DNA variation among seven New Zealand sheep breeds

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ABSTRACT

Genetic variation among 7 sheep breeds was investigated using 10 DNA probes. No significant differences were observed between Romneys, Coopworths and Perendales at any of the loci examined. Significant variation was observed between Romney related breeds and Merinos at several loci. At the haemoglobin locus (Hb), a probe which appears to detect alleles coding for HbA and/or HbB as classified by protein electrophoresis, failed to detect alleles in all samples. The missing allele appeared predominant in Texel DNA samples, but was also seen in Romneys, Coopworths and Merinos. A new allele was detected in Texel DNA with a probe coding for the β subunit of follicle-stimulating hormone (FSH-β). This allele was observed at a high frequency in the Texel population and may be Texel specific. Similarity of allele frequencies between the Gotlands and Finns at many loci suggests that these two breeds may be related. Texels and Merinos do not appear to be closely related to any of the breeds studied.

Keywords Allele frequency, genetic linkage, gene mapping, restriction fragment length polymorphism (RFLP).

INTRODUCTION

A genetic linkage map of the sheep genome is to be constructed as part of a New Zealand national programme. This map of linked genetic markers is required as a tool for the study of genetic factors influencing production characteristics, and to identify DNA markers linked to major genes of economic importance. The map can be used to efficiently direct the search for markers linked to major genes, define the chromosomal location of the gene, and to find the approximate location of the gene by using flanking markers from the same region. A comprehensive genetic map of the human genome is available (Donis-Keller *et al.*, 1987) and there is a high degree of conservation between regions of the genetic maps of mouse, human and cattle (Womack and Moll, 1986; O'Brien *et al.*, 1988). This conservation of syntenic groups is being utilised to produce a provisional map of the bovine genome (Fries *et al.*, 1989). Since comparative karyotyping suggests that the cattle and sheep maps will be similar (Hediger, 1988), it is reasonable to assume that the same syntenic groups in sheep may also be conserved. While information from other species can direct mapping programs, specific map information on the sheep genome is required to determine the exact genetic distances between locations and specific genetic rearrangements in sheep chromosomes.

In order to assemble the map, high levels of DNA variation will be required in both parents and offspring. Since many DNA markers are not polymorphic, they cannot be used in mapping programmes. Exploitation of natural genetic diversity may provide the increased variability in the progeny that is required for genetic linkage anlysis.

The aim of these experiments was to examine genetic variation in a group of New Zealand and exotic sheep breeds using a variety of RFLP markers. The 4 New Zealand breeds selected were Romney (Rom), Coopworth (Coop), Perendale (Per) and Merino (Mer). The three exotic imported breeds were Texel (Tex), Finnish Landrace (Finn) and Gotland (Got).

MATERIALS AND METHODS

Animals

Romney, Coopworth and Merino samples were collected

from animals located at Invermay Agricultural Centre, Mosgiel. Perendale female samples were also collected from Invermay, while Perendale male samples were obtained from a private farm in North Otago. Blood from Texels and Finns was collected from Ruakura Agricultural Centre, Hamilton. Gotland blood was supplied by Lamb XL, Bulls. Ten unrelated animals from each breed (5 males and 5 females) were sampled. Heparinised blood samples were collected, placed on ice and sent to the laboratory within 24 hours. Every effort was made to select animals that were unrelated, to ensure that the maximum number of alleles and allele frequencies at any locus would fairly represent that population. An additional 15 samples (10 Finns and 5 Texels from Ruakura) were required to complete the study.

Probes

Recombinant DNA probes are listed according to locus, probe name and source reference (Table 1).

TABLE 1 Probe summary: loci examined and source reference of probes used in this study. (MET c-met proto-oncogene, LH luteinizing hormone, FSH follicle-stimulating hormone).

LOCUS	PROBE	REFERENCE
Human MET oncogene	pMET 5,D,H	ATCC *
Human MYCN oncogene	pNB1	ATCC *
Bovine Cytochrome Oxidase	B Cox VIIc	Seelan *et al.*, 1989.
Ovine anonymous sequence	pV4(a)	Crawford A. (*pers.comm.*)
Goat $\phi\beta^x$, $\phi\beta^y$ and $\phi\beta^z$ Haemoglobin	PG4EC3HA3	Townes *et al.*, 1984.
Bovine LH-β	LH7-sp65	Maurer 1985.
Bovine FSH-β	FSH-β	Maurer and Beck 1986.
Ovine inhibin	Inhibin βA	Crawford *et al.*, 1987.

* ATCC American Type Culture Collection.

RFLP Analysis

DNA was extracted from heparinised blood samples using a Proteinase K digestion followed by high salt precipitation (Montgomery and Sise, 1990). DNA samples (3 µg) were digested using 3-5 units of enzyme per µg of DNA under the manufacturer's recommended conditions. Restriction enzymes were chosen to include those that detect a high rate of polymorphisms in human DNA samples (Devor, 1988). The enzymes were *Bam*HI, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, and *Taq*I (Boehringer Mannheim). The restricted DNA was run in 0.8% (w/v) agarose gel in Tris-acetate buffer (5 mM Tris-acetate, 1 mM EDTA, pH 7.8) at 25V/ 40mA for 17 hr. The gels were photographed under UV light, and transferred to Hybond N+ (alkaline transfer, Amersham International, Amersham, Bucks, UK) according to the manufacturer's recommended procedures.

Recombinant cDNA and genomic probes were verified by restriction analysis and isolated insert labelled with [α-^{32}P] dCTP by nick translation (Rigby *et al.*, 1977). Filters were pre-hybridised for at least 30 min in 6xSSC (1xSSC = 15 mM sodium citrate, 150 mM sodium chloride), 5x Denhardt's (100x Denhardt's = 2% ficol, 2% polyvinlypyrrolidone, 2% BSA), 0.5% sodium dodecyl sulphate (SDS), 20 µg/ml denatured sonicated herring sperm DNA at 63°C and hybridised overnight after addition of the labelled probe. The filters were rinsed in 2xSSC, 0.1% SDS at 63°C (15 min and 30 min) and washed once in 0.5xSSC, 1.0% SDS at 63°C for 10 min. Washed filters were autoradiographed using Kodak XAR-5 films at -80°C for 2-5 days. Filters were reprobed after stripping twice in 0.1xSSC, 0.1% SDS heated to 100°C and poured over the filters at 15 min intervals.

Data Analysis

Results for the allele frequencies of all breeds at individual loci were analysed using a Chi square distribution analysis (Steel and Torrie, 1980).

RESULTS

Seven probes coding for 5 separate loci which were known to be polymorphic in Romneys and Merinos were scored directly in the 7 breeds. No differences were observed at the bovine cytochrome oxidase locus when scored using a *Bam*HI polymorphism. The polymorphism detected with pV4(a), a random sheep genomic probe, also showed no variation in DNA digested with *Taq*I. However, there was significant

TABLE 2 Allele frequencies for *Msp*I and *Taq*I polymorphisms at pV4(a) locus.

		Rom	Coop	Per	Mer	Got	Finn	Tex
pV4(a) *Msp*I								
	A1	0.20	0.10	0.33	0.15	0.25	0.45	0.50
	A2	0.80	0.90	0.67	0.85	0.75	0.55	0.50
Alleles scored		20	20	18	20	20	20	20
Chi-square	13.5*							
pV4(a) *Taq*I								
	A1	0.20	0.10	0.22	0.05	0.05	0.20	0.25
	A2	0.80	0.90	0.78	0.95	0.95	0.80	0.75
Alleles scored		20	20	18	20	20	20	20
Chi-square	6.5 NS							

variation between the breeds for a polymorphism detected with the same probe in samples digested with *Msp*I (Table 2).

At the MYCN locus a human genomic probe was used to score a *Bgl*II polymorphism. Allele frequencies differed significantly ($p<0.001$) among the seven breeds. The Merinos (A1 = 0.69, A2 = 0.31) had a higher frequency of the A1 allele compared with Romneys (A1 = 0.33, A2 = 0.67). No copies of the A1 allele were observed in 10 Finn DNA samples. A further 8 Finns were subsequently screened, and no copies of the A1 allele were detected.

A syntenic group of three human probes from the MET locus was screened and allele frequencies calculated (Table 3). Romneys, Coopworths and Perendales appear similar at all three loci. Merinos, Gotlands and Finns show similar allele frequencies at the pMET 5 (*Taq*I) and pMET H (*Bgl*II) loci, while at pMET D (*Msp*I and *Taq*I) the allele frequencies of the Gotlands appear more like the Romney derived breeds.

A goat cDNA probe PG4EC3HA3 (PG4), coding for the $\phi\beta^x$, $\phi\beta^y$ and $\phi\beta^z$ goat globin genes was used to screen Romneys and Merinos for polymorphisms at the sheep β globin locus. Polymorphisms were identified with 9 out of 10 enzymes used including *Pvu*II and *Pst*I. When samples restricted with *Pvu*II or *Pst*I were screened against individuals of known haemoglobin type, a clear two allele pattern was seen allowing animals to be scored as HbA, HbAB or HbB (Fig. 1).

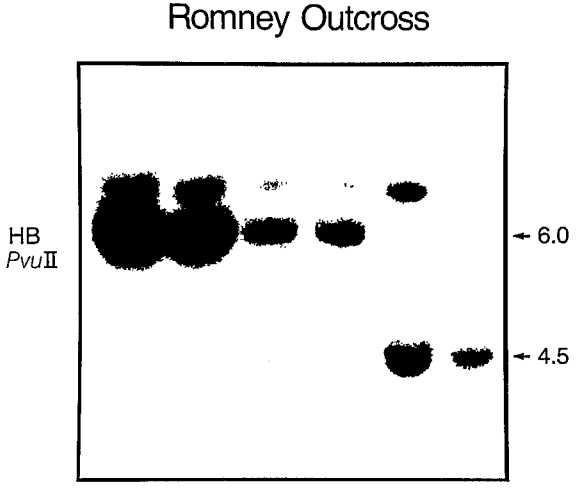

FIG 1 Six Romney outcross animals of known haemoglobin type restricted with *Pvu*II and hybridised with a probe coding for β-haemoglobin (PG4EC3HA3). Lane 1&2 = HbA, Lane 3 = HbAB, Lane 4 = HbA, Lane 5&6 = HbB.

*Pvu*II (A1=6.0 kb, A2=4.5 kb) was selected to

TABLE 3 Allele frequencies at pMET 5, pMET H and pMET D loci.

		Rom	Coop	Per	Mer	Got	Finn	Tex
pMET 5 *TaqI*								
	A1	0.22	0.30	0.28	0.75	0.72	0.75	0.65
	A2	0.78	0.70	0.72	0.25	0.28	0.25	0.35
Alleles scored		18	20	18	20	18	20	20
Chi-square	27.3***							
pMET H *BglII*								
	A1	0.75	0.75	0.72	0.35	0.35	0.20	0.55
	A2	0.25	0.25	0.28	0.65	0.65	0.80	0.45
Alleles scored		20	20	18	20	20	20	20
Chi-square	24.3***							
pMET D *TaqI*								
	A1	0.75	0.70	0.72	0.35	0.83	0.20	0.55
	A2	0	0.15	0.06	0	0.06	0.15	0.15
	A3	0.25	0.15	0.22	0.60	0.11	0.65	0.30
Alleles scored		20	20	18	20	18	20	20
Chi-square	32.3***							

score haemoglobin type. When DNA from all 7 breeds was hybridised with the PG4 probe, 12 animals including 7 Texels, 2 Merinos, 2 Coopworths and a Perendale appeared to have neither the A1 or A2 alleles. No whole blood samples were available from these individuals to score haemoglobin type by protein gel electrophoresis. Additional experiments with a variety of restriction enzymes confirmed that the banding pattern for these individuals differed from the banding pattern observed with the other samples. Individuals were scored according to the *Pvu*II banding pattern. The A1 allele represents the allele coding for HbA and A2 represents HbB.

Seven out of 10 Texels showed no bands in *Pvu*II digests with the haemoglobin probe (PG4). The absence of bands was much higher in this breed than other breeds studied. No Gotlands or Finns were seen to be missing alleles. These 2 breeds appear to be predominantly HbA type, with only 1 copy of the HbB (A2) allele in 40 Finn sheep. No copies of the A2 allele were seen in 8 Gotland samples.

Three probes were used to screen 4 breeds, including Gotlands, Finns, Texels and Coopworths, against a 10 enzyme panel to identify new polymorphisms not detected in Romneys and Merinos. Coopworths were included as a control. No polymorphisms had been identified with the β subunit of luteinising hormone (LH-β) or the βA subunit of inhibin (inhibin βA). No new polymorphisms were detected when these probes were screened in the exotic sheep breeds.

The β subunit of follicle stimulating hormone (FSH-β) contains an insertion/deletion polymorphism which is approximately 2 kb in size and located downstream from exon 3 (Montgomery *et al.*, 1990). This insertion/deletion polymorphism was also found to be present in the exotic breeds. When Texel DNA was hybridised with the FSH-β probe an additional band was seen with 9 out of 10 enzymes tested. These bands (which were not observed in any of the other breeds)

were seen in 9 out of 10 sheep with 6 enzymes (Figure 2) and 8 out of 10 sheep with 3 enzymes. A partial restriction map of the FSH-β gene around exon 3 has shown that there is a restriction site for *Eco*RI between exon 2 and 3, and a second site downstream from the insertion/deletion polymorphism. The presence of an additional 4.3 kb band with *Eco*RI suggests that this band is not associated with exon 3, or the insertion/deletion polymorphism, as no reduction in the size of the *Eco*RI insertion/deletion alleles has been observed.

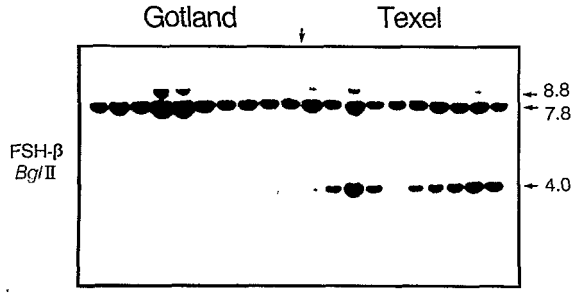

FIG 2 DNA from 10 Gotland and 10 Texel sheep restricted with *Bgl*II and hybridised with FSH-β.

In addition to the Texel specific polymorphism, a new *Rsa*I polymorphism was identified in the Coopworths and exotic breeds. This polymorphism was subsequently scored in all breeds, and allele frequencies calculated ($P<0.01$). The Gotlands (A1 = 0.95, A2 = 0.05) and Finns (A1 = 1.00, A2 = 0.0) appear to have a higher frequency of the A1 allele than other breeds (Merinos A1 = 0.55, A2 = 0.45, and Romneys A1 = 0.75, A2 = 0.25).

DISCUSSION

The objective of this study was to examine genetic variation among a group of seven sheep breeds present in New Zealand. Significant variation was seen at a number of loci. However, regions of DNA around two important genes were found to be invariant in all the sheep breeds studied, with no polymorphisms detected for the β subunit of LH, or inhibin βA. These two hormones, which are involved in the reproductive pathway, have been strongly conserved throughout evolution. Mature inhibin βA subunits from human, porcine, bovine and murine inhibins have been shown to be identical (Ying 1988), and genomic blots of bovine sperm DNA restricted with *Bam*HI, *Eco*RI and *Hin*dIII, hybridised with LH-β cDNA probe show single bands of a similar size to those seen in sheep (Virgin *et al.*, 1985). It appears from the absence of RFLP's associated with these genes that there has also been conservation of non-coding regions surrounding the genes.

Significant variation was observed around three loci known to be polymorphic in sheep. Differences were observed between Merinos and Romney related breeds at both the MYCN and MET loci. Texels, a recently imported breed, appear to differ from the New Zealand domestic breeds at these loci, and also at the haemoglobin locus. One interesting feature of the results was the demonstration of a variant at the haemoglobin locus that does not conform to the proposed model of the goat beta globin locus (Townes *et al.*, 1984). This variant appears to be present at a high frequency in the Texel population, however the nature of the variant has not been fully characterised. Gotlands and Finns were very similar at both the haemoglobin and FSH-β (*Rsa*I) locus. At the haemoglobin locus, both Finns and Gotlands were predominantly HbA type (A1 allele) with a very low frequency of the A2 allele.

A new polymorphism was identified at the FSH-β locus in Texel DNA. No attempt has yet been made to characterise the additional bands, as this would require the construction of a complete restriction map around the FSH-β locus. Since the extra bands were observed with 9 out of 10 enzymes it seems unlikely that they are due to a series of point mutations. One explanation could be that an additional insertion/deletion polymorphism is present at a high frequency in the Texel population. However, one individual has additional bands with only 6 out of 9 enzymes, which is inconsistent with the presence of a simple insertion/deletion.

Results from this study show that there is some variation at the DNA level between breeds of sheep present in New Zealand. The results suggest 4 groups; Romneys, Coopworths and Perendales were similar at all loci, Gotlands and Finns grouped together based on similarities at a number of loci, and Merinos and Texels

which do not appear to be closely related to any of the other breeds studied. Further work is required to adequately characterise the genetic distance between the sheep breeds. Increased sample numbers combined with an increase in the number of polymorphic loci tested, would provide a more accurate assessment of this natural genetic diversity.

Efficient mating designs for linkage mapping exploit fixed genetic differences in the two parental strains to produce F1 progeny that are heterozygous at many loci and informative in backcross analysis (O'Brien *et al.*, 1988). Results from this study show that there was little evidence for fixed differences between the seven breeds studied. The only breed specific difference was the additional bands observed with the FSH-β probe in the Texels. No new breed specific alleles were observed for any other probes, and differences observed between breeds were due to differences in the frequency of alleles present. Taken together these results show that fixed genetic differences are unlikely to exist at large numbers of loci among the 7 breeds examined.

The recent development of new types of genetic markers such as the highly informative micosatellite markers (Crawford *et al.*, 1990) and single strand conformation polymorphisms (Orita *et al.*, 1989), will provide markers that are more informative for both linkage mapping and the examination of genetic variation at specific loci (such as LH-β and inhibin βA) that are not polymorphic. Natural genetic diversity in combination with new marker technology, should ensure the rapid generation of the sheep gene map.

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