BIOLOGY OF REPRODUCTION **64,** 216–221 (2001)

Evidence That an Imprinted Gene on the X Chromosome Increases Ovulation Rate in Sheep

George H. Davis,[1,2] Ken G. Dodds,[2] Roger Wheeler,[3] and Nigel P. Jay[4]

Invermay Agricultural Centre,[2] Mosgiel, New Zealand
Woodlands Research Station,[3] Invercargill, New Zealand
Animal and Food Sciences Division,[4] Lincoln University, Canterbury, New Zealand

ABSTRACT

Ovulation rate records from 1311 female progeny of 50 Coopworth rams were used to study the inheritance of ovulation rate in a screened high prolificacy sheep flock. Breeding values (BV) for ovulation rate for 33 sires used within the screened flock and ovulation rate deviations for a further 17 sires progeny tested in commercial flocks suggest that a major gene (*Woodlands* gene) for ovulation rate with a non-Mendelian inheritance pattern is segregating in a family line. Rams assigned as carriers of the putative gene did not produce carrier sons (zero of three), and this coupled with the observation that daughters of carrier rams had ovulation rates of 0.39 (standard error of difference [SED] = 0.06) higher than contemporaries without a significant increase in the variance of log ovulation rate strongly suggests that the gene is on the X chromosome. The evidence suggests that the gene is also maternally imprinted because ovulation rate data indicate that it is expressed where females inherit a paternal allele but is silenced when inherited on a maternal allele. Maternal granddaughters of carrier rams had mean ovulation rates that were only 0.02 (SED = 0.06) higher than noncarrier ewes from the same flock. Furthermore, carrier dams expressing the gene (paternal allele) had 24 sons, none of which had female offspring that expressed the gene, whereas carrier dams not expressing the gene (maternal allele) had 7 out of 17 sons that had female progeny expressing the gene. There is no evidence of the infertility that occurs in homozygous ewes carrying the X-linked Inverdale gene. Collectively, these results suggest the existence of a novel gene for prolificacy located on the X chromosome that is maternally imprinted. The *Woodlands* gene was only expressed upon paternal inheritance from carrier males that were the progeny of nonexpressing carrier dams. The gene was not expressed in ewes that received it from either carrier dams (expressing or nonexpressing) or from carrier males that were the progeny of expressing carrier dams.

ovulation

INTRODUCTION

Production traits are typically considered as the result of many genes acting primarily in an additive manner, and this assumption is the basis of most breeding strategies. The identification and use of specific major genes for production traits in livestock enables an increased rate of genetic improvement [1]. There is evidence of several major genes affecting prolificacy in sheep [2–8]. The *Booroola* gene has been mapped to sheep chromosome 6 [9], whereas the *Inverdale* gene is located on sheep chromosome X [10]. We have been investigating a putative gene influencing ovulation rate in a flock of New Zealand Coopworth sheep.

Intensive screening among recorded sheep flocks throughout New Zealand commenced in 1979 and led to the establishment of flocks (hereafter called the screened flocks) of highly prolific Coopworth, Romney, and Perendale sheep at the Invermay Agricultural Centre. The 212 foundation ewes in the screened flocks were selected on the basis of high litter size [11], 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. Records from the Romney flock, and subsequent progeny testing of rams, identified the X-linked *Inverdale* gene. In heterozygous females the *Inverdale* gene increases ovulation rate by about 1.0 and litter size by about 0.6 [7] but causes small streak ovaries leading to infertility in homozygous ewes [12, 13].

In 1984, high ovulation rates were measured among the daughters of one of the foundation Coopworth rams (79-754) used in 1982, and this was reflected in his subsequent high breeding value (BV) for ovulation rate (+0.74). Descendants of ram 79-754 have been designated the *Woodlands* family line. A program of progeny testing (PT) males of the *Woodlands* family, by mating these with unrelated ewes in commercial flocks, was initiated in 1987. The objective was to determine whether a new major gene for ovulation rate was present in this family, using records from the screened flock and the progeny test flocks.

MATERIALS AND METHODS

The experimental procedures reported in this study were carried out in accordance with the 1987 Animal Protection (Codes of Ethical Conduct) Regulations of New Zealand after approval was granted by the AgResearch Invermay Animal Ethics Committee.

Selection following the initial screening was based on BV for ovulation rate that was calculated using an animal model with heritability of 0.2 and repeatability of 0.3. Ovulation rates were measured by laparoscopy [14] twice at 1.5 yr of age and once or twice in each successive year. All females were retained until laparoscoped twice at 1.5 yr of age, and those with the highest BVs for ovulation rate were selected as flock replacements. The sire BVs, which excluded male progeny information and had been calculated on the basis of multigenic inheritance of autosomal genes, included all records of ovulation rate, whereas the PT ovulation rate records analyzed were those measured at 1.5 yr of age before any females were culled.

Within the screened flock from 1982 to 1997, *Woodlands* sires (ram 79-754 plus 32 descendants) were designated either as expressers or as noncarriers of the putative gene based on their BV. Although ovulation rate is only ex-

[1]Correspondence: George H. Davis, Invermay Agricultural Centre, Puddle Alley Road, Private Bag 50034, Mosgiel, NZ. FAX: 64 3 489 9024; e-mail: davisg@agresearch.cri.nz

Received: 14 June 2000.
First decision: 7 August 2000.
Accepted: 22 August 2000.
© 2001 by the Society for the Study of Reproduction, Inc.
ISSN: 0006-3363. http://www.biolreprod.org

pressed in females, we use the term expresser males to identify carrier males that have daughters with increased ovulation rates because in the discussion we propose that there are also silent carrier males that have daughters showing no increase in ovulation rate. Ram 79-754 and five of his descendants with high BVs (range: +0.40 to +0.68) were designated expressers, and the remaining 27 male descendants with BVs from −0.40 to +0.26 were designated noncarriers. The mean number of female progeny per ram was 23.3 (SEM ± 1.4).

From 1987 to 1997 the PT program involved 19 *Woodlands* rams (including two that had also been used within the screened flock) that were each mated to unrelated ewes in commercial flocks. The female progeny (mean per sire = 28.6 ± 1.4) were retained until 1.5 yr of age when ovulation rate was measured by laparoscopy twice with an interval of 18–21 days between measurements. Three *Woodlands* rams with daughters having mean ovulation rates that were 0.33 to 0.39 (mean = + 0.36) higher than contemporaries were designated expressers and 16 rams with daughters ranging from mean = −0.12 to +0.17 compared with contemporaries were classified as noncarriers.

Woodlands rams were assigned to one of three categories depending on their relationship to an expresser male ancestor. Category 0 was the sons of expresser rams (zero females in the line between the ram and his carrier ancestor), category 1 was the maternal grandsons of an expresser ram (one female in the line between the ram and his carrier ancestor), and category 2 was no closer than maternal great grandsons of an expresser ram (at least two females, and no males, in the line between the ram and his carrier ancestor).

Statistical Analysis

Ewes born in the screened flock from 1982 onward were classified with a number of indicator variables (genotype classes). Each of these variables represented a line of descent from an expresser carrier ram (five different pathways in total), and the variable was set to one if the ewe had that ancestry and was set to zero otherwise. An additional genotype class was created for ewes that were progeny of expresser rams, and whose dam descended from an expresser ram. Ewes in this category are potentially homozygous for the gene, and their other genotype classes were set to zero.

Ovulation rates at 1.5 yr of age were analyzed in a mixed model that included ewe as a random effect. Year of measurement, measurement time within year, and the genotype classes were included as fixed effects. Initially, interactions between genotype classes (where relevant) were fitted, but these were dropped as none was significant. Heterogeneous variances were allowed for the ewe effects and residual effects. Preliminary analyses indicated that there was no effect on variances of genotype classes that included descent through a noncarrier ram. Therefore heterogeneous variances were modeled only for the remaining genotype classes.

A segregation analysis was performed to compare autosomal, X-linked, and imprinted models of inheritance. The analysis calculated the likelihood of obtaining the observed set of ram genotypes under each possible model. It was assumed that the gene was not present in animals that did not descend from the founder. Because of the unusual inheritance models, these calculations were performed manually.

TABLE 1. Segregation among the sons of two half-sib ewes in the *Woodlands* family; the mother (82-268) of both dams was a daughter of carrier sire 79-754.

Dam	Son	Progeny ovulation rate deviation	Classification
85-852	85-516	+0.26	Carrier
85-852	89-626	−0.17	Noncarrier
85-852	90-27	+0.53	Carrier
85-852	90-28	+0.37	Carrier
85-852	92-487	−0.13	Noncarrier
85-852	93-300	−0.15	Noncarrier
86-66	90-164	+0.39	Carrier
86-66	90-165	+0.10	Noncarrier
86-66	92-90	+0.12	Noncarrier
86-66	93-704	+0.39	Carrier

RESULTS

There were no expresser rams among the three category 0 rams and the 24 category 1 rams. However, among the 17 category 2 rams, there were seven expressers (41%). In addition, there was one ram that was assigned both categories 0 and 1, three rams that were assigned categories 0 and 2, and one that was assigned categories 1 and 2. The only expresser in these multicategory rams was one that was categories 0 and 2. The classification of noncarrier assigned to the two rams that had been used in both the screened flock and in the progeny tests was consistent across flocks.

The 102 daughters of expresser rams in the screened flock (excluding 14 from dams that were also carriers) had a mean ovulation rate 0.39 (standard error of difference [SED] = 0.06) higher than Coopworth contemporaries. There were 113 maternal granddaughters of expresser rams, sired by noncarrier rams, and their mean ovulation rate was 0.02 (SED = 0.06) higher than contemporary Coopworth ewes. In addition, there were 67 maternal great granddaughters of expresser rams, sired by noncarrier rams, which had a mean ovulation rate 0.01 (SED = 0.06) higher than contemporary Coopworths.

The 341 daughters of category 1 rams, all classified noncarriers, had a mean ovulation rate no higher than contemporary Coopworths (difference = 0.00; SED = 0.04), and the 198 daughters of these 341 ewes, sired by noncarrier rams, had a mean ovulation rate 0.01 (SED = 0.04) lower than contemporary Coopworths.

Within the screened flock there were also 14 daughters of a *Woodlands* expresser ram crossed with a *Woodlands* carrier ewe. All 14 had functional ovaries and their mean ovulation rate was 0.39 (SED = 0.16) higher than contemporary Coopworths, which was identical to the contemporary progeny of *Woodlands* expresser rams crossed with noncarrier ewes.

All groups (except daughters of expresser sires) had ewe and residual variance estimates that were not significantly different from those of the control. The daughters of expresser sires had a higher ($P < 0.05$) ewe variance than the controls (0.39 vs. 0.25; SED = 0.06). When the log of the ovulation rate was analyzed, no groups had a significantly higher variance than the controls.

Two half-sib maternal granddaughters (85-852 and 86-66) of the founder carrier sire (79-754) had six and four sons retained for breeding, respectively (Table 1). Half the sons of each ewe had progeny with high ovulation rates and were classified as expressers of the gene. This evidence

TABLE 2. Comparison of X-linked model having a maternal imprint that is only removed when inherited by a male from a silenced carrier female, with other inheritance models.

Case	Founder genotype[a]	Model[b]	$Log_{10}L$[c]	Odds[d]
1	WW	A	$-\infty$	
2	WW	AS_{fm}	$-\infty$	
3	W+	A	−14.57	6×10^7
4	W+	AS_{fm}	−9.57	553
5	W+	AS_f	−9.35	336
6	W/Y	X	−14.83	1×10^8
7	W/Y	XS_{fm}	−6.83	1
8	W/Y	XS_f	−7.05	1.66

[a] W, *Woodlands*; +, wild-type; Y, Y chromosome.
[b] A, autosomal; X, X-linked; S_f, silenced female (heterozygous expresser females pass imprinted allele to all daughters but to no sons); S_{fm}, silenced female and male (heterozygous expresser females pass imprinted allele to half their progeny of each sex). The same odds exist for this model as the male offspring-specific transmission-ratio model where expresser females pass their paternal allele to half their daughters but to no sons.
[c] $Log_{10}L$, log_{10}(likelihood).
[d] Odds ratio of case 7 compared to the current case.

of genetic segregation included two full-sib sons (90-164 and 90-165) of ewe 86-66. The daughters of one son had ovulation rates 0.29 (SED = 0.096; $P < 0.01$) higher than the other (Table 1).

Records from 193 progeny of the daughters of five expresser sires were examined to investigate whether the sexes of the progeny differed from the expected 50% males and 50% females. There were 102 males (52.8%) and 91 females (47.2%), which is not significantly different from the expected 50% of each sex (binomial test: $P = 0.47$).

The 2-yr-old carrier ewes that inherited the gene from their sire in the screened prolific flock had a mean ovulation rate at the cycle of mating of 2.65 (SEM = 0.07) and a mean litter size of 2.11 (SEM = 0.07). The distribution of litters within this group was 15.3% singles, 60.0% twins, 23.5% triplets, and 1.2% quadruplets.

For progeny of expresser sires in the screened flock, yearling liveweight, yearling fleece weight, and 1.5-yr-old mating weight averaged 38.2 kg (SEM = 0.54), 2.55 kg (SEM = 0.12), and 60.9 kg (SEM = 0.89), respectively. The respective values for contemporary progeny of noncarrier rams were 37.2 kg (SEM = 0.57), 2.44 kg (SEM = 0.33), and 60.5 kg (SEM = 0.83). None of these differences between expressers and noncarriers was statistically significant ($P > 0.05$).

The segregation analysis (Table 2) confirmed that the data are most likely under the X-linked maternally imprinted model where the imprint is only removed when inherited by a male from a silenced carrier female. The data were 336 times more likely under this model than under the best autosomal model.

DISCUSSION

Results from the three category 0 sires, whereby an expresser male does not pass the gene to his sons, are consistent with a single gene or closely linked group of genes on the X chromosome. In total, seven rams had expresser sires (category 0 = 3; category 0 and 1 = 1; category 0 and 2 = 3), and the only expresser ram amongst this group also had an expresser maternal great grandsire (i.e., categories 0 and 2). Based on the category 2 results, it seems probable that this category 0 and 2 sire inherited the gene from his maternal great grandsire. Among daughters of expresser rams, the higher ovulation rate (+0.39) was not associated with a significant increase in the variance for log ovulation rate (an increase in coefficient of variation). There was therefore no evidence for segregation of the *Woodlands* gene among the daughters of expresser rams, which is consistent with a prolificacy gene on the X chromosome [7]. Furthermore, the segregation analysis (Table 2) supported X-linked inheritance compared with autosomal inheritance.

It appears most likely that the gene is expressed in a parent-of-origin-dependent manner (imprinted), being expressed from only the paternally inherited allele (maternally imprinted), because there was a high ovulation rate in daughters of expresser rams compared with a normal ovulation rate in daughters of these high ovulating carrier dams. Genomic imprinting is the condition in which the parental alleles of a gene are differentially expressed. Typically, in an imprinted gene there is transcription of the allele inherited from one parent but not the other. The effect of imprinting is that the gene is turned off, which renders the genome functionally hemizygous at the specific locus. Over 30 imprinted genes were discovered in mice and humans during the 1990s [15]. It is known that one of the X chromosomes undergoes a process of inactivation in somatic cells during early development [16] but neither of the patterns that have been described, random X inactivation and paternal X inactivation [17], explain why the putative *Woodlands* gene appears to be expressed in all females inheriting the paternal allele but none of those inheriting the maternal allele.

This is the first report of an imprinted gene on the sheep X chromosome, and it is currently the only imprinted gene shown to affect ovulation rate. There was no evidence of pleiotropic effects on either liveweight or fleece weight, as the differences between carriers and noncarriers were small and not significant ($P > 0.05$). There is a maternally imprinted locus on the human X chromosome affecting cognitive function in females with Turner's syndrome [18] and paternal inheritance of a fragile X chromosome leading to an increased incidence of premature ovarian failure [19]. Two other imprinted genes have been identified in sheep, but neither is on the X-chromosome. The insulin-like growth factor-2 gene on ovine chromosome 21 is maternally imprinted [20], while the *Callipyge* muscular hypertrophy gene, which is only expressed in heterozygous individuals that paternally inherit the *Callipyge* allele (due to a polar overdominance effect), has been mapped to ovine chromosome 18 [21].

Our hypothesis is that the expresser dams of category 1 sires pass the gene to 50% of their sons and daughters, but the gene is silenced. The expression of a gene affecting ovulation rate is sex-limited, but the data show that no category 1 sires have progeny with increased ovulation rates. This could be explained on the basis that 50% of these category 1 sires are silent carriers. This inheritance is shown in Figure 1 and implies a novel maternal imprinting effect whereby the imprint is only removed when inherited by a male from a silenced carrier female. Sire R (Fig. 1) has a silenced maternal grand-sire and could therefore be either an expresser (gene inherited from a silenced carrier female) or a silenced carrier (gene inherited from a dam that had inherited the gene paternally), and further progeny test data are needed to determine which of these inheritance patterns is involved.

To know whether some category 1 sires are silent carriers we need to identify expresser descendants. The infor-

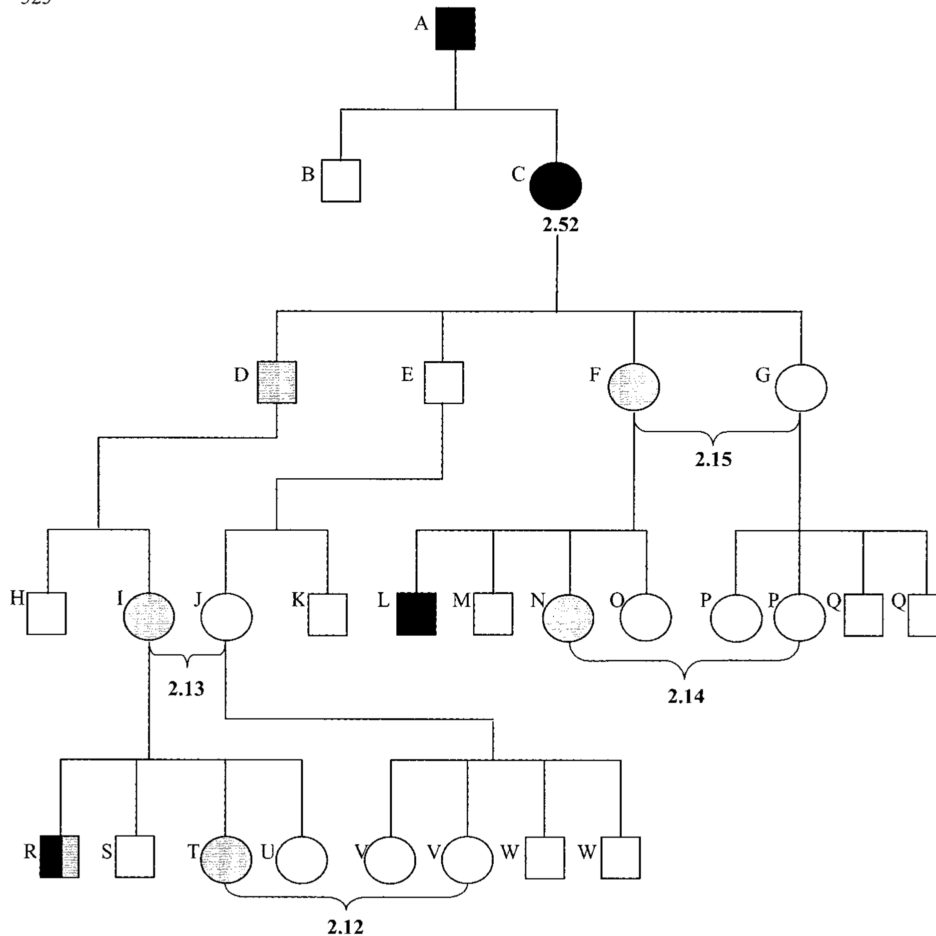

FIG. 1. Schematic multigeneration family showing descendants of an expresser male (A). Numbers indicate mean ovulation rates of all relevant descendants of expresser rams. Open squares (male) and circles (female) denote noncarriers. Solid squares and circles denote carriers in which the gene is expressed (males assigned on the basis of their daughters expressing the gene), and shaded squares and circles denote carriers in which the gene is silenced. Category 0 males are represented by sire B, category 1 males by sires D and E, and category 2 males by sires L, M, and Q. Because silenced carriers and noncarriers cannot be distinguished by phenotype, bracketed ovulation rates are the combined means for carriers in which the gene is silenced and noncarriers. The figure shows inheritance according to the X-linked model with a maternal imprint that is removed when inherited by a male from a silenced carrier female that has inherited the gene maternally. Sire R is shown as solid and shaded because he could be either an expresser (gene inherited from a silenced carrier female) or a silenced carrier (gene inherited from a dam that had inherited the gene paternally), depending on which of these inheritance patterns is involved. The male offspring-specific transmission-ratio model where expresser females pass their paternal allele to half their daughters and no sons is illustrated by excluding sire D and his descendants; and the male offspring-specific transmission-ratio model where expresser females pass their paternal allele to all their daughters and no sons is illustrated by excluding sire D and dam G plus their descendants.

mative animals would be the maternal great-grandsons of a silent carrier category 1 sire because carriers in this generation would have inherited the gene from their dam's maternal X chromosome and it would be expected that the gene would be expressed in their female progeny. There was only one sire used in the screened flock that was the maternal line great grandson of a category 1 sire and on the basis of his daughters' ovulation rate he was a noncarrier. Half of the category 1 males would be expected to be silent carriers, but these cannot be distinguished phenotypically from the noncarriers because both would produce daughters that show no increase in ovulation rate. Hence, on average, daughters of half the category 1 rams would be carriers, one in four granddaughters and only one in eight great-grandsons.

A search of litter size records in the Lincoln University flock in which sire 79-754 was bred (there were no records of ovulation rate) does provide some evidence that the gene is carried by category 1 rams. Sire 79-754 was used in this flock in 1981 prior to his use in the screened flock, and his 38 2-yr-old daughters had a mean litter size 0.36 higher than the mean of their cohorts. Progeny means for litter size from the other nine sires used in 1981 (n = 10–22 progeny per sire) ranged from 0.16 below to 0.11 above the cohort mean. His maternal line great grandsire (71-475) had daughters that had a mean litter size 0.11 higher than their cohort mean, and daughters of the 11 contemporary sires had means ranging from −0.20 to +0.22. This suggests that if the daughters of 71-745 were carriers, the gene was silent. The dam of 71-475 had 19 lambs in seven lambings, including four sets of triplets and the first set of quadruplets recorded in the flock. Her sire (67-44) had 19 daughters with a mean litter size 0.39 higher than daughters of six contemporary sires (range −0.16 to +0.03), which is consistent with 67-44 being an expresser. In Figure 1 this would be represented by the gene passing from A (representing 67-44) to C, D (representing 71-475), I and T, with sire 79-754 being the son of ewe T.

An alternative explanation for the observed inheritance pattern is that the consistent results from the 24 category 1

sires suggest that a dam inheriting the paternal *Woodlands* allele does not transmit it to her sons, i.e., no sire D's produced (Fig. 1). Transmission ratio distortion is defined as a statistically significant departure from Mendelian transmission, regardless of its basis [22]. The alternative hypothesis involves complete transmission-ratio distortion, as a dam inheriting the paternal allele does pass the allele to at least some of her daughters, although they do not express the gene. This contrasts with the X-linked *Inverdale* prolificacy gene where a dam inheriting the gene either paternally or maternally, passes it to half her progeny of both sexes, and the carrier daughters all express the gene [7]. Parental origin-dependent, male offspring-specific transmission-ratio distortion has been observed at loci in a specific region of the human X chromosome [23], but the distortion was incomplete as 61.7% of males inherited grandpaternal alleles versus 38.3% that inherited grandmaternal alleles.

If transmission-ratio distortion is male-offspring specific, the female offspring would be unaffected, with half inheriting the grandpaternal allele and half inheriting the grandmaternal allele. The segregation analysis (Table 2) was unable to compare this model with the model having a maternal imprint that is only removed when inherited by a male from a silenced carrier female. This is because in the absence of progeny testing or a DNA marker for the gene it is not possible to differentiate the phenotype of noncarrier males and the putative silent carrier males. The possibility that the grandpaternal allele was passed to all female offspring (i.e., no G's produced; Fig. 1) was also investigated. A segregation analysis of these daughters, on the basis of whether or not they produced carrier sons, was inconclusive, with the odds ratio suggesting that the data were slightly more probable (1.2×) if 50% of these ewes carried the allele than if 100% carried the allele.

Another possible explanation for the observation that there were no carrier category 1 males with daughters having increased ovulation rates was that there could have been lethality in hemizygous carrier males. Strong segregation distortion due to a male-lethal effect has been found for several X chromosome markers in mouse interspecific backcrosses [24], and placental hypotrophy where the conceptus was male, frequently leading to fetal death, has been observed in mouse interspecific hybrids by Zechner et al. [25], who also noted that one of the determinants of placental growth has been mapped to the mouse X chromosome. If there was lethality in all hemizygous carrier males, the number of males would be expected to be half the number of females because only the 50% noncarrier males would survive. However, the 53% males and 47% females recorded among the 193 progeny of carrier ewes was not indicative of any lethal effect in hemizygous carrier males.

The presence of carriers among the *Woodlands* category 2 sires is evidence that the maternal imprint is removed when the silenced allele is passed from a carrier dam to her son. With an X-linked gene, all daughters of a carrier sire would be expected to be carriers and subsequently 50% of their daughters and 25% of their grandsons. Although the observed 41% (7 of 17) carriers was higher than the expected 25%, the difference was not significant (binomial test: $P = 0.21$). Records from the sons of half-sib ewes 85-852 and 86-66 also provide compelling evidence of genetic segregation. Five of their 10 sons were classified as carriers (Table 1), and these included a set of full-sibs where one was a carrier and the other a noncarrier. Because there are both carrier males and females, mitochondrial inheritance was excluded as sperm contribute very few mitochondrial DNA molecules to the zygote and random replication probably reduces this contribution to zero in most individuals [26].

Half of the 14 daughters of an expresser ram crossed with a heterozygous carrier ewe would be expected to be homozygous. If the effect of the gene is additive for ovulation rate, like the *Booroola* prolificacy gene in sheep [2], this group of females would be expected to have a higher mean ovulation rate than the heterozygous progeny of an expresser ram. Alternatively, if the inheritance involved polar overdominance as has been observed in the *Callipyge* muscling gene in sheep [21], the mean ovulation rate of these females would be lower than heterozygotes. The evidence suggests that the increased ovulation rate of the 14 ewes (+0.39) was the same as contemporary heterozygous progeny of carrier rams crossed with noncarrier ewes. There was no evidence of segregation for ovulation rate among the 14 ewes, as the between-ewe variation (0.18) was not higher than in the progeny of expresser rams across noncarrier ewes (0.39; SED = 0.10). Furthermore, all 14 had functional ovaries, which shows that in homozygous ewes the gene does not impair ovarian development in the manner observed in ewes homozygous for the X-linked *Inverdale* gene [12, 13]. A homozygous female that had an expresser sire, as distinct from a silenced carrier sire, would be expected to have one allele that expresses (paternal) and one allele that is silenced (maternal). Thus, a homozygous female would be expected to have the same ovulation rate as a heterozygous female expressing the gene, which is consistent with our limited data from the 14 ewes bred from two carrier parents. Although the sample of ewes is small, these results are consistent with the hypothesis that the gene is on the X chromosome and in females the paternally inherited allele is expressed but the maternally inherited allele is silenced.

The carrier status of sons of homozygous ewes has not been determined. If the paternally inherited copy is expressed and the maternally inherited copy silenced, half the sons will inherit their dam's expressing copy and half her silenced copy. According to our hypothesis the allele will be silenced in sons inheriting the expressing copy but expressed (imprint removed) in sons inheriting the silenced copy. Thus, only 50% of the sons would have daughters with increased ovulation rates. However, if the total absence of expressers in the category 1 rams is due to complete transmission-ratio distortion, it would then be expected that all sons of a homozygous dam would be carriers because they would inherit the silenced (imprinted) allele from the dam's maternal X chromosome.

The mean effect of the gene on ovulation rate (+0.39) is less than the *Booroola* (+1.65), *Java* (+1.30), *Cambridge* (+1.25), *Inverdale* (+1.00), and *Olkuska* (+0.70) prolificacy genes in sheep [5–7, 27, 28]. The distribution of single, twin, triplet, and quadruplet births among daughters of expresser sires was the same as occurs in other prolific flocks with an equivalent mean litter size, but where no major gene is known to be segregating [29]. This differs from the pattern seen among progeny of a sire heterozygous for the autosomal *Booroola* gene [29] but is typical of the distribution of litter size where a major gene for prolificacy, such as *Inverdale,* is located on the X chromosome [30] and is being passed from a male to all daughters.

The Coopworth breed was developed by interbreeding Border Leicester and Romney sheep [31]. The Lincoln University flock was founded in 1958, and our study of the litter size records back to its foundation has revealed that

sire 67-44 was the first Coopworth sire with female progeny having a much higher mean litter size than their contemporaries. Because the Lincoln University flock has subsequently been the source of Coopworths for many industry flocks [31], it is likely that the *Woodlands* gene is widely distributed in New Zealand sheep flocks. Determination of the frequency of the gene and its targeted use in industry flocks must await the discovery of DNA markers located close to the gene or the gene itself.

Because the inheritance pattern of the *Woodlands* gene is different from the *Inverdale FecX* locus, we have assigned the locus *FecX2* and the allele symbol $FecX2^W$ in accordance with the guidelines established by the Committee on Genetic Nomenclature of Sheep and Goats [32]. Thus female genotypes are $FecX2^W/FecX2^W$, $FecX2^W/FecX2^+$, and $FecX2^+/FecX2^+$; and male genotypes $FecX2^{W/Y}$ and $FecX2^{+/Y}$.

ACKNOWLEDGMENTS

We thank Messrs. M.V. Howarth, C. Matheson, P. Reid, M. Hishon, G.D. Bruce, and Miss S. Clarke for technical assistance; Dr. P.F. Fennessy and Mr. J.C. McEwan for helpful discussions.

REFERENCES

1. Smith C. Utilization of major genes. In: Land RB, Robinson DW (eds.), Genetics of Reproduction in Sheep. London: Butterworths; 1985: 151–158.
2. Davis GH, Montgomery GW, Allison AJ, Kelly RW, Bray AR. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. NZ J Agric Res 1982; 25:525–529.
3. Hanrahan JP, Owen JB. Ovulation rate in Cambridge ewes. Anim Prod 1985; 40:529.
4. Jonmundsson JV, Adalsteinsson S. Single genes for fecundity in Icelandic sheep. In: Land RB, Robinson DW (eds.), Genetics of Reproduction in Sheep. London: Butterworths; 1985: 159–168.
5. Bradford GE, Quirke JF, Sitorius P, Tiesnamurti II, Bell FL, Fletcher IC, Torell DT. Reproduction in Javanese sheep: evidence for a gene with large effect on ovulation rate and litter size. J Anim Sci 1986; 63:418–431.
6. Radomska MJ, Martyniuk E, Klewiec J, Knothe A. Inheritance of high prolificacy of the Olkuska sheep (preliminary results). J Agric Sci Fin 1988; 60:597–598.
7. Davis GH, McEwan JC, Fennessy PF, Dodds KG, Farquhar PA. Evidence for the presence of a major gene influencing ovulation rate on the X-chromosome of sheep. Biol Reprod 1991; 44:620–624.
8. Bodin L, Elsen JM, Poivey JP, San Cristobal-Gaudy M, Belloc JP, Eychenne F. Hyper-prolificacy in the French Lacaune sheep breed. Proc World Congr Genet Appl Livest Prod 1998; 27:11–14.
9. Montgomery GW, Crawford AM, Penty JM, Dodds KG, Ede AJ, Henry HM, Pierson CA, Lord EA, Galloway SM, Schmack AE, Sise JA, Swarbrick PA, Hanrahan V, Buchanan FC, Hill DF. The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. Nat Genet 1993; 4:410–414.
10. Galloway SM, McNatty KP, Cambridge LM, Laitinen MPE, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW, Beattie AE, Davis GH, Ritvos O. Growth differentiation factor 9B mutations cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat Genet 2000; 25:279–283.
11. Davis GH, McEwan JC, Howarth MV, Kelly RW, Paterson AT. Reproductive performance of progeny of prolific Romney, Coopworth and Perendale sheep. Proc NZ Soc Anim Prod 1987; 47:89–91.
12. Davis GH, McEwan JC, Fennessy PF, Dodds KG, McNatty KP, O W-S. Infertility due to bilateral ovarian hypoplasia in sheep homozygous ($FecX^I$ $FecX^I$) for the Inverdale prolificacy gene. Biol Reprod 1992; 46:636–640.
13. Braw-Tal R, McNatty KP, Smith P, Heath DA, Hudson NL, Phillips DJ, McLeod BJ, Davis GH. The ovaries of ewes homozygous for the X-linked Inverdale gene ($FecX^I$) are devoid of secondary and tertiary follicles but contain many abnormal structures. Biol Reprod 1993; 49: 895–907.
14. Kelly RW, Allison AJ. Measurement of ovulation rates by laparoscopy and effects on reproductive performance. Proc NZ Soc Anim Prod 1976; 36:240–246.
15. Kelsey K. The hows and whys of imprinting. Trends Genet 2000; 16: 15–16.
16. Lyon MF. Possible mechanisms of X chromosome inactivation. Nature 1971; 232:229–232.
17. Moore T, Haig D. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 1991; 7:45–49.
18. Skuse DH, James RS, Bishop DVM, Coppins B, Dalton P, Aamodt-Leeper G, Bacarese-Hamilton M, Creswell C, McGurk R, Jacobs PA. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. Nature 1997; 387:705–708.
19. Hundscheid RDL, Sistermans EA, Thomas CMG, Braat DDM, Straatman H, Kiemeney LALM, Oostra BA, Smits APT. Imprinting effect in premature ovarian failure confined to paternally inherited fragile X mutations. Am J Hum Genet 2000; 66:413–418.
20. McLaren RJ, Montgomery GW. Genomic imprinting of the insulin-like growth factor 2 gene in sheep. Mamm Genome 1999; 10:588–591.
21. Cockett NE, Jackson SP, Shay TL, Farnir F, Berghmans S, Snowder GD, Nielsen DM, Georges M. Polar overdominance at the ovine *callipyge* locus. Science 1996; 273:236–238.
22. Montagutelli X, Turner R, Nadeau JH. Epistatic control of non-Mendelian inheritance in mouse interspecific crosses. Genetics 1996; 143: 1739–1752.
23. Naumova AK, Leppert M, Barker DF, Morgan K, Sapienza C. Parental origin-dependent, male offspring-specific transmission-ratio distortion at loci on the human X chromosome. Am J Hum Genet 1998; 62: 1493–1499.
24. Biddle FG. Segregation distortion of X-linked marker genes in interspecific crosses between *Mus musculus* and *M. Spretus.* Genome 1987; 29:389–392.
25. Zechner U, Reule M, Orth A, Bonhomme F, Strack B, Guenet J-L, Hameister H, Fundele R. An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrids. Nat Genet 1996; 12:398–403.
26. Birky CW. Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc Natl Acad Sci U S A 1995; 92:11331–11338.
27. Piper LR, Bindon BM, Davis GH. The single gene inheritance of the high litter size of the Booroola Merino. In: Land RB, Robinson DW (eds.), Genetics of Reproduction in Sheep. London: Butterworths; 1985: 115–125.
28. Glazko VI, Owen JB, Ap Dewi I, Axford RFE. An association of haemoglobin protein (HBB) with ovulation rate in Cambridge sheep. Anim Sci 1997; 64:279–282.
29. Davis GH, Kelly RW, Hanrahan JP, Rohloff RM. Distribution of litter sizes within flocks at different levels of fecundity. Proc NZ Soc Anim Prod 1983; 43:25–28.
30. Davis GH, Dodds KG, McEwan JC, Fennessy PF. Liveweight, fleece weight and prolificacy of Romney ewes carrying the Inverdale prolificacy gene ($FecX^I$) located on the X-chromosome. Livest Prod Sci 1993; 34:83–91.
31. Clark VR. Evolution and establishment of the Coopworth breed 1950–1991. In: Davis GH (ed.), Lab Coats to Gumboots. New Zealand Society of Animal Production, Occasional Publication No. 13; 1994: 107–133.
32. Luavergne JJ, Dolling CHS, Renieri C (eds.). Mendelian Inheritance in Sheep 1996. Camerino: University of Camerino Press; 1996: 1–214.