Karyotyping Booroola gene carriers

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ABSTRACT

Karyotype analysis, used for screening chromosomal anomalies in humans and livestock, is being evaluated as a means of identifying sheep carrying the Booroola fecundity gene. This has involved comparing the chromosomes of metaphase spreads from animals heterozygous for the Booroola gene (Fec^{B+}) to screen for numerical or physical abnormalities between the chromosome pairs using Giemsa (GTG) banding. A number of anomalies have been observed from 18 karyotypes of 3 Fec^{B+} rams and 4 Fec^{B+} ewes. The anomalies were spread throughout most chromosomes, but none were present in all karyotypes. No alteration in chromosome number was observed. These preliminary results suggest that the Booroola mutation is not the result of a major chromosomal alteration.

Keywords Sheep, Booroola, karyotype analysis, chromosomes, GTG-banding.

INTRODUCTION

The Booroola fecundity gene (Fec^{B}), inherited as a single autosomal gene with a major effect on fecundity (Piper *et al.*, 1985), is being used as a model for molecular genetic studies. The objective of this research is to develop a rapid and accurate test that would identify Fec^{B} carriers. One approach is to use cytogenetics, the study of chromosomes, to screen heterozygous (Fec^{B+} or B+) animals.

Karyotype analysis is used routinely in human cytogenetics to screen for chromosomal abnormalities such as Down's syndrome. Qin and Halnan (1989) and Halnan (1989) have reviewed cytogenetic studies of sheep and cattle covering animals of abnormal phenotype. These tended to be associated with sex chromosome abnormalities. In other cases whole chromosome changes can occur without an effect on phenotype or reproduction (Bruére and Ellis, 1979). An exception is the fusion of chromosomes 1 and 29 in cattle associated with a decrease in fertility (Weber *et al.*, 1989). Karyotyping of lambs with ovine hereditary chondrodysplasia showed no chromosomal differences (Vanek *et al.*, 1988) and there are no reported cases of karyotype analysis being successfully utilised to select animals with improved productive traits.

The inheritance pattern of the Fec^{B+} gene would suggest that one chromosome in an as yet undetermined pair will contain the Fec^{B} gene in B+ animals. The detection of a difference, due to the presence of the Fec^{B} gene, in the banding pattern of one chromosome of a pair would be extremely useful for identifying Fec^{B} gene carriers. The aim of this study is to karyotype the chromosomes of known B+ rams and ewes from the Invermay flocks using GTG-banding and compare chromosome pairs for physical differences.

MATERIALS AND METHODS

Blood samples were taken from Booroola and Booroola Romney animals, known to be B+ carriers, via jugular venipuncture into sodium heperanised vacuum tubes. Whole blood microcultures were set up in duplicate within 4 to 6 hours of collection to obtain chromosome spreads. Blood (0.3 ml) was added to 5 ml of RPMI 1640 medium (GIBCO BRL), supplemented with 15% fetal calf serum (GIBCO BRL), 0.3 mg/ml L-glutamine (GIBCO BRL), 100 IU penicillin (Boehringer Mannheim), 100 µg/ml streptomycin (Boehringer Mannheim) and 10 µg/ml concanavalin A (Boehringer

Mannheim). The cultures were incubated in 10 ml screw-capped tubes with flat sides (Nunc) for 72.5 hours at 37°C until harvest. After 48 hours of culture the division of lymphocytes was synchronised as described by Di Berardino *et al.* (1989). The cells were exposed to Colcemid (Boehringer Mannheim, 0.1 µg/ml final concentration) 1 hour before harvest.

The cultures were centrifuged at 400g for 8 min and the supernatant aspirated. The cells were resuspended in 8 ml of warm 75 mM KCl for hypotonic treatment at 37°C over 15 min. Two drops of fixative (methanol-acetic acid 3:1) were added and the suspension was centrifuged at 400g for 5 min and aspirated. The cells were resuspended in 4 ml of fixative, left at room temperature for 15 min and centrifuged as before. The fixative step was repeated twice with the cells finally resuspended in 0.3 ml of fixative. Three drops of the fixed cell solution were spread onto clean, wet microscope slides and air dried.

For GTG-banding (Seabright, 1971) the slides were placed overnight in a 60°C oven, rinsed in 150 mM NaCl and treated with a trpysin solution (Difco, 0.1% final activity in 150 mM NaCl) for 2 min 40 s. The slides were rinsed twice in 150 mM NaCl and placed horizontally on a slide rack. Working Leishman's stain [0.6 ml of 0.2% Leishman's stain (BDH) and 3.0 ml of Gurr's pH 6.8 buffer] was poured on to each slide and left for 7 min. The slides were washed with tap water, air dried and mounted with DPX.

Chromosome spreads were photographed under a Zeiss Axioplan microscope with a 100X Neofluar oil immersion lens using Kodak technical pan 2415 film. Enlargements were produced on Agfa Brovira Speed glossy paper. Individual chromosomes were cut out and karyotyped according to the descriptions of Ford *et al.* (1980). For karyotype analysis the chromosome pairs were visually inspected for anomalies in conformation and their GTG-banding pattern.

RESULTS

A chromosome spread of a B+ ram is shown in Figure 1 and its karyotype is presented in Figure 2. In this example, obvious differences in the GTG-banding pattern can be seen as a contraction of chromosome 1, an overlap between chromosomes 5 and 12 and poor spreading of chromosomes 9 and 16. More subtle differences are a missing light band in chromosome 13 and an additional dark band in chromosome 15.

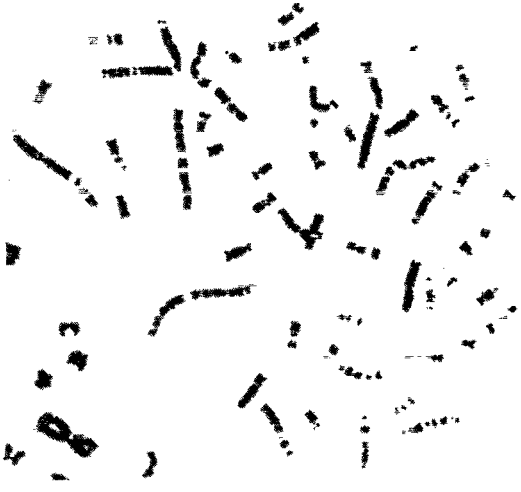

FIG 1 GTG-banded metaphase spread from a Fec^{B+} ram (x750).

Karyotype analysis has been done on 18 karyotypes from 3 B+ rams and 4 B+ ewes. Chromosomal anomalies, similar to those described above, totalled 114. A third of these were due to bands which appeared to be either additional or missing. The remaining two-thirds were due to whole chromosomes that were either missing, twisted or did not stain well. No single consistent anomaly was found between the chromosome pairs of these B+ animals.

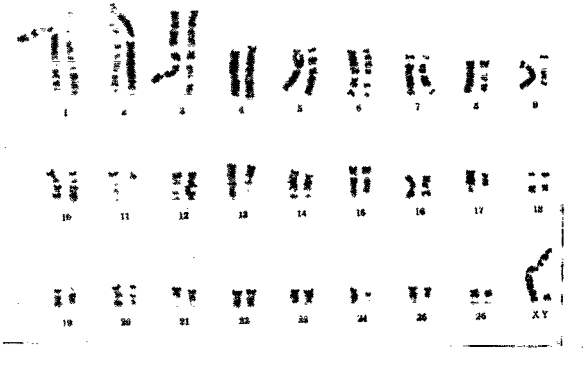

FIG 2 Karyotype of chromosomes of a Fec^{B+} ram from the metaphase spread of Figure 1 (x1,500).

DISCUSSION

Karyotype analysis has been used successfully in human medicine, as described by Jonasson (1986), and cattle breeding (Weber *et al.*, 1989) when identifying chromosomal anomalies with detrimental effects. This is the first report of attempting karyotype analysis to identify a positive effect on sheep production. Karyotype analysis of 7 B+ animals has yet to show any consistent anomaly in GTG-banding which could be used to identify Fec^B gene carriers.

One major problem is the resolution of GTG-banding as each band represents a large region of the chromosome. Since the resolution of GTG-banding is low and only gross changes in the chromosomal conformation would be detected these initial results suggest the Fec^B gene does not involve a large change in the conformation of a chromosome. The resolution could be improved by producing elongated prometaphase spreads (Di Berardino *et al.*, 1989). Other chromosomal staining methods are available as described by Jonasson (1986), but these would not have any advantage in resolution over GTG-banding. Many anomalies occur by virtue of the preparation of chromosomes onto microscope slides. These include missing and overlapping chromosomes and chromosomes which become twisted or kinked. The staining procedure can also produce anomalies in the intensity of staining as chromosomes lying about the edge of a spread tend to stain lightly.

It is possible that the Fec^B gene has arisen by a small mutation in or around a single gene which would not be seen by karyotype analysis. Such mutations can occur spontaneously and be propagated through a flock if it is favoured by selection as in the case of higher ovulation rates with Fec^B gene carriers. Even if a gene duplication or translocation event has occurred it may not be detected by GTG-banding unless a much larger region of the chromosome around the gene has also been duplicated or translocated.

This study is continuing and it is intended to screen 10 karyotypes each from 5 B+ rams and 5 B+ ewes. The analysis of a hundred karyotypes should give a better perspective on the usefulness of karyotyping to identify Fec^B gene carriers. If an obvious mutation is found by karyotype analysis this would assist the selection of Fec^B gene carriers and enhance the search for identifying the Fec^B gene and understanding how the Fec^B gene operates.

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