MAPPING GENES FOR DISEASE RESISTANCE IN DEER

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Abstract

Recent advances in animal molecular genetics offer the potential to identify genes which determine innate resistance or susceptibility to disease New Zealand deer are a unique resource for identification of such disease genes because the species, subspecies and hybrids which are farmed show major differences in their susceptability to disease We are constructing a gene map of deer in order to determine, in interspecies hybrids, exactly which chromosome segments originate from each parental species By following the inheritance of chromosome segments and resistance or susceptibility in hybrids we will locate the regions of deer chromosomes carrying genes for disease resistance In other animals, such genetic analyses have identified two gene regions which determine susceptibility to tuberculosis and other diseases, the major histocompatibility complex (MHC) and the Bcg locus We describe, in particular, our work mapping the MHC chromosome region in deer, and our progress in characterising in detail (gene sequencing) the variation in MHC-DRB and Bcg genes in red deer

Introduction

Genetic markers which indicate a predisposition to disease are becoming important tools in medical diagnosis and treatment In farmed animals, this technology introduces new possibilities for limiting the incidence and spread of disease through selective breeding of resistant animals Many of the developments in medical genetic technology are directly applicable to animals Indeed, one of the major means of studying human disease is to examine the genetics of disease in animals, usually the mouse or rat and occasionally farmed animals (Lui *et al.* 1993)

This paper describes our research on genetic marker technology in deer and outlines how this technology could be used to identify genes associated with resistance or susceptibility to major deer diseases such as malignant catarrhal fever (MCF), internal parasitism and tuberculosis Our research involves two quite different approaches The first is to use a gene map of deer to identify the genes which determine the differences between deer species and subspecies in disease resistance and other traits The second approach is to examine, in deer, genes which have been shown to be associated with resistance to diseases in other mammals This latter approach concentrates particularly on the genes which may be involved in resistance or susceptibility to tuberculosis

Deer species and subspecies differ in disease resistance and gene markers.

New Zealand farmed deer are notable in that the "varieties" of deer are not breeds but evolutionarily distinct species and subspecies or hybrids between these taxa. The four major types of deer farmed in New Zealand are European red deer (eg: *Cervus elaphus scotticus*), North American wapiti (eg: *Cervus elaphus manitobensis*), wapiti x red deer hybrids, and fallow deer (*Dama dama*). There are also small numbers of Père David's deer (*Elaphurus davidianus*), sika (*Cervus nippon*), rusa (*Cervus timorensis*) and sambar (*Cervus unicolor*). Of these, Père David's deer and sika form fertile hybrids with red deer. We have characterised genetic differences between these species and subspecies using blood proteins. This work has provided insights into the evolutionary relationships of deer species (Emerson and Tate 1993) and identified Père David's deer x red deer hybrids as one of the widest known mammalian hybrids in which both male and female offspring (F1 generation) hybrids are fertile (Tate *et al.* 1992).

Our recent work has focused on characterising DNA differences between red deer, Père David's deer and wapiti. This is because of the actual and potential importance of hybrids between these species to the deer industry and their value in gene mapping and genetic analysis (Fennessy and Mackintosh 1992). Table 1 shows the data for one class of DNA marker (restriction fragment length variants) which we have examined. Compared to protein methods, the DNA methods have been particularly effective in identifying differences between red deer and Père David's deer and have revealed some new markers distinguishing the more closely related wapiti and red deer.

The deer species farmed in New Zealand also show major differences in traits and, in particular, the susceptibility to disease. Table 2 (from Mackintosh 1990) summarises these differences. Possibly the most dramatic difference is in the variation in susceptibility to MCF, a gammaherpes virus carried by sheep which inevitably leads to death once the infection is established in deer (Reid 1990). Père David's deer, rusa, and sika are highly susceptible and so cannot be maintained in close proximity to sheep. Fortunately MCF has not been a major problem in first cross (F_1) hybrids between red deer and Père David's deer and the progeny of these animals mated to red deer (backcross hybrids). Of over 200 Père David hybrid animals born at Invermay since 1988, only 5 have died from MCF.

Another major difference in disease resistance among farmed deer is that wapiti succumb to a wide range of diseases to which the con-specific red deer are relatively resistant. Of the diseases listed in Table 2 fading elk syndrome, preputial prolapse and enzootic ataxia are occasional syndromes either restricted to or, in the case of enzootic ataxia, much more prevalent in pure wapiti or animals with a high proportion of wapiti genes. In contrast wapiti appear to be generally more susceptible than red deer to lungworm infection, gut parasitism, ryegrass staggers and dietary scour and these show a range of susceptibility in hybrids. Anecdotal evidence suggests the incidence of disease increases more or less in relation to the proportion of wapiti genes.

Table 1: Genetic markers differences between red deer, wapiti and Père David's deer

	No. loci of tested	No. of loci with differences between species	
		red deer - wapiti	red deer - Père David
Protein differences[1]	45	4	21
DNA differences (RFLV)	134	20	123

RFLV - restriction fragment length variants. [1] Data from Emerson and Tate (1993) and Tate *et al.* (1992).

Table 2: Summary of the relative disease susceptibility of deer species found in New Zealand (from Mackintosh 1990).

Disease	Very Susceptible	Susceptible	Resistant
Malignant catarrhal fever	PD, S, T, V	R, W,	F
Yersiniosis	---	R, W	F
Facial eczema (sporidesmin)	F	R, W	---
Ryegrass staggers	W	R,	---
Enzootic ataxia	W	R,	---
Lung worm	R(c) W(c) F(c)	W(a)	R (a), F(a)
Gastrointestinal nematodes	W(c)	W(a), R(w)	R (a), F(a)
Prepupital ulceration and prolapse	W		R, F
"Dietary scour"	W		R, F
"Fading elk syndrome"	W		R, F

Species: F, fallow; PD, Père David's deer; R, red deer; S, sika; T, Rusa; V, white tailed deer; W, Wapiti.
Age: c, weaner calves; a, adults.

Deer hybrids are a unique resource for linking markers to disease resistance

The differences in disease resistance of deer species farmed in a common environment clearly indicate a genetic basis for resistance Interspecies hybrid pedigrees are the key resource required for further genetic analysis This approach, termed "genetic linkage" is a standard technique used in experimental animals, typically mice, where highly inbred resistant and susceptible strains are crossed and gene markers are tested for linkage to resistance, or susceptibility, in a variety of cross-bred animals (eg, Shurr *et al* 1989)

The criteria for a successful linkage experiment are firstly, strains which show wide divergence in traits, secondly, large numbers of animals (eg, 200) of an appropriate inter-strain or interspecies cross (eg, a backcross or intercross), thirdly, good definition and measurement of traits and last but not least, a large number of genetic markers (Lander and Botstein 1989) In Père David hybrids, MCF is a good candidate for linkage studies in that the clinical signs of the disease and outcome (death) are dramatic and easily measurable While existing pedigrees, which always involve crosses to red deer may not be appropriate, future pedigrees which involve intercrossing of hybrids may provide very useful data In wapiti x red deer pedigrees, lungworm infection, internal parasitism, ryegrass staggers and dietary scour are all potential candidates and appropriate backcrosses could be produced by mating F_1 wapiti x red deer hybrids to pure wapiti or red deer However, in each case defining a standardised disease challenge and improving measurement of infection will be crucial

Our work has focused on developing a comprehensive set of genetic markers for use in deer so that each part of each chromosome can be checked for association with disease resistance or other traits Red deer, wapiti and Père David's deer have 34 pairs of chromosomes Our aim is to generate sufficient deer gene markers to determine, in hybrids between these taxa, exactly which chromosome segments and genes are derived from each species

The deer genetic map

To ensure that each segment of each chromosome can be tested with markers a genetic map is required The map records to which chromosomal group the marker belongs and the arrangement of markers within each group We are generating a primary deer gene map using backcross hybrid pedigrees produced by mating F1 Père David x red deer sires to red deer These animals are one of the widest known interspecies hybrids with fertile offspring They are ideal for constructing a gene map because it is very easy to find markers which distinguish Père David's deer and red deer (Table 1) To date we have tested over 150 markers in pedigrees which involve 123 backcross progeny of three F1 hybrids Linkage analyses among the markers place them into 28 putative chromosomal groups and define the order of markers within each group Current work is focusing on filling regions of the deer map where there are no markers and also adapting the markers from the Père David hybrids for use in other crosses

Figure 1: The use of species specific markers to determine the origin of the MHC region in 121 1/4 Pere David x 3/4 red deer backcross hybrids (see text for explanation).

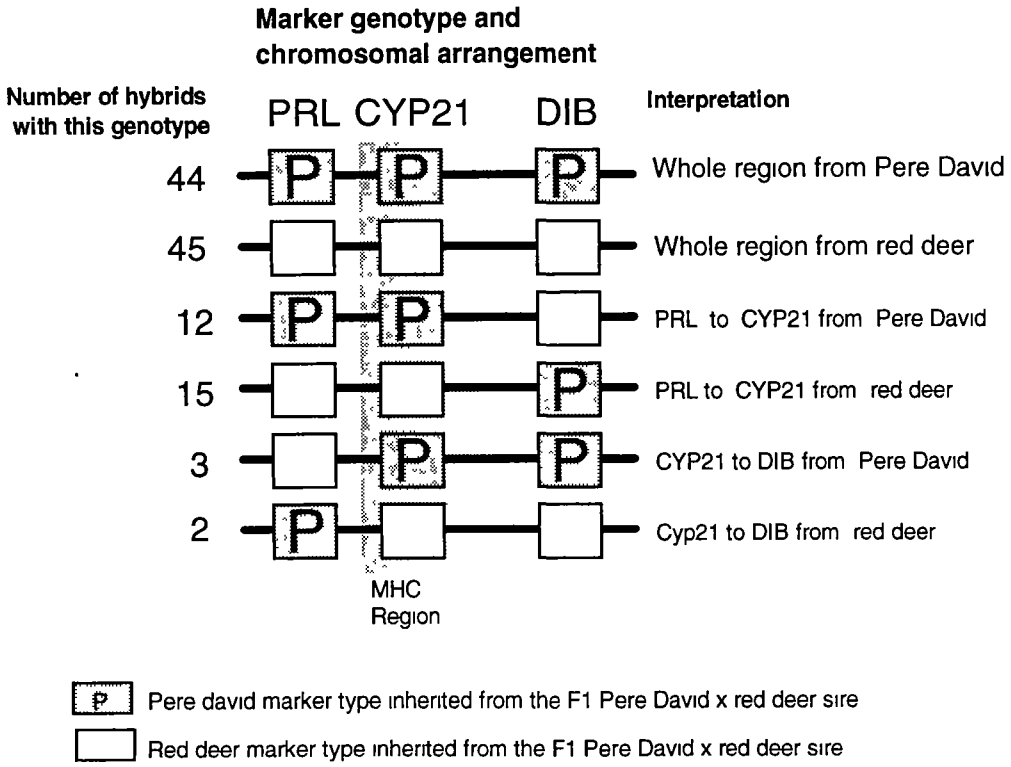

Figure 1 gives an example of the use of the gene map to determine the species of origin of a particular chromosome segment in deer hybrids. The segment chosen for the example spans the major histocompatibility complex (MHC) which is a dense cluster of genes involved in the immune reaction to disease. These genes include the so called "MHC Class 1" genes which encode the transplantation antigens that are found on the cell surface and cause rapid rejection of skin and organ grafts between individuals. MHC Class II genes code for proteins which present antigens to the immune system. The area also contains genes for proteins which transport these proteins to the surface of cells and genes for some proteins involved in the complement system. The figure shows a deer chromosome (depicted as a horizontal line) on which three marker genes have been mapped. They are prolactin (PRL), cytochrome P450 steroid 21 alpha hydroxylase (CYP21) and an MHC associated gene identified in cattle, BoLA-DIB. These markers were chosen for the example because they bracket a conserved mammalian chromosomal region containing the MHC.

Each of the markers have a distinctively different "type" in Père David's deer and red deer when DNA is analysed using restriction enzymes. Figure 1 lists the arrangement of markers which we found in 121 progeny of three F_1 Père David x red deer sires. In all cases the mother of the hybrids was a red deer thus the complementary chromosome (not shown) was always from a red deer. The letter P indicates that a Père David marker was inherited from the F1 hybrid sire while a blank square indicates that only red deer markers were found. The markers demonstrate that 44 backcross calves inherited a copy of the entire MHC region from their Père David grandsire while in 45 backcross calves only red deer genes were found. Genetic recombination in the paternal gametes resulted in the

remaining 32 animals inheriting combination of red deer and Père David genotypes.

These markers provide the potential to test whether the genes of the MHC region determine the differences in disease resistance between deer species. To take a purely hypothetical example, if all the calves inheriting the Père David MHC died of MCF while those inheriting the red deer MHC did not, it would be clear evidence that genes in the Père David MHC were responsible for this species susceptibility to the disease and the markers could then be used to select only animals with a red deer MHC for further breeding. On the other hand, if there was no association between MCF susceptibility and the MHC, it would exclude this region as a candidate and markers from other deer chromosomes could be examined. In reality, these particular animals, which are essential to our gene mapping program, have not been intentionally exposed to MCF or any other disease. The point of the example is to show how the gene marker technology we are developing will be used to identify and select for disease resistance genes in deer.

Genes which determine susceptibility to tuberculosis

The gene mapping approach described above is very powerful where clear differences in disease resistance or susceptibility can be demonstrated and appropriate crosses can be made. However, some economically important diseases do not show appropriate variation in deer. Bovine tuberculosis (*Mycobacterium bovis*) is a prime example in which there is, as yet, no evidence of different susceptibility in different deer species.

Genes determining susceptibility to *M. bovis* have been identified in other species and offer good candidates for the study of genetic resistance in deer. In crosses between strains of mice which are resistant and susceptible to mycobacterial infections, genetic mapping experiments have identified a region of mouse chromosome 1 termed the "Bcg locus" which is strongly linked to susceptibility. The Bcg locus affects the ability of macrophages to eliminate phagocytised intracellular parasites in the early phase of infection (Schurr *et al.* 1989). Recently a gene for the Bcg locus has been identified and the DNA sequence determined. From the structure it appears to be a membrane transporter gene possibly important in nitrate transport (Vidal *et al.* 1993). How important this gene is in resistance or susceptibility of other species to *M. bovis* is unknown but it is certainly a strong candidate for having a role in resistance in other species including deer. We have begun to search for this locus in deer. Once it has been isolated we will search for any naturally occurring variation within the farmed deer populations and, if variation is found, we will test to see if any variants are associated with resistance or susceptibility to tuberculosis.

In the later phase of mycobacterial infection association between the course of infection and MHC class II genes (see above) has been found (Brett *et al.* 1992). Our initial focus has been on one of the genes in the MHC region, the Class II DRB. Genetic characterisation of these genes is complex because within the MHC there are a large number of similar genes, only some of which are functional, and within each functional gene there are typically many different variants. One explanation for this high degree of variation is that it has been caused by the selection pressure of a diverse array of diseases acting on the population. This theory suggests that the particular variants of these genes may be associated with resistance or susceptibility to specific diseases such as tuberculosis

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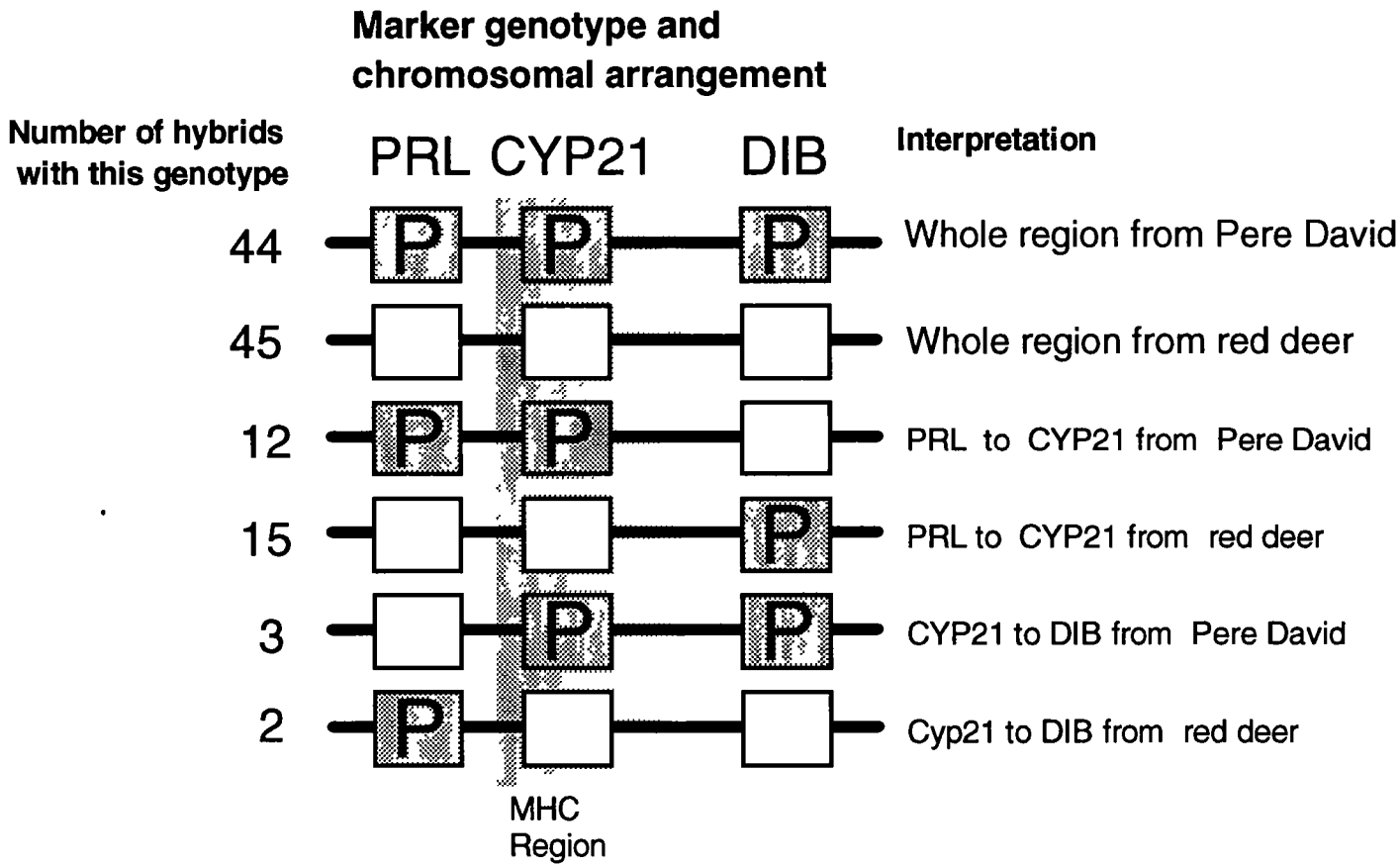

(Potts and Wakeland 1990).

We have examined the functional DRB genes from 50 unrelated deer using DNA sequencing. The results show that in red deer there are at least two functional MHC-DRB genes with, typically, three or four different DRB sequences per animal. In total we have identified 49 different sequences. Work is now concentrating on a rapid way of typing the sequence variants and examining associations between the variants and the response of deer to tuberculosis.

Outlook

The advances in the field of animal molecular genetics allow us to follow, using DNA markers, the inheritance of genes and whole regions of chromosomes with previously unavailable precision. Experiments following the inheritance of markers in animal pedigrees have identified genes responsible for disease resistance and we expect the genetic basis of many more traits and diseases will be described in the future. These discoveries have the potential to revolutionise animal breeding because resistant animals could be selected more rapidly using genetic markers without exposing them to disease. The work described here is an important step toward application of this technology in deer and provides basis to take advantage of the further advances in the field. We believe the combination of the deer gene map, interspecies hybrid pedigrees and the development of techniques to examine candidate genes for disease resistance in deer will identify useful gene markers predictive of resistance or susceptibility to major deer diseases.

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