MARKER GENES AND PRODUCTION CHARACTERS IN MERINO SHEEP

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SUMMARY

We are involved in mapping the sheep genome using family linkage studies. Data are being collected on two generation families in two merino flocks held at the CSIRO Division of Animal Production in Armidale.DNA has been extracted from over 700 animals and Southern blots have been prepared with a panel of eight restriction enzymes. Using a bank of ovine probes we have detected RFLPs at a number of loci. These include the high-sulphur, ultra-high-sulphur and high-glycine-tyrosine keratins, immunoglobulin light chains, growth hormone, prolactin, alpha tubulin, alpha FSH and growth hormone receptor.

INTRODUCTION

For the last half century at least scientifically based animal breeding has relied upon a biometrical approach. Heritabilities, selection indices and breeding values have been applied to a number of domestic animals to improve productivity. This biometrical approach reflects the dominance of statistical and population approaches in genetics generally which was apparent until the early sixties. From that time on, molecular biolo-gy has become progressively more and more influential. The genetic material of any organism can now be analysed and manipulated with a variety of powerful techniques. The last decade or so has seen the application of these techniques to domestic animals and plants.

These advances in molecucar genetics over the past decade make it possible to identify particular genes or chromosomal regions and even to map entire genomes. Of course, this is no small task. The enormity of the human genome project has been well docu-mented. Very little work has been carried out on the sheep genome, with much more interest being shown in other animal species. This is due, in part, to the difficulties associated with such outbreeding organisms and the large size of the genome and also because sheep are not as economically important in other countries. Mapping of the sheep genome is a realistic goal for Australian researchers. We can use the findings of other animal mapping projects, especially those of cattle. It has been shown that there is a high degree of conservation of linkage groups between cattle and sheep.

It is imperative for Australia to keep up with other countries in this type of research and essential that the benefits to the sheep industry be realised here. This joint project between Macquarie University and CSIRO Division of Animal Production at Armidale is aimed at mapping regions of the sheep genome that may have some effect on important production characters such as fecundity, wool weight, fibre diameter, carcass weight and so on. We expect that this approach could radically affect animal breeding. Genes that affect production characters (quantitative trait loci or QTLs) that can be identified directly can be used to improve selection methods. Genetic markers that are linked to the QTL can also be used through

Marker Assisted Selection (MAS) (Gelderman et al. 1985; Soller and Beckman 1982).

MATERIALS AND METHODS

Restriction fragment length polymorphism (RFLP) analysis, as described by Gogolin-Ewens et al., (1990) was performed using DNA extracted from sheep blood samples. The Harvey computer program (Harvey, 1964) was used to do between alleles within sires ANOVA as a preliminary test for linkage between production characters and marker loci. In essence, the model tests whether the allele transmitted by the ram to his progeny influences the production character in question in these progeny. The analysis allowed for effects of sire, sex, parity of dam, age and type of birth.

RESULTS AND DISCUSSION

We are investigating a number of canditate genes to see if genetic variation at these loci is related to variation in production characters. The initial stage of this research, detection of RFLPs which indicate genetic variation, is well under way. The candidate loci looked at so far include the principal genes which direct wool production, which are the keratin and keratin-associated-protein genes, a number of hormone genes that affect growth and reproduction, two of the immunoglobulin genes and the ribosomal genes that are central to protein production. A total of 19 gene probes have been used and 12 have yielded definite positive results with two more probable. Details of these RFLPs are shown in Table 1.

We now have 12 genetic markers that can be used to test for any relation to production characters. Six of these markers are in the keratin-associated-protein (KAP) genes. Interestingly no variation was detected in the keratin genes themselves which suggests that variation in wool characters, such as staple length or fibre diameter, may be determined by variation in other loci, for example the ultra high sulphur, high sulphur and/or high glycine tyrosine genes.

We are in the process of large scale genetic typing of two merino flocks, Medium Peppin and Medium Non-Peppin, maintained for this study by CSIRO Armidale. The medium peppin flock consists of 20 single sire groups with 8 to 20 dams and offspring in each comprising 650 animals. The medium non-peppin flock consists of 10 unrelated rams, two half-sib groups of 12 offspring each and one sire group with 10 dams and offspring. The typing will provide data on the inheritance of the RFLPs, for linkage analysis between single gene markers and between QTLs and markers and any relationship with production characters. Production data has been collected for all members of the medium peppin flock and includes lambing data i.e. birthweight, weaner weight, type of birth, age of dam etc., and sampling data i.e. greasy wool weight, clean fleece weight, fibre diameter, hogget weight and so on. Linkage analysis between growth hormone and weight parameters (birthweight, weaner weight and hogget weight) and between the ultrahigh-sulphur cuticle and fleece parameters (greasy wool weight, clean fleece weight and fibre diameter) has been carried out on a number of sire-group families and preliminary results for growth hor-mone vs. weaner weight are shown in Table 2.

As can be seen from Table 2 there is no significant association between a particular allele at the growth hormone locus and mean weaner weights of offspring. To date the results of all analysis performed have not been significant. However, the investigation is in reality a pilot one, and a very much larger body of data is needed to make definitive tests for linkage.

Table 1. Results of DNA sequence polymorphism searching in sheep

Name of gene or gene family	Probe Symbol	Probe Origin	Position in human genome	Polymorphisms with RE	Provisional Estimation of No. of loci*	Linkage analysis**
Wool protein genes						
Keratin A	IFI	B.Powell	17q21-33	None found***	Multigene family	
Keratin B	IFII	B. Powell	12	None found***	Multigene family	
Ultra high sulphur cuticle	UHS cut.	B. Powell	11q13/ 11p15	BamHI, Bgl II Hind III, Rsa I	3-4? 1 diallelic system	Yes
Ultra high sulphur cortex	UHS cort.	B. Powell	Not known	BamHI, TaqI, Hind III(?)	3-4?	
High sulphur keratin B2	PSK10	K. Ward	Not known	TaqI, PvuII, Bgl II	2-4? complicated polymorphism pattern	
High sulphur keratin BIIIA	HSBIIIA	B. Powell	Not known	None found	Multigene family	
High sulphur keratin IIIB	HSBIIIB	B. Powell	Not known	BamHI, PstI, RsaI, Hind III, TaqI	1-2	
High glycine tyrosine 1F	HGT1F	B. Powell	Not known	BamHI(?), PstI(?), RsaI(?)	1	
High glycine tyrosine 1C2	HGT1C2	B. Powell	Not known	None found		
High glycine tyrosine II	HGTII	B. Powell	Not known	BamHI	1	Yes
Hormone genes						
Growth hormone	GH	T. Adams	17q22-24	PvuII, TaqI	1 loci with several alleles	Yes
Growth hormone Receptor	GHR	T. Adams	5p13-p12	TaqI	2-3	
Prolactin	PRL	T. Adams	6p23-23.1	TaqI	1	
α follicle stimulating hormone	αFSH	T. Adams	Not known	BamH1?	1	
Placental lactogen	PL	T. Adams	17q22-q24	BamHI,PvuII,TaqI	1	
Immunoglobulin genes						
Immunoglobulin lambda light chain	SLC	K. Beh	22q11.1-2	BamH1, PvuII, TaqI, RsaI	Multigene family	
Immunoglobulin kappa light chain	SKC	K. Beh	2p12	TaqI	1	
Other genes						
Ribosomal RNA	Xenopus	K. Jones (?)	13p, 14p, 15p,	None found	Multigene family with concerted evolution	
α-tubulin	TUBA	T. Adams	2q	TaqI,PvuII,BamHI	2-3?	

* Based upon results of Southern blotting.
** Preliminary analysis of linkage with production characters performed.
*** Low level variation found with RsaI, PvuII and BamHI; however, the possibility that it represents presence of pseudogenes and not variation at the keratin loci themselves has not been excluded.

Table 2. Preliminary results of linkage analysis between growth hormone and weaner weight.

Sire	Allele transmitted	No.progeny	Weaner wt. mean	S.E.	p-value
3033	3	11	13.36	.73	
	1	6	14.30	.97	.4625
3053	2	4	14.21	1.20	
	1	5	14.80	1.11	.6973
3076	2	6	13.11	1.00	
	1	4	13.58	1.24	.7496
3271	2	5	14.20	1.15	
	1	5	15.08	1.12	.5438
3284	2	6	14.68	1.02	
	1	2	16.09	1.34	.3898
3360	1	9	14.73	.88	
	3	2	17.69	1.64	.0945
3406	3	4	12.29	1.14	
	2	13	15.75	.75	.0091 *

* When allowance is made for the number of tests performed this result is not significant (Cooper, 1968).

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