Evaluation of the ovine *callipyge* locus: IV. Genotypic effects on reproductive traits[1,2]

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ABSTRACT: A resource population of ewes derived from Dorset and Romanov grandparents was generated over 5 yr to provide genotypic and phenotypic data to estimate effects of each *callipyge* genotype on component traits of reproduction. Effects on ovulation rate were estimated from data recorded by examination of ovaries from serially slaughtered ewe lambs (n = 174) born in 1994 and 1995. Productivity and longevity through 5 yr of age were recorded on a total of 265 F_2 ewes produced in 1996 through 1998. Number of lambs born, birth weights, and weaning weights of lambs were calculated for each ewe. Weaning weight was recorded for each lamb and adjusted for age to 56 and 32 d for dam- and nursery-reared lambs, respectively. Longevity was determined as a binary trait based on the presence or absence of the ewe in the breeding flock at 5 yr of age. Ewes generated 937 records for breeding weight and 925 records for conception rate, which was determined on ewes exposed and still present at the start of each lambing season. Seven hundred seventeen records were analyzed for traits based on the number of ewes lambing. Genotypes for the causative *callipyge* mutation were determined using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assay. Each ewe was classified into 1 of 4 genotypes; $N^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, or $C^{Mat}C^{Pat}$, where N represents wild type and C represents the mutant allele. The mean ovulation rate of the ewe lambs genotyped $N^{Mat}N^{Pat}$ was greater ($P < 0.02$) than that exhibited by $N^{Mat}C^{Pat}$; however, the estimated difference of 0.25 ovum in the ewe lambs did not affect the number born. The live weight of $N^{Mat}C^{Pat}$ ewes at breeding was 2.5 kg less than that of other genotypes ($P < 0.01$). The *callipyge* genotype class did not significantly affect conception rates, fecundity, maternal ability, or ewe longevity. Maintaining a ewe flock to produce homozygous mutant rams for use in terminal sire mating systems presents no unusual problems from a reproduction standpoint.

Key words: *callipyge*, reproduction, sheep

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INTRODUCTION

A single base mutation, altering a highly conserved segment within the intergenic region between imprinted genes DLK1 and MEG3 on ovine chromosome 18, has been identified as the causal polymorphism for the *callipyge* muscle hypertrophic phenotype (Freking et al., 2002; Smit et al., 2003). Dramatic effects of the mutation, favorable for slaughter and carcass traits but unfavorable for meat tenderness, were documented previously (Jackson et al., 1997b; Freking et al., 1998b, 1999).

The DLK1 gene is highly expressed in preadipocytes, inhibits their differentiation, and is normally suppressed once the differentiation process begins (Smas and Sul, 1993). Functional analysis of the tissues from lambs of each genotype indicated that the mutation in the paternally derived heterozygous state caused abnormal postnatal maintenance of elevated expression of DLK1, specific to fast-twitch muscle fibers (Charlier et al., 2001; Murphy et al., 2005). These temporal and spatial associations of altered gene expression make a strong argument that a varied DLK1 transcript level, and ultimately the level of the protein, is directly responsible for the hypertrophic condition.

A complete evaluation of all aspects of lifetime productivity is required to assess the relative merits of using specific genetic variation in a breeding program. In addition to the meat tenderness antagonism, any decreased reproductive fitness would be a potential cost

[1]Mention of trade names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the same by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Number of F_2 ewes and traits measured by year of birth and *callipyge* genotype

Year of birth	Phenotypic traits measured	*Callipyge* genotype[1]			
		$N^{Mat}N^{Pat}$	$N^{Mat}C^{Pat}$	$C^{Mat}N^{Pat}$	$C^{Mat}C^{Pat}$
1994	Ovulation rate	27	24	14	13
1995	Ovulation rate	40	28	20	8
1996	5-yr productivity	29	25	18	14
1997	5-yr productivity	31	36	12	13
1998	5-yr productivity	37	13	29	8

[1]N = wild type allele; C = *callipyge* allele. Superscripts Mat and Pat represent maternal and paternal origin, respectively.

component to consider while maintaining a terminal sire population fixed for the *callipyge* mutation. Our specific experimental objective was to estimate the effects of each *callipyge* genotype on reproductive traits to test the null hypothesis that this mutation has no effect on reproduction.

MATERIALS AND METHODS

Animal Population, Flock Management, and Traits

Experimental procedures were approved and performed in accordance with US Meat Animal Research Center animal care guidelines. Animals in the study were produced as part of a Dorset-Romanov F_2 resource population created to evaluate *callipyge* genotypic effects (Freking et al., 1998a). Contemporary sets of F_2 ewe lambs segregating for the *callipyge* mutation were produced in each of 5 yr from 1994 through 1998 (Table 1). Ewe lambs produced in 1994 and 1995 from 8 F_1 sires (7 paternally derived heterozygotes and 1 homozygous normal) were part of a serial slaughter design (ranging from 149 to 280 d of age) to estimate genotypic effects of the *callipyge* locus on slaughter, carcass, and meat quality traits (Freking et al., 1998b, 1999). Female reproductive tracts were removed in the abattoir, and both ovaries were examined. The total number of corpora lutea was recorded for each ewe. A total of 174 of 191 ewe lambs showed evidence of puberty, which allowed ovulation rate to be recorded from the estrous cycle at the time of slaughter.

Ewe lambs produced in 1996 through 1998 from 4 F_1 sires (all paternally derived heterozygotes) were maintained as a single flock and were evaluated for reproductive performance through 5 yr of age. Ewes born in 1996 lambed from 1997 through 2001; ewes born in 1997 lambed from 1998 through 2002; and ewes born in 1998 lambed from 1999 through 2003. Progeny produced in 1997 and 1998 were the result of inter se matings of ewes to F_2 Dorset × Romanov rams of each of the 4 *callipyge* genotypes, which were generated to test polar overdominance gene action in a complete 16-cell mating design (Leymaster and Freking, 1999). Random samples of F_2 ewes were assigned to single-sire mating pens with an F_2 ram of known *callipyge* genotype. Progeny produced from 1999 through 2003 were the result of multisire matings to Suffolk rams. Each breeding season was 35 d in length. In 1996 and 1997, ewes were exposed to rams in early December. In 1998, 2001, and 2002, ewes were exposed during October, whereas in 1999 and 2000, exposures were in August, which was less than optimal because of facility limitations. Variation in reproductive performance caused by seasonality is completely confounded with the interaction between the ewe's year of birth and the age of the ewe.

Each trait was considered a trait of the ewe. Culling of ewes was practiced only for reasons that adversely affected their ability to produce or care for lambs. Primary culling reasons were mastitis and poor health (total of 88% of culls). The weight of each ewe was recorded at the beginning of each breeding season. Conception rate (0, 1) was determined for ewes exposed and still present at the start of each lambing season. The number of lambs born and birth and weaning weights of lambs were recorded. Ewes giving birth to triplets or quadruplets were generally limited to rearing only 2 lambs.

Excess lambs (17% of lambs born) were artificially reared in a nursery. All male lambs were castrated at approximately 14 d of age. All lambs were offered ad libitum access to a total mixed creep ration (2.90 Mcal of ME/kg of DM with 17.5% CP) by approximately 14 d of age. At weaning (56 d for lambs reared by the ewe and 32 d for lambs reared in the nursery), the number of lambs reared by the ewe and in the nursery was tabulated separately. Weaning weight was recorded for each lamb and was adjusted for age to 56 and 32 d for dam- and nursery-reared lambs, respectively. Litter weights of lambs reared naturally and in the nursery were calculated separately for each ewe. Longevity of the ewe was determined as a binary trait based on the presence or absence of the ewe in the breeding flock at 5 yr of age.

Data collected on 265 ewes entering the breeding flock were analyzed for longevity and productivity through 5 yr of age. These ewes generated 937 records for breeding weight and 925 records for conception rate. A total of 717 records was available for traits based on number of ewes lambing.

Genotypic Data

A blood sample was obtained from each ewe, and DNA was extracted following standard protocols (Miller et al., 1988) or using Gentra Generation Capture kits (Gentra Systems Inc., Minneapolis, MN). Genotypes for the *callipyge* (adenine to guanine transition) mutation were determined using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assay described in detail by Freking et al. (2002). For ewes that were heterozygous for the mutation and also derived from heterozygous matings, flanking marker in-

formation was required to determine paternal and maternal origin of alleles. Genotypic data were collected for 6 microsatellite loci (TGLA122, ILSTS054, MCM38, CSSM18, OY3, and OY15), flanking both sides of the causative mutation. Primer pair information, locus map positions, and genotyping procedures have been described in detail elsewhere (Freking et al., 1998a). Grandparental (Dorset or Romanov) phase information was determined at the *callipyge* locus position from CHROMPIC output using CRIMAP version 2.4 software (Washington University School of Medicine, St. Louis, MO). Each ewe was classified into 1 of 4 genotypes ($N^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, or $C^{Mat}C^{Pat}$), where N represents the wild type adenine base and C represents the mutant guanine base; the superscripts indicate maternal (Mat) or paternal (Pat) origin. Romanov alleles were all wild type, and Dorset alleles were either *callipyge* or normal, discriminated by phase-known information from the F_1 sires of the F_2 ewes.

Statistical Analysis

Data were analyzed with the mixed model analysis of variance procedure of SAS (SAS Inst., Inc., Cary, NC). The model for ovulation rate included fixed effects of year (1994 and 1995), *callipyge* genotype ($N^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, and $C^{Mat}C^{Pat}$), and the 2-way interaction. Genotype-specific, second-order polynomials of slaughter age were also fitted. Sire (n = 8) of the ewe was included as a random effect. The Satterthwaite option was used to approximate denominator df associated with the random effect of sire. Gene action was tested by 3 orthogonal, 3-df contrasts to partition the associated 9 df into additive (1, 0, 0, and –1), dominance (–1, 1, 1, and –1), and reciprocal genetic effects (0, 1, –1, and 0).

The model for ewe longevity included fixed effects of ewe year of birth (1996, 1997, and 1998), *callipyge* genotype ($N^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, and $C^{Mat}C^{Pat}$), and the 2-way interaction. Sire (n = 4) of the ewe was fitted as a random effect. Models for ewe productivity traits were as described for longevity with the exception that ewe age (1, 2, 3, 4, and 5 yr) was also fitted as a fixed effect along with all possible 2-way interactions; age was treated as a repeated measure effect of ewe within year of birth and *callipyge* genotype. A compound symmetry structure was assumed for the residual (co)variance matrix. Contrasts to test additive, dominance, and reciprocal effects were performed to partition the 3 df associated with *callipyge* genotypes.

RESULTS

General

Effects of year of birth of ewe, age of ewe, and 2-way interactions among the fixed effects are not presented. Year of birth of the ewe only affected breeding weight and the number of lambs weaned by the ewe ($P < 0.05$). Results for this effect would not be expected to be repeatable because of the limited sample sizes used here. The interaction of age of ewe with *callipyge* genotype rarely approached significance. The age of ewe was highly significant for all traits.

Table 2. Number of corpora lutea by *callipyge* genotype[1]

Callipyge genotype[2]	Number corpora lutea[3]
$N^{Mat}N^{Pat}$	1.98 ± 0.083
$N^{Mat}C^{Pat}$	1.73 ± 0.091
$C^{Mat}N^{Pat}$	1.79 ± 0.107
$C^{Mat}C^{Pat}$	1.82 ± 0.136

[1]Means ± SEM for F_2 ewe lambs slaughtered at 23 to 38 wk of age.
[2]N = wild type allele; C = *callipyge* allele. Superscripts Mat and Pat represent maternal and paternal origin, respectively.
[3]Least squares means adjusted to an average age of 216 d.

Ovulation Rate

Callipyge genotype was associated with a decreased ovulation rate of ewe lambs ($P < 0.05$). Least squares means by genotype adjusted to the average slaughter age of 216 d are presented in Table 2. There was a lack of evidence for differential effects between the reciprocal heterozygous classes (3 df, $F = 1.62$, $P = 0.19$); therefore, traditional sets of contrasts (additive, dominance, and reciprocal) to test gene action were considered appropriate. There was no evidence of an additive effect (3 df, $F = 0.56$, $P = 0.65$), but there was an indication of dominance (3 df, $F = 2.45$, $P = 0.06$). Inspection of the least squares means shows that heterozygous genotypes were not intermediate to the homozygous genotypes but were similar to $C^{Mat}C^{Pat}$. However, any ovum difference among *callipyge* genotypes in ewe lambs did not result in an increased number born at any ewe age (see subsequent data).

Breeding Weight

Callipyge genotype affected weight of ewes at breeding ($P < 0.001$; Table 3). Reciprocal heterozygotes differed (1 df, F = 7.73, $P = 0.006$). Under this situation, a different set of 3 orthogonal contrasts were derived that included paternal polar overdominance as a description of gene action (Freking et al., 1998a). These contrasts of $N^{Mat}N^{Pat}$, $N^{Mat}C^{Pat}$, $C^{Mat}N^{Pat}$, and $C^{Mat}C^{Pat}$ effects defined additive (1, 0, 0, and –1), maternal dominance (–1, 0, 2, and –1), and paternal polar overdominance (–1, 3, –1, and –1) models of gene action, respectively. The additive (1 df, $F = 2.38$, $P = 0.13$) and maternal dominance (1 df, $F = 0.04$, $P = 0.85$) contrasts did not contribute significantly to variation in breeding weight, whereas the effect of paternal polar overdominance was detected (1 df, $F = 12.26$, $P < 0.001$). The $N^{Mat}C^{Pat}$ genotype was 2.5 kg lighter at breeding, on average, than other *callipyge* genotypes.

Table 3. Summary of analyses of variance of traits measured through 5 yr of age by *callipyge* genotype[1]

Trait	No. of observations	Variance component			Least squares means				Average SEM	Level of significance
		Sire of ewe	Ewe age	Residual	$N^{Mat}N^{Pat}$	$N^{Mat}C^{Pat}$	$C^{Mat}N^{Pat}$	$C^{Mat}C^{Pat}$		
Breeding wt., kg	937	1.31	20.30	28.88	58.4	55.2	57.6	57.0	0.94	0.001
Conception rate, %	925	0.13	4.71	11.59	78.0	78.6	78.1	67.8	4.43	0.20
Number born	717	0.01	0.02	0.45	2.01	2.02	2.13	2.08	0.08	0.43
Litter birth wt., kg	717	0.37	0.33	4.45	8.03	7.99	8.13	8.52	0.37	0.39
Ewe-reared lambs, no. weaned	717	0.01	0.01	0.39	1.43	1.35	1.35	1.47	0.08	0.34
Nursery-reared lambs, no. weaned	717	0.01	0.01	0.23	0.27	0.30	0.31	0.30	0.05	0.92
Ewe-reared litter 56-d wt., kg	717	4.79	9.18	81.77	23.18	21.51	20.82	23.76	1.45	0.08
Nursery-reared litter 32-d wt., kg	717	0.22	0.06	22.17	2.74	3.10	3.01	3.09	0.48	0.87
Ewe longevity,[2] %	265	0.67	—	23.03	41.9	46.0	52.3	64.4	7.74	0.13

[1]N = wild type allele; C = *callipyge* allele. Superscripts Mat and Pat represent maternal and paternal origin, respectively.
[2]Percentage of ewes entering the system as ewe lambs that remained alive 30 d after the beginning of the fifth lambing season.

Conception Rate

There was little evidence that the *callipyge* genotype influenced ($P = 0.20$) conception rate (Table 3). Ewes genotyped as $C^{Mat}C^{Pat}$ had the lowest conception rates numerically (10% less than other genotypes). Limited sample size resulting in high standard errors for this trait did not allow us to detect this difference as significant.

Productivity to Weaning

Callipyge genotype did not contribute significantly to variation for measures of ewe productivity to weaning (Table 3). Despite the significant differences among genotypes in ovulation rate of ewe lambs, there were no observed differences among genotypes in number of lambs born per ewe lambing at any ewe age ($P = 0.98$ for interaction of ewe age × genotype). All 4 genotypes averaged just over 2 lambs born per ewe lambing. Because of the nearly equivalent litter sizes at birth, litter birth weights were also similar across all genotypes. No difference was detected in lamb sex ratio, which was nearly 50% for each sex across the ewe genotypes. Maternal ability was evaluated to weaning and revealed no significant effect of genotype on number or weight of lambs weaned. The extra 0.3 nursery lambs per ewe lambing also did not differ by genotype. Growth rate and survival of progeny from ewes of all 4 genotypes were similar, indicating that maternal ability was not influenced by *callipyge* genotype.

Longevity

An evaluation of ewe longevity calculated at 5 yr of age is reported by genotype (Table 3). *Callipyge* genotype did not contribute significantly to variation in the percentage of ewes still present in the breeding flock 30 d after the beginning of the fifth lambing season. Similar to the conception rate information, small sample sizes and large standard errors for the specific trait prevented detection of genotypic effects. However, inspection of the least squares means indicated that the lowest percentage of ewes maintained in the flock was from the $N^{Mat}N^{Pat}$ ewes (41.9%) and the highest percentage was from those genotyped as $C^{Mat}C^{Pat}$ (64.4%).

DISCUSSION

Before the current study, no experimental data have reported effects of the *callipyge* mutation on reproductive traits. Because of its unique gene action (paternal polar overdominance), implementation of a mating system that maximizes production of lambs with *callipyge* muscle hypertrophy would require the use of homozygous *callipyge* rams as terminal sires. Reproductive performance and replacement rates associated with maintaining a ewe flock of this genotype can have an impact on the costs of production of terminal sires. Extreme leanness may influence reproduction, as adipose tissue can convert androgens to estrogen and it is also used to store steroid hormones (Frisch, 1987). Because mutation in the homozygous state does not alter carcass composition (Freking et al., 1998b), there is less concern of this specific biological antagonism when implemented as part of a terminal sire mating system. Once the appropriate terminal sire population has been created using marker-assisted introgression, there would be no impact of carcass leanness on reproduction attributable to the *callipyge* locus. Additionally, the abnormal postnatal maintenance of DLK1 transcripts to higher prenatal levels has only been observed in fast-twitch muscle fibers of the $N^{Mat}C^{Pat}$ genotype and is not considered a global condition for all tissues (Murphy et al., 2005). Increased expression of DLK1 (70-fold over controls) has been identified from microarray analysis of human uterine fibroids, or leiomyomata, which are primarily benign, myometrium cell tumors found in up to 70% of reproductive-age women (Tsibris et al., 2002). Concern about reproductive failure in sheep because of uterine myometrial tumors, which exhibit abnormally high levels of DLK1 in the tissue myometrium would be reduced if considering only a flock of $C^{Mat}C^{Pat}$ ewes.

Differences were detected among genotypes for ovulation rate of ewe lambs at slaughter. Gene action was estimated to be complete dominance for the C allele; however, a 0.25 ovum difference of the $N^{Mat}C^{Pat}$ compared with the $N^{Mat}N^{Pat}$ genotype at the early age evaluated is not likely to be biologically important. In $N^{Mat}C^{Pat}$ ewes, dietary protein is preferentially converted to carcass lean to the detriment of other tissues such as fat, wool, and internal organ growth. During the prepubertal growing phase, dietary inputs are preferentially allocated toward muscle growth, and the resultant lower degree of fatness associated with the $N^{Mat}C^{Pat}$ genotype could delay onset of puberty. Data for ovulation rate in this study were not standardized to a common estrous cycle because of the serial slaughter design. A reasonable explanation of the lower ovulation rate of the $N^{Mat}C^{Pat}$ genotype would be that this group had gone through fewer estrous cycles than other *callipyge* genotypes at the average age of 216 d. Alternatively, another locus on chromosome 18 near *callipyge* might have an effect on ovulation rate, where Romanov alleles exceed the Dorset alleles for ovum shed.

Live weight of the $N^{Mat}C^{Pat}$ ewes at breeding was less than other genotypes. Although significant growth rate differences among genotypes during the finishing stages have not been reported, this result is consistent with previous research that identified lower live weight at slaughter age end points. This weight difference was primarily attributed to lower pelt weight, less kidney fat, and lighter liver and intestinal organ weights for the $N^{Mat}C^{Pat}$ genotype (Koohmaraie et al., 1995; Jackson et al., 1997a; Freking et al., 1998b). Consistent with lighter weights of internal organs, daily feed intake was reduced in this genotype compared with normal lambs (Jackson et al., 1997a). Lower body weights and feed intakes, thus lower maintenance costs, would be considered beneficial in the female breeding population. However, extreme carcass leanness to the detriment of reproductive fitness can have dramatic negative impact on economic returns to a production system. Differences in breeding weights approached 2.5 kg, but this lower BW did not have an impact on subsequent fertility or productivity. As indicated previously, the relevant issue for a terminal sire population would be performance of $C^{Mat}C^{Pat}$ ewes, which did not differ from $N^{Mat}N^{Pat}$.

Callipyge genotype did not influence component traits of overall reproductive performance. Conception rate, number born, and maternal ability were similar for all genotypes. Growth rate and survival of progeny were also similar regardless of genotype of the dam. One additional component trait to overall flock productivity is ewe longevity. Replacement rates within a ewe flock are a cost associated with maintaining a production system. Performance of young ewes is typically less than that of mature ewes (3 to 5 yr of age); therefore, the higher the percentage of replacement ewes required to maintain a flock, the greater the costs of production. In this experiment, ewes were allowed an opportunity to lamb annually for 5 consecutive yr. Although differences among genotypes in percentage of ewes maintained after the 5-yr evaluation period seemed substantial (ranging from 64.4 to 41.9%), the large standard errors for this trait did not allow us to detect genotype as a significant source of variation. Longevity was at least as high for $C^{Mat}C^{Pat}$ ewes as for any other genotype. Despite dramatic differences in expression of DLK1 in muscle fibers associated with the *callipyge* mutation, there appeared to be no correlated long-term impact on health status or other measures of reproductive ability or fitness.

The current experiment indicated limited or no effects on component traits of reproduction and fitness. However, the documented adverse effects on meat tenderness still require an intervention strategy to ensure a favorable eating experience by consumers of loin chops (Koohmaraie et al., 1995; Freking et al., 1999). A genetic solution or an easily adaptable postmortem intervention strategy (Duckett et al., 1998; Koohmaraie et al., 1998; Solomon et al., 1998) that solves the tenderness antagonism could have great impact on lean meat production in sheep. Experiments are underway at the US Meat Animal Research Center (Clay Center, NE) to investigate genetic solutions for the tenderness antagonism that would facilitate incorporation of *callipyge* into terminal sire production systems.

IMPLICATIONS

An investigation of effects of the *callipyge* mutation on major components of life cycle productivity has been completed. The performance of ewes to 5 yr of age, representing each of the 4 *callipyge* genotypes, provided data to compare aspects of ewe productivity. In general, there was a lack of evidence to conclude that there was any biologically important influence of this mutation on reproduction or fitness. It was concluded that maintaining a ewe flock that was fixed for the mutation to produce rams for a terminal sire mating system would present no unusual problems from a reproduction standpoint. Industry adaptation of the *callipyge* phenotype is limited only by the antagonism with longissimus muscle tenderness.

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