuals with respect to the disease resistance trait, and to genotype these individuals with respect to a limited number of markers from the region shown by linkage analysis to contain the DRL (Barreiro and Quintana–Murci, 2010).

Theoretical studies show that over truly small chromosomal regions (< lcM), considerable linkage disequilibrium can be expected in populations with small 'effective' breeding size, as is typical for many farm animals. In dairy cattle, for example, although milking cows number in the many millions, only a limited number of sires are used to reproduce the population, so that the effective breeding size of the population is rather small. In this context, the authors note that on the average, with a marker spacing of 2 cM, a DRL will be within 0.5 cM of the nearest markers; while with a marker spacing of 1 cM, a DRL will be within 0.25 cM of the nearest markers. For many farm animal populations, these distances are small enough for linkage disequilibrium to be present. To uncover this, the chromosomal regions to which the DRL have been mapped must be saturated with many markers. One or more of these may then be in strong linkage disequilibrium with the DRL. This is detected by a population–wide association test, similar to that carried out when testing the possibility that a candidate gene is a DRL. Since linkage disequilibrium is expected over small chromosomal regions only, a positive association test means that the DRL must be close to the marker in question. Here too, the power of the association test is increased by basing the test on marker haplotypes, rather than single polymorphic sites (Stranger et al., 2011).

Experimental Studies of Within–Population Linkage Mapping of Disease Resistance Loci

Due to the difficulty of collecting good disease records, comprehensive within–population linkage mapping studies have not yet been carried out for DRL, although a number of studies have been carried out where the genetic markers have been restricted to candidate genes, such as the MHC. In many dairy cattle populations, breeding values for milk somatic cell counts are estimated and used as a correlated trait for mastitis resistance. Whole genome scans for the presence of DRL for somatic cell scores are currently being conducted in several laboratories, and a limited granddaughter design study of this trait has recently been published. In the Scandinavian countries, breeding values for veterinary treated diseases, including mastitis, are available and can be utilised in DRL analysis (Stinchcombe and Hoekstra, 2008).

Selective Genotyping and Selective DNA Pooling

In many of the designs shown above, DRL mapping is based on population–wide linkage disequilibrium. These include linkage mapping in F2, BC, chromosome design and AIL populations, and association testing of candidate genes or of markers saturating a DRL–containing region. In these cases, the number of individuals genotyped for given power can be decreased considerably by genotyping only individuals from the most resistant and most susceptible phenotypic tails of the entire sample population, a procedure termed 'selective genotyping'. Furthermore, theoretical studies show that virtually all of the mapping information in the two tails of the population is contained in the difference in marker allele frequencies between the two tails. Experimental studies show that the relative frequency of micro satellite alleles genotyped by PCR amplification can also be detected in pooled DNA samples. This means that quantitative genotyping of a small number of pooled DNA samples can be substituted for individual genotyping of large populations. This procedure, termed 'selective DNA pooling', has been used successfully in mice to map polygenes affecting obesity and a Mendelian locus affecting muscular development (Andersson and Georges, 2004).

For within–population linkage mapping, selective DNA pooling loses much of its effectiveness since the pooling must be implemented separately for each family. Nevertheless, when family sizes are large, selective DNA pooling can still provide very significant reductions in genotyping costs. Thus, in an 11 marker linkage mapping search for loci affecting milk protein percentage involving 7 large sire families of Israel Holstein dairy cattle, a 100–fold reduction in genotyping was achieved relative to individual selective genotyping (Devlin and Roeder, 1999).

Strategies for Cloning Disease Resistance Genes

The information on the chromosomal location of a locus affecting disease susceptibility can be exploited in practical breeding programmes by MAS, as described below. However, the molecular cloning of such genes and the identification of mutations causing the phenotypic effect are justified from a scientific point of view, as such identification will advance the basic knowledge concerning disease resistance. These attempts are also justified from a practical point of view, since a direct test of a causative mutation is more straightforward and easy to use than linked markers. A comprehensive review of strategies and methods applicable to cloning trait genes in farm animals is provided by Andersson and only a brief overview is given here. Positional candidate cloning is a very powerful approach to clone trait genes. In this procedure, a trait locus is first assigned to a specific chromosomal region by linkage mapping. One then tries to discover whether this chromosomal region harbours any gene which, on the basis of its known function, can be assumed to cause the actual phenotypic effect. This strategy has turned out to be very successful for cloning trait genes in humans and mice and will be the prime strategy once a complete human gene map is available in the near future. The number of coding sequences assigned to the genetic maps of farm animals is still in the few hundreds, which is insufficient for efficient positional candidate cloning. However, comparative mapping information can be taken advantage of in the search for candidate genes. This is denoted 'comparative positional candidate cloning'. A clear demonstration of this

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approach is the recent identification of the causative gene for the dominant white phenotype in pigs, which is a form of pigmentation disorder. The locus was first assigned to a region of pig chromosome 8 which shares homology with parts of human chromosome 4 and mouse chromosome 5 (Dove, 2005; Chevin et al., 2008).

The actual regions harbour the KIT gene, encoding the mast/stem cell growth factor receptor, and mutadons in this gene causes pigmentation disorders in humans and mice. Subsequent molecular characterisation revealed the presence of a KIT gene duplication which showed a complete concordance with the dominant white phenotype. If positional candidate cloning fails, there still remains the possibility to clone the gene by pure positional cloning. The critical issue is to map the trait locus very precisely, as it is hardly meaningful to start a positional cloning attempt if the region in which the gene is located exceeds about 1 million bp (which is expected to harbour on average approximately 25 genes in the mammalian genome). In farm animals, it may be necessary to clone additional marker loci from the actual region to be able to improve the localisation. The next step is to produce a contig of large insert clones (BACs and/or YACs) spanning the region between the two closest markers showing recombination with the trait locus. Then all coding sequences present in the region must be identified and each of them evaluated as possible candidate genes by expression and sequence analyses. Positional cloning is obviously a very demanding task. The strategy has been used successfully a number of times in humans and the mouse but the success has been facilitated by the presence of excellent pedigree material, dense genetic maps and often by chromosomal rearrangements in some families. No gene has yet been isolated by pure positional cloning in any farm animal. The cloning of genes affecting disease resistance with a polygenic inheritance will clearly be a formidable task due to the poor precision in QTL mapping (Murray and Anderson, 2000; Ron and Weller, 2007).

Marker–Assisted Selection and Marker–Assisted Introgression

Once the genomic analysis phase has been successfully completed, information will be available on the map location and effect of the DRL (if the DRL have been identified through linkage mapping); while, if the DRL have been identified by association testing or positional cloning of candidate genes, there will be information on the known genes corresponding to the DRL. It is important to realise that identifying a DRL and mapping that DRL to a specific chromosomal location is not in itself sufficient to permit MAS. For this, it is essential to move beyond the genomic analysis to determine coupling linkage associations between specific alleles at the marker locus and specific resistance/susceptibility alleles at the DRL. Once this objective (termed 'determining marker/DRL phase') has been achieved, the specific form of the MAS programme will depend primarily on the source of resistance alleles at the DRL, whether within the commercial population itself, or in an exotic population from which they must be introduced by introgression into the commercial population (Howard et al., 2001; Meuwissen et al., 2001).

Determining Marker/Disease Resistance Loci Phase

Approaches and possibilities for the determining marker/DRL phase depend largely on the methods used for identifying then DRL. Association tests automatically provide information on the marker/DRL phase, as does linkage mapping in crosses between populations that differ in resistance status, but this is not the case for within–population linkage mapping, which provides information on DRL location but not on the marker/DRL phase. Each of these situations will now be considered in turn (Terwilliger, 1995; Barton and Keightley, 2002).

Association Tests

Between genetic variants and differences of traits on a population scale genetic association studies assess correlations. Until recently on the genetic side there are relatively few deoxy ribo nucleotide (DNA) variants. In the past few years however approximately two million such polymorphisms have been identified. The phenotypes assessed in association studies on the side of traits include status of disease. Such width of information regarding genetics and depth of phenotypic measures holds promise considerably for identification of correlation between genotype and disease (Drews and Ryser, 1997). By their very nature, association tests based on candidate gene analysis identify associations between specific marker alleles or haplotypes at the candidate gene and the contribution of the candidate gene to the resistance/susceptibility status of the animal. Similarly, association tests based on short–range population–wide linkage disequilibrium (as a follow up to a within–population linkage mapping study) identify within population associations between specific marker alleles or haplotypes in a very limited chromosomal region and the resistance/susceptibility allelic state of presumed DRL present in that region. Thus, this information is immediately available for utilisation in MAS programmes in the population within which it was demonstrated. As linkage between marker and causative mutation is very close in both cases, the marker/DRL phase can be considered to be constant over many generations and need not be re–established each generation. For candidate gene analysis, unless the causative mutation has been identified, association between specific marker alleles and resistance status cannot be automatically assumed to hold in different populations. However, if intragenic haplotypes have been defined at the candidate gene, it is highly plausible that identical haplotypes in different populations are identical by descent from the same ancestor. In this case, the haplotypes can also be presumed to carry the same functional mutation (although it is possible that the functional mutation arose after the populations diverged). For short–range disequilibrium analysis, there is a greater likelihood that the marker/DRL phase may differ in different populations, and hence any effects should be restudied in each population (Glazier et al., 2002; Frank, 2003).

Linkage Determination in Cross Populations

Linkage equilibrium as well as disequilibrium are the terms that are used for the chance of coinheritance of alleles at various loci. In random association alleles are said to be in linkage equilibrium. In mapping of genes linkage is used. Since the beginning of the 20th century this concept has been used (Lander and Schork, 1994). When marker/DRL linkage is determined in a cross population, the mapping exercise will automatically provide information on the marker/DRL phase. As this is long–range mapping, the region to which the DRL is mapped will normally span 10 to 20 cM or more. Thus, the marker/DRL phase can be expected to differ in different resistant populations. In

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addition, the large chromosomal region in which the DRL may be located poses special problems in MAS programmes (Goldstein and Weale, 2001; Oldenbroek et al., 2007).

Linkage Determination within Populations

When marker/DRL linkage is determined by means of a within–population linkage analysis using designs based on half–sib or full–sib families, the basic assumption is that the population is in long–range linkage equilibrium, so that the marker/DRL phase will differ in different chromosomes and families. Hence, within–population linkage mapping, while it provides information as to DRL effect and general location, does not provide information as to the marker/DRL phase (Gibson and Weir, 2005).

This must be determined separately for the chromosomes of each individual. Possibilities for determining the marker/DRL phase for individuals depend very strongly on the size of the individual families within the mapping population. When family sizes are small to moderate (up to 100 progeny per family), definite marker/DRL phase assignments within individual families ordinarily will not be possible, except for DRL having very large effects. This is compounded by the fact that family designs, even when based on a relatively large total mapping population (approximately 10,000 individuals), will only map a DRL to a confidence interval of 20 to 40 cM. In such situations, therefore, linkage mapping of DRL can best be thought of as a preliminary step to fine mapping through candidate gene or short–range linkage disequilibrium analysis. Moreover, there is an expectation that future statistical–genetic developments, based on multi–generation animal–model approaches, will eventually enable more accurate assessment of DRL location and of marker/DRL phase for individual chromosomes, even in this problematic situation (Cheung Spielman, 2002; www.animalgenome.org).

When family sizes are large, however, the determination of the marker/DRL phase with fair to high accuracy within individual families will be possible. This will certainly be the case for the large half–sib daughter families of artificial insemination sires in dairy cattle and full–sib families in fish, which can easily exceed 1,000 or more progeny. The same will also be true for DRL of moderate to large effect at the upper size limit of half–sib families in poultry and pigs (approximately 200 progeny). But this will require individual phenotyping of the members of each family, thus enormously increasing the phenotyping load. Genotyping should not be a major burden as this can be achieved through selective DNA pooling, as described above (Christiansen and Sandoe, 2000).

Chromosome Design Mapping within Populations

When within–population mapping is carried out by means of a chromosome design, the marker/DRL phase will be known with high accuracy for the founder parents of the mapping population and for their descendants within the mapping population. The marker/DRL phase will not, however, be known for the individuals making up the bulk of the population, and a chromosome design will obviously not provide the marker/DRL phase information for other populations (Adam, 2002; Yu and Buckler, 2006).

In this aspect a classical example is analysis of Israeli Holstein sire families for QTL effects on chromosome 6 for various milk production traits by a daughter design. The families with effect significantly have been genotyped for upto ten markers additionally. By comparative mapping possible candidates for the major quantitative gene has been determined. Within a sequence of 2 mega base pair (Mbp) tweleve genes have been identified (Ron et al., 2001).

Marker–Assisted Selection within a Population

For most farm animals, selection among males can be much more intense than among females. In addition, many of the productivity traits come to expression in females only, so that early selection among males is limited to pedigree or family information. In this case, it is possible to produce many more males of approximately equal estimated breeding value at an early age than are needed for reproductive activity. This opens a powerful window of opportunity for MAS for DRL. Thus, in most animal species, if the marker/DRL phase can be established on a population–wide basis through candidate gene analysis or short–range linkage disequilibrium, MAS for disease resistance can proceed forward vigorously, primarily by selection among young males. This will rapidly increase the frequency of resistance alleles in the population. It has to be noted that for candidate gene analysis, allele effects on resistance must be established only once, and selection thereafter is on the basis of marker genotype alone. Thus, heavy investment in phenotyping a single generation will be feasible, as the benefits will flow for many generations. The same applies for short–range linkage disequilibrium studies (www.agroedu.net). When the marker/DRL phase is established through within–population family studies, MAS is at present only feasible for those species (such as dairy cattle or fish) in which very large half–sib or full–sib families can be produced. In this case, the marker/DRL phase can be established for the parent individuals in the family. In the case of half–sib families, selection will then be made on the basis of the marker allele transmitted from the evaluated parent to his progeny. In this case, however, information on the marker/DRL phase is lost rapidly across generations. This is due in part to recombination between marker and DRL and in part to loss of ability to track the parental origin of a marker received by an individual: for example, if the individual and both parents are heterozygous for the same pair of marker alleles. Both of these problems are markedly reduced when dealing with multiallelic markers tightly linked to the DRL, or with multimarker haplotypes bracketing the region containing the DRL. In this case, if the DRL has been mapped to a small region (for example, 5 to 10 cM), the marker/DRL phase will be maintained for a number of generations. Thus, if the population is fully pedigreed, information on marker/DRL linkage for a significant proportion of the chromosomes segregating in the population will build up over time, enabling effective early MAS among males, as described above. A programme of this sort will require a large amount of phenotyping. This is required for the initial DRL mapping, and will need to be carried out consistently for some appreciable portion of the new sires entering the population each year, to maintain and renew information on the marker/DRL phase against its continual degradation by the mechanisms described above. However, continued phenotyping and genotyping will contribute to a continual decrease in the CI of, DRL map location, which will in turn decrease recombination between marker and DRL and, more importantly, will open opportunities for uncovering candidate genes or short–range population–wide linkage

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disequilibrium (Rosyara et al., 2007 & 2009; www.lifetechnologies.com).

When family size is small (< 200), linkage mapping in a large total population will be able to locate and map DRL but, unless DRL effects are very large, it will not be possible with present statistical methodologies to establish marker/DRL phase in most families. Thus, as stated, in this case linkage mapping should be viewed as a stepping–stone to candidate gene identification, or to a search for short–range population–wide linkage disequilibrium. However, this is an active area of statistical QTL mapping research, thus there is a possibility that over the next few years the appropriate statistical methods will become available. In some instances, where good phenotyping information on disease resistance has been collected, it may be worthwhile storing DNA samples of the phenotyped individuals, on the likelihood that the statistical techniques needed to use this information for mapping and for establishing the marker/DRL phase will be developed in the near future (Bovenhuis and Meuwissen, 1996; Rhee, 2005; Khatkar et al., 2004).

When within–population linkage mapping has been carried out by means of a chromosome design, the situation becomes one of marker–assisted introgression into the main population from a sub–population. This will, therefore, be discussed in the following section on marker–assisted introgression (Collard and Mackill, 2007).

Marker–Assisted Introgression from a Donor (Resource) Population

Marker–assisted introgression (MAI) will be employed when resistance alleles at one or more DRL have been identified in a donor population, mapped with respect to a marker locus or marker haplotype, and the marker/DRL phase has been established. In this case, the DRL can be introgressed into the recipient population by repeated backcrossing, as in a classical introgression programme, except that instead of challenging and selecting for resistance in each generation, selection is performed on the basis of marker–allele status only. Thus, the entire problem of selecting under challenge for disease resistance is avoided. Furthermore, by actively selecting against donor markers that characterise the unwanted portion of the donor genome, the number of backcross generations required to eliminate donor genome from the recipient population can be reduced by 1 or 2. Finally, donor genome that is closely associated with the DRL can be eliminated by selecting for recombinant chromosomes carrying only minimal donor chromosome flanking the DRL (Law and Archibald, 2000). Thus, all of the problems associated with introgression of resistance alleles detailed in the introduction section find a satisfactory solution in the framework of MAI. Here too, however, the details of the programme will depend greatly on the kind of linkage information available on the marker/DRL phase in the donor. MAI will be most effective when the DRL has been identified by candidate gene analysis. In this case, selection at all stages of the introgression will be performed on the basis of the marker allele or haplotype characteristic of the resistant allele at the DRL. Selection against donor marker alleles covering the remainder of the genome, and screening for recombinants between DRL and recipient chromosomes which carry minimal donor genome, will speed the retrieval of recipient productivity status. In particular, since selection in the intercross stages can be based on the genomic testing of males at an early age, this should not interfere with later, phenotype–based selection for productivity traits. The use of a marker or marker haplotype that is not present in the recipient population to monitor the target DRL will be preferable. Otherwise, there will be progressive dilution of the donor DRL in each generation of backcrossing and selection, in proportion to the relative frequency of the donor marker in the recipient population. As a result of the effectiveness of the MAS procedure, any deleterious allele tightly linked to the donor DRL will be retained and brought to fixation in the intercross population along with the DRL. To reduce the likelihood and severity of such incidents, the introgression programme must be initiated using a number of donor parents. Although this will increase the overall likelihood of introducing a severely deleterious gene in tight linkage to the DRL, it will prevent the deleterious allele from reaching high frequencies in the intercross population and will provide opportunities to select against it while retaining resistance (Kruglyak, 1999; Appels et al., 2004; Chan, 2005).

More or less the same considerations will apply if the DRL has been mapped either by population–wide linkage disequilibrium or by linkage mapping with respect to one or more linked markers. Again, selection in the backcross and intercross generations of the introgression programme will be based on the markers associated with the target DRL, rather than on disease resistance itself. Clearly, in order to avoid losses of the DRL by recombination between DRL and marker, it will be necessary to introgress a donor chromosome segment that brackets the CI. Much will therefore depend on the width of the CI to which the DRL has been mapped. If the CI is small, a two–marker haplotype will be sufficient in principle. However, this will increase possibilities that the marker alleles making up the haplotype are also segregating in the recipient population. This will cause difficulties in distinguishing the introgressed donor segment from recipient genome, and recombinants from parental types. Thus, a multi–marker haplotype will be preferable. As the CI of the DRL map location becomes greater, possibilities for double recombination and consequent loss of the desired DRL allele also increase, so that selection on the basis of a multi–allelic haplotype covering the introgressed donor chromosome segment becomes even more necessary. Introgressing a larger chromosomal segment carries additional penalties. In each backcross generation, the proportion of recombinant haplotypes among all donor haplotypes is equal to the total width of the marker bracket encompassing the CI. If only a single DRL is targeted, this is not too worrisome, since even for a very large bracket, for example 30 cM, sufficient recombinant haplotypes will remain to continue the backcross procedure. However, if two or more DRL are targeted, the proportion of individuals carrying parental–type donor chromosome segments at all DRL drops precipitously with increased CI. Thus, with three targeted DRL and confidence intervals of 30 cM per locus, only (0.35) 3 = 0.04 of BC individuals will be carrying all three non–recombinant DRL segments. This will require taking individuals carrying only one or two donor DRL segments to continue the backcross programme, with consequent reduction in donor DRL frequency at the initiation of the intercross generations. There will also be loss of informative

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haplotypes due to recombination during the intercross generations. Both of these factors will increase the number of intercross generations required for fixation of resistance DRL alleles. Finally, the larger the introgressed donor segment, the greater the risk of 'chromosomal drag', leading to the incorporation of deleterious donor productivity or fitness alleles in the intercross population. All of the above lead to the conclusion that it is imperative in an introgression programme to work with DRL that have been mapped to a narrow (for example, < 5 cM) CI. An attractive possibility is to continue with DRL mapping within the framework of the backcross programme itself. In the BC generations, the individuals chosen to continue the introgression procedure are heterozygous at the DRL, and thus essentially represent an F1 individual. Consequently, each of the backcross generations is the equivalent of a BC1 population with respect to mapping power. Further, refinement of DRL map location can be achieved by continued challenge and phenotyping with respect to resistance in the BC generations themselves. This is increased by the map expansion at the DRL generated by the backcrossing procedure. In addition, in the BC generations, the required challenge and consequent disease incidence does not interfere with improvement of productivity, since this is achieved by the continued introduction of recipient genome. Thus, if mapping is continued over three BC generations it should be possible to markedly narrow the CI of DRL map location. If this is done, then by the time the intercross generations within which selection for productivity must be initiated are reached, loss of DRL allele information due to recombination, and 'chromosome drag' due to associated donor chromosome segments, can both be minimised (Smith, 1994; Hospital et al., 2000; Gomez–Raya et al., 2013).

USE OF MOLECULAR MARKERS TO ENHANCE RESISTANCE OF LIVESTOCK TO DISEASE

For the future potential use although the ideal is conservation of all breeds of livestock the financial as well as physical and human resources are very unlikely to be available. Therefore it is required to take decisions for allocating resources that are finite for the purpose of conservation. One of the goal for conservation will be retaining the diversity to be used in future at its maximum limit. There is absence of information completely on the distribution of genetic polymorphisms that are useful potentially among breeds. On the phenotypes of the breeds of livestock in developing world very much limited information is available. Molecular markers thus in the short term basis provides estimates of genetic diversity that are most easily available within as well as between a given set of breeds. At all stages of the process of decision making importantly there is use of informations on molecular markers. It includes the final decision on whether or not there will be collection of further marker informations within the programme of final genetic improvement. Integrated strategy must be employed while utilizing molecular markers which help in assessing genetic diversity as a tool to design studies on disease genetics along with simultaneous detection and exploitation of genetic variation in resistance. A major role can be played by this strategy to understand the genetic control of resistance to infectious diseases and to solve practical issues that can undermine potentially the sustainable development of livestock production systems (Simianer et al., 2003; Gibson and Bishop, 2005)

Use of Molecular Marker Diversity in Conservation Decisions

Although the ideal would be to conserve all breeds of livestock for future potential use, the financial, physical and human resources are very unlikely to be available to do that. Decisions will therefore have to be taken on how to allocate finite resources for conservation. One goal of conservation will be to retain the maximum amount of diversity for potential future use. There is an almost complete absence of information on the distribution of potentially useful genetic polymorphisms among breeds, and only very limited information exists on phenotypes of developing–world breeds. In the short term, therefore, molecular marker information provides the most easily obtainable estimates of the genetic diversity within and between a given set of breeds.

Weitzman proposed a method for optimal allocation of finite resources for conservation to maximise the future inter–population diversity of wildlife species. This method has recently been adapted to conservation of livestock breeds and extended to incorporate predictions of extinction probabilities and to utilise combinations of molecular marker and phenotypic data. An alternative approach, designed to maximise a combination of genetic diversity within and between populations, has also been developed. These methods will require further development to deal with the complex reality of decision taking in conservation, but already provide a sound justification for collecting molecular marker data to map the global diversity of livestock species (Gilligan, 2001; Magnusson, 2005).

These breeds have evolved to allow livestock production in a wide range of situations, including some of the most stressful environments inhabited by humans. These naturally evolved genetic characteristics provide a coherent basket of sustainable solutions to disease resistance, survival and efficient production that have often been ignored in the drive to find technological and management solutions to individual problems of livestock production in low–input systems. It is estimated that 35% of mammalian breeds and 63% of avian breeds are at risk of extinction, and that one breed is lost every week. The performance, adaptation and disease resistance of the vast majority of breeds in developing countries have not been systematically recorded, and little of the information that does exist is in an easily accessible form. Moreover, the majority of livestock genetic diversity is found in the developing world, where documentation is most lacking and the risk of extinction is highest and increasing. Molecular genetic markers can be used to estimate the genetic diversity within and between a set of breeds. Such information has been collected in a number of projects and used both to map the geographic distribution of livestock genetic diversity and to infer movements of livestock following domestication. Such information is of great scientific interest. Until recently, however, it has not been clear how information on molecular genetic marker genotypes can contribute to the utilisation of livestock genetic diversity (Soller and Andersson, 2004; Battle et al., 2010).

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Use of Molecular Marker Diversity in Decisions on Utilisation

Population genetics theory has long predicted that, under a given selection pressure, evolution will pick different genetic solutions in populations that are isolated from each other. Essentially, selection acts on the variation available, and this variation will vary between populations. The more genetically distinct are any two populations, the greater the likelihood that they will contain distinct genetic polymorphisms and the greater the chance that selection will lead to fixation of different genetic solutions to the same problem in the two populations. Experimental support for this theory exists in model species, and most recently also for the case of trypanosomiosis tolerance in livestock (Dekkers, 2004).

While there is enormous variation in levels of resistance to disease, there are many cases where no breed has achieved complete resistance. Trypanotolerance in cattle and gastrointestinal helminth resistance in sheep are good examples, where breeds exist that are able to survive and produce under disease challenge but still perform better in the absence of the disease. It would be desirable to produce animals with even higher levels of resistance to disease, which would be able to thrive under the highest challenge in the absence of other disease control measures. There are well–documented examples of several distinct breeds of a given species having evolved partial resistance to a given disease. Given the general lack of information on the characteristics of livestock breeds, there are probably many more undocumented examples. A good example is gastrointestinal parasite resistance in sheep, with at least eight breeds of sheep having been recorded as having some degree of enhanced resistance compared to exotic breeds developed in other environments (Nash and Freeman, 2004; Dominik, 2005).

In order to identify the best possible genotype for each of a range of production environments, the ideal situation would be to test all breeds with potentially useful characteristics, and all their crosses in each production environment. In practice such testing is not feasible, due to economic and logistical limitations, and increasingly also the difficulties imposed by issues related to sovereignty. Over livestock germplasm. What would be feasible in many cases would be to undertake testing of just two breeds from different countries. Many countries would see the advantage of a reciprocal exchange of germplasm with another country, which could overcome sovereignty concerns in many cases. Given that considerable time and money will be involved in the testing, the critical question is which two breeds would maximise the probability of being able to develop a better genotype. Obviously, choice of breeds will involve careful examination of existing data on breed characteristics and the environments under which they evolved. But where further improvement of a particular trait such as helminth resistance is desired, one consideration would be the likelihood that two breeds have evolved different mechanisms of resistance and that a higher level of resistance could therefore readily be developed from a cross between them. In this case breeders would seek breeds with suitable phenotypes, which are as genetically distant from each other as possible (Ruane and Colleau, 1995; Phillips and Belknap, 2002).

Use of Molecular Markers to Confirm the Hypothesis of Different Mechanisms of Genetic Control

Having brought two breeds together for testing in a given environment, on the hypothesis that they carry different mechanisms for genetic control of a desirable trait such as helminth resistance, it will be important to test that hypothesis before proceeding with a breeding programme. A suitable method for testing the hypothesis is to perform a genome–wide QTL interval mapping based on anonymous genetic markers in the F2 and/or backcrosses between the two breeds. Depending on whether or not the hypothesis is confirmed, the size of the QTL detected, the performance of the pure breeds and the F2 or backcrosses, an informed decision can then be taken on a suitable genetic improvement programme. The outcome might be to utilise one of the purebreds, to develop a crossbreeding programme, to develop a new breed through selection from crossbred or backcross populations, or to introgress QTL from one breed to the other. An informed decision can also be taken on whether or not the genetic improvement programme would incorporate marker–based selection. This decision will depend not just on the potential value of the marker information, but also on the cost and logistics of collecting and using the marker information in the genetic improvement programme (Beuzen et al., 2000; Thomson, 2003; de Koning, 2008).

CONCLUSION AND FUTURE PERSPECTIVES

The strategies of disease resistance breeding that have emerged recently still requires to attain maturity. It is true that in comparison to other economic traits little breakthrough has been achieved in disease resistance studies. In order to uncover the disease resistance genes novel genomic as well as systems biological techniques like high throughput sequencing as well as gene function analysis are found to be helpful thereby further strengthening the studies of disease resistance. The multitude of applications of genomic studies conducted in farm animals continues to attract audiences. The genomic data of cattle can now be used by the meat industry for confirming the quality of meat that is produced. In order to determine the disease resistance genes various companies are using genomic informations. Enterprises like xenotransplantation (wherein there is transfer of animal tissues or organs in humans) will be aided by genomic work in farm animals. For satiating organ donor shortage certainly animal organs may be used someday but in this area genomic work is still in its early stage. By identifying causal variants along with expansion of such studies into populations of diverse ancestry scientists will be able to facilitate biological understanding further along with population genetics of complex traits. Implementation of new technologies as well as analytic approaches for integrating diverse data types will help in accelerating pace of discovery of variants that are trait–associated. Lastly but not the least it is believed that improvement in the field of disease resistance breeding will benefit the future livestock industry.

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