Polar Overdominance at the Ovine *callipyge* Locus

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An inheritable muscular hypertrophy was recently described in sheep and shown to be determined by the *callipyge* gene mapped to ovine chromosome 18. Here, the callipyge phenotype was found to be characterized by a nonmendelian inheritance pattern, referred to as polar overdominance, where only heterozygous individuals having inherited the *callipyge* mutation from their sire express the phenotype. The possible role of parental imprinting in the determinism of polar overdominance is envisaged.

In 1983, a ram with muscular hypertrophy (Fig. 1) was reported in a flock of Dorset sheep. This phenotype was transmitted to some of the offspring, and subsequent matings of hypermuscled male descendants of the founder ram and normal ewes demonstrated a 50%-50%, sex-independent segregation of the trait. It was postulated that a dominant mutation (*CLPG* instead of the normal *clpg* allele) at the autosomal *callipyge* locus was responsible for this hypertrophy. The *callipyge* locus was subsequently mapped to the distal part of ovine chromosome 18 (*1*).

For further characterization of the *callipyge* syndrome, matings were performed between either normal rams (*clpg/clpg*, unrelated to the founder sire) or callipygous male descendants of the founder sire (*CLPG/clpg*), and callipygous ewes descendant of the same founder ram (*CLPG/clpg*). A nonmendelian segregation pattern of the callipyge phenotype (*2*) was evident from these crosses.

All 35 offspring from the first cross (*clpg/clpg* ♂ × *CLPG/clpg* ♀) were normal; none had muscular hypertrophy typical of the callipyge phenotype ($\chi^2_1 = 35$, $P < 0.0001$). Analysis of microsatellite markers (*3*) spanning chromosome 18, however, demonstrated the expected 50%-50% mendelian segregation of the corresponding maternal chromosome segment in these pedigrees (Table 1, mating A). Therefore, the data clearly demonstrated the nonequivalence of reciprocal crosses, because *CLPG/clpg* ♂ × *clpg/clpg* ♀ matings gave a 50%-50% sex-independent segregation ratio of the callipyge and normal phenotypes, whereas *clpg/clpg* ♂ × *CLPG/clpg* ♀ matings yielded normal offspring exclusively. The callipyge phenotype of $CLPG^{Pat}/clpg^{Mat}$ animals compared with the normal phenotype shown by $clpg^{Pat}/CLPG^{Mat}$ individuals reveals the "polar" nature of the *CLPG* mutation, that is, the influence of parental origin on its phenotypic effect. (The superscripts Pat and Mat, respectively, indicate the paternal and maternal origin of the alleles at the *callipyge* locus.)

The second type of cross, namely, matings between heterozygous callipygous rams and ewes (*CLPG/clpg*), yielded 51 offspring. Fifteen (29%) of these were phenotyped as callipygous and 36 (71%) as normal. Obviously, these numbers differ significantly ($\chi^2_1 = 56.5$, $P < 0.0001$) from the expected 75% callipygous versus 25% normal proportions expected for the segregation of a dominant mutation in an F_2 generation. However, analysis of the chromosome 18 microsatellite genotypes of these offspring revealed a clear pattern (Table 1, mating B), unlikely to have occurred by chance alone ($\chi^2_3 = 31.97$, $P < 0.0001$). All but two individuals with genotype $CLPG^{Pat}/clpg^{Mat}$, $clpg^{Pat}/CLPG^{Mat}$, or $clpg^{Pat}/clpg^{Mat}$ exhibited the expected phenotype as deduced from previous matings, that is, callipygous, normal, and normal, respectively. In addition, all seven inferred $CLPG^{Pat}/CLPG^{Mat}$ offspring were normal in appearance, showing no signs of muscular hypertrophy. The normal phenotype of $CLPG^{Pat}/CLPG^{Mat}$ animals indicates that the "inactivation" of the $CLPG^{Mat}$ allele dominates the "activation" of the $CLPG^{Pat}$ allele. Therefore, the *callipyge* locus is characterized by a type of overdominance, where only heterozygous individuals having inherited the *CLPG* mutation from their sire express the phenotype.

To more fully exploit the available data, we performed a multipoint linkage analysis under the hypothesis of polar overdominance at the *callipyge* locus with all 51 offspring generated from $CLPG^{Pat}/clpg^{Mat}$ × $CLPG^{Pat}/clpg^{Mat}$ matings. The lod score curve obtained is shown in Fig. 2B and compared with the most recent mapping data as obtained from $CLPG^{Pat}/clpg^{Mat}$ ♂ × $clpg^{Pat}/clpg^{Mat}$ ♀ matings (Fig. 2A). (The lod score is the logarithm of the odds ratio for linkage.) The most likely positions of the *callipyge* gene, with associated lod scores of 9.52 and 55.61, respectively, are in good agreement, validating our hypothesis of polar overdominance.

The reversible nature of the polarity at the *callipyge* locus was further examined by generating offspring from phenotypically normal rams, carrying either (i) one ($clpg^{Pat}/CLPG^{Mat}$) or (ii) two ($CLPG^{Pat}/CLPG^{Mat}$) copies of the *CLPG* mutation, mated to normal ewes (*clpg/clpg*, unrelated to the founder sire). Twenty-three lambs were obtained from the first type of mating involv-

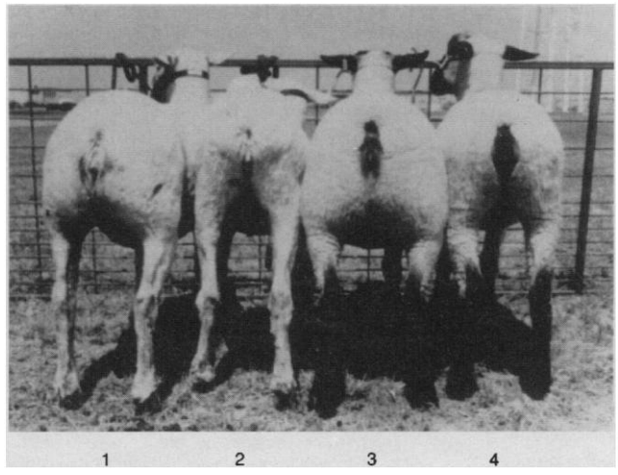

Fig. 1. The callipygous (animals 1 and 3) compared with the normal (animals 2 and 4) phenotype.

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ing five different carrier $clpg^{Pat}/CLPG^{Mat}$ rams. (Four of these carrier rams resulted from *clpg/clpg* ♂ × $CLPG^{Pat}/clpg^{Mat}$ ♀ crosses, and one from a mating between a $CLPG^{Pat}/clpg^{Mat}$ ram and ewe.) Thirteen (56.5%) of these lambs were classified as callipyge and 10 (43.5%) as normal, pointing toward reactivation of the *CLPG* mutation after passage through the male germ line. Moreover, genotyping these offspring confirmed linkage between the chromosome 18 markers and the callipyge phenotype in these crosses. The association between the segregation of the chromosome 18 microsatellite markers and the callipyge phenotype ($\chi^2_3 = 10.21$, $P < 0.025$) is evident from Table 1, mating C.

It is noteworthy, however, that the proportion of "recombinant" individuals (17.4%) seems considerably higher in these matings compared with previous estimates. Intriguingly, three of the four observed inconsistencies occur within one pedigree. This is illustrated as well by the apparent heterogeneity of the two-point lod scores obtained between the most informative, tightly linked marker, CSSM18, and the *callipyge* locus (*4*). Whereas the association between the chromosome 18 markers and the callipyge phenotype is clear in three of the five pedigrees that show no recombination between CSSM18 and *callipyge*, the association seems to be broken in the other two. Applying Morton's test for lod score heterogeneity (*5*) yields a χ^2 value of 8 for four degrees of freedom, corresponding to $P < 0.1$, suggestive of admixture of heterogeneous families. This would also explain why in a multipoint analysis we obtained the highest lod score (2.23) when allowing for 10% "misclassification" in both phenotypic classes (Fig. 2C). The nature and significance of this linkage heterogeneity needs to be further scrutinized.

Matings between two $CLPG^{Pat}/CLPG^{Mat}$ rams and *clpg/clpg* ewes yielded 33 lambs, of which 30 (91%) were classified as callipyge and 3 as normal (Table 1, mating D). It should be noted that the grandparental origin of the *CLPG* mutation could not be determined in these matings, because both $CLPG^{Pat}/CLPG^{Mat}$ rams were homozygous for the markers closely linked to the *callipyge* locus. In the absence of any evidence for segregation distortion of the corresponding chromosome segment in other crosses, these data strongly suggest reactivation of the paternal $CLPG^{Mat}$ alleles in these matings as well. However, the generation of three normal offspring from these crosses suggests that the reversibility of the *callipyge* polarity is not absolute.

The observation of a parent-of-origin–dependent, heterozygote-specific phenotype is in some ways reminiscent of *P* element–mediated hybrid dysgenesis in *Drosophila* (*6*) and the mouse "polar lethality" syndrome in DDK ♀ × "alien" (non-DDK) ♂ matings (*7*). It is well established that the parent-of-origin effect observed in the case of hybrid dysgenesis is due to a *P* element–encoded repressor of transposition, present or absent in the ooplasm of P or M strains, respectively. Initially, a conceptually related but single-locus model (containing two tightly linked genes, *Om* and S) was proposed to explain polar lethality, on the basis of the incompatibility between a hypothetical DDK-specific oocyte factor and an "alien"-specific spermatozoa factor (*7*). Subsequent studies confirmed the single-locus prediction of the model, positioning the *Om* gene on mouse chromosome 11 (*8*). The postulated oocyte and spermatozoa factors, however, remain hypothetical.

Contrary to hybrid dysgenesis and polar lethality, a zygotic event as the cause of polarity of the callipyge segregation pattern seems unlikely given the tissue specificity of the callipyge muscular hyperplasia. A dominant negative mutation resulting in functional homomultimers but defective heteromultimers, or some form of interallelic complementation, could account for the observed heterozygote-specific phenotype but not for its polar character.

Sapienza *et al.* (*8*), in particular, proposed that parental imprinting at the *Om* (ovum mutant) locus might explain the observed mode of inheritance. The same proposal could be made for the *callipyge* locus in sheep. The fact that animals homozygous for the *Om* or *CLPG* mutation are not expressing the lethality or muscular hypertrophy, respectively, appears to be in

Table 1. Offspring from conventional $clpg^{Pat}/clpg^{Mat}$ rams and callipygous $CLPG^{Pat}/clpg^{Mat}$ ewes (mating A), callipygous $CLPG^{Pat}/clpg^{Mat}$ rams and ewes (mating B), phenotypically normal $clpg^{Pat}/CLPG^{Mat}$ rams and conventional $clpg^{Pat}/clpg^{Mat}$ ewes (mating C), and phenotypically normal $CLPG^{Pat}/CLPG^{Mat}$ rams and conventional $clpg^{Pat}/clpg^{Mat}$ ewes (mating D), sorted by phenotype and inferred genotype at the *callipyge* locus given linked marker genotypes. The genotype at the *callipyge* locus was predicted from the genotype at the two microsatellite markers that are the most closely linked to *callipyge* (CSSM18 and IDVGA30), assuming no recombination between the *callipyge* locus and these markers. For some offspring, additional information from flanking markers was used, assuming no double recombination in the interval GMBT16–TGLA122. All the offspring were not informative for the segregation of the *callipyge* locus with these criteria, which explains the difference between the number of offspring reported in this table compared with the total number of offspring produced for the different types of crosses.

Inferred genotype	Offspring of phenotype	
	Callipygous	Normal
Mating A: $clpg^{Pat}/clpg^{Mat}$ ♂ × $CLPG^{Pat}/clpg^{Mat}$ ♀		
$clpg^{Pat}/CLPG^{Mat}$	0	12
$clpg^{Pat}/clpg^{Mat}$	0	9
Mating B: $CLPG^{Pat}/clpg^{Mat}$ ♂ × $CLPG^{Pat}/clpg^{Mat}$ ♀		
$CLPG^{Pat}/CLPG^{Mat}$	0	7
$CLPG^{Pat}/clpg^{Mat}$	11	1
$clpg^{Pat}/CLPG^{Mat}$	0	11
$clpg^{Pat}/clpg^{Mat}$	1	9
Mating C: $clpg^{Pat}/CLPG^{Mat}$ ♂ × $clpg^{Pat}/clpg^{Mat}$ ♀		
$CLPG^{Pat}/clpg^{Mat}$	10	1
$clpg^{Pat}/clpg^{Mat}$	3	9
Mating D: $CLPG^{Pat}/CLPG^{Mat}$ ♂ × $clpg^{Pat}/clpg^{Mat}$ ♀		
$CLPG^{Pat}/clpg^{Mat}$	30	3

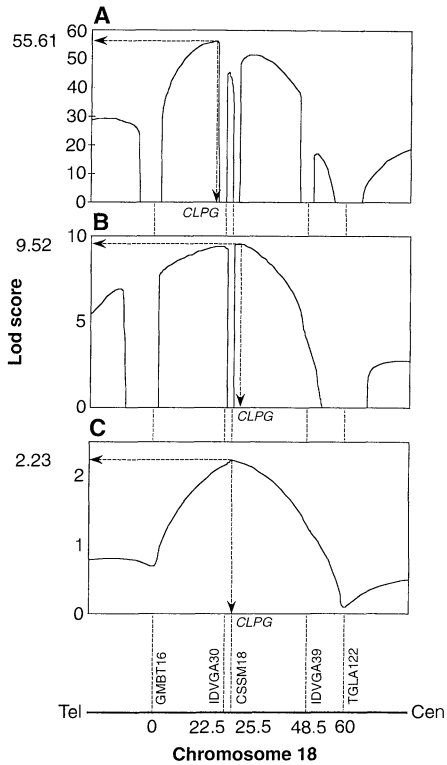

Fig. 2. Multipoint lod score curves obtained from (**A**) $CLPG^{Pat}/clpg^{Mat}$ ♂ × *clpg/clpg* ♀ matings, (**B**) $CLPG^{Pat}/clpg^{Mat}$ ♂ × $CLPG^{Pat}/clpg^{Mat}$ ♀ matings, and (**C**) $clpg^{Pat}/CLPG^{Mat}$ ♂ × *clpg/clpg* ♀ matings. The marker map for the distal end of ovine chromosome 18 was constructed from the matings of (A) with the ANIMAP programs (*15*). The lod score curves in (A) and (C) were obtained with the LINKMAP option of the FASTLINK programs (*16*), whereas the lod score curve in (B) was obtained with customized programs accounting for the polar overdominance hypothesis in F_2 crosses (*17*). As much familial information as possible was included to infer the proper marker allele phase in the parents; only the offspring, however, contributed information for the segregation of the *callipyge* locus, that is, the lod score values were uniformly zero when all offspring phenotypes were considered as unknown. Complete penetrance was assumed in (A) and (B), but 10% misclassification was allowed in (C). Tel, telomere; Cen, centromere.

conflict with the general rule observed so far that parental imprinting results in transcriptional silencing of one of the parent-of-origin–specific alleles (9). A number of molecular models that assume parental imprinting can, however, be considered to fit the observed segregation pattern. One of these, also proposed by Sapienza *et al.* (8) to explain the DDK syndrome, postulates a mutation (*CLPG*) that would switch the parent-of-origin–specific expression pattern from male to female or vice versa. Indeed, if one assumes that the *clpg* allele is paternally expressed, whereas the *CLPG* allele is maternally expressed, only $CLPG^{Pat}/clpg^{Mat}$ individuals would not express the gene, thus explaining their unique phenotype. An alternative hypothesis corresponding to the existence of two tightly linked genes (*A* and *B*), one of these (*A*) being paternally expressed and coding for a trans-acting suppressor of the other one (*B*), would explain the data as well, with the product of the *B* gene causing the callipyge phenotype. If the assumption is made that the *CLPG* mutation would be a deletion involving both genes, only $CLPG^{Pat}/clpg^{Mat}$ individuals would generate the product of gene B and therefore express the muscular hypertrophy.

It is noteworthy that the regions homologous to the distal part of ovine chromosome 18 correspond to the distal part of mouse chromosome 12 and the distal part of human chromosome 14. Evidence for parental imprinting has been presented for the corresponding chromosomal regions in both organisms: in mice, both maternal and paternal duplications of the region cause early embryonic lethality (*10*), whereas in humans, uniparental disomy of chromosome 14 has been associated with mental retardation and multiple congenital anomalies (*11*). More refined comparative mapping, however, is needed to establish the potential relevance of this observation.

Whereas the polar overdominance model explains the majority of our observations, some of the inconsistencies between the phenotype and callipyge genotype as inferred from marker data remain puzzling. This is particularly the case for the relaxation of the linkage association with chromosome 18 markers observed after reactivation of the $CLPG^{Mat}$ to $CLPG^{Pat}$ mutation. Although we cannot exclude the possibility that this observation is a result of trivial phenotypic misclassification, this hypothesis is hardly convincing because a recombination rate as low as 6% was found with the closest microsatellite marker for more than 600 offspring issued from $CLPG^{Pat}/clpg^{Mat}$ ♂ × $clpg^{Pat}/clpg^{Mat}$ ♀ matings [(*1*) and Fig. 2], putting an upper limit of 6% misclassification in these crosses. Likewise, such a high level of genotypic misclassification as a result of undetected recombinations between the closest markers (CSSM18 and IDVGA30) or double recombinants in the GMBT16–TGLA122 interval seem unlikely (see the legend to Table 1).

If one assumes that parental imprinting occurs at the *callipyge* locus, the four individuals with normal phenotype, although having inherited the *CLPG* mutation from their $clpg^{Pat}/CLPG^{Mat}$ or $CLPG^{Pat}/CLPG^{Mat}$ sire, might be the result of incomplete erasure of the grand-maternal imprint. It would be of interest to determine the grandparental origin of the *CLPG* mutation for the three phenotypically normal offspring of the $CLPG^{Pat}/CLPG^{Mat}$ sires, predicted to be grand-maternal under the hypothesis of incomplete imprint erasure. As previously mentioned, this cannot yet be done with the available markers. The capacity to erase the maternal imprint could itself be under the genetic control of modifier "imprintor" loci either in the sire (*12*) or transmitted by the ewes (*13*).

The occurrence of four offspring with an inferred *clpg/clpg* genotype but callipygous phenotype is more difficult to understand. Because the segregation of the *callipyge* locus accounted for virtually all trait variance in the $CLPG^{Pat}/clpg^{Mat}$ ♂ × $clpg^{Pat}/clpg^{Mat}$ ♀ matings (*1*), a two-locus model is difficult to fit to the data. One could postulate either a transposition of the *callipyge* locus in some $clpg^{Pat}/CLPG^{Mat}$ sires or the conversion of the paternal $clpg^{Pat}$ allele by its $CLPG^{Mat}$ homolog, possibly by a trans-sensing effect (*14*). The latter two hypotheses can be tested and are under scrutiny.

In addition to the fundamental interest in demonstrating such a nonmendelian segregation pattern, this finding illustrates the importance in agricultural genetics of dissecting production traits into their individual components. Most production traits are classically considered as the manifestation of a variable number of genes acting primarily in an additive mode, and breeding strategies are most often based on these assumptions. Obviously, conventional selection programs could not deal appropriately with genes exhibiting polar overdominance. For instance, it would be impossible to fix the callipyge phenotype by selecting hypertrophied parents in subsequent generations. From the point of view of population genetics, polar overdominance generates balanced polymorphism at the corresponding loci. On the basis of our model, however, we could predict that nonexpressing $CLPG^{Pat}/CLPG^{Mat}$ males mated to $clpg^{Pat}/clpg^{Mat}$ females might produce 100% callipyge offspring. This prediction has essentially been confirmed in at least two $CLPG^{Pat}/CLPG^{Mat}$ ♂ × $clpg^{Pat}/clpg^{Mat}$ ♀ matings yielding 91% callipygous offspring (Table 1, mating D).

Finally, the polar overdominance model might help to explain complex inheritance patterns observed in other organisms including humans. Linkage analysis performed under the polar overdominance model might help to uncover previously undetected causative loci.

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2. Sheared animals were phenotyped as callipygous or normal after repeated visual examination by two experienced observers, each dealing with one flock (N.E.C. and S.P.J.). All animals were appraised every 2 weeks from the animal's birth until 5 months of age. Animals exhibiting an ambiguous phenotype at 5 months were dropped from further analysis. Phenotypic classification was performed before genotyping, because most animals were slaughtered by the time genotypes became available.
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