DNA MARKERS FOR ASEASONALITY AND MILK PRODUCTION IN SHEEP

R. G. Mateescu and M.L. Thonney

Department of Animal Science
Cornell University
Ithaca, New York

Introduction

Knowledge about genetic markers linked to genes affecting quantitative traits can increase the selection response of animal breeding programs, especially for traits that are difficult to improve when using traditional selection (Meuwissen and Goddard, 1996; Meuwissen and Van Arendonk, 1992). The seasonal breeding pattern that is commonly observed in temperate sheep breeds is a major obstacle to increasing the intensity of sheep production. Sheep breeds differ in timing and duration of breeding (Notter D.R., 1992; Nugent et al., 1988; Vincent et al., 2000) but less is known about genetic variation within breeds for traits associated with seasonal breeding. There-fore, selection of sheep for aseasonal reproduction is based upon observing the animals' pheno-type, which often lacks accuracy and can be done only after animals reach sexual maturity. Similarly, selection for milk production is difficult for at least two reasons. First, measuring the trait takes time and money (females need to reach maturity before expressing the trait and the lactation period lasts more than 200 days so repeated measurements are necessary to accurately assess the phenotype). Second, milk yield is a sex limited trait; therefore the males, the most important path for selection because of their higher reproductive capacity, can be evaluated only based on the phenotype of their female progeny. Furthermore, for both traits the exact molecular nature of the target genes remains essentially unknown.

Phenotypic variation usually is continuous instead of discrete and conditioned by allelic variation at several (and sometimes many) genetic loci (genes), each with a relatively small effect. Char-acteristics such as breeding out-of-season or milk production, where phenotypic variation is con-tinuous and determined by the segregation at multiple loci, are referred to as quantitative traits and they have a polygenic inheritance. The individual loci controlling a quantitative trait are re-ferred to as polygenes or quantitative trait loci (QTL). For many years, animal breeding schemes have operated without knowledge of the actual genes underlying the traits under selection, and genetic evaluations were entirely based on phenotypic data. In the last 15 years techniques have been developed which enable scientists to detect genes. Molecular mark-ers, small pieces of DNA which can be genotyped easily and used to follow the transmission of chromosomal segments from parents to offspring, are used to identify and map QTLs. With advances in molecular biol-ogy, the identification of QTLs is now possible and is likely to lead to more efficient breeding programs. The identification of QTLs can increase the accuracy of selection by providing more information to predict an animal's breeding value. Also, genetically superior individuals can be identified early in life, perhaps even before they are born. This will greatly reduce the generation interval and increase the rate of genetic progress per year.

The variability of timing and duration of breeding, as well as the successful selection for asea-sonality indicates that breeding out-of-season is under genetic control (Notter D.R., 1992), but very little is known about the identity of the genes responsible for this genetic variation. Several candidate genes potentially involved in breeding out-of-season are melatonin receptor(s), prolac-tin, and the genes implicated in the circadian clock.

Several candidate genes potentially involved in milk production traits are the casein cluster (Fitzgerald et al., 1997), b-lactoglobulin (Hill et al., 1997), as well as segments of chromosomes 6 (Spelman et al., 1996) [distinct from the casein locus 14 (Coppieters et al., 1998)] and 20 (Arranz et al., 1998). These candidate genes and chromosomal regions have been identified to affect milk production traits in dairy cattle. In sheep, there is only one study on QTLs influencing milk traits, and this study confirms the presence of a QTL on chromosome 6 (Diez-Tascon et al., 2001).

A project at the Cornell Sheep Farm is being carried out to examine the sheep genome for muta-tions related to candidate genes and will scan the genome for markers of QTLs likely to increase milk production and to allow aseasonal breeding. The specific objectives of this re-search are to:

1. Identify QTLs associated with aseasonality and milk production using a candidate gene ap-proach to determine for each gene the allele that results in the most valuable phenotype.
2. Identify QTLs associated with aseasonality and milk production using whole genome screen-ing to localize genes using marker-QTL associations.

Experimental Plan

Two approaches will be used to identify the QTLs. The first strategy will use the candidate gene approach, which consists of studying genes potentially involved in the physiological pro-cess. The second method, known as positional cloning, will use markers covering the whole genome to localize the genes affecting the trait of interest using marker-QTL associations. The positional approach is based on mapping QTLs to progressively narrower chromosomal regions, using a series of microsatellite markers.

Breeding design

The basic methodology for mapping QTLs involves using a cross between two breeds that differ substantially in the quantitative trait(s) of interest. An experimental population of animals was created by crossing Dorset ewes from the Cornell Sheep Farm and East Friesian rams from Old Chatham Sheepherding Company (OCSC), a sheep dairy near Albany, NY. The Cornell Dorset ewes are non-dairy sheep that have been selected for aseasonality and prolificacy, while the East Friesian dairy breed is known for high milk production but poor aseasonal breeding. The two pa-rental populations are adequate for this project, with one line predominantly likely to carry fa-vorable alleles and the other line predominantly likely to carry unfavorable alleles with respect to the two traits of interest. During August 2000, October 2000, January 2001 and March 2001, 72 F1 rams and 86 F1 ewes were produced. The F1 ewes were purchased by OCSC to be

placed in the milking flock and bred back to East Friesian rams. About 200 backcross animals will be gen-erated to identify QTLs for milk yield using the candidate gene and whole genome scan approach.

Eight F1 rams were used to breed the entire flock of 240 Dorset ewes during the October 2001 breeding season to produce about 125 backcross females in Spring 2002. These females will be used in Spring 2003 to identify QTLs for aseasonality using the candidate gene and whole ge-nome scan approach. Another group of backcross females will be produced in 2003.

Measurement of milk production

Milk production in the East Friesian x F1 backcross ewes will be recorded weekly in the OCSC data base. A genetic evaluation of the OCSC flock was performed by Professor P.A. Oltenacu (Cornell University) and breeding values for milk production for all animals were estimated. A test-day animal model accounting for age and season effects was used. After milk production records are accumulated on the backcross ewes, their breeding values will be com-puted using the test-day animal model and correlated with candidate gene differences and mark-ers for QTLs to identify potential DNA indicators for selection.

Measurement of aseasonality

Aseasonality in the yearling F1 x Dorset backcross ewes will be determined by measuring pro-gesterone (a spike in blood progesterone indicates that a ewe is in estrus) using four blood sam-ples prior to the start of the spring 2003 breeding season on March 15. Blood samples will be taken twice a week during the last two weeks of February to detect estrus in nonstimulated ewes and again during the first two weeks of March when the presence of vasectomized rams should help to stimulate estrus activity (this is a common management practice). The vasecto-mized rams will be brisket-painted to leave marks on any ewes that are mounted. On March 15, intact rams (also brisket-painted) will be introduced to the ewes and the ultimate test will be the num-ber lambs a ewe produces in the August-September 2003 lambing season. Whether a ewe lambs and how many lambs she delivers after breeding in March 2003 is a key determinant of aseason-ality because there is evidence that part of the aseasonal response is the ability to main-tain preg-nancy after a spring conception (Pope et al., 1989).

DNA collection and processing

Blood samples will be collected on all the animals in the parental populations and back-crosses shortly after weaning and the DNA will be extracted using a Qiagen kit. Appropriate primers will be used to amplify microsatellite markers to verify parentage of all animals.

Identification of QTLs associated with aseasonality and milk production using a candidate gene approach

Using the information from published marker maps, a panel of microsatellite markers will be identified for each candidate gene (so that the markers are located inside the gene or within 5 cM of the gene). These markers will be tested in all the animals in the parental lines. Only those markers

that are polymorphic (the individuals carry different alleles at each locus) will be used in the experiment. Our goal is to use five to six microsatellite markers for each candidate gene.

The analysis of the candidate genes for aseasonality will be carried out using the animals that result from the F1 x Dorset backcross. These animals will be scored for their genotype at the marker loci for each of the candidate genes and their phenotype for aseasonality. The analysis of the candidate genes for milk production will be carried out using the animals that result from the East Friesian x F1 backcross which will be scored for their genotype at the marker loci for each of the candidate genes and their phenotype for milk production.

Identification of QTLs associated with aseasonality and milk production using a whole ge-nome scan approach

The current genetic linkage map for sheep consists of more than 1800 microsatellite markers of which about 1,000 with known map locations are well-suited for a genome scan (Archibald et al., 2001). About 120 markers placed approximately 20 cM apart throughout the entire genome will be used, which should provide a good chance of detecting QTLs anywhere in the genome. These markers will be tested in all the animals in the parental lines and only those markers that are po-lymorphic (the parents carry different alleles at each locus) will be subsequently used in the ex-periment. All the informative markers will then be genotyped in both backcrossed populations (F1 x Dorset and East Friesian x F1).

Microsatellite marker genotyping

The microsatellite markers will be PCR-amplified using fluorescently labeled primers. The samples will be then separated on vertical acrylamide gels and ABI Genescan analysis software will be used to analyze the fragment sizes. The use of fluorescently labeled primers permits the analysis of four different PCR reactions in only one gel lane. Genotyping at the marker loci will be carried out at the Cornell BioResource Center.

Data analysis

Our data will consist of a set of markers genotyped on each individual and values of the pheno-typic trait also measured on each individual. Because our markers belong to a linkage map and their position on the map is known, we will use the interval mapping method described by (Lander and Botstein, 1989) in which sets of linked markers are analyzed simultaneously. The method uses maxi-mum likelihood estimation and provides a likelihood ratio test for the presence of a QTL between the markers considered. If there is a QTL effect at a specific location in the genome there will be an association between the trait values and the interval analysis linked to that location.

Expected Results

We expect to identify QTLs that can be used to improve our understanding of the basic biology of reproduction and milk production and to intensify selection for these traits. Identified QTLs will then be used in genetic evaluation programs to select for higher milk production and ability to breed out of season.

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