

Breech Strike Genetics

This Project is a collaborative research effort of CSIRO Agriculture, Armidale, NSW and Department of Agriculture and Food WA supported by Australian Wool Innovation Limited

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With completion of the Breech strike genetics projects at Armidale, this is the last newsletter in this series. The experimental program for the project was complete in 2015 once the 2014 drop progeny were recorded to yearling age. Since then, the focus has been on statistical analysis of the entire dataset and reporting to AWI. Here we include a summary of the genetic analyses and implications for the wool growing industry. We also report on some of the other activities that were undertaken in the last few years for the study.

The sheep are not going to disappear just yet. The breeding flock will become part of the base ewe flock of the New England site Merino Lifetime Productivity Project and will continued to be recorded for certain traits for the next couple of years.

The 2014 drop progeny were the last to be fully recorded up to yearling age. The last few years of the project were a little challenging climatically for breech strike genetics work at Armidale. We went through a particularly dry period in 2013 and 2014. The flock was supplementary fed continuously from mating in early April 2013 to the end of lambing in October 2014. Alongside that, the 2013-14 fly season was particularly long; running from mid-October 2013 to late June 2014.

We have conducted a genomics project using the Ovine High Density (600K) beadchip, results of which are summarised here. Dr Sonja Dominic, the CSIRO scientist who conducted the analysis regards this work as a 'comprehensive first pass' at investigating the genomics of breech flystrike.

We have also been collecting wool and skin samples from animals in the Armidale flock for collaborative work with DAFWA and UWA on skin bacterial populations of Resistant and Susceptible sheep, and work on wool odour.

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Figure 1. Armidale Breech Strike Genetics flock ewes and lambs on the plots at lambing 2014

Project summary of results 2005-2015

an extract from the Executive summary of the Project final report

- AWI projects on breech strike genetics ran at the CSIRO property Chiswick, Armidale for 10 years between 2005 and 2015.
- During that time the flock was fully pedigreed, and comprehensively measured for a wide range of conformation and production traits. The flock was managed under flystrike challenge conditions (i.e. in the absence of preventative chemicals) in order to record breech strike incidence. Through the strategic use of industry link sires and inclusion in Merino Select, this flock makes a valuable contribution for benchmarking and encouragement of uptake of breeding for breech strike resistance in industry.
- Interim phenotypic and genetic parameters for breech strike, flystrike indicators, and production traits in this flock have been reported in earlier newsletters and in reports to AWI. The Final Report includes revised phenotypic and genetic parameters using the entire dataset, and in due course this will be reported in the scientific literature. In summary, results are;
- Breech strike at weaner, yearling and adult age has low to moderate heritability (0.16 - 0.26). *This means that selection can be used to reduce breech strike, but it might be better and simpler to use an indirect selection criterion rather than breech strike itself*
- Breech strike of weaners, yearlings and adults have moderate to high genetic correlations (0.26 - 0.92). *This means that animals that get struck as young sheep are likely to get struck as older animals, so cull them.*
- Many potential indirect selection criteria for breech strike were evaluated. Breech wrinkle, dag, urine and breech cover have suitable combinations of phenotypic and genetic parameters which make them appropriate candidates as indirect selection criteria for breech strike.
- ***The most suitable indirect selection criterion for breech strike for fine wool sheep in the summer rainfall environment is breech wrinkle (heritability 0.36, genetic correlation with breech strike 0.42).***
- Merino Select has provided across-flock ASBV's for these three traits since 2009 and they remain relevant and suitable to aid selection for improved breech strike resistance in Merinos. *If you are a ram buyer, look for rams with negative ASBV values for breech wrinkle breech cover and dag to improve your flock's breech strike resistance.*
- There are some moderately unfavourable associations between indirect selection criteria for breech strike and economically important production traits. The largest, and most important of these are the genetic correlations between adult clean fleece weight and breech wrinkle (0.38), and between adult fibre diameter and breech wrinkle (-0.23). *It is important to note that while these associations are not so strong as to preclude concurrent genetic gain in both breech strike resistance and fleece traits, they are an impediment to adoption of breeding objectives aimed at improving wool quality, quantity and breech strike resistance of Merinos.*
- There are also many favourable associations between the indirect selection criteria for breech strike and production traits, particularly those involving body weight and variation in fibre diameter. *So there are additional advantages to be had in some production traits by selecting animals that are plainer and barer.*
- Earlier recommendations to industry regarding inclusion of breech strike into Merino breeding programs generally remain unchanged. ***That is, the indirect selection criterion of choice should be dependent upon your sheep type, breeding objective, and production environment (climate).***

Flystrike Results in 2013-14

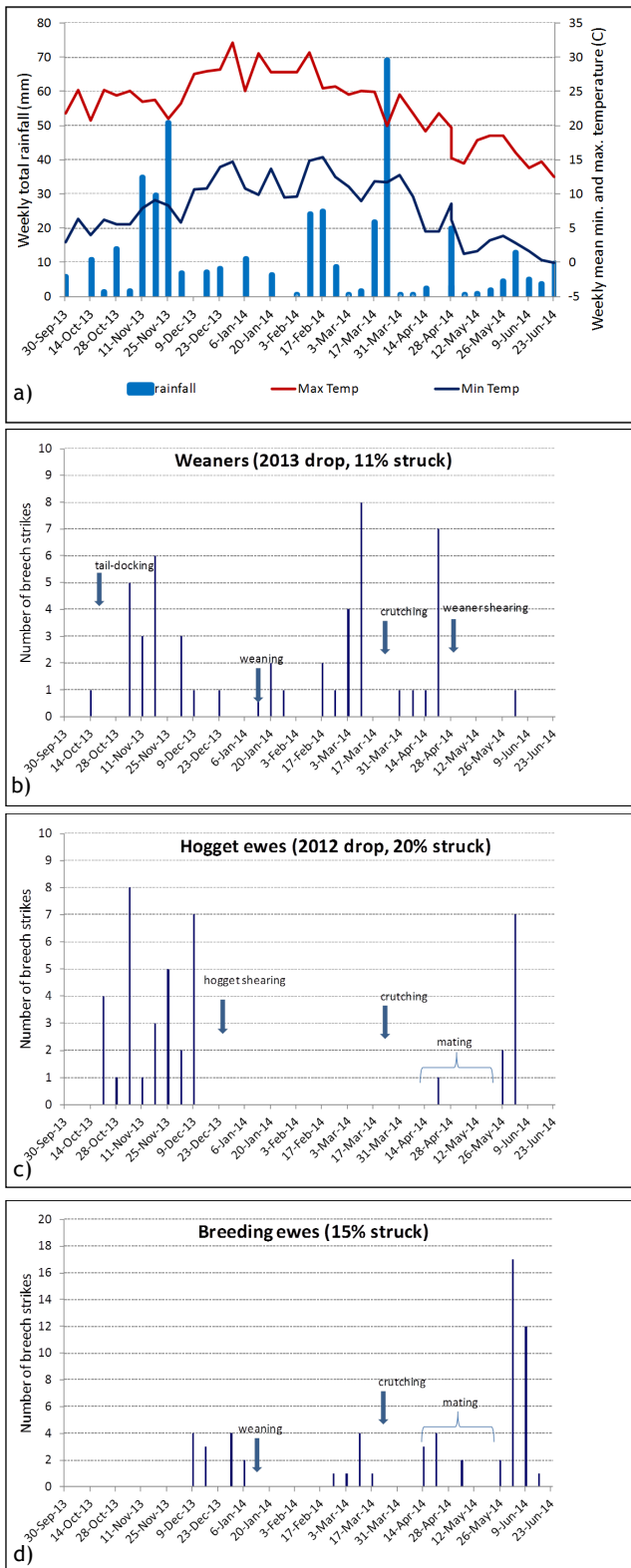


Figure 2. a) Rainfall and temperature during the 2013-14 flystrike season at Armidale, NSW, with corresponding flystrike incidence in b) mixed-sex weaners sheep, c) hogget ewes, and d) breeding ewes.

In the New England environment the fly season is largely governed by the combined effects of rainfall and temperature with flies active in the period from spring to autumn. In most years there is a run of at least moderate frosts by the end of April. Fly activity ceases with that, and recommences again the following spring.

The 2013-14 flystrike season at Armidale turned out to be an anomaly. Climatically, it was an unusual year and that, combined with the management program of the sheep, resulted in a fly strike season that ran from mid-October to late-June. Rainfall was low, and autumn temperatures were particularly mild. Rainfall during the fly season was approximately two-thirds of the long term average for the period and much of that came in just a few major rain events. This kept the flystrike rates low to moderate for most of the usual fly season (October to April inclusive). However, there was no run of hard frosts until mid-June, and hence, fly activity continued to that time (Fig 2 a)). As a result of those rainfall and temperature patterns, the overall flystrike rates in the flock were moderate (11-20% across the different sheep classes), but the fly season was very long.

Both the 2012 and 2013 drop progeny were involved in a separate, post-weaner fleece traits study that meant they were shorn at times of year when they would not normally be shorn (December 2013 and April 2014 respectively). These out-of-season shearings resulted in seasonal flystrike patterns that vary from what we would expect if they were shorn as yearlings, which is standard practice.

The 2013 drop weaners (Fig 2b)) demonstrated flystrike in accordance with the weather pattern up until they were shorn in April 2014. There were several rain events in November, with accompanying strikes. It was dry through December, January and February, with few strikes. The flystrike rate began to increase in March, but was halted by crutching. Flystrike peaked again in April, just before shearing.

The 2012 drop hogget ewes (Fig 2c)), experienced high breech strike rates through the spring and until they were shorn in December. Normally, if shorn as yearlings they would have been in short wool in the spring, and the flystrike rate would probably have been lower than it was here. The December shearing, and then crutching kept the flies out of those ewes throughout the summer and early autumn.

The breeding flock (Fig 1d)) exhibited only low flystrike rates throughout the spring, summer and autumn in accordance with the low rainfall. The protection provided by crutching in March was not sufficient to get the ewes past the usual flystrike danger period because the frosts came very late in 2013. This was also the case for the ewe hoggets.

Breech flystrike genomics

Background

The question was: Are genomic tools useful for genetic improvement of breech strike resistance and indicator traits?

The preliminary answer: Potentially yes, because...

- Flystrike and indicators are heritable
- There are genetic correlations between flystrike and several indicator traits
- Flystrike is a difficult trait to record in industry
- Genomics is potentially an approach that could fast-track genetic gain by enabling selection at young age, and selection where the trait isn't well expressed all of the time.

The proposed method was to use genome wide association study (GWAS) to find associations between the animals genotype and physical characteristics. Genotype information can be used in different ways depending upon whether there are few, some or very many significant associations.

In this area of research, available technology moves very quickly. In the period between when we proposed the project to AWI and the work commenced, available technology moved from the Ovine 50K to 600K beadchip.

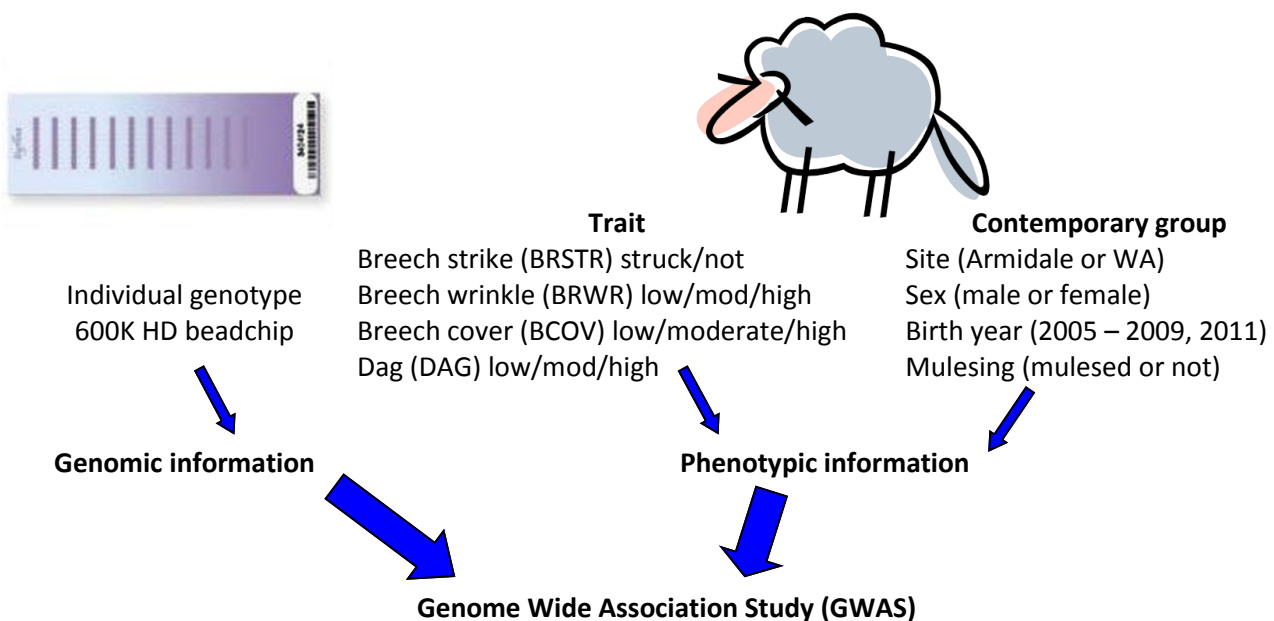
Hence, considerable lead-up time and effort was spent working out, through various modelling and simulation exercises, the most efficient approach for genotyping given the available resources.

Method

We used about 950 animals born across several years in the Armidale and WA breech strike genetics flocks (although more were from the Armidale flock). Animals were assigned to groups based on 4 traits of interest (see diagram below), as well as contemporary groups. There were groups of animals that fitted almost every contemporary group and trait combination, but a few missing cells. Armidale only had low DAG, and WA only had low BRWR.

Individuals were genotyped using the Ovine 600K beadchip. The genotype and phenotype (or physical) information was combined in a GWAS. GWAS enables us to look for an association between each SNP (single nucleotide polymorphism, or each single little piece of genomic information) and a certain phenotype (physical characteristic) of each sheep. A significant association between the genotype and phenotype occurs at the population level when the same allele at a particular SNP is consistently associated with a certain phenotype across families in that population.

The GWA identifies how many associations there are between genomic and physical characteristics and where they are on the genome.



Breech flystrike genomics (cont.)

Results and Discussion

The result in graph form of the GWAS is a so-called 'Manhattan plot'. We have one of these for each of the 4 traits. I've only shown the one for BCOV here (Fig 4). It shows the association between the trait and each of the 600K SNP along the sheep genome (26 chromosomes). The higher the peak, the more evidence for an association. We are looking for 'clusters' with peaks. In this data, we see lots of peaks, but no real standouts.

The horizontal line on the graph is the significance threshold. For this job, it was set to be very strict. BCOV was the only one of the 4 traits with SNP above that significance threshold. This is probably because there was most variation in BCOV – BRSTR is a binomial trait (struck/not), there was little variation in BRWR in WA, and little variation in DAG at Armidale. Initially there were 612 significant SNP for BRSTR, but once the significance threshold was imposed, there were none.

For these traits, we have established there are lots of SNP of small effect. This suggests that genomic information on breech strike in the future might be useful in the form of genomic estimated breeding values (GEBV). Genomics assisted selection relies on a reference population that is both genotyped and well phenotyped (measured), and that has good genetic links to industry flocks. Then, other animals out in industry can be genotyped, and even if they aren't measured for a particular trait, can be evaluated for that trait via a GEBV.

To have confidence in this method, validation is required to confirm that associations between SNP and a trait in the experimental population hold up in industry populations. At the moment, we don't have a validation population for breech strike. We have done preliminary validation work by splitting the existing dataset – using part as the reference population and part as the validation population. The result is that currently the accuracy of GEBVs for breech strike are not sufficiently high for use by industry. To rectify that, more animals would need to be genotyped and recorded for breech strike. This would not necessarily all need to be using high density genotypes (high cost). For some animals low density genotypes (lower cost) may suffice.

While genotyping is expensive, phenotyping for breech strike is also an expensive exercise – it takes a long time, a lot of labour, and is at the whim of the prevailing environment. However, selection for breech strike resistance using indirect criteria such as breech wrinkle is relatively more cost effective.

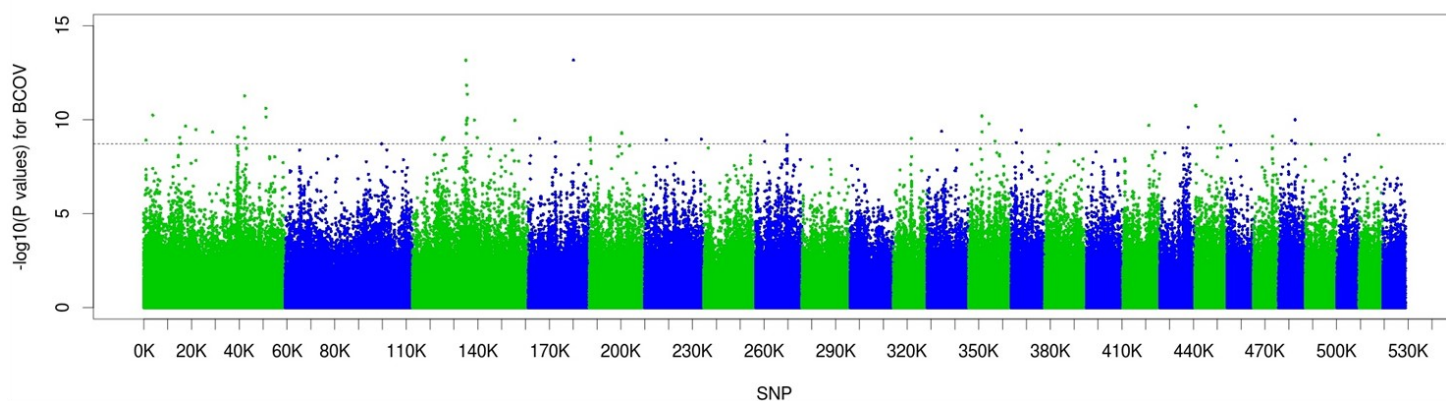


Figure 4. Manhattan plot from single SNP regression for breech cover (BCOV), the line indicates the Bonferroni corrected $P > 0.001$.

Summary

We don't have a genomics tool for breech strike yet, but it may come in the future. The next step in this work is additional analytical work using the genotypes we already have to determine whether an alternative statistical method may be more appropriate. There is currently no follow-on collaborative work in this area, but CSIRO is pursuing haplotype based analysis and sequence analysis of a particular gene identified in the haplotype analysis work. We haven't discounted the prospect of a low density targeted SNP panel. We continue to collect DNA from the research flocks for potential use at a later date.

Does tail-docking method have an effect on breech strike?

A study was conducted to determine whether the method of tail-docking of lambs affected flystrike rates in later life. The study was conducted over 2 consecutive drops of lambs (2012 and 2013 drop) and we have flystrike data on both drops in their first (weaner) and second (yearling) flystrike challenge seasons.

Method

In both years lamb marking was conducted in mid-October (median marking age 30 days). An experienced lamb-marking contractor (Lenehan Contracting) was engaged to conduct the tail-docking.

Lambs were pre-assigned to one of four treatment groups that were balanced for selection line, sire, sex, birth-rearing type, age of dam, and age. Lambs were weighed at to marking. In each year there were at least 100 lambs in each treatment group and at least 50 each of males and females. Treatment groups were tail-docking methods, being;

- cold knife (COLD),
- 'regular' hot docking iron (HOT),
- elastrator ring (RING),
- and Te Pari Patesco hot docking iron (TEPARI)

For lambs docked using elastrator rings, the tail was also cut off approximately 3cm below the ring, which was standard practice by that contractor.

The large majority (in excess of 95%) of all strikes in this flock are regarded to have started on the breech (below tail) rather than on the tail itself.

Results

In most of the year-sex-age classes the differences in breech strike rates were not significant (Fig 5). Exceptions were for COLD being associated with greater breech strike in 2012 drop female weaners, and 2013 drop male yearlings, and for TEPARI being associated with greater breech strike in 2013 drop female weaners.

In some other cases there can appear to be quite large differences in strike rates, but this is associated with the group sizes and the nature of the trait—that breech strike is a 'yes/no' trait—either struck or not, rather than a continuous scale like lots of production traits, and that environmental effects on breech strike rates are large. These factors together mean there are large confidence limits around the breech strike rates.

There does not appear to be any consistent association between tail docking method and breech strike rate of weaners or yearlings.

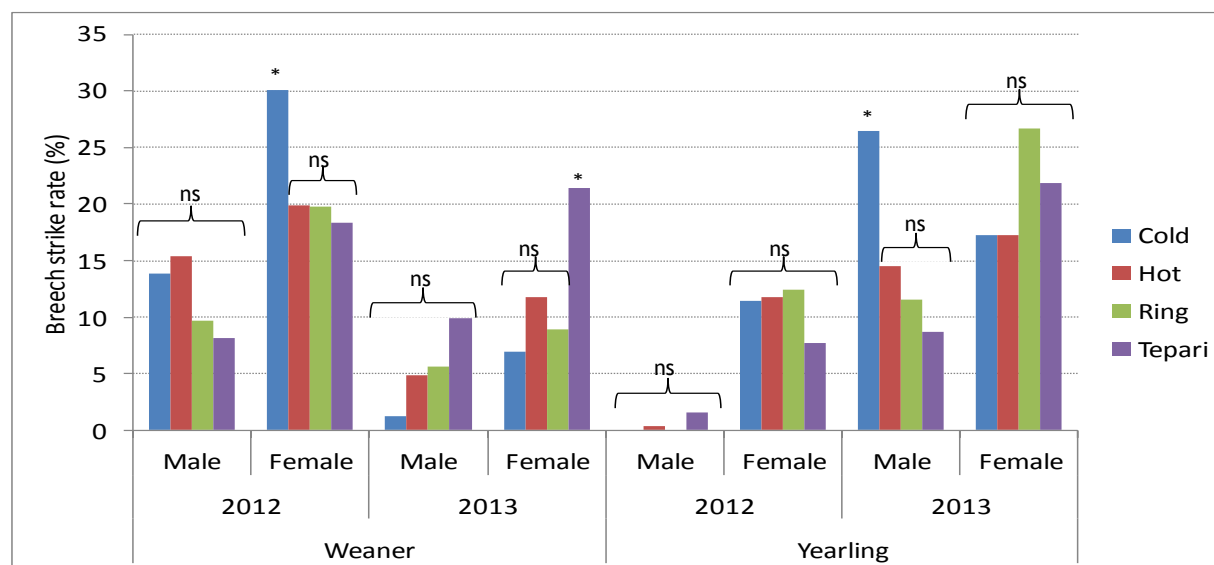


Figure 5. Breech strike rates among weaners and yearlings using 4 tail-docking methods.

Conclusion

Flystrike rates among the tail-docking methods ranked differently across birth years, sexes and ages. Hence, there is no consistent evidence that tail-docking method affects subsequent flystrike rates.

An observation survey of the breech strike genetics flock breeding ewes in 2014

During 2014 we were fortunate to have a visiting French agriculture student working with us, Marie-Alice Bauland. One of the things Marie did while she was here was take a set of photographs of the ewes when we put them on the plots for lambing. We used those photos for a survey exercise around ability to discriminate, simply based on physical appearance, between the selection lines.

Mating and lambing are the only times of year when the ewes are split into the two selection lines, Resistant and Susceptible. In 2014 the ewes were split across 32 plots. They were on the point of lambing at the time the photos were taken, so their wrinkle characteristics may be biased by that. Only one person (Heather) knew the allocation of ewes to plots, and for that reason she did not participate in the survey.

Members of the Project team viewed the photos and were to decide whether the ewes in each plot were Resistant or Susceptible. Several other CSIRO staff members not involved with the Project did the same. Nobody got all 32 groups correct. Happily, I (Jen) was correct on all but 2 plots, as was Marie. Everyone else who took part was correct with at least 70% of the groups.

This suggests to me that its not all that difficult, even for those unfamiliar with these particular sheep, to distinguish those that are more or less likely to get flystruck, just by looking at their wrinkle and wool cover characteristics.

The ewes in the selection lines are different in their average wrinkle and wool cover characteristics, and the differences between lines are greater for wrinkle than wool cover (Table 1). However, as you can see from Fig 6, there is certainly overlap between the lines – there are some plain sheep in the Susceptible line and some wrinkly sheep in the Resistant line. The overall differences in wrinkle and wool cover however, translate into very large differences in breech strike. *As an example, in the 2014-15 fly season which ran from October 2014 to March 2015, there was 2% breech strikes in the Resistant line ewes, and 33% breech strikes in the Susceptible line ewes.*

Table 1. Selection line means for key wrinkle and wool cover traits for the breeding flock in 2014, measured at yearling age

Breeding flock 2014	Neck wrinkle	Body wrinkle	Breech wrinkle	Crutch cover	Breech cover	Breech strike 2014-15 (%)
Resistant line mean	2.3	1.7	2.1	3.4	4.0	2
Susceptible line mean	3.0	2.6	3.4	3.9	4.4	33
Overall Mean	2.6	2.2	2.8	3.6	4.2	18
Mean difference between lines	0.7	0.9	1.3	0.5	0.4	31

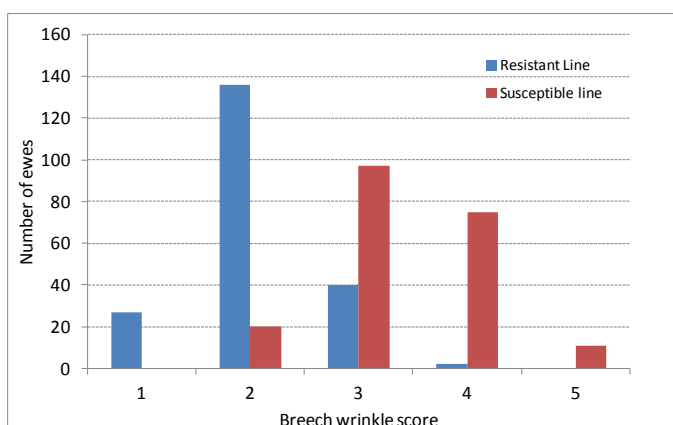


Figure 6. Frequency distribution of ewes in the Resistant and Susceptible lines in 2014. The majority of Resistant line are breech wrinkle score 2, while the Susceptible line are mostly 3 or 4.



Figure 7. Marie and Heather on the 2nd day of Spring heading out to do lambing rounds. Despite appearances, the weather was mostly fairly mild at lambing in 2014!

Examples of the Armidale breec strike flock ewes at lambing in 2014

Resistant line ewes →
↓



Susceptible line ewes

↙ ↓



Earlier issues of this newsletter outlined the project background, objectives and design, along with progress reports and interim results. Copies are available from Heather Brewer using details below or go to http://www.wool.com/Grow_Animal-Health_Flystrike-prevention.htm

Breec Strike Genetics is produced by
CSIRO Agriculture Flagship
FD McMaster Laboratory, New England Highway, Armidale NSW 2350
Jen Smith: 02 6776 1381, jen.smith@csiro.au
Heather Brewer: 02 6776 1385, heather.brewer@csiro.au

DAFWA
Johan Greeff: 08 9368 3624
jgreeff@agric.wa.gov.au
John Karlsson: 08 9821 3221
jkarlsson@agric.wa.gov.au

AWI
Geoff Lindon
geoff.lindon@wool.com

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