

## DNA Tests in Prolific Sheep from Eight Countries Provide New Evidence on Origin of the Booroola (*FecB*) Mutation<sup>1</sup>

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### ABSTRACT

Recent discoveries that high prolificacy in sheep carrying the Booroola gene (*FecB*) is the result of a mutation in the BMP1B receptor and high prolificacy in Inverdale sheep (*FecX'*) is the result of a mutation in the *BMP15* oocyte-derived growth factor gene have allowed direct marker tests to be developed for *FecB* and *FecX'*. These tests were carried out in seven strains of sheep (Javanese, Thoka, Woodlands, Olkuska, Lacaune, Belclare, and Cambridge) in which inheritance patterns have suggested the presence of major genes affecting prolificacy and in the prolific Garole sheep of India, which have been proposed as the ancestor of Australian Booroola Merinos. The *FecB* mutation was found in the Garole and Javanese sheep but not in Thoka, Woodlands, Olkuska, Lacaune, Belclare, and Cambridge sheep. None of the sheep tested had the *FecX'* mutation. These findings present strong evidence to support historical records that the Booroola gene was introduced into Australian flocks from Garole (Bengal) sheep in the late 18th century. It is unknown whether Javanese Thin-tailed sheep acquired the Booroola gene directly from Garole sheep from India or via Merinos from Australia. The DNA mutation test for *FecB* will enable breeding plans to be developed that allow the most effective use of this gene in Garole and Javanese Thin-tailed sheep and their crosses.

gene regulation, ovary, ovulation

### INTRODUCTION

Studies of the inheritance patterns of ovulation rate and litter size in prolific flocks have shown that major genes for prolificacy are segregating in Booroola (*FecB*) [1, 2] and Inverdale (*FecX'*) sheep [3]. There is evidence that sev-

eral other strains of prolific sheep also have segregating major genes. These strains include Cambridge (*FecC*) [4], Thoka (*FecI*) [5], Javanese (*FecJ*) [6], Olkuska [7], Belclare [8], Lacaune [9], and Woodlands (*FecX2*) [10] sheep.

*FecB* is an autosomal dominant gene with a large effect on ovulation rate. Piper et al. [11] concluded that the effect of *FecB* was additive for ovulation rate with each copy of *FecB* increasing ovulation rate by about 1.6, but a multiplicative model was a better fit to the data of Davis et al. [12], with each copy increasing ovulation rate by 90%. *FecX'* is a dominant gene located on the X-chromosome. The ovulation rate of *FecX'/FecX'* ewes is about 1.0 higher than that of *FecX+/FecX+* ewes [3], but *FecX'/FecX'* ewes have small nonfunctional streak ovaries and are infertile [13].

Only recently have researchers discovered that high prolificacy in Booroola sheep is the result of a mutation in the BMP1B (ALK6) receptor [14] and high prolificacy in Inverdales is the result of a mutation in an oocyte-derived growth factor gene, *BMP15* [15]. These discoveries make it possible to use DNA testing to determine whether other prolific strains of sheep carry the mutations, without the need for the pedigree information required for the previous marker tests in Booroola and Inverdale sheep [16, 17].

The Garole sheep from India (Fig. 1) is a prolific microsheep found in the hot, humid rice paddies of the southern part of West Bengal, particularly in the coastal belt of Sundarbans. These sheep average only 10–14 kg adult live weight and have a reported mean litter size of 2.27 in the Sundarbans region [18] but have a lower mean litter size of 1.74 in the semiarid environment of the Deccan plateau of Maharashtra [19]. Turner [20] suggested that the highly prolific Booroola Merinos can be traced back to an early Australian flock known to include prolific Bengal sheep. In 1792, 10 Bengal ewes and 2 rams arrived into Australia from Calcutta, and a further shipment of about 100 Bengal sheep arrived in the following year. Through the Australian Stud Merino Flock register, Turner [20] established a possible early link between Booroola and Bengal sheep; the Egelabra strain of Merinos to which the Booroola Merino belonged can be traced back to the flock of the Rev. Samuel Marsden, who was also one of the early flock owners of

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FIG. 1. Ear-tagging adult Garole microsheep in the Sundarbans region of West Bengal, India.

Bengal sheep. The highly prolific Garole microsheep of West Bengal have many of the fleece and body characteristics reported for the early Bengal sheep in Australia [21], and the two names possibly refer to the same sheep or very closely related breeds.

Here, we report the results of recently developed DNA tests for the Booroola and Inverdale mutations in prolific Javanese, Thoka, Woodlands, Olkuska, Lacaune, Belclare, Cambridge, and Garole sheep.

## MATERIALS AND METHODS

### Prolific Sheep Flocks and Test Sample Collection

Javanese ewes are assigned the *FecJ<sup>F</sup>/FecJ<sup>F</sup>* genotype if they have at least one ovulation or litter size record of three but no records of more than three but are assigned the *FecJ<sup>F</sup>/FecJ<sup>F</sup>* genotype if they have at least one ovulation or litter size record of four or more [22]. Pedigree and/or litter records from the 12 Javanese ewes sampled indicated that eight ewes were likely to be homozygous (*FecJ<sup>F</sup>/FecJ<sup>F</sup>*) and four to be heterozygous (*FecJ<sup>F</sup>/FecJ<sup>+</sup>*) for this gene. Twelve Thoka ewes were sampled, and six of these ewes were assigned a heterozygous genotype (*FecI<sup>F</sup>/FecI<sup>+</sup>*) for the Thoka gene on the basis that each had at least one litter of three or more lambs [5]. The other six Thoka ewes were designated noncarriers (*FecI<sup>+</sup>/FecI<sup>+</sup>*) because none had a litter size record exceeding two. The 12 Woodlands ewes comprised 10 heterozygous (*FecX2<sup>W</sup>/FecX2<sup>+</sup>*) daughters of a progeny-tested carrier (*FecX2<sup>W/Y</sup>*) ram plus 2 progeny-tested *FecX2<sup>W/Y</sup>* rams [10]. The 12 Olkuska ewes were evaluated for ovulation rate three times per year, and all had at least two records of three or more ovulations, indicating that they were carriers of the putative Olkuska gene [23]. The 10 Lacaune rams had all been progeny tested and were heterozygous for the Lacaune gene on the basis of high litter size variability among daughters [9]. The 13 Belclare and 8 Cambridge ewes each had at least two ovulation records, excluding measurements as lambs. There is no consistent ovulation rate criterion for determining carrier status for the major genes responsible for the exceptional ovulation rates observed in the Belclare and Cambridge flocks. Clearly, the occurrence of three corpora lutea is not a suitable criterion since such an event is not uncommon in sheep populations under lowland conditions in Ireland or Great Britain. The minimum value would have to be at least five corpora lutea. On this basis, the set of Belclare ewes included seven carriers and the Cambridge set included five carriers. The 12 Garole ewes sampled all had at least one record of three or more lambs. After the test results were known for the 12 Garole sheep (Table 1), additional Garole ewes ( $n = 25$ ) with smaller

TABLE 1. Litter records for *FecB<sup>B</sup>/FecB<sup>B</sup>* Garole ewes.

Ewe no.	No. lambs/litter	Mean litter size
14	1, 2, 2, 3, 2	2.0
54	2, 1, 3	2.0
107	1, 2, 2, 3, 4, 3, 1, 3	2.3
158	2, 2, 2, 2, 4, 3, 2	2.4
169	1, 1, 2, 2, 3, 4	2.2
201	2, 3, 1, 1	1.8
533	3	3.0
582	3	3.0
34704	1, 3	2.0
34711	2, 3	2.5
34731	1, 4	2.5
G-9	1, 2, 2, 3, 1, 3, 1, 2, 3, 3, 2	2.1

litter sizes were sampled and tested for evidence of genetic segregation at the *FecB* locus in this breed.

Blood samples were collected in 10-ml heparinized vacutainers from Thoka (Iceland), Woodlands (New Zealand), and Lacaune (France) sheep or as a few drops of blood on FTA paper (Whatman BioScience, Cambridge, UK) from Garole (India), Javanese (Indonesia), Olkuska (Poland), Belclare (Ireland), and Cambridge (Ireland) sheep. All samples from outside New Zealand, except Lacaune, were consigned to the AgResearch commercial DNA testing laboratory (Genomnz) at the Invermay Agricultural Centre, with the appropriate import approval from the New Zealand MAF. The Lacaune samples were tested at the INRA Genetique Cellulaire laboratory (Toulouse, France). The initial sampling of Javanese, Woodlands, Olkuska, Belclare, Cambridge, and Garole flocks involved ewes that all had records of high ovulation rate or litter size. The samples from Belclare ewes represented individuals in a subflock derived from ewes with exceptional ovulation records [8], and the Cambridge ewes were from the prolific flock previously described by Hanrahan [8]. The Belclare and Cambridge ewes tested included individuals with ovulation rates at the upper end of the range for each population and were presumptive carriers of a major gene. The Thoka samples were from both putative carrier and noncarrier ewes, and the Lacaune samples were from progeny-tested rams that were all heterozygous for the Lacaune gene.

### Preparation of Blood on FTA Paper

Blood samples on FTA paper were prepared using FTA Purification Reagent (Whatman BioScience) following the manufacturer's instructions. FTA punches (1.2 mm) were washed three times with 100  $\mu$ l of FTA Reagent and twice with TE (10 mM Tris, 1 mM EDTA, pH 8.0) and then rinsed with 70% ethanol prior to air drying. One FTA punch was used per polymerase chain reaction (PCR), and an unused FTA punch was prepared as a negative control.

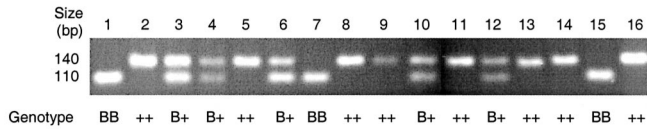
### Forced Restriction Fragment Length Polymorphism PCR

PCRs were carried out using a modification of the forced restriction fragment length polymorphism (RFLP) method described by Wilson et al. [14]. The primer TestR15 has been engineered to introduce a point mutation resulting in PCR products from the BMP1B receptor (BMP1BR) gene with the Booroola mutation containing an *Ava*II (New England Biolabs, Beverly, MA) restriction site (G GACC), whereas products from non-carriers lack this site. Genomic DNA (~100 ng) was amplified as described by Wilson et al. [14]. For FTA samples, DNA from single 1.2-mm punches was amplified in a 25- $\mu$ l reaction volume using an alternative primer set, 5'-CCAGAGGACAATAGCAAAGCAA-3' (TestF2) and 5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3' (TestR15). The amplification was carried out using 35 cycles at 94°C for 15 sec, 60°C for 30 sec, and 70°C for 30 sec followed by 72°C for 5 min and 99°C for 15 min. The 190-base pair (bp) product was then digested using *Ava*II, and the resulting products were separated by electrophoresis on a 3.5% agarose gel and visualized with ethidium bromide. Booroola products digest to yield a 160-bp fragment, whereas noncarrier products remain uncut, at 190 bp.

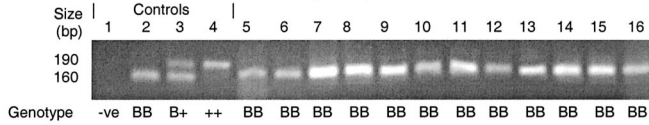
### TaqMan Allelic Discrimination Assay

Animals were tested for *FecX<sup>I</sup>* using the TaqMan allelic discrimination (AD) assay. The assay takes advantage of the 5' exonuclease activity of Taq DNA polymerase to cleave off allele-specific probes (fluorescently

## A) Forced RFLP analysis of Booroola and non-Booroola sheep samples.



## B) Forced RFLP analysis of Garole sheep samples.



## C) Forced RFLP analysis of Javanese sheep samples.

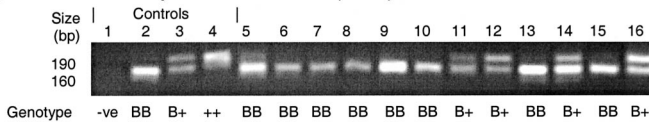


FIG. 2. Forced RFLP analysis to identify BB (*FecB<sup>B</sup>/FecB<sup>B</sup>*), B+ (*FecB<sup>B</sup>/FecB<sup>+</sup>*), and ++ (*FecB<sup>+</sup>/FecB<sup>+</sup>*) genotypes in Booroola, Garole, and Javanese sheep. **A)** PCR using purified genomic DNA with primers TestR15 and F12 [14] produces a 140-bp product (Booroola samples) before *Ava*II digestion. **B** and **C)** PCR from FTA papers using primers TestR15 and TestF2 that produce a 190-bp product (Garole and Javanese samples) before *Ava*II digestion.

labeled with reporter dyes FAM and VIC), which are then hybridized to template DNA during PCRs. An increase in one or both of the fluors determines the genotype at that locus. FTA punches were first amplified in a 20- $\mu$ l reaction using primers 5'-GGCAGTATTGCA-TCGGAAGTTCC-3' (Bmp21) and 5'-CATGATTGGGAGAATTGAGACC-3' (Bmp20) to produce a 205-bp product containing the Inverdale mutation. FTA paper was not added directly to the TaqMan AD assay because it interferes with the fluorescence. Amplification was carried out at 94°C for 5 min; then 35 cycles of 94°C for 30 sec, 62°C for 30 sec, and 72°C for 30 sec; followed by a final extension of 72°C for 4 min. Resulting products (5  $\mu$ l) were separated by electrophoresis on a 2% agarose gel and visualized with ethidium bromide to confirm amplification. All products were diluted by 1:1000 and added as template to the TaqMan AD assay.

The PrimerExpress program (Perkin-Elmer, Applied Biosystems Division, Melbourne, Australia) was used to design primers and probes for the TaqMan AD assay. The sequences of the primers and allele-specific probes are as follows: 5'-GGGCTGAAAGTAACCAAGTTC-3' (INV-TaqmanForward), 5'-CCCGAGGACATACTCCCTTACA-3' (INV-TaqmanReverse), FAM-5'-CACCTTTTCAAGTCAGCTCCAGCA-3' (ProbeINV-T), and VIC-5'-CACCTTTTCAAGTCAGCTCCAGCA-3' (ProbeINV-A). Each TaqMan AD reaction was in 22  $\mu$ l and contained 900 nM primers, 200 nM ProbeINV-T, 100 nM ProbeINV-A, 11  $\mu$ l TaqMan Universal PCR Master Mix (Perkin-Elmer), and 2  $\mu$ l of either genomic DNA (~100 ng) or diluted Bmp20/Bmp21 PCR product. Amplification was carried out on an ABI 770 machine (Perkin-Elmer) at 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 94°C for 15 sec and 62°C for 1 min. Fluorescence was measured on the ABI 770 machine after amplification was complete. Data were analyzed and genotype was determined with the Sequence Detector software (Perkin-Elmer).

## Single-Stranded Conformational Polymorphism Analysis

The *FecB* mutation was tested in Lacaune sheep by single-stranded conformational polymorphism (SSCP) as described by Mulsant et al. [24]. *BMP15* genotypes of Lacaune sheep were determined by SSCP analysis of a 310-bp fragment (upper: 5'-CATGATGGGCTGAAAGTAAC-3', lower: 5'-GGCAATCATAACCTCATACTCC-3'). Amplification was carried out for 30 cycles in a 40- $\mu$ l reaction mixture, with 50 ng of genomic DNA and 1.5 mM magnesium and an annealing temperature of 56°C. PCR fragments were analyzed by SSCP in polyacrylamide gels with overnight migration at 15 V/cm, 15°C.

TABLE 2. Litter records and genotype of Javanese ewes.

Ewe no.	No. lambs/litter	Mean litter size	Genotype
6074	1, 4	2.5	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
7015	2, 2, 2	2.0	<i>FecB<sup>B</sup>/FecB<sup>+</sup></i>
7046	2, 1, 2	1.7	<i>FecB<sup>B</sup>/FecB<sup>+</sup></i>
7056	2, 3	2.5	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
7062	2, 2	2.0	<i>FecB<sup>B</sup>/FecB<sup>+</sup></i>
7258	4, 3	3.5	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
8109	2	2.0	<i>FecB<sup>B</sup>/FecB<sup>+</sup></i>
9022	0	0	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
9046	2	2.0	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
9059	3	3.0	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
9060	2	2.0	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>
9081	2	2.0	<i>FecB<sup>B</sup>/FecB<sup>B</sup></i>

## RESULTS

All 12 Garole ewes initially sampled were homozygous (*FecB<sup>B</sup>/FecB<sup>B</sup>*) for the Booroola gene (Fig. 2), but none carried the *FecX<sup>I</sup>* mutation. The mean ( $\pm$ SEM) litter size of the 12 ewes was 2.3 ( $\pm$ 0.1), and the mean number of records per ewe was 4.3 ( $\pm$ 0.9). The individual records are shown in Table 1.

Booroola test results were obtained from a further 25 Garole ewes that had records of only single and/or twin births. The mean litter size was 1.7 ( $\pm$ 0.1), and the mean number of records per ewe was 2.4 ( $\pm$ 0.3). Twenty-two ewes were homozygous (*FecB<sup>B</sup>/FecB<sup>B</sup>*), and three were heterozygous (*FecB<sup>B</sup>/FecB<sup>+</sup>*).

The litter size records of the Javanese sheep are shown in Table 2, and pedigree records suggested that the 12 ewes would all carry at least one copy of a putative major gene. The test results showed that four ewes were heterozygous for *FecB*, which agreed with their assignment on the basis of pedigree, and the other eight ewes were homozygous for *FecB* (Fig. 2). None of the four heterozygous ewes had produced more than two lambs, whereas four of the eight homozygous ewes had produced triplets or quadruplets. The four homozygous ewes that had not produced triplets or quadruplets were only 2 yr old and had only a single lambing record or had not yet lambed. None of the Javanese sheep carried the *FecX<sup>I</sup>* mutation.

No ovulation rate records were available for the Thoka ewes, but the 12 ewes had an average of 3.7 records of adult litter size. The six putative heterozygous ewes had at least one record of triplets, and their mean litter size was 2.43 ( $\pm$ 0.14). The six putative noncarriers had no records of triplets, and their mean litter size was 1.69 ( $\pm$ 0.08). Neither the *FecB* nor *FecX<sup>I</sup>* mutations were present in any of the Thoka sheep.

The 10 Woodlands ewes had five to eight ovulation records, and each had at least three records of three or more ovulations. All were classified as carrying and expressing one copy of the imprinted Woodlands gene; these ewes had a high mean ovulation rate (3.10;  $\pm$  0.16) and were daughters of a progeny-tested *FecX<sup>2</sup><sup>W/Y</sup>* sire. None of these ewes and neither of the two progeny-tested *FecX<sup>2</sup><sup>W/Y</sup>* sires carried the *FecB* or *FecX<sup>I</sup>* mutations.

The 12 Olkuska ewes were all classified as carrying the Olkuska gene on the basis of their high ovulation rates. There were 3–18 records per ewe, and all had at least two records of three or more ovulations. The mean ovulation rate of the 12 ewes was 3.35 ( $\pm$ 0.29). None of the Olkuska ewes carried the *FecB* or *FecX* mutations.

The *FecB* and *FecX<sup>I</sup>* mutations were absent in all of the heterozygous Lacaune males. In addition, the *BMP1BR*

(*ALK6*) locus was excluded by linkage analysis of microsatellite *BMS2508* in the Lacaune backcross population (lod = -2.86, at a recombination frequency of 0.15).

The 13 Belclare ewes had mean ovulation records ranging from 2.18 to 4.78, and on the basis of these records at least 7 ewes could be classified as carriers of a major gene using the criterion of at least one record five or more ovulations. Test results showed that none of these ewes carried the *FecB* or *FecX<sup>I</sup>* mutations.

The eight Cambridge ewes had mean ovulation records ranging from 2.50 to 8.75, and on the basis of these records at least five ewes could be classified as carriers of a major gene. None of these ewes carried the *FecB* or *FecX<sup>I</sup>* mutations.

## DISCUSSION

The Garole sheep tested in this study came from two regions in northeastern India. Twenty-seven ewes were descended from 12 rams and were part of a group of 32 ewes purchased from six villages in the Sundarbans region in 1993 and 1994 as described by Nimbkar et al. [19]. The remaining 10 ewes were purchased in 1999 from markets in an area about 70 km distant from the sources of the first purchases. All of the 27 ewes from the first group were *FecB<sup>B</sup>/FecB<sup>B</sup>*, including 18 ewes that had never produced triplets, one of which had only lambed singles at each of her three lambings. In the group of 10 ewes from the later purchase, 7 were *FecB<sup>B</sup>/FecB<sup>B</sup>* and 3 were *FecB<sup>B</sup>/FecB<sup>+</sup>*. These results are strong evidence that *FecB* has been fixed in the first group of sheep but not in the second group. Both groups of sheep had the phenotype typical of pure Garoles, including horned males.

The results show that *FecB* is present at a high frequency in the Garole sheep sampled and suggest that *FecB* is fixed (all sheep homozygous carriers of the gene) in some Garole populations. It seems highly unlikely that the gene could become fixed in a population in the absence of ovulation rate records and DNA marker tests. The fact that sheep are practically all owned in small flocks of less than 10 sheep also precludes progeny testing as a method of fixing the gene. Thus, it is possible that the homozygous genotype (*FecB<sup>B</sup>/FecB<sup>B</sup>*) is the original genotype for this breed, and the presence of some heterozygous individuals in one of the groups sampled could be the result of an outcross. Two breeds that could have been used for outcrossing are the Shahabadi and Chottanagpuri. However, both of these breeds are larger than Garoles and males are polled; therefore, any crossbreeding would need to have occurred many generations ago for these characteristics to have been bred out. Further extensive sampling and DNA analysis would be required to test this hypothesis.

It is estimated that in 1993 there were about 50 000 Garole sheep in West Bengal [18]. These prolific microsheap, which are very well adapted to grazing in wet conditions, are reared solely for meat, and their coarse fleece is never shorn. The Garole phenotype bears practically no resemblance to the Booroola Merino, which under Australian dryland conditions is about three times the adult live weight (35–40 kg) and produces 3.5–4.5 kg clean fleece weight of fine wool with a fiber diameter of 20.5–22.5  $\mu\text{m}$  [21]. The *FecB* gene has apparently survived about 40–50 generations of breeding from the imported Bengal (Garole) sheep without specific selection for prolificacy until the first ewe with triplets was observed in the Sears brothers "Booroola" flock about the mid 1940s. Confirmation that a major gene for prolificacy is present in Garole sheep has impor-

tant implications for crossbreeding with local breeds, which has been recommended for the extensive and harsh sheep-rearing environments in Maharashtra [19].

Bradford and Inouu [25] suggested that on the basis of the similarity of the effect and the postulated origin of the Booroola gene, the *FecJ* gene segregating in the Javanese sheep might also be the same as *FecB*. Roberts [26] estimated the frequency of the *FecJ<sup>F</sup>* allele in Javanese Thin-tailed sheep at 0.34. The results from the present study confirm that *FecB* is segregating in the Garut strain of the Javanese Thin-tailed sheep, and the complete agreement between the assigned *FecJ* genotype for *FecJ<sup>F</sup>/FecJ<sup>F</sup>* and *FecJ<sup>F</sup>/FecJ<sup>+</sup>* ewes and the DNA results for *FecB* genotypes shows that *FecJ* and *FecB* are the same locus. These results suggest that the *FecJ* locus in Javanese Thin-tailed sheep should be designated *FecB* and similarly that the *FecJ<sup>F</sup>* allele should be designated *FecB<sup>B</sup>* in recognition of the discovery that the Javanese and Booroola sheep carry the same mutation.

Javanese sheep may have acquired the Booroola gene from Australian Merinos. Merkens and Soemirat [27] reported that the Garut strain of the Javanese Thin-tailed sheep could have come from crosses between local sheep, Merino and Kaapstadt, about 1865. The Merinos that were used for crossing were imported from Australia in the 1860s and bred to Garut sheep for wool, meat, and manure production. Merino rams were given to the Regent of Limbangan (Garut) and others, who started to cross them with the local sheep. However, it is more probable that the Booroola gene came directly from India; some livestock, such as the Jamunapari goat from Ettawah, were brought directly to Indonesia from India. Trade between India and Indonesia was firmly established by the first century A.D. and flourished in the Sumatran coastal state of Shrivajaya from the 7th to 12th centuries [28]. Bradford and Inouu [25] suggested that the thin-tailed sheep of Java probably originated from the India/Bangladesh region of Asia. Reports that Javanese Thin-tailed ewes weigh only 17–30 kg [26] and have a phenotype resembling Garoles, including the characteristic of earlessness segregating in the population, are consistent with the hypothesis that there had been earlier crossbreeding with the Garole microsheap.

The effect of one copy of *FecB* on ovulation rate in the Javanese sheep is about 0.8 [29], which is only half that reported for *FecB* in other breeds by Piper et al. [11]. The difference could be due to a lower prolificacy potential from the background genotype, to environmental factors such as the relatively low nutritional value of the tropical forages available to these ewes, or to a combination of these factors. Litter sizes of ewes that were noncarrier, heterozygous, or homozygous for *FecJ<sup>F</sup>* have been measured at 1.24, 1.95, and 2.59, respectively [22]. Bradford and Inouu [25] considered that homozygous ewes had too many lambs for Indonesian farming conditions and suggested that heterozygous ewes would be best under good conditions and noncarriers would be best under poorer farming conditions. The discovery that *FecB* is segregating in Javanese sheep and the availability of a DNA test to identify carriers opens up the opportunity for the effective use of *FecB* to improve sheep production in Indonesia by matching carrier and non-carrier animals to the appropriate management level.

The absence of *FecB* in the Thoka, Woodlands, Olkuska, Lacaune, Belclare, and Cambridge flocks indicates that other mutations affecting ovulation rate must be present in these sheep. Different mutations in an autosomal gene are likely to be responsible for the increase in ovulation rate

of about 1.2 in Thoka sheep [30] and 1.0 in Olkuska sheep [23] and the high ovulation rate (5.8) in Lacaune sheep [31]. Although the mutation has not been identified, there is strong evidence from the size of the effect and the mode of inheritance that *FecX2* is a separate mutation on the X-chromosome of Woodlands sheep [10]. The inheritance of prolificacy in the Belclare and Cambridge flocks has been described as a somewhat inconsistent pattern, and the hypothesis of a one-locus model may be too simple to explain the pattern of variation in these two populations [8]. However, progeny test data for both Cambridge and Belclare rams have indicated that the variability in ovulation rate cannot be explained by simple polygenic inheritance [32].

There is no evidence that any of the other sheep studied are related to the Merino, Garole, or Javanese sheep in which *FecB* has been identified. The Thoka family line was identified in Icelandic sheep. The Woodlands gene was found in the Coopworth breed, which was established by interbreeding Romney and Border Leicester sheep in New Zealand. Olkuska sheep were established by crossing long-wool Polish sheep with Pomeranian, Friesian, and Holstein sheep about 100 yr ago, and Lacaune are long-established dairy and meat sheep in Roquefort, France. The Belclare and Cambridge breeds were established by screening prolific ewes from several breeds in Ireland and Great Britain, respectively, and in both cases there was a significant incorporation of genetic material from the Finnish Landrace breed, which appears to have high prolificacy inherited quantitatively.

The finding that none of the sheep sampled carried *FecX1* was not unexpected. The effect of the Thoka gene on litter size (+0.6) and 15% sterility in female progeny of a carrier ram mated to carrier ewes [5] were suggestive of the Inverdale gene, but inheritance studies support the hypothesis that the gene is autosomal. The Woodlands gene (*FecX2*) is X-linked, but the smaller effect on ovulation rate coupled with imprinting effects are evidence that the *FecX2* locus is separate from *FecX*. Sterility has also been observed in some Belclare [33] and Cambridge [8] ewes, and a model proposing an autosomal gene similar to Booroola and an X-linked gene similar to Inverdale has been proposed for the Cambridge sheep [34].

The discovery of the *FecB* mutation in Garole sheep strongly supports the theory that the Booroola gene was introduced into Australia through Garole sheep late in the 18th century. Knowledge that *FecB* is present in prolific Garole and Javanese sheep will allow breeding strategies to be developed that maximize the benefits of increased prolificacy in these breeds and their crosses.

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