INVESTING IN GENOMICS – A NEW ERA FOR SMALL STOCK BREEDING

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Genomic services provided to Small Stock breeds associated with SA Stud Book allows breeders to take the next step in boosting the prediction of an animal's true genetic merit, ensuring pedigree integrity and unravelling the status of single genes within your flock. Breeders unfamiliar with genomics and genomically enhanced breeding values (GEBVs) must be wondering what the benefit of genotyping your stud animals truly is. This short article will hopefully illustrate the impact of genotyping an animal, the effect of parentage verification and how genomics is integrated into a genetic evaluation to enhance the accuracy of breeding values.

Parentage Verification

The use of DNA Microsatellite markers for Parentage Verification is widespread across the livestock industry in South Africa, but has limitations that can result in the incorrect identification of an animal's parentage. The DNA Microsatellite test is not a true confirmation of parentage, but rather an exclusion of who is not the parent. The low number of markers used (12–25) may result in more than one animal being nominated as the possible sire of the tested progeny, especially if a breeder is using rams that are highly related to each other due to a common ancestor.

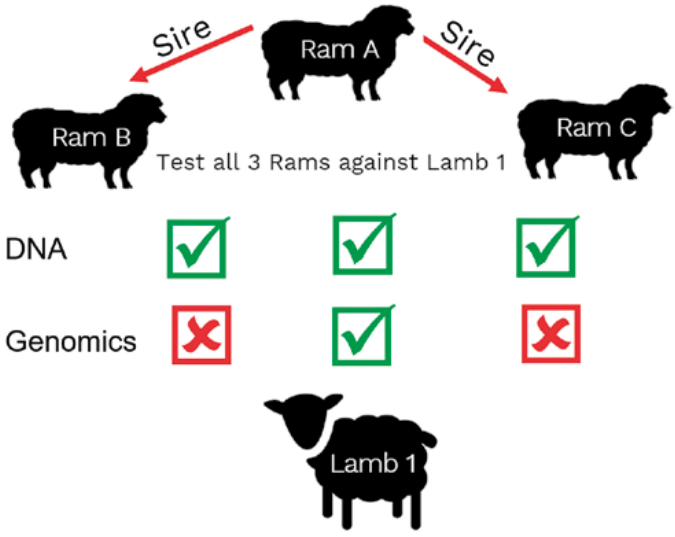

Figure 1: This picture indicates how DNA micro-satellites would indicate that all 3 sires could possibly be the sire of Lamb 1, while only the genomics can truly confirm that Ram A is the correct sire of Lamb 1.

Genomics, which is currently the most powerful commercially available tool for parentage verification, uses around 15 000 to 25 000 markers (SNPs) to correctly identify the animals that are directly related to each other. Using a stringent 1% mismatch threshold, this means that a parent will be excluded as the true sire when 150 to 250 markers are not in alignment with the progeny they are tested against. The analysis of opposing homozygotes, illustrated in Table 1, allows for the confirmation that the tested animals are indeed directly related to each other.

Table 1: How parentage is determined using opposing homozygotes for genomically tested animals.

	SNP 1	MATCH?	SNP 2	MATCH?	SNP 3	MATCH?	SNP 4	MATCH?
LAMB 1	AA		BB		BB		AA	
SIRE A	AA	☑	BB	☑	BB	☑	AA	☑
SIRE B	BB	☒	AA	☒	AA	☒	AA	☑

The concept of opposing homozygotes is simply illustrated above, with two sires being tested against Lamb 1. The genomic marker for an animal can only be AA, AB or BB, with the informative marker being the homozygotes or fixed markers (AA or BB). Sire A is a positive match for all four markers as he can only pass on an A marker to his progeny at SNP 1 and SNP 4, and a B marker at SNP 2 and 3. Sire B has 3/4 conflicts as at SNP 1 he can only pass on the B marker, while Lamb 1 is AA meaning, he has a mismatch for SNP 1 against Lamb 1. The number of mismatches is summed and if it exceeds 1% (150 – 250 markers), this will arise in a sire conflict. Parentage is verified when the number of mismatches is therefore less than 1%.

Genetic Relatedness using Genomics

Apart from accurate parentage verification and knowing the status of specific single-gene mutations, how does genomics benefit the breeder's ability to more accurately select his top animals? Research on genomics in beef and dairy cattle, as well as small stock breeds, indicates that genomics has the biggest impact on traits that are lowly heritable, sex-limited and only measured later in life.

The integration of genomic data into a genomic evaluation does one simple thing, it fills in the gaps of the pedigree. The pedigree will at times indicate that animals within the same breed are not related, as breeders may not yet have genetically linked their flocks by using other breeders' animals, which is known to be a limitation of using simple mendelian inheritance as the pattern of inheritance across generations. Animals within the same breed will be inherently related to each other, as they are the same species (in this case sheep) and of the same breed (Merino), indicating that they will indeed share a similar genetic makeup. Table 2 showcases how the pedigree matrix uses simple mendelian inheritance to estimate genetic relatedness, while Table 3 showcases the genomic matrix using the 60 000 markers in conjunction with the pedigree matrix, to refine and truly estimate the relatedness between the animals.

Table 2: Genetic relatedness between animals using the pedigree and conventional mendelian inheritance.

	MER_1	MER_2	MER_3	MER_4	MER_5	MER_6
MER_1	1.00					
MER_2	0.00	1.00				
MER_3	0.25	0.0625	1.00			
MER_4	0.00	0.00	0.00	1.00		
MER_5	0.125	0.0625	0.125	0.00	1.00	
MER_6	0.50	0.03125	0.50	0.00	0.0625	1.00

Animal 6 is the Sire of both Animals 1 and 3, which is indicated by the 50% relatedness estimated between them. This makes Animal 1 and 3 half-siblings, meaning that it is assumed they share 25% of the same genetic makeup from the sire's side. Animal 5 is a close relative to Animals 1 and 3, with the pedigree estimating that they are 12.5% related. Animal 4 has no pedigree links to any of the other animals; thus it is assumed that they are 0% related to the rest of the animals in Table 1.

Table 3: Genetic relatedness between animals combining the pedigree and the genomic information to estimate the true genetic relationships between these animals

	MER_1	MER_2	MER_3	MER_4	MER_5	MER_6
MER_1	1.00					
MER_2	0.06	1.00				
MER_3	0.315	0.08	1.00			
MER_4	0.04	0.03	0.07	1.00		
MER_5	0.146	0.05	0.095	0.01	1.00	
MER_6	0.50	0.02	0.50	0.04	0.085	1.00

By comparing Table 2 with Table 3, it can be seen that the genomics has corrected the assumed genetic relationships to realised genetic relationships. Animals 1 and 3, who are half-siblings, are now indicated to be 31.5% related, meaning they inherited more similar genetics from the sire than was originally assumed. The genomics has revealed that Animal 5 is more related to Animal 1 (14.6%) than to Animal 3 (9.5%), while Animal 4 is no longer not linked to the other animals, but is between 1% - 7% related to them. Genomics allows the BLUP analysis to make connections between genetically related animals that the conventional pedigrees are unable to make and thus aids in accurately predicting an animal's genetic potential not just from animals it is directly related to.

Single Genes available through Genomic Testing

The horn/poll mutation (located on the *RXFP2* gene) was verified in an Australian study as being 70% accurate in identifying polledness in Merino sheep, with the Polled variant showing dominance over the Horned variant. So far we have verified 28/29 phenotypes with corresponding genotypes. PP rams (double-polled) were completely polled (no scurs) or showed the "Bud" phenotype. PH rams (carriers of the polled and horned genes) were polled with scurs, indicating possible epistasis or incomplete penetrance of the gene. HH rams were always phenotypically horned. One ram tested HH genomically, but was phenotypically polled. The last batch of genotypes from the Cape Wools project in conjunction with over 1 500 horn/poll phenotypes will allow for a more comprehensive study on the horn/poll phenotypes, and let us verify this mutation in our population.

Milk related mutations include *FABP4* and *DGAT*. *FABP4* is a mutation that is known to increase butterfat production in milk as well as increases the fat thickness and inter-muscular fat of the carcass. DGAT1 in sheep has a similar effect as seen in dairy cattle breeds: It increases butterfat production, butterfat and protein percentages while decreasing milk and milk-protein production.

Double muscling in sheep was identified in the Texel breed on the GDF8 gene, which has a translational effect on the myostatin protein, thus causing a lower production in myostatin, and causes muscular hypertrophy. A lack of myostatin in sheep causes an increase in muscle size (higher dressing %) and a decrease in fat deposition (carcass and IMF). The effects observed at a phenotypic level include a minor impact on live weight and growth performance, while a significant effect can be seen on muscling and fatness at slaughter. This mutation has already been identified in 3 SA breeds, indicating this mutation is not novel to the Texel breed and comes from an ancestral population. Unlike in beef cattle, the double-muscling mutation in sheep does not break the myostatin protein, but rather limits the amount that is produced.

Transmissible Spongiform Encephalopathy, known as Scrapie, is a neurological disorder which is similar to Mad Cow Disease. A prion, simplified as a corrupted protein, is transmissible between sheep through ingestion or (in)direct contact with an infect placenta, birth fluids and milk. No animal is immune to this prion, but animals do show varying levels of resistance depending on what combination of genes they may carry. A haplotype, which is different from a single gene, consists of a small segment of DNA that is inherited together from each parent. The ARQ haplotype is the original or wild-type mutation and shows normal resistance. ARR shows the highest levels of resistance against Scrapie and we at SA Stud Book encourage breeders to use animals with at least one copy of ARR in breeding programs in order to increase its frequency within the flock. The VRQ haplotype shows high levels of susceptibility and is the combination we don't advise breeders to breed with. The SA Merino population has a high frequency of the ARR variant, with 50% of the SA Ovine Genomic Population carrying at least 1 copy of the ARR variant. VRQ only appears in 7% of the population and may already be indirectly selected against.

Ovine Progressive Pneumonia (OPP), also known as Lentivirus, is caused by a retrovirus that targets lungs, joints, nervous system and udder of sheep breeds. There is no treatment and or vaccine and because of this, it can be thought of as "Sheep HIV". Afflicted animals can be seen to have fever, cough and nasal discharge, while the end of life is characterised by loss of body condition ("thin ewe syndrome"). Serological tests are ongoing around the world, picking up Lentivirus in all populations tested. As seen with Scrapie, a mutation that confers resistance to this retrovirus has been identified on the TMEM154 gene. The wild type variant is known as "E", while the mutation conferring resistance is "K". 38% of tested sheep in South Africa carry 2 copies of K, giving them the most resistance to the retrovirus. 85% of the population carry at least 1 copy of K.

The use of genomics is ushering in a new era of stud breeding, especially for breeders who may have closed flocks or are new members of their society. The recording of performance and fertility traits will not be replaced by genomics, as genomics only assists in boosting the pedigree side of the BLUP equation. Phenotype will therefore remain King in Animal Breeding, while Genomics is the Queen the King has always been looking for.