Mapping production traits in farm animals : the Booroola (FecB) locus

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SUMMARY

The Booroola (*FecB*) locus has been mapped to sheep chromosome 6. Markers flanking this gene were identified in sheep x hamster somatic cell hybrids containing chromosomes 6 and 24. Partial genomic libraries were constructed from the cell lines, sheep-specific clones were selected from the libraries and microsatellite markers developed. Four markers mapped to sheep chromosome 6 and one marker mapped to sheep chromosome 24, demonstrating that chromosome-specific clones were generated from the hybrid cell lines and further defining the position of the *FecB* locus on sheep chromosome 6.

INTRODUCTION

Searching for genes of large effect by genome mapping methods will allow us to identify the molecular basis of production differences in livestock. One example demonstrating an important step in this process is the recent mapping of the Booroola fecundity gene (*FecB*) in sheep (Montgomery et al., 1993). The approach used illustrates several features of livestock mapping projects. Linkage was identified with a combination of anonymous markers and known genes. Once linkage was established, extensive use was made of comparative gene maps to define the position of the gene. Detailed mapping of the region containing the gene will require the development of chromosome-specific libraries.

The *FecB* locus, that increases ovulation rate in sheep, is linked to markers for secreted phosphoprotein 1 (SPP1, human chromosome HSA4q11-21) and epidermal growth factor (EGF, human chromosome HSA4q25; Montgomery et al., 1993). The linkage group including EGF, *FecB* and SPP1 has recently been assigned to sheep chromosome 6 (Montgomery et al., 1994). Markers from the linkage group were mapped to somatic cell hybrids containing the Robertsonian translocation t1 (*rob* 6;24) that includes chromosome 6 (Montgomery et al., 1994).

The aim of these studies was to confirm the presence of markers flanking *FecB* in the hybrid panel and generate additional markers specific to sheep chromosome 6 to assist in fine mapping around the FecB locus.

MATERIALS AND METHODS

Somatic cell hybrids. and genomic libraries. DNA was extracted from hybrid cell lines as described previously (Burkin et al., 1993). DNA was partially digested with Sau 3A and cloned into either pSuperCos1 or pBluescript (Stratagene, La Jolla). Libraries were plated out and sheep-specific clones detected using total sheep genomic DNA as a probe. Positive clones were subsequently screened with hamster Cot1 DNA (Gibco-BRL) and (AC)n (Pharmacia) to select sheep clones with dinucleotide repeats. Positives were subcloned and sequenced.

Marker analysis. DNA probes for alpha-S1-casein (CSN1S1), platelet derived growth factor receptor alpha (PDGFRA), SPP1 and EGF detect RFLPs at homologous sheep loci (Montgomery et al., 1993). Southern blotting and microsatellite analysis used standard procedures described previously (Montgomery et al., 1993,Montgomery et al., 1992).

Flocks for linkage analysis. Linkage between marker loci was analysed in 9 three-generation pedigrees bred by embryo transfer (range 6-17 off-spring) as part of an international resource for linkage studies in sheep. The breeds included as grandparents were Texel, Coopworth, Merino, Romney and Perendale. Lod scores for linkage between pairs of markers were calculated using MENDEL (Lange et al., 1988).

RESULTS

Sheep-specific bands for markers flanking the *FecB* gene (PDGFRA, OarAE101, SPP1 and EGF) were present in the three hybrid cell lines containing chromosome 6 (Table 1) and in the sheep control DNA (data not shown). These bands were not present in the other cell lines containing different sheep chromosomes (Table 1) or in hamster DNA.

Partial genomic libraries were constructed from DNA extracted from the cell lines containing chromosome 6. Few clones in the libraries contained sheep DNA, while most clones contained hamster DNA.

TABLE 1
Sheep-specific bands for markers in the hybrid cell lines

Cell line Chromosomes	a 1q	b 1q,9,10	c 9,10	d 3,6,24	e 6,24	f 3	g 3q
EGF	-	-	-	+	+	-	-
OarAE101	-	-	-	+	+	-	-
SPP1	-	-	-	+	+	-	-
PDGFRA	-	-	-	+	+	-	-

Discriminating between sheep and hamster clones was easier with the large DNA fragments in the cosmid vectors. Five sheep-specific markers were generated from the first clones screened out of the libraries. Four clones mapped to sheep chromosome 6 and one clone mapped to sheep chromosome 24 (the other chromosome involved in the t1 translocation).

These markers mapped outside of the region containing FecB, but extended the linkage group on this chromosome. One marker (OarEL13) mapped into the region of the casein genes and showed no recombinants with CSN1S1 (Zmax=7.53). A second marker (OarJMP1) was located in the region between CSN1S1 and markers closer to the *FecB* locus. OarJMP1 was significantly linked to OarEL13, PDGFRA, OarAE101 (Table 2).

TABLE 2
Pairwise linkage data to OarJMP1

	Recombination fraction							
Locus	0.05	0.10	0.15	0.20	0.30	0.40	Z_{max}	Θ
PDGFRA	4.88	8.97	10.31	10.43	8.62	4.91	10.49	0.18
OarAE101	-8.65	-1.64	1.66	3.36	4.12	4.23	4.26	0.28
OarEL13	-5.97	2.75	6.56	8.26	8.24	5.29	8.70	0.25
CSN1S1	0.50	1.95	2.49	2.63	2.25	1.32	2.63	0.20

DISCUSSION

The region of the sheep genome containing markers flanking the *FecB* mutation in sheep is contained within somatic hamster x sheep hybrid cell lines selected for the Robertsonian translocation t1 (*rob* 6;24) that includes chromosomes 6 and 24 (Burkin et al., 1993). Sheep-specific microsatellite markers selected from the hybrid cell libraries mapped to sheep chromosome 6 and 24.

Two new markers confirm the assignment of the *FecB* locus to sheep chromosome 6 (Montgomery et al., 1994). OarEL13 maps to the region of the casein genes with no recombinants between OarEL13 and CSN1S1. Linkage between *FecB*, OarAE101, OarJMP1 and OarEL13 spans the region from *FecB* to the casein genes that map to sheep chromosome 6 (Ansari et al., 1992).

The new markers demonstrate that the chromosome-specific clones were generated from the hybrid cell lines and thirteen markers have now been assigned to sheep chromosome 6. The next step in identifying the mutation responsible for the increased ovulation rate in Booroola gene carriers will not be easy. There are no obvious candidate genes in the immediate region of the *FecB* locus. Identifying the mutation by positional cloning will require the identification of new markers much closer to the gene and a physical map of the region.

We thank D.J. Burkin and C. Jones, Eleanor Roosevelt Institute, Colorado, USA for provision of somatic cell hybrids.

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