University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Papers and Publications in Animal Science

Animal Science Department

1998

Evaluation of the Ovine *Callipyge* Locus: I. Relative Chromosomal Position and Gene Action

B. A. Freking
U.S. Meat Animal Research Center, Clay Center, NE, brad.freking@ars.usda.gov

J. W. Keele
Roman L. Hruska U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE

C. W. Beattie
Roman L. Hruska U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE

S. M. Kappes
Roman L. Hruska U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE

T. P.L. Smith
U.S. Meat Animal Research Center, tim.smith@ars.usda.gov

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/animalscifacpub

Part of the Animal Sciences Commons

Authors

B. A. Freking, J. W. Keele, C. W. Beattie, S. M. Kappes, T. P.L. Smith, T. S. Sonstegard, Merlyn K. Nielsen, and Kreg A. Leymaster

Evaluation of the Ovine *Callipyge* Locus: I. Relative Chromosomal Position and Gene Action[1,2,3]

B. A. Freking*,†,[4], J. W. Keele*, C. W. Beattie*,[5], S. M. Kappes*, T.P.L. Smith*, T. S. Sonstegard*,[6], M. K. Nielsen†, and K. A. Leymaster*,[7]

*Roman L. Hruska U.S. Meat Animal Research Center, ARS, USDA, Clay Center, NE 68933 and †Department of Animal Science, University of Nebraska-Lincoln 68583-0908

ABSTRACT: Genotypic and phenotypic data were collected to estimate chromosomal position of the *callipyge* (CLPG) gene and to test gene action. Nine Dorset rams of extreme muscling phenotype and 114 Romanov ewes composed the grandparent generation of a resource flock of 362 F_2 lambs segregating at the *CLPG* locus. The parent generation consisted of eight F_1 sires and 138 F_1 dams. The F_2 lambs were serially slaughtered in six groups at 3-wk intervals starting at 23 wk of age to allow comparisons at different end points. A linkage group of 25 marker loci (mean of 708 informative meioses per marker) spanning 87.2 cM was developed and improved the previous known coverage and precision of marker order and interval distance from available maps of ovine chromosome 18. Probabilities of each *CLPG* genotype were calculated at 1-cM intervals (0 to 107 cM). Statistical models included effects of year, sex, sire, regressions on genotypic probabilities, and genotype-specific linear and quadratic regressions on appropriate covariates. Orthogonal contrasts of *CLPG* genotypic effects evaluated additive, maternal dominance, and paternally derived polar overdominance models of gene action. The most parsimonious model did not include the additive and maternal dominance genetic contrasts. From analyses of four key traits, a consensus for position of *CLPG* was obtained at 86 cM relative to the most centromeric marker. An F-test with 3 df representing polar overdominance was maximum at position 86 cM (F = 407.4; $P < .00001$) with leg score as the dependent variable. These results are consistent with assignment of the *CLPG* locus to the telomeric region of chromosome 18 and support the polar overdominance model of gene action proposed by Cockett et al. (1996). Furthermore, recombinant individuals with definitive phenotypes confined the position of *CLPG* to a 3.9-cM interval, facilitating positional cloning experiments.

Key Words: *Callipyge*, Sheep, Carcass Composition, Genes

Introduction

A Dorset ram born in 1983 in the Moffat flock near Piedmondt, OK, conferred postnatal development of an extreme, heavy-muscled phenotype to some of his descendants. Phenotypic ratios of progeny derived from designed matings were consistent with a new mutant allele with nonrecessive gene action at a single, autosomal locus (Jackson et al., 1997). The locus, designated *callipyge* (**CLPG**), mapped to the telomeric region of ovine chromosome 18 (Cockett et al., 1994). The gene action of *CLPG* was further elucidated by Cockett et al. (1996) based on limited numbers of offspring from five different mating combinations, where the maternal and paternal alleles were inferred from five DNA markers on chromosome

18. The term *polar overdominance* was used to describe the genetic model in which only heterozygous individuals that were receiving the newly identified allele from their sire expressed the unique phenotype.

Callipyge phenotypic effects were previously estimated from data collected on heterozygous (mutant *CLPG* allele inherited from the sire) and noncarrier lambs, with each lamb subjectively classified as expressing either the *callipyge* or normal phenotype. Experiments used relatively few animals, and the lambs were typically slaughtered at similar ages or live weights, which limited statistical inference. Estimates of effects of all four *CLPG* genotypes, with inferences over wide ranges of biological and economical end points, are needed to provide complete information to sheep industries in the United States and abroad. The objectives for this experiment were to increase marker density on ovine chromosome 18, to refine the chromosomal position of *CLPG*, and to test different models of gene action; this information is prerequisite for precise estimation of *CLPG* genotypic effects.

Materials and Methods

Population Structure

Nine rams of the Dorset breed were identified based on visual appraisal of muscle mass and pedigree relationships that traced back within four generations to the presumed founder ram (Solid Gold) from the Moffat flock. Romanov ewes (n = 255), assumed to be noncarriers of the mutant allele, were exposed to the nine Dorset rams in single-sire mating pens during two distinct 35-d breeding seasons in the fall of 1992. Each F_1 lamb was subjectively classified as expressing either the *callipyge* or the normal phenotype at 4, 8, and 12 wk of age; results demonstrated that each Dorset sire was heterozygous at the *CLPG* locus. All healthy dam-reared F_1 ewe lambs, regardless of phenotype, from the first lambing group were retained for breeding (n = 105). The F_1 ewe lambs that expressed the *callipyge* phenotype were preferentially selected from the second lambing group, resulting in an additional 47 ewe lambs available for breeding in 1993. Two-thirds of the 152 F_1 ewe lambs were evaluated as *callipyge* phenotype, and the remaining one-third was classified as normal phenotype. Therefore, the frequency of the mutant *CLPG* allele in these ewe lambs was approximately .33. Eight F_1 ram lambs evaluated as exhibiting the *callipyge* phenotype were used as sires of the next generation. Two replicates of F_2 lambs (n = 432) were born to 138 F_1 ewes during 1994 and 1995. Three of the F_1 sires were used during both years of matings. One F_1 sire used only in the second year was ultimately shown to not carry the mutant allele, and it thus produced no detectable informative meioses for the *CLPG* locus.

Phenotypic Data Collection

Routine data collected on individuals included date of birth, sex, lambing difficulty, date and cause of mortality, type of birth-rearing, defects, and weights recorded at birth and at 8 (weaning), 14, and 20 wk of age. Ranges in lambing dates were 37 and 38 d for each year of F_2 lamb production, and the appropriate data were recorded when a contemporary weaning group averaged the target age. Ram lambs were castrated shortly after birth, and lambs unable to be naturally reared were slaughtered. Following weaning, lambs were allowed ad libitum access to standard diets in a feedlot setting.

The F_2 ewe and wether lambs were serially slaughtered at 23, 26, 29, 32, 35, and 38 wk of age (n = approximately 30 per age group per year). Live weight was recorded the morning of slaughter on lambs given continual ad libitum access to feed and water. Pelt (including feet), kidney-pelvic fat, liver, and hot carcass weights were measured. Ovaries of ewe lambs were examined for pubertal status and number of corpora lutea. Following a 24-h chill, carcass weight was again recorded and width of the carcass recorded at the widest points of the shoulder and rump using callipers. A subjective leg muscling score, normally scaled from 1 (low cull) to 15 (high prime), was assigned to each of the carcasses after sorting them on the rail from heaviest- to lightest-muscled. In this sample, scores varied from 9 to 17 because some carcasses exhibited degrees of muscling beyond the high prime classification. Carcasses were split along the dorsal midline, and the right-side weight was recorded. Carcass length was measured from the anterior edge of the first rib to the anterior edge of the aitch bone. Metacarpal bone length was measured. Fat depths were recorded at two locations: at the midpoint over the longissimus muscle between the 12th and 13th ribs and at the midline of the fourth sacral vertebra.

To evaluate distributions of carcass components, the right side was cut into three sections: anterior (shoulder, neck, foreshank, and breast), midsection (loin, rib, and flank), and posterior (sirloin, leg, and hindshank). Each section was weighed separately. The anterior section and midsection were separated between the fifth and sixth ribs. The posterior section was separated from the midsection between the second-to-last and last lumbar vertebrae. The midsection was split between the 12th and 13th ribs, where depth, width, surface area, and subjective marbling score (0 = devoid; 200 = traces; 400 = small; 600 = moderate) were evaluated at the surface of the longissimus muscle. Complete sections were frozen and subsequently ground separately to determine chemical composition by proximate analysis of water, protein, ether extract, and ash components. Weight of fat-free lean or other components on a whole carcass basis were derived from the sums of the three sections.

Genotypic Data Collection

Forty-four primer pairs from 42 distinct loci previously mapped to either ovine chromosome 18 or the homologous bovine chromosome 21 were evaluated in this population. Information on the 25 useful markers in this population is summarized in Table 1. One locus was a polymorphism of restriction sites for the enzyme *Msp*I in an amplified region of the IGF-1R locus initially described by Moody et al. (1996) as a *Taq*I bovine polymorphism. The allele products for this locus were resolved by electrophoresis of digested samples on a 3% agarose gel and visualized by UV light following ethidium bromide staining. Primers designed around microsatellite sequences from either bovine or ovine species were used at remaining loci.

The lack of highly informative microsatellite primer pairs near the telomeric region of ovine chromosome 18 prompted isolation of additional microsatellite loci using available markers and an ovine yeast artificial chromosome (**YAC**) library (Broom and Hill, 1994). The primer pair for the BMS1561 marker was used to screen the library and isolate two unique YAC clones containing this locus. Two microsatellite markers (OY3, OY5) were subsequently developed from one of the YAC clones using the procedures of Sonstegard et al. (1998). This clone has been physically assigned by fluorescence in situ hybridization to ovine chromosome 18 and is near but not at the telomere (unpublished data) with no evidence of chimerism.

Microsatellite loci were amplified by PCR with direct incorporation of $[\alpha\text{-}^{32}P]dATP$ using standard protocols (de Gortari et al., 1997). Three microsatellite loci (CSSM18, TGLA122, OARVH54) were alternatively end-labeled $[\gamma\text{-}^{32}P]$ using a kinase reaction with the forward primer to reduce sub-banding and enhance accuracy of scoring these loci. Standard protocols for thermal cycling and electrophoresis conditions have been reported (Bishop et al., 1994; de Gortari et al., 1997).

Genotypes were independently scored twice using an entry screen system similar to that described by Rohrer et al. (1994) and entered into the USDA-MARC relational database (Keele et al., 1994) from which data could be easily retrieved for linkage analyses. After several loci had been genotyped, it became apparent that several pedigree and(or) DNA source errors existed. This occurrence was anticipated given the high reproductive rates of the Romanov and F_1 ewes and the penning protocols during the time of lambing; natural cross-fostering events could not be prevented. Six (4%) F_1 ewes and 34 (8%) F_2 lambs had genotypes not possible given the parental information observed. Extraction of DNA from a different tissue source was conducted for these individuals and pedigree/DNA source errors were corrected when possible. Genotypes from two X-linked microsatellites were used to assist correct parentage identification.

Information from six F_2 lambs was excluded from the final analysis because parentage could not be determined from currently available data.

Linkage Group Analyses

Linkage analyses were performed using CRI-MAP Version 2.4 software (Green et al., 1990). Initially, two-point logarithm of odds (**LOD**) tables were computed for all pairs of loci. The phase-unknown likelihood tolerance value, to test the null hypothesis of independent segregation, was set at a LOD of 3.0, and recombination rates were assumed equal in the two sexes. A total of 25 loci exceeded these minimum criteria for linkage and were subjected to multiple point analyses.

The BUILD option was used to construct the initial linear locus order. The tolerance levels used to discard alternative locus orders were set at highly stringent levels (LOD of 5.0) for phase known and phase unknown data. The remaining loci were placed into the linkage group by order of informative meioses using the ALL option with a lower tolerance level (LOD of 3.0) for rejection of locus orders. The FLIPS(n) option was used throughout the process of building the linkage group to evaluate all permutations of n consecutively ordered loci relative to the current reference order (n varied from 2 to 5). The CHROMPIC option was used to identify unlikely crossover events not substantiated by changes in phase of adjacent loci regardless of genetic distance. The genotypes contributing to these events were amplified and scored a second time to resolve discrepancies and errors in interpretation. This is the process suggested by Buetow (1991) and, it proved valuable in identifying genotypic errors.

Genotypic Probabilities

A FORTRAN program was written to calculate conditional probabilities and analyses of variance to predict the position and test gene action of *CLPG*. An approach described by Haley et al. (1994) was implemented to evaluate evidence for the presence of the *CLPG* locus at sequential positions along the chromosome 18 linkage group. The display of grandparental origins of alleles generated by CHROMPIC for each chromosome 18 gamete in the F_2 generation with the phase choice having the highest likelihood was used to calculate conditional probabilities for each animal for each genotypic class at the *CLPG* locus. Conditional probabilities for the presence of the mutant *CLPG* allele (**C**) were determined on each gamete contributing to an F_2 individual at 1-cM intervals in the linkage group as a function of the recombination rates with the two informative, flanking marker loci. The frequency of **C** in Romanov ewes was assumed to be 0, and genotypes of the Dorset

Table 1. Marker loci information for ovine chromosome 18

Locus	Primer pair sequences 5' → 3'	Annealing temperature °C	Approximate size, bp	F_1 parent heterozygosity[a]	Relative position, cM	Primer reference
IGF-1R	CCCAATGGATTGATCCTCATGT GCTGTGTAGTTCCCTGGGTT	60	625[b]	.475	0	Moody et al. (1996)
BMS1117	TGTGTGCTCTCACACATGC AACCAAAGCAGGGATCAGG	56	100	.798	3.6	Stone et al. (1995)
BM3413	TCCCTGGTAACCAATGAATTC CAATGGATTTGACCCTCCC	58	180	.817	12.9	Bishop et al. (1994)
AGLA233	TGCAAACATCCACGTAGCATAAATA GCATGAACAGCCAATAGTGTCATC	48	230	.153	20.9	Georges and Massey (1992)
ILSTS052	CTGTCCTTTAAGAACAAACC TGCAACTTAGGCTATTGACG	56	180	.923	25.2	Kemp et al. (1995)
BMS1494	TCTGGAGCTTGCAAAAGACC AATGGATGACTCCTGGATGG	58	230	.499	28.9	Stone et al. (1995)
BP33	AGGCCTCGAATTCCTCTCC AACCAGCAGTCTTGCTCTCTC	58	280	.515	30.7	Bishop et al. (1994)
OARCP134	TAAGTGGGATTTCATCTTTCCGGGATC TGTCAAGAAACACGGTGCTGTAAG	48	100	.858	31.1	Pierson et al. (1994)
OARVH54	ATGGTTACTGAATGGCTGCCTAACCC CCTTAGGAACTAAATGTGCACTTGTATGTG	56	110	.581	31.8	Pierson et al. (1994)
IDVGA-45	GTGGTGGCAAAGAGTCAGA AACAGCCCTGATTTCCATA	56	180	.967	34.6	Mezzelani et al. (1995)
MCM148	ACACGGCTGAGTGACTGAACA TATTCCTGCCCTGCAGATAGG	60	200	.489	37.0	Smith et al. (1995)
UWCA4	CAGCTTGAAATGAGTATGTCACC CAACAAGAAAGCAGAGACTCC	60	100	.906	43.7	Sun et al. (1992)
BMS2815	TGATATTCAAACTCAATGAACCC CTTGCATATGCTCATCATTATCA	56	100	.897	45.3	Stone et al. (1997)
BM7243	TCTAGATAATGCATATGTGCCTGT AAAATAGGCTGTCAGTCACAAGA	56	150	.901	46.5	Freking et al. (1998b)
BMC5221	AGGGCAGAGAACAGGCATTC CTTCTTTGCAGCACAGTTTC	56	200	.974	58.1	Bishop et al. (1994)
OARHH47	TTTATTGCAAACTCTCTTCCTAACTCCACC GTAACTTATTTAAAAAAATATCATACCTCTTAAGG	56	140	.996	59.8	Henry et al. (1993)
TGLA337	TTTGTTAAGGATAGTAGGCTACT GCTCTTCCCTTGGTTTCCTTG	56	110	.420	60.1	Georges and Massey (1992)
TGLA122	CCCTCCTCCAGGTAAATCAGC AATCACATGCAAATAAGTACATAC	58	140	1.000	67.4	Georges and Massey (1992)
ILSTS054	GAGGATCTTGATTTTGATGTCC AGGGCCACTATGGTACTTCC	56	140	.738	71.4	Kemp et al. (1995)
MCM38	TGGTGAATGGTGCTCTCATACCAG CAGCCAGCAGCCCTCTAAAGGAC	56	150	1.000	72.0	Hulme et al. (1994)
CSSM18	AGGAATTCCCTCTAGAAAAGCAGGC TGTGCATAATTTGTCGTCCGGA	58	120	.887	83.3	Cockett et al. (1994)
IDVGA-30	TGAATATGTCTCTCGAGTGCATCT TTCTTCCAGCCCTTCTCCAGAGC	56	120	.928	84.1	N. E. Cockett (personal communication)
BMS1561	ACCCACATGTTTGGGAGG AGGGAAAGGCCAAAGCAC	60	140	.029	87.2	Freking et al. (1998b)
OY3	TTTTGGTTTCACTTATTGTTTGAG CTCTGTCTAAAGAAGCTTTTCCC	58	160	.837	87.2	This article
OY5	CTACAGTCCACGGCCAC GGATAATGATAGCTCCCTGGC	54	140	.733	87.2	This article

[a] Calculated as [heterozygosity of F_1 male parents (n = 8) + heterozygosity of F_1 female parents (n = 138)]/2.
[b] Full-length product before analysis of differential restriction sites by enzyme MspI.

rams at the *CLPG* locus were heterozygous (frequency of **C** = .5). The CHROMPIC output from the linkage analysis established the grandparental source (Dorset or Romanov) of alleles at the marker loci for the F_2 individuals. The conditional probability of an F_2 lamb's chromosome carrying **C** is the product of the probabilities that the F_1 parent inherited this allele from a Dorset ram and that same allele was subsequently inherited by the F_2 individual.

Probabilities of F_1 sires being heterozygous carriers of **C** were based on progeny test information. Seven of eight F_1 sires were assigned probabilities of unity of being heterozygous carriers. The remaining sire was given a probability of null based on 41 progeny all of normal phenotype and output from the linkage analysis, which indicated that this sire was in opposite phase with respect to the telomeric region of its paternal chromosome 18 compared to a paternal half-sib F_1 sire shown to be a carrier. Probabilities that F_1 dams were heterozygous carriers of **C** were calculated from the flanking genotypic information at each 1-cM position on the linkage group. The conditional probability of an F_1 ewe's paternal chromosome carrying **C** is the product of probabilities that the Dorset sire inherited this allele from its own sire ($P = 1$) and that the same allele was subsequently inherited by the F_1 ewe. This latter probability varies dependent on the presence or absence (indicating a crossover event) of paternal grandsire alleles flanking each position.

Probabilities that maternal and paternal chromosomes carried **C** were then used to calculate **CC**, **CN**, **NC**, and **NN** *CLPG* genotypic probabilities for each F_2 lamb, where **C** represents the mutant *CLPG* allele, **N** represents the normal allele(s), and the paternal allele of the genotype is listed first. For example, at position 86 cM, one lamb had probabilities of .0003, .9995, .0000, and .0002 for **CC**, **CN**, **NC**, and **NN** genotypes, respectively. Genotypic probabilities must sum to one within animal, creating a dependency.

Statistical Analyses

Based on empirical distributions, four traits that discriminated between *callipyge* and normal phenotypes were analyzed to position *CLPG* and to evaluate different models of gene action. The regression of loin-eye area on carcass weight was used to describe muscle size. An objective measurement of carcass shape was rump width regressed on carcass length, whereas a subjective assessment of carcass shape was leg muscling score regressed on carcass weight. The regression of carcass fat-free lean (carcass water plus carcass protein) on carcass fat provided a comparison of carcass components.

Traits were analyzed using a statistical model that included effects of year, sex, and sire to partially account for environmental and polygenic effects.

Regressions on **CC**, **CN**, **NC**, and **NN** probabilities (**NN** effect was set to zero to remove the dependency) and genotype-specific second-order polynomials of appropriate covariates (described above) were also estimated. A single contrast involving *CLPG* genotypic effects provided a 9-df F-test (341 residual df) to evaluate significance of the full genotypic model. Analyses of traits were conducted at 1-cM intervals starting at the most centromeric marker and extending 20 cM beyond the most telomeric marker. The F-statistic from each analysis was plotted against the position. The position that maximized the F-statistic for a given trait was taken as the position of the *CLPG* locus.

After positioning the *CLPG* locus for each trait, different models of gene action were evaluated to partition the 9 df associated with the full genotypic model. A set of three orthogonal contrasts (3 df per contrast) of genotypic regressions and genotype-specific linear and quadratic polynomial effects was developed. Contrasts of **CC**, **CN**, **NC**, and **NN** effects defined traditional additive (1, 0, 0, and −1), dominance (−1, 1, 1, and −1), and reciprocal heterozygote (0, 1, −1, and 0) models of gene action, respectively. If the reciprocal heterozygote contrast was significant, the dominance contrast was misleading because the effect of one heterozygous genotype was underestimated and the other heterozygous genotype was overestimated. Under this situation, a second set of orthogonal contrasts was derived to include the polar overdominance hypothesis of Cockett et al. (1996). Three orthogonal contrasts of **CC**, **CN**, **NC**, and **NN** effects defined additive (1, 0, 0, and −1), maternal dominance (−1, 0, 2, and −1), and paternally derived polar overdominance (−1, 3, −1, and −1) models of gene action, respectively.

Results

Ovine Chromosome 18 Map Development

A linkage group of 25 loci flanking 87.2 cM of ovine chromosome 18 was constructed. Relative genetic distances arranged from the most centromeric to the most telomeric are presented in Table 1. Seven microsatellite loci (BMS1117, AGLA233, MCM148, ILSTS054, IDVGA-30, OY3, and OY5) and the PCR-RFLP for IGF-1R are new linkage assignments for ovine chromosome 18 compared to the most recent sheep linkage map (de Gortari et al., 1998). There were no discrepancies in locus order, and there was remarkable conservation of interval distances for the 16 markers in common with the homologous bovine chromosome 21 linkage group (Kappes et al., 1997). In contrast, the interval of 37.5 cM between TGLA122 and IDVGA-30 of Cockett et al. (1996) is more than twice the distance reported herein (16.7 cM). Like-

Table 2. Summary of *CLPG* position analyses

Dependent variable	Covariate	Full genotypic model					Parsimonious model	
		Position, cM	Maximum F-test[a]	Additive[b]	Maternal dominance[b]	Polar overdominance[b]	Position, cM	Polar overdominance[c]
Loin-eye area	Carcass weight	87	62.1	.50	1.97	164.41	91	183.0
Rump width	Carcass length	86	38.3	2.13	1.57	110.66	86	106.6
Leg score	Carcass weight	86	136.4	1.42	.86	371.78	86	407.4
Carcass fat-free lean	Carcass fat	87	52.4	.19	2.68	145.27	94	152.7

[a]Maximum F-test (9 df) associated with full genotypic model (three genetic contrasts and interaction of each contrast with linear and quadratic covariate terms).

[b]F-test (3 df) associated with additive, maternal dominance, or polar overdominance genetic contrasts and interactions with linear and quadratic covariate terms tested at the same relative position as the full genetic model.

[c]Maximum F-test (3 df) associated with polar overdominance effect and interactions with linear and quadratic covariate terms.

wise, de Gortari et al. (1998) estimated 97 cM between BM3413 and BMS1561, compared with 74.3 cM in the present research, a difference of 23%. Differences in interval distances can be attributed to differences in family-specific recombination rates, genotypic data errors, and lack of precision of the estimated interval owing to sparse coinformative data. There were 1,082 possible informative meioses per marker for animals genotyped in this population. The mean number of informative meioses per marker on the linkage group was 708 (range 30 to 967) with similar contributions by males (54.3%) and females (45.7%). On average, genetic distance relationships between any two loci in this linkage group were determined from 482 coinformative meiotic events.

The distribution of recombination events per gamete transmitted by F_1 parents to F_2 lambs indicated that 37% of gametes exhibited no crossovers within the 87-cM flanked region; 47% had a single crossover event; and only 16% had two, three, or four observed crossovers. Because gametes without crossovers do not help position the *CLPG* locus, only 63% of the gametes were informative for positioning. However, only 12.8% of the sheep were completely uninformative, in regard to *CLPG* position, at maternal and paternal chromosomes. This expected lack of recombination is the underlying genetic basis for imprecise positioning of an unknown locus, regardless of size of the effect in pedigrees extending only three generations. Occurrences of fortuitous recombination events close to the locus of interest have low probability in a single generation of segregation, and yet they are necessary to break up strong disequilibrium. The frequency of useful crossover events is primarily a function of experiment size.

Position and Gene Action of the Callipyge *Locus*

The most likely position of *CLPG* based on the full genotypic model ranged from 86 to 87 cM for the four traits (Table 2). The maximum values for 9-df F-tests varied from 38.3 for rump width to 136.4 for leg score.

The 3-df contrast of reciprocal heterozygotes was significant for each trait (results not tabulated). Therefore, F-statistics for the three orthogonal contrasts derived to evaluate the polar overdominance model are presented for each trait. The additive and maternal dominance contrasts did not contribute significantly to the variation of any trait. A 3-df F-test would need to exceed a value of 8.0 to reach the genome-wide level of significance ($P = .05$).

Because of the lack of evidence for differential effects among **CC**, **NC**, and **NN** genotypes, the additive and maternal dominance genetic effects were excluded to achieve a parsimonious model that included only the polar overdominance effect. The polar overdominance effect was estimated by regression on the difference between the **CN** probability and the pooled probability of **CC**, **NC**, and **NN** genotypes. The interaction of this effect with linear and quadratic covariate terms was also estimated. Analyses were again conducted at 1-cM intervals as described above. The profile of the polar overdominance F-test for leg score is presented in Figure 1. The position of *CLPG* based on the parsimonious model was less consistent among traits compared to results of the full genotypic model (Table 2). The 3-df F-test from the reduced model varied from 106.6 for rump width to 407.4 for leg score. These 3-df maximum F-values far exceed the genome-wide threshold value of 8.05, which is the value of F that satisfies $[(C + 2\rho g\gamma_1 F)P] = .05$ (Lander and Kruglyak, 1995), where $C = 27$ chromosomes, $g = 30$ morgans, $\rho = 1$, and P is the probability that values from an F-distribution with $\gamma_1 = 3$ numerator df and 347 denominator df exceed a value of F. The results of this experiment and the study conducted independently by Cockett et al. (1996) would represent a case of a locus that meets the Lander and Kruglyak (1995) criterion for confirmed linkage.

Recombination Evidence to Limit Flanked Region Containing the Callipyge *Locus*

Physical recombination evidence in rams with known *CLPG* genotype (progeny test) placed *CLPG*
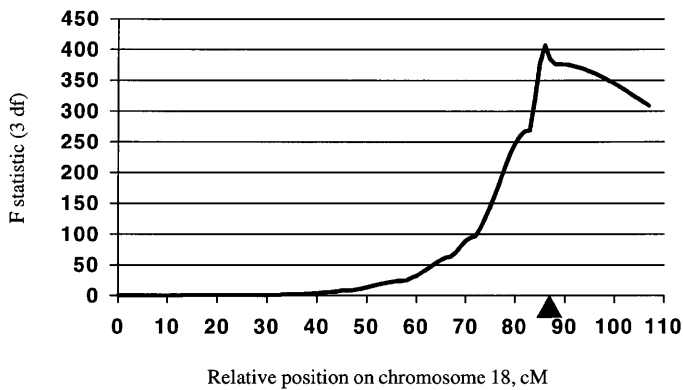
Figure 1. Profile of F-statistics for leg score regressed on carcass weight.

centromeric of the OY3-OY5-BMS1561 haplotype. The single fortuitous recombination event occurred between CSSM18 and the three-marker haplotype group and was detected on the paternally derived chromosome of one of the Dorset grandsires (9212042, Figure 2). A different haplotype of allele combinations from these four markers was in coupling phase with the mutant *CLPG* allele when transmitted to his progeny compared to the combination transmitted to progeny of a paternal half-sib Dorset ram (9212092) also used as a grandsire in this population. Thus, F_1 progeny from these two Dorset rams, with the same paternal grandparental (9012500) origin of the **C** allele, transmitted different marker alleles in coupling phase with the **C** allele at the OY3-OY5-BMS1561 loci but the same marker allele at the CSSM18 locus.

Recombination evidence also placed *CLPG* telomeric of CSSM18. Three F_1 dams and four F_2 lambs expressed definitive *callipyge* phenotypes, implying that their *CLPG* genotypes were **CN**. Their maternal chromosomes were of Romanov origin. The centromeric regions of their paternal chromosomes through and including the CSSM18 locus were of Romanov origin in the four F_2 lambs and of paternal grandam origin (Dorset, **N** allele) in the three F_1 dams. Recombinations in the interval between CSSM18 and OY3-OY5-BMS1561 were detected on the paternal chromosomes, indicating that the telomeric region must contain the **C** allele transmitted by Dorset grandsires. These results jointly position *CLPG* telomeric of CSSM18 and centromeric of OY3-OY5-BMS1561, which restricts the *CLPG* locus to a 3.9-cM interval between positions 83.3 and 87.2 in this linkage group. Individuals recombinant in this interval contribute to positional cloning experiments.

Discussion

A previous experiment determined linkage of the *CLPG* locus to ovine chromosome 18 (Cockett et al.,

1994). A genetic model of paternal polar overdominance was proposed (Cockett et al., 1996) to explain the relationship between *callipyge* phenotypes and genotypes. We confirm the hypothesis of paternal polar overdominance. The most probable position of the locus is within a 3.9-cM interval on chromosome 18 bounded by CSSM18 and OY3-OY5-BMS1561. In contrast to Cockett et al. (1994), we found the most likely interval containing *CLPG* to be telomeric, rather than centromeric, of CSSM18, which is at position 83.3 cM in this linkage group. The OY3-OY5-BMS1561 haplotype defines the telomeric boundary and represents the closest linkage of marker loci to the location of *CLPG*.

Regardless of the approach used, qualitative or quantitative, paternal polar overdominance was identified as the most likely genetic model. Reciprocal heterozygote genotypes expressed different phenotypes (polar), and the two homozygous genotypes produced similar, normal phenotypes (over-
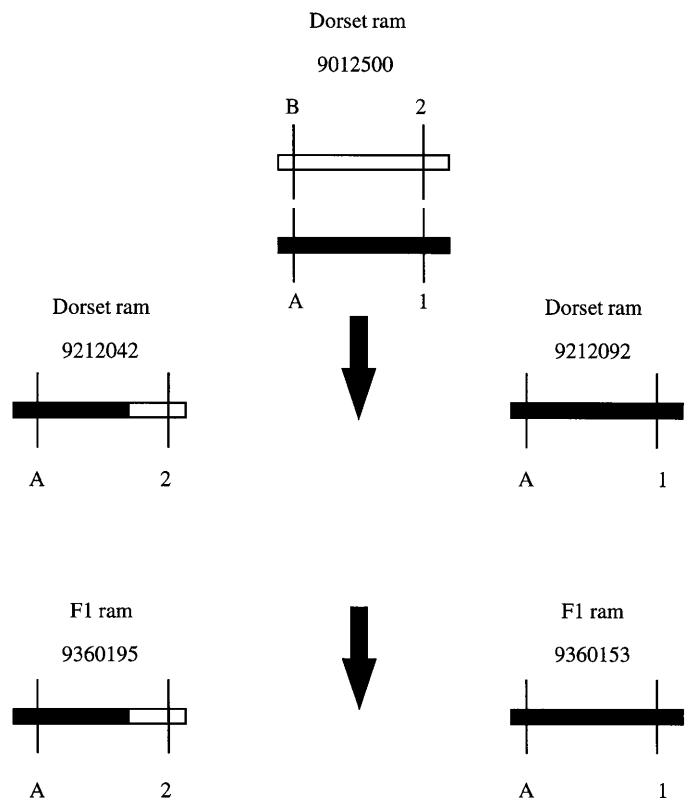
Figure 2. Transmission of paternally derived gametes showing a recombination event in the 3.9-cM interval for Dorset grandsire 9212042, which provides evidence that *CLPG* is centromeric of the right flanking haplotype. Solid bars represent gametes containing the mutant *callipyge* allele. All sires were known heterozygotes of the mutant *callipyge* allele based on progeny test. Left flanking marker (CSSM18) alleles are represented by letters, and the right flanking haplotype (OY3-OY5-BMS1561) alleles by numbers.

Table 3. Predicted percentages of offspring that show the *callipyge* phenotype for each possible mating combination[a]

		Ram			
		CC Normal	**CN** *Callipyge*	**NC** Normal	**NN** Normal
Genotype/phenotype					
Ewe					
CC	Normal	0	0	0	0
CN	*Callipyge*	50	25	25	0
NC	Normal	50	25	25	0
NN	Normal	100	50	50	0

[a]**C** represents the mutant *callipyge* allele. **N** represents the wild-type normal allele(s).

dominance). Inferences concerning the actual expression of the different alleles for the gene(s) at the *CLPG* locus are speculative until it is positionally cloned and the variable alleles determined. Statements made in the literature about animals that express the *CLPG* gene refer to the expression of the phenotype rather than to unique gene expression on the molecular level. It is perhaps just as likely that the phenotype is caused by lack of normal gene expression or function at the *CLPG* locus. Evidence indicates that extreme phenotypic variants in musculature can be the result of loss of function, or disrupted function, of alleles at a single locus that act as regulators of normal muscle development (Fujii et al., 1991; Grobet et al., 1997; Kambadur et al., 1997; McPherron et al., 1997).

A genetic model built on the premise of a switch in the parent-specific origin expression pattern of an imprinted region and that null expression at the locus would cause the unique phenotype was suggested to explain the polar, preimplantation-embryo lethal phenotype known as the "DDK syndrome" in mice (Sapienza et al., 1992). Recent experiments led to the rejection of this hypothesis as the putative genetic model for the DDK syndrome (Pardo-Manuel de Villena et al., 1997). However, as discussed by Cockett et al. (1996), the "reverse imprinting" model could still be used to explain the segregation patterns for the *callipyge* phenotypes. The chromosome-specific methylation patterns, which are indicative of imprinted regions, can be tested when the locus is positionally cloned.

The unusual gene action of *CLPG* will require structured industry use of specific mating systems. Table 3 presents the expected percentage of progeny exhibiting the *callipyge* phenotype from all possible mating combinations in a two-allele (mutant *CLPG* vs wild type) single-locus model with paternal polar overdominance as the form of gene action. Only 6 of the 16 cells have been directly tested with reported experimental data. Experiments to completely evalu-

ate these combinations are complicated because three genotypes (**CC**, **NC**, and **NN**) have the same normal phenotype. Until the *CLPG* locus is cloned, inference of *CLPG* genotypes must be made using flanking markers and previously determined within-family phase information. The only mating combination expected to produce progeny that all express the *callipyge* phenotype is mating **CC** rams to **NN** ewes; both genotypes are associated with normal phenotypes. Cockett et al. (1996) reported exceptions to this expectation, but assumed no recombination between *CLPG* and their two most closely linked markers.

Effects of the *CLPG* locus exceed any previously reported net breed effects on carcass composition (Freking et al., 1998a). Terminal sire mating systems with homozygous or heterozygous sires are most likely implementation scenarios. Specialized terminal sire lines can be developed in approximately 5 yr by introgression of the **C** allele into genetic backgrounds of industry choice. Initial matings would require use of known **CC**, **CN**, or **NC** rams and visual evaluation of male progeny to identify carriers for the next generation of the backcross. In 4 yr of backcross matings, turning one generation per year, heterozygous males and females (**CN**) would exist that contain on average 15/16 of the genetic background of choice. *Inter se* mating will produce 25% of progeny (12.5% males) as *callipyge* homozygotes (**CC**). The positional cloning of the *CLPG* locus becomes a relevant issue at this point because homozygous progeny need to be differentiated from two other genotypes (**NC** and **NN**) with the same normal phenotype. Use of DNA-based assays to directly evaluate the allelic differences at the *CLPG* locus reduces costs associated with progeny testing a large number of rams and is more accurate than using flanking markers. When family phase information is known, currently available markers are useful to haplotype the locus with an acceptable error rate.

Use of the **C** allele would not be limited to mating schemes involving homozygous terminal sire systems. Heterozygous sires could be used in smaller flock mating systems in which progeny that express the *callipyge* phenotype are targeted for slaughter and noncarrier progeny are available for selection as replacement females. Any increase in frequency of the **C** allele in the ewe flock will decrease the percentage of progeny that expresses the hypertrophied phenotype.

Multiple-tier stratified breeding structures, such as exist in the United Kingdom, may be able to use different ram genotypes at different tiers. In the U.K. system, hill breeds are maintained in pure-breeding flocks and produce ewes for the upland sector. These ewes are crossed to longwool breed rams for the production of crossbred ewes subsequently used by the lowland sector in terminal sire matings. Heterozygous sires perhaps fit into this system as hill breed and

longwool breed rams for the production of *callipyge* ewe and wether lambs for slaughter and normal phenotype ewes for breeding use by all three sectors. The half-bred ewe lambs that do not exhibit the *callipyge* phenotype could then be mated to homozygous **CC** rams in the terminal sire portion of this mating system to improve carcass composition in the lowland sector. The large effects of the **C** allele may justify use of heterozygous and(or) homozygous rams in a wide variety of breeds and production systems, but careful monitoring of allelic frequency in ewes is critical to maintain expected performance levels.

Implications

A resource flock of 362 F_2 lambs provided the carcass and genotypic data necessary to refine the position of the *callipyge* (*CLPG*) locus to a 3.9-cM interval of chromosome 18. Efforts to positionally clone the gene responsible for this phenotype are greatly enhanced by this information. Individuals recombinant in this specific interval allow further refinement of map position. Gene action at the *CLPG* locus is characterized by unique muscle hypertrophy and low carcass fatness in heterozygous animals that inherited the allele from their sire. Other genotypes have a normal phenotype. Marker-based information could replace progeny-testing programs to provide sheep for mating systems that optimize production of the *callipyge* phenotype.

Literature Cited

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S.L.F. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, J. Yoo, and C. W. Beattie. 1994. A genetic linkage map for cattle. Genetics 136:619–639.

Broom, M. F., and D. F. Hill. 1994. Construction of a large-insert yeast artificial chromosome library from sheep DNA. Mamm. Genome 5:817–819.

Buetow, K. H. 1991. Influence of aberrant observations on high-resolution linkage analysis outcomes. Am. J. Hum. Genet. 49: 985–994.

Cockett, N. E., S. P. Jackson, T. L. Shay, F. Farnir, S. Berghmans, G. D. Snowder, D. M. Nielsen, and M. Georges. 1996. Polar overdominance at the ovine *callipyge* locus. Science (Wash., DC) 273:236–238.

Cockett, N. E., S. P. Jackson, T. L. Shay, D. Nielsen, S. S. Moore, M. R. Steele, W. Barendse, R. D. Green, and M. Georges. 1994. Chromosomal localization of the *callipyge* gene in sheep (*Ovis aries*) using bovine DNA markers. Proc. Natl. Acad. Sci. USA 91:3019–3023.

de Gortari, M. J., B. A. Freking, R. P. Cuthbertson, S. M. Kappes, J. W. Keele, R. T. Stone, K. A. Leymaster, K. G. Dodds, A. M. Crawford, and C. W. Beattie. 1998. A second generation linkage map of the sheep genome. Mamm. Genome 9:204–209.

de Gortari, M. J., B. A. Freking, S. M. Kappes, K. A. Leymaster, A. M. Crawford, R. T. Stone, and C. W. Beattie. 1997. Extensive genomic conservation of cattle microsatellite heterozygosity in sheep. Anim. Genet. 28:274–290.

Freking, B. A., J. W. Keele, M. K. Nielsen, and K. A. Leymaster. 1998a. Evaluation of the ovine *callipyge* locus: II. Genotypic effects on growth, slaughter, and carcass traits. J. Anim. Sci. (In press).

Freking, B. A., R. T. Stone, M. J. de Gortari, and A. M. Crawford. 1998b. Chromosomal assignment by linkage of nineteen unassigned bovine microsatellites using ovine reference populations. Anim. Genet. (In press). 29:000–000.

Fujii, J., K. Otsu, F. Zorzato, S. DeLeon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, and D. H. MacLennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science (Wash., DC) 253:448–451.

Georges, M., and J. Massey. 1992. Polymorphic DNA markers in bovidae (World Intellectual Property Org. Geneva). WO Publ. No. 92/13102.

Green, P. K., K. Falls, and S. Crooks. 1990. Documentation for CRI-MAP, version 2.4. Washington University School of Medicine, St. Louis, MO.

Grobet, L., L.J.R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset, and M. Georges. 1997. A deletion in the bovine *myostatin* gene causes the double-muscled phenotype in cattle. Nat. Genet. 17:71–74.

Haley, C. S., S. A. Knott, and J. M. Elsen. 1994. Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 136:1195–1207.

Henry, H. M., J. M. Penty, C. A. Pierson, and A. M. Crawford. 1993. Ovine microsatellites at the OARHH35, OARHH41, OARHH44, OARHH47, and OARHH64 loci. Anim. Genet. 24:223.

Hulme, D. J., J. P. Silk, J. M. Redwin, W. Barendse, and K. J. Beh. 1994. Ten polymorphic ovine microsatellites. Anim. Genet. 25: 434–435.

Jackson, S. P., R. D. Green, and M. F. Miller. 1997. Phenotypic characterization of Rambouillet sheep expressing the *Callipyge* gene: I. Inheritance of the condition and production characteristics. J. Anim. Sci. 75:14–18.

Kambadur, R., M. Sharma, T.P.L. Smith, and J. J. Bass. 1997. Mutations in *myostatin* (GDF8) in double muscled Belgian Blue and Piedmontese cattle. Genome Res. 7:910–915.

Kappes, S. M., J. W. Keele, R. T. Stone, R. A. McGraw, T. S. Sonstegard, T.P.L. Smith, N. L. Lopez-Corrales, and C. W. Beattie. 1997. A second generation linkage map of the bovine genome. Genome Res. 7:235–249.

Keele, J. W., J. E. Wray, D. W. Behrens, G. A. Rohrer, S.L.F. Sunden, S. M. Kappes, M. D. Bishop, R. T. Stone, L. J. Alexander, and C. W. Beattie. 1994. A conceptual database model for genomic research. J. Computat. Biol. 1:65–76.

Kemp, S. J., O. Hishida, J. Wambugu, A. Rink, M. L. Longeri, R. Z. Ma, Y. Da, H. A. Lewin, W. Barendse, and A. J. Teale. 1995. A panel of polymorphic bovine, ovine and caprine microsatellite markers. Anim. Genet. 26:299–306.

Lander, E. S., and L. Kruglyak. 1995. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat. Genet. 11:241–247.

McPherron, A. C., A. M. Lawler, and S. J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature (Lond.) 387:83–90.

Mezzelani, A., Y. Zhang, L. Redaelli, B. Castiglioni, J. L. Williams, S. S. Toldo, G. Wigger, R. Fries, and L. Ferretti. 1995. Chromosomal localization and molecular characterization of 53 cosmid-derived bovine microsatellites. Mamm. Genome 6:629–635.

Moody, D. E., D. Pomp, and W. Barendse. 1996. Linkage mapping of the bovine insulin-like growth factor-1 receptor gene. Mamm. Genome 7:168–169.

Pardo-Manuel de Villena, F., A. K. Naumova, A. E. Verner, W. H. Jin, and C. Sapienza. 1997. Confirmation of maternal transmission ratio distortion at *Om* and direct evidence that the maternal and paternal "DDK syndrome" genes are linked. Mamm. Genome 8:642–646.

Pierson, C. A., A. J. Ede, and A. M. Crawford. 1994. Ovine microsatellite at the OARHH30, OARHH51, OARVH54, OARCP88, OARCP93, and OARCP134 loci. Anim. Genet. 25: 294–295.

Rohrer, G. A., D. W. Behrens, and J. W. Keele. 1994. A simplified procedure for entry of raw genotypic data. J. Computat. Biol. 1: 111–119.

Sapienza, C., J. Paquette, P. Pannunzio, S. Albrechtson, and K. Morgan. 1992. The polar-lethal *Ovum mutant* gene maps to the distal portion of mouse chromosome 11. Genetics 132:241–246.

Smith, A. J., D. J. Hulme, J. P. Silk, J. M. Redwin, and K. J. Beh. 1995. Thirteen polymorphic ovine microsatellites. Anim. Genet. 26:277–278.

Sonstegard, T. S., S. M. Kappes, J. W. Keel, and T.P.L. Smith. 1998. Refinement of bovine chromosome 2 linkage map near the *mh* locus reveals rearrangements between the bovine and human genomes. Anim. Genet. 29. (In press).

Stone, R. T., S. M. Kappes, J. W. Keele, and C. W. Beattie. 1997. Characterization of 109 bovine microsatellites. Anim. Genet. 28:5–9.

Stone, R. T., J. C. Pulido, G. M. Duyk, S. M. Kappes, J. W. Keele, and C. W. Beattie. 1995. A small-insert bovine genomic library highly enriched for microsatellite repeat sequences. Mamm. Genome 6:714–724.

Sun, H. S., M. R. Dentine, and B. W. Kirkpatrick. 1992. A polymorphic microsatellite (UWCA4) detected on bovine chromosome 21. Anim. Genet. 24:149.