FURTHER EVIDENCE OF NON-MENDELIAN INHERITANCE AT THE *FECX2* LOCUS IN A PROLIFIC SHEEP FLOCK

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INTRODUCTION

Intensive screening for prolificacy among recorded sheep flocks throughout NZ, which commenced in 1979, led to the establishment of flocks of highly prolific Coopworth, Romney and Perendale breeds. From 1981, these flocks (hereafter called the screened flocks) were managed at the Woodlands Research Station in Southland, NZ. The 212 foundation ewes in the screened flocks were selected on the basis of high litter size (Davis *et al*., 1987) and the 85 foundation rams were selected from similar high prolificacy backgrounds. Subsequent selection within each screened flock was on the basis of ovulation rate. The maternally imprinted *Woodlands* gene (*FecX2*), which in heterozygous ewes increases ovulation rate by about 0.4, was discovered in the Coopworth flock by Davis *et al*. (2001a). The origin of *FecX2* in the Coopworth screened flock traced back to a ram from the Lincoln University Coopworth flock where the breed originated. This paper reports the discovery of a second line of Coopworth sheep in the screened flock showing the same pattern of inheritance of prolificacy.

MATERIALS AND METHODS

Selection following screening was based on breeding values (BV) for ovulation rate, which were calculated using an animal model with heritability of 0.2 and repeatability of 0.3. Ovulation rates were measured by laparoscopy twice at 1.5 years of age and once or twice in each successive year. All females were retained until laparoscoped twice at 1.5 years of age and those with the highest BVs for ovulation rate were selected as flock replacements. The progeny ovulation rate records analysed were those measured at 1.5 years of age before any females were culled, but the BVs, which excluded male progeny and had been calculated on the basis of multigenic inheritance of autosomal genes, included all records of ovulation rate. Litter size was recorded at the subsequent lambing. Contemporary Coopworth ewes in the screened flock not carrying the *Woodlands* gene were compared as controls.

In 1989, a Coopworth ram (#87-226) that was the maternal grandson of prolific foundation ewe #75-1367 from the flock of Mr J.A. Metherell was used in the screened flock. Ewe #75-1367 was a very small framed Coopworth ewe weighing about 45 kg, but in her four lambings she produced she produced a set of twins and three sets of triplets. In 1991, daughters of ram #87-226 were observed to have high ovulation rates, and in 1993 a progeny test programme commenced with male descendants of ewe #75-1367 (the *Metherell* line). Within the screened flock, eight rams from the *Metherell* line produced 192 daughters from 1989 to 1996.

Progeny testing (PT) of 24 *Metherell* line rams, including seven sons of a putative carrier ram, was carried out in commercial flocks where each ram was single-sire mated to unrelated ewes.

The 744 daughters were retained until 1.5 years of age when ovulation rate was measured twice with an interval of 18-21 days between measurements.

Rams were assigned to one of three categories depending on their relationship to a putative carrier male ancestor. Category 0 were the sons of carrier rams (0 females in the line between the ram and his carrier ancestor), category 1 were the maternal grandsons of a carrier ram (1 female in the line between the ram and his carrier ancestor) and category 2 were no closer than maternal great grandsons of a carrier ram (at least 2 females, and no males, in the line between the ram and his carrier ancestor). Four of the *Metherell* line rams used early in the breeding programme in the screened flock were maternal grandsons (2) or maternal great grandsons (2) of foundation ewe #75-1367 and these were also designated as category 2 sires.

Ewes born in the screened flock from 1985 onwards, and not descendants of *Woodlands* sires, were classified into one of four classes: daughter of Metherell ram, maternal granddaughter of Metherell ram, maternal great granddaughter of Metherell ram, or a control ewe. Ovulation rates at 1.5 years of age were analysed in a mixed model that included ewe as a random effect, and year, measurement time within year, and the genotype class as fixed effects. Heterogeneous variances, according to genotype class as inferred from phenotype, were allowed for the ewe (σ^2_u) and residual effects.

RESULTS

On the basis of their BV, the eight rams in the screened flock fitted into two groups with one group of three rams having BVs ranging from +0.22 to +0.32 (carriers) and the other group of five rams with BVs from –0.40 to +0.07 (non-carriers). There was a mean of 24.0 (SE = 2.9) female progeny per *Metherell* sire.

The 24 PT *Metherell* rams had a mean of 31.0 (SE = 1.6) progeny per sire. Two of these rams had daughters with mean ovulation rates that were 0.22 and 0.29 higher than contemporaries, and were designated carriers. The other 22 had daughters with mean ovulation rates ranging from -0.30 to +0.12 compared with contemporaries, and were classified as non-carriers.

From the screened flock and the PT there were a total of nine *Metherell* category 0 rams and 12 *Metherell* category 1 rams, none of which had daughters with increased ovulation rates (Table 1). However, there were 11 category 2 rams from the screened flock and the PT, of which five (45%) were classified as *Metherell* carriers on the basis of their BV or ovulation rate deviation.

The 61 1.5-year-old daughters of the category 2 carrier rams in the screened flock had a mean ovulation rate of 2.46 (SEM = 0.08; σ_u = 0.58). The 49 maternal granddaughters and 39 maternal great granddaughters of the category 2 carrier rams had mean ovulation rates of 2.19 (SEM = 0.06; σ_u = 0.47) and 2.24 (SEM = 0.07; σ_u = 0.52) respectively. Contemporary control Coopworth ewes had a mean ovulation rate of 2.17 (SEM = 0.02; σ_u = 0.49). Mean litter size in daughters of category 2 rams was 0.18 (SED = 0.10) higher than in contemporary control Coopworth ewes, whereas maternal granddaughters and maternal great granddaughters had mean litter sizes that were 0.03 (SED = 0.09) higher and 0.05 (SED = 0.11) lower than contemporary control respectively.

Table 1. Incidence of carrier sires according to their relationship to a carrier sire ancestor in *Metherell* and *Woodlands* lines (category 0 = son; category 1 = maternal grandson; category 2 = maternal great grandson).

Group	Category 0	Category 1	Category 2
Metherell line sires	0/9	0/12	5/11
Woodlands line sires[A]	0/3	0/24	7/17

[A] From Davis *et al*., (2001a)

Records from one female progeny of a *Metherell* carrier sire x *Metherell* carrier dam, nine female progeny of a *Metherell* carrier sire x *Woodlands* carrier dam, and eight female progeny of a *Woodlands* carrier sire x *Metherell* carrier dam showed that all had fully functional ovaries.

DISCUSSION

Results from the nine category 0 sires showing no increase in the ovulation rate of their progeny is suggestive of a single gene or closely linked group of genes on the X-chromosome, which is also a feature of the inheritance of the *Woodlands* gene described by Davis *et al.* (2001a).

The increased mean ovulation rate coupled with only small changes in the standard deviation of ovulation rate among daughters of a carrier ram suggests that there was no genetic segregation for the putative gene among these females. A similar effect was found among sheep carrying the *Woodlands* gene (Davis *et al*., 2001a) and this is also characteristic of a prolificacy gene on the X chromosome as observed for the *Inverdale* gene by Davis *et al*. (1991).

The finding (Table 1) that none of the 12 category 1 sires had daughters with increased ovulation rates is also consistent with the inheritance pattern of the *Woodlands* gene. This is indicative of an imprinting effect whereby sons inheriting the gene from a dam in which the gene is expressed, do not have daughters expressing the gene.

The classification of 5 of the 11 category 2 sires as *Metherell* carriers (Table 1) shows that where a ewe has maternally inherited the gene she passes it to about half of her sons. With an X-linked gene, all daughters of a carrier sire would be expected to be carriers, and subsequently on average 50% of their daughters and 25% of their grandsons.

The mean ovulation rate of the daughters of the category 2 carrier rams was 0.27 higher than the maternal granddaughters. A difference of +0.39 had been previously detected between ewes expressing the *Woodlands* gene and those in which the gene is silenced (Davis *et al*., 2001a). There were only small differences in mean ovulation rate between the maternal granddaughters, maternal great granddaughters and control flock, which supports the hypothesis of a maternally imprinted gene with the same inheritance as the *Woodlands* gene. The litter size difference between the different groups reflected the differences in ovulation rate and showed that ewes expressing the gene produce about 0.2 more lambs than non-expresser and control ewes.

Ewes homozygous for Inverdale ($FecX^I/FecX^I$) or Hanna ($FecX^H/FecX^H$), as well as those with one copy of both these alleles ($FecX^I/FecX^H$) have non-functional streak ovaries and are infertile (Davis *et al*., 2001b). It is known that ewes that are homozygous carriers of the *Woodlands* gene have fully functional ovaries (Davis *et al*., 2001a), and the results from the 17 daughters of *Metherell* x *Woodlands* parents showed that these also had fully functional ovaries.

As with many Coopworth flocks in New Zealand, it is known that rams purchased from Lincoln University were used in the Mr Metherell's flock during the 1970s. There is therefore a possible link indicating that *Woodlands* and *Metherell* may be exactly the same mutation. However, it is unknown whether any of the Lincoln University rams used in Mr Metherell's flock carried the *Woodlands* gene or indeed, whether ewe #75-1367 was sired by one of these rams. The recent discovery that although *Inverdale* ($FecX^I$) and *Hanna* ($FecX^H$) had the same inheritance pattern and produced the same phenotype (Davis *et al*., 2001b), they are separate mutations (alleles) of the same gene (Galloway *et al*., 2000), has prompted us to retain the *Metherell* and *Woodlands* sheep as separate lines until the mutation(s) can be identified. The *Woodlands* gene has been assigned the locus symbol *FecX2* and allele symbol $FecX2^W$ (Davis *et al*., 2001a). Because the *Metherell* family line appears to have the same inheritance pattern and similar effect to the *Woodlands* gene we have assigned it the same locus symbol but have chosen the symbol $FecX2^M$ for the putative *Metherell* allele.

CONCLUSION

The ovulation rate records of 936 female progeny of 32 rams descended from a prolific Coopworth ewe in a screened high prolificacy flock indicate an allele (*Metherell*) for prolificacy with the same inheritance pattern as the *Woodlands* allele. The effect of the *Metherell* allele is to increase ovulation rate by 0.2 - 0.3. This is further evidence of a maternally imprinted X-linked gene in Coopworth sheep, which also has a second imprinting effect whereby in order for ewes to express the gene their sire must inherit the gene from a dam in which the gene is silent. Further work is required to determine whether the *Metherell* and *Woodlands* alleles are different mutations.

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