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Gene by environment interaction for reproduction traits in Australian sheep

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Abstract

This study had two aims. It firstly established the existence or absence of genotype x environment (GxE) interactions in Merino sheep in the Information Nucleus flock and secondly, if GxE interactions were present, determined genomic regions associated with robustness of sire performance for reproduction traits across environments. Analyses demonstrated the presence of GxE interactions of only small effect and no conclusive evidence of associations with underlying genomic regions with robustness in reproduction trait was found. Despite the lack of significant SNP associations with robustness for reproduction traits, it was concluded that the random regression approach in combination with genomic information offer new approaches to explore GxE interactions in production traits with higher heritability. Novel measurements techniques for reproduction traits might disentangle the complex phenotype and describe the underlying genetic processes more precisely than can currently be done with the traditional phenotypes.

Executive summary

Reproduction is one of the most important profit drivers in the Merino industry, but genetic progress is generally difficult to achieve due to low heritabilities of the breeding objective traits associated with reproductive performance. It is unclear if the low heritability is due to a lack of precision in the definition of the environment, or the actual phenotype or because other sources of variance such as non-additive genetic effects or variation due to genotype x environment (GxE) interactions are important.

If GxE interaction is a major source of phenotypic variation for reproduction traits, a selection strategy for robust genotypes could lead to more consistent performance in reproduction performance and lamb survival. This approach could lead overall to more robust and consistent production performance, buffering periods of sub-optimal environmental conditions.

This study investigated two aspects. Firstly, the existence GxE interactions in Merino sheep in the Information Nucleus Flock were explored. Different analysis approaches were taken to define the environmental descriptor to explore if different definitions facilitate separation of genetic and environmental variances and their interactions. Confirming the results from previous analyses, some evidence of the existence of small GxE interactions for reproduction traits was found, but they only accounted for small proportions of phenotypic variance. All analyses yielded low heritabilities.

The second aspect of this study explored a random regression approach to determine the robustness of sire performance across environments and subsequently determine underlying genomic regions and / or genes for this characteristic in a genome wide association study (GWAS). This approach has been suggested by Lillehammer et al. (2009) who detected underlying genes for robustness for milk production traits. This study demonstrated that the variability of sires' reproductive performance across environments was low and therefore it was unlikely that a GWAS would detect significant SNPs of large effect on reproductive performance and conclusive evidence for underlying genes. Rather, the results indicated that robustness in reproduction across environments in Merino sheep of the INF is influenced by a large number of genes of small effect.

Genomic information certainly offer a wider range of approaches to explore GxE interactions compared classical quantitative genetic analysis methods and have the potential to provide tangible solutions to industry, such as marker tests or genomic breeding values for robustness of production traits. No conclusive evidence of marker associations with robustness for reproduction traits were detected in this study. However, for production traits where GxE interactions have been shown to be important, the random regression approach coupled with a GWAS could potentially be a useful methodology to determine genes underlying robustness. Also, novel measurements techniques for reproduction traits might disentangle the complex phenotype and describe the underlying genetic processes more precisely than can currently be done with the traditional phenotypes.

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1. Background

Reproduction is one of the most important profit drivers in the Merino industry, but genetic progress is generally difficult to achieve due to low heritabilities of the breeding objective traits associated with reproductive performance (Safari et al., 2005). Major genes with large effects on ovulation rate have been detected (Davies, 2005), but marker-assisted-introgression programs have largely been unsuccessful due to complicated inheritance patterns, large effects and gene interactions. Low heritabilities could be due to several reasons, one reason being that traditionally measured traits, such as number of lambs born and weaned, might be insufficient descriptors of the genetic components of the complex underlying processes of reproduction. An alternative reason could be that other sources of variance such as non-additive genetic effects or variation due to genotype x environment (GxE) interaction are important.

Few studies have investigated GxE interactions for reproduction traits in sheep. Significant sire x season interactions were reported for lambing performance of crossbred ewes of the Maternal Sire Central Progeny Test (Fogarty et al. 2001). A study on the Trangie Weight Selection Experiment (Amores et al. 1999) reported significant interactions of selection line with location and year, suggesting an effect on relative reproductive performance. Notter et al. (2000a, b) found little evidence for GxE interactions for reproduction traits, lamb growth and ewe body size in cross bred sheep. The question arises if GxE interactions do not exist or if the definition of the environmental variable is imprecise in the context of interactions with genotypes. An alternative definition of the environment to the traditional categorization of environment as a physical or temporal entity, such as “flock” or “year”, might be a more appropriate categorization to distinguish between environments in a relevant manner.

Large data sets are required to explore the existence of GxE interactions comprehensively. The Information Nucleus Flock (INF) has been designed with the objective to estimated genetic and phenotypic parameters, including GxE interactions for production traits (van der Werf et al. 2010), and common sires were used across a wide range of environments. Genetic and phenotypic parameters for lamb survival and behavioural traits of the lamb were estimated from INF data (Brien et al. 2010). Lamb survival to 3 days of age and to weaning showed low heritability of $h^2=0.014 \pm 0.008$ and $h^2=0.01^{\pm}0.007$ respectively. Further genetic and phenotypic parameters for reproduction traits in yearling, maiden and adult ewes were estimated in maternal cross and Merino ewes (Newton et al., 2014). Heritability traits for all reproduction traits were low ranging from $h^2=0.00 \pm 0.06$ for fertility in adult Merino ewes to $h^2=0.20 \pm 0.05$ for number of lambs born in maternal cross ewes at yearling age. A GxE interaction study for carcase and meat quality traits (Hagan, 2012) found significant sire x flock interactions for the majority of traits, explaining up to 69% of the phenotypic variance. Genetic correlations for carcase and meat quality traits across two of the INF sites expressed GxE interactions. GxE interactions for reproduction traits have not been investigated in the INF.

If GxE interaction is a major source of phenotypic variation for reproduction traits, a selection strategy for robust genotypes could lead to more consistent performance in reproduction performance and lamb survival. This approach could lead overall to more robust and consistent production performance, buffering periods of sub-optimal environmental conditions.

2. Project objectives

This study explored the existence of GxE interactions for reproduction traits using different methods of analysis on INF data, which also includes the alternative definitions of the environmental descriptor. Sufficient variation associated with GxE interaction in any of the analyses is followed up by a genome wide association study to determine potential underlying genes that are important for robustness in reproduction traits in Australian sheep.

The project objectives are

1. Assess genotype x environment interaction in the classical quantitative ways
2. Develop a suitable production based measure that represents the environment for the INF data
3. Identify SNP / genomic regions that have variable and consistent effects on reproductive performance and lamb survival (as trait of the ewe) across environments in the INF (Stop/Go)
4. Provide SNP targets for selection to improve robustness of reproductive performance in sheep
5. Identify underlying genes and their role in environmental sensitivity/robustness and / or reproductive performance in sheep

3. Methodology

3.1 Data

3.1.1 Phenotypes

Data originated from the Information Nucleus Flock (INF), including records on yearling, maiden and adult reproductive performance. The INF comprises 8 sites (INF01 to INF08) covering temperate and Mediterranean climates in New South Wales, Victoria, South Australia and Western Australia, with different rainfall distribution and patterns across all sites. The sites have been described by Hagan (2012). Reproduction traits were recorded between 2007 and 2011 on Merino and maternal cross ewes. Milestone 2 of this project explored the data and the most comprehensive data set was based on Merino sheep only. Focussing on only Merino sheep and excluding any complexities due to multiple breeds removes influencing factors of different genotype effects of breeds in the later analysis steps. The Merino sheep data set of the INF contained 5,097 ewe records.

Reproduction traits included number of lambs born (nlb) and weaned (nlw), fertility (fert), fecundity of lambs born per ewe lambing (fecund), number lambs weaned per ewe joined (nlwej), and pregnancy scan result (pregscan), recorded at an age between 1.5 and 2.5 years, labelled as maiden (“m” prefix), and as adult (“a” prefix) greater than 2.5 years. Number of reproduction records at yearling age, defined as less than 1.5 years, were low for Merino ewes and were not included in the analysis. Not all ewes had reproductive records and the number of records differed between traits. Appendix 1 provides an overview of number of records. More detail on the reproduction trait data can be found in Newton et al. (2014) and van der Werf et al. (2010). Based on the number of records, this study focused on nlb, nlb and pregscan. For some of the analyses a further trait called “mean weight of lambs born (mean_lambwt)" was created, which is the average weight of all lambs born per ewe and lambing event.

Full pedigree was recorded on the INF ewes. Other information included date of birth, year of birth (2007 to 2011), birth type, rearing type, INF site (IN01 – IN09), age of dam (between 2 and 8 years of age), sire breed (Merino or Poll Merino) and birth and rearing type (single, twin or triplet).

3.1.2 Environmental descriptor

The GxE interaction analyses required environmental descriptors that reflect a combination of climatic and environmental conditions that affect reproduction. In one analysis approach "INF site" (IN01 – IN08) was used as environmental descriptor.

In a second approach, pre-joining condition score (pj_cond) of the ewe, recorded as a score ranging from 1 (low) to 5 (high), and pre-joining weight (pj_wt) were used to form an environmental descriptor, because it was assumed that prevailing pasture conditions at each site might not reflect feeding status appropriately with supplementary feeding occurring during feed shortages. Numbers of records are presented in Table 1.

Table 1. Total number of records for reproduction traits, pre-joining weight and condition scores Merino ewes in the Information Nucleus flock.

	pj_wt	pj_cond
nlb	3,641	3,468
nlw	3,641	3,468
pregscan	3,617	3,444
mean_lambwt	2,013	1,944

The environmental descriptor was formed by adjusting pj_cond or pj_wt for fixed effects and effect of sire. Then, the data set was split based on residual pj_cond or pj_wt into ewes with residual pj_cond/pj_wt below ($\text{pj\_cond}_{\text{low}}$/$\text{pj\_wt}_{\text{low}}$) or above ($\text{pj\_cond}_{\text{high}}$/$\text{pj\_wt}_{\text{high}}$) the mean. Five percent of records above and below the mean were discarded, to clearly distinguish the high and low environments.

3.1.3 Genotype data

SNP data of 147 Merino sires from the INF were used in the association study. Data included 48,603 quality controlled SNP across 26 chromosomes. SNP positions from Sheep Genome Version 3.1 were used for the extraction of the underlying genes.

3.2 GxE interactions for reproduction traits

3.2.1 Sire x flock interaction

Data was organised in two ways. Firstly, traits were analysed individually within maiden and adult expressions (Model 1a). Secondly, maiden and adult trait expressions were organised as repeated records (Model 1b). Univariate analyses were run using ASReml (Gilmour et al. 2002), fitting sire and sire x INF site interaction as random effects. The variance component of the sire x INF site interaction provides evidence for the existence and size of a GxE interaction. For Models 1a and b, fixed effects tested included contemporary group, which is a combination of birth year (2007-2011) and INF site (IN01-IN08), age of dam and sire breed (Polled Merino or Merino). Joining length (in days) was confounded with the contemporary group effect and therefore not fitted. The model used in this study is similar to one of the models used by Newton et al. (2014), with the main difference of being a sire model in this study. Only significant effects were fitted in the final model. The effect of “contemporary group” was significant for all traits. The effect of “age of dam” was not significant for most traits, with the exception of mpregsan. The effect of “sirebreed” was only significant for mnlw and mpregscan. Model 1b fitted contemporary group as defined above and also pre-joining date to adjust for the effect of maiden and adult lambing.

3.2.2 Correlation between traits expressed in different environments

A bivariate animal model (Model 2) was used in a second approach to explore GxE interaction as the genetic correlation between the same trait expressed in two different environments (Falconer, 1952). Rather than using “INF site” as environmental descriptor, two production environments were defined by high and low pj_cond/pj_wt as outlined in previous paragraph 3.1.2. The genetic correlation (r_g) between the two traits (e.g. NLB_{low} and NLB_{high}) reflects the existence of a GxE interaction. If $r_g < 1$ between $NLBh_{igh}$ and NLB_{low}, it would indicate the existence of a GxE interaction. The same fixed effects and covariates as in Models 1a and b were fitted and animal was fitted as a random effect. All analyses were conducted using ASReml (Gilmour et al. 2002).

3.2.3 Determine potential genes that underlie GxE interaction

A three-step analysis was applied to determine genes that potentially underlie GxE interaction. The analysis was modelled on an approach published by Lillehammer et al. (2009), where successfully genes for robustness in dairy traits were identified. The approach involved firstly a random regression analysis. The slope of the regression describes the robustness of the sire genotype across environments. The next step is a genome wide association study (GWAS) on the sire effect on the slope if sufficient variance warranted this step. Significant SNPs from the GWAS were searched against the Ovis aries 3.1 Ensembl gene annotation (Version 1.74) to identify potential underlying genes in a third step.

3.2.3.1 Random regression analysis (Step 1)

A random regression sire model was run using the mean deviation in pj_cond/pj_wt of individual INF sites from the overall mean of all sites (mean INF site deviation) as environmental descriptor. Mean INF site deviations were sorted by increasing order to create an environmental trajectory. The model included a random regression of sire on mean INF site deviation in pj_cond/pj_wt by fitting the interaction of sire and the first order Legendre polynomial on mean INF site deviation in pj_cond/pj_wt. A permanent environmental effect was fitted as a constant across parities and environments. Individual residual diagonal variance structures were specified for each INF flock. Analyses were conducted using ASReml (Gilmour et al, 2002).

3.2.3.2 Genome wide association study (Step 2)

The second step was a GWAS using single SNP regression on the sires' effects on slope and intercept. Sire effects for the slope were used as dependent variable in a single SNP regression analysis. SNP were fitted individually as fixed effect and sire was fittedas random effect. Sire relationships were accounted for by fitting a numerator relationship matrix based on a genetic group pedigree. All analyses were conducted in ASReml (Gilmour et al. 2002).

3.2.3.3 Gene browser

Genes that were the closest to the position of significant SNP ($P < 0.001$) were extracted from NCBI Reference sequence and Ensembl. Genes were followed up on GeneCards, NCBI, Ensembl and through Google searches to determine their full name and function and their significance and known function in the physiological processes of reproduction.

4. Results

The mean flock deviations for pj_cond and pj_wt demonstrated differences between sites (Table 2). Differences in weight might have been expected due to the difference in size of the ewe type at different sites, however, despite a common management strategy to manage ewes to the same condition score, differences can be observed between flocks. Reproductive performance of Merino ewes of the INF is described in Table 3. All traits had a minimum of 0 and a maximum of 3. The number of records for singles, twins and triplets for all traits and INF sites are summarised in Appendix 1. Reproductive performance was lower in maiden than adult ewes in the data set. The mean reproductive performance indicated that more lambs were scanned than were actually born, which would be a combination of scanning error and foetal losses. Losses also occurred between lambing and weaning, which is demonstrated in the lower means for nlw compared to nlb.

Table 2. Mean INF site deviation in pre-joining condition score (pj_cond) and weight (pj_wt) of ewes.

INF site	pj_cond	pj_wt
IN01	0.64	-6.15
IN02	0.10	14.85
IN03	0.31	9.93
IN04	-0.19	-1.74
IN05	-0.22	-7.89
IN06	-0.13	-1.99
IN07	-0.10	4.26
IN08	-0.41	-11.27

Table 3. Descriptive statistics of reproduction traits for maiden and adult Merino ewes in the INF.

	Mean	Min	Max	Stddev
mnlb	0.81	0	3	0.73
anlb	0.88	0	3	0.84
mnlw	0.58	0	3	0.70
anlw	0.76	0	3	0.77
mpregscan	1.06	0	3	0.65
apregscan	1.29	0	3	0.71

4.1 Sire x flock interaction (Model 1)

4.1.1 Maiden and adult reproduction traits (Model 1a)

Heritabilities estimated with a sire model were low ranging from h^2=0.10-0.25 when no interaction variance component was fitted (Table 4). Fitting a sire x flock interaction in the model reduced the additive genetic variance component and therefore the heritabilities (Tables 5), as is demonstrated by comparing the variances presented in Table 4 and 5. Heritabilities were low and ranged from h^2=0.00 – 0.15. The trait anlb resulted in very low genetic variance, which after rounding resulted in a h^2=0.00.

The variance components associated with sire x flock interaction were small, but in some cases larger or at least the same as the additive genetic variance. The variances of the interaction accounted for between 8-21% of the phenotypic variation.

Table 4. Phenotypic (σ^2_p), additive genetic (σ^2_a) and error variances (σ^2_e) and heritabilities (h^2) for reproduction traits in Merino ewes estimated without fitting an interaction variance component.

	σ^2_p	σ^2_a	σ^2_e	h^2
mnlb	0.41	0.09	0.39	0.22 ± 0.05
anlb	0.47	0.05	0.46	0.10 ± 0.04
mnlw	0.39	0.07	0.39	0.15 ± 0.04
anlw	0.41	0.05	0.40	0.12 ± 0.05
mpregscan	0.40	0.10	0.37	0.25 ± 0.05
apregscan	0.47	0.04	0.16	0.09 ± 0.04

Table 5. Phenotypic (σ^2_p), additive genetic (σ^2_a), sire x flock interaction (σ^2_{SxF}) and error variances (σ^2_e) and heritabilities (h^2) estimated with Model 1a for reproduction traits in Merino ewes, split into maiden (m) and adult (a) traits.

Traits	σ^2_p	σ^2_a	σ^2_{SxF}	σ^2_e	h^2
mnlb	0.41	0.03	0.06	0.34	0.08 ± 0.05
anlb	0.47	0.00	0.10	0.37	0.00 ± 0.00
mnlw	0.39	0.03	0.03	0.35	0.07 ± 0.05
anlw	0.41	0.01	0.05	0.36	0.02 ± 0.05
mpregscan	0.40	0.06	0.04	0.34	0.15 ± 0.06
apregscan	0.47	0.01	0.04	0.43	0.02 ± 0.05

4.1.2 Reproduction traits as repeat measure (Model 1b)

In Model 1b, expressions of reproduction traits at maiden and adult age were treated as repeated records. Results were very similar to the results from Model 1a (Tables 5 and 6), where maiden and adult traits were analysed separately. Heritabilities were low. Model 1b also analysed the trait mean lambwt born, which had higher phenotypic and genetic variances and the heritability, even though still low, it was slightly higher than for the other traits. Variances associated with sire x flock interaction were low, but again in most cases higher than the additive genetic variances, and accounted for between 4-10% of the phenotypic variance.

Table 6. Phenotypic (σ^2_p), additive genetic (σ^2_a), sire x flock interaction (σ^2_{SxF}) and error variances (σ^2_e) and heritabilities (h^2) estimated with Model 1b for reproduction traits in Merino ewes.

	σ^2_p	σ^2_a	σ^2_{SxF}	σ^2_e	h^2
nlb	0.48	0.01	0.05	0.42	0.03±0.04
nlw	0.46	0.04	0.02	0.40	0.08±0.04
pregscan	0.50	0.04	0.03	0.43	0.08±0.04
mean_lambwt	1.01	0.07	0.06	0.80	0.16±0.06

4.2 Correlation between traits expressed in different environments (Model 2)

Model 2 defined the environmental descriptor differently to Models 1a and b, treating pre-joining condition score or body weight of the ewe as the production environment. The correlation (r_g) of the same trait expressed at high and low body condition score / body weight indicates the level of GxE interaction. Using high and low pj_cond as environmental descriptor, all traits showed correlations that deviated from 1, which indicated the presence of a GxE interaction (Table 7). All correlations were associated with high standard errors and the correlations therefore do not present conclusive evidence for the existence or absence of a GxE interaction.

Table 7. Correlations (r_g) between the same reproduction trait in ewes with high ($trait_{high}$) and low ($trait_{low}$) pre-joining condition score (pj_cond) and associated heritabilities in the high (pj_cond_{high}) and low (pj_cond_{low}) environments (Model 2).

pj_cond	σ^2_a		σ^2_e		h^2		r_g
	low	high	low	high	low	high	low/high
nlb	0.06	0.06	0.28	0.33	0.17±0.04	0.14±0.04	0.66±0.22
nlw	0.03	0.06	0.31	0.32	0.09±0.04	0.15±0.04	0.60±0.28
pregscan	0.10	0.08	0.30	0.36	0.24±0.05	0.19±0.05	0.80±0.16
mean_lambwt	0.33	0.17	0.52	0.39	0.39±0.07	0.19±0.07	0.84±0.24

Results for the genetic correlations were similar when using pj_wt as environmental descriptor, genetic correlations were higher but were still below 0.9 for nlb, nlw and pregscan (Table 8). Standard errors were large and as in previous results, there is no conclusive evidence for the existence or absence of a GxE interaction for any of the traits.

Table 8. Correlations (r_g) between the same reproduction trait in ewes with high ($trait_{high}$) and low ($trait_{low}$) pre-joining body weight (pj_wt) and associated heritabilities in the high (pj_wt_{high}) and low (pj_wt_{low}) environments (Model 2).

pj_wt	σ^2_a		σ^2_e		h^2		r_g
	low	high	low	high	low	high	low/high
nlb	0.06	0.06	0.34	0.47	0.15±0.04	0.12±0.05	0.76±0.21
nlw	0.05	0.03	0.33	0.45	0.12±0.04	0.06±0.04	0.84±0.34
pregscan	0.12	0.60	0.29	0.44	0.29±0.04	0.12±0.04	0.87±0.17
mean_lambwt	0.23	0.14	0.65	0.80	0.26±0.07	0.15±0.07	0.97±0.27

Heritabilities (Table 7 and 8) were low to moderate in high and low production environments and higher than heritabilities from Models 1a and b. Heritabilities were similar for nlb and nlw across the high and low production environments, irrespective of the environmental descriptor. For pregscan and mean_lambwt, heritabilities were higher in the low environment, due to higher genetic variances, reaching a heritability of 0.39 for pj_cond_{low}. The moderate heritabilities were higher than current literature estimates (Safari et al., 2005).

4.3 Determine potential genes that underlie GxE interaction

Random regression analyses were conducted for all traits and environmental descriptors to determine the variance associated with the slope of the regression, which expresses the sensitivity of sires' performance to the environment. Slope variances were low for all traits (Table 9). The results show some evidence of a GxE interaction, but of very small effect. Slope variances were slightly higher when pj_wt was used as an environmental descriptor compared to pj_cond.

Table 9. Variances of the residual (σ^2_e) slope (σ^2_{slope}) and intercept (σ^2_{int}) and their covariances between intercept and slope ($\sigma_{int/slope}$) from random regression models (Model 3) fitting pj_cond or pj_wt as environmental descriptor.

pj_cond	σ^2_e	σ^2_{int}	σ^2_{slope}	$\sigma_{int/slope}$
nlb	0.50	0.016	0.0003	0.002
nlw	0.45	0.020	0.0008	0.003
pregscan	0.49	0.029	0.0024	-0.001
mean_lambwt	0.90	0.110	0.0140	-0.025
pj_wt	σ^2_e	σ^2_{int}	σ^2_{slope}	$\sigma_{int/slope}$
nlb	0.48	0.044	0.0220	0.027
nlw	0.43	0.034	0.0087	0.014
pregscan	0.47	0.045	0.0130	0.016
mean_lambwt	0.91	0.12	0.0100	0.018

The traits nlb had the highest slope variance followed by mean_lambwt and pregscan using pj_wt as environmental descriptor. The trait mean_lambwt had also the highest slope variance with pj_cond as environmental descriptor. Using the results of the analysis of Models 1a,b and 2 as supporting evidence on which traits to follow up with a GWAS, nlb and nlw appeared to show slightly stronger indications for GxE interaction than the other traits. The traits mean_lambwt using pj_cond as environmental descriptor and nlb and mean_lambwt using pj_wt as environmental descriptor were followed up with a GWAS. Considering the low variances associated with the sire effect on the slope of any of these traits, any significant SNPs would only have very small effects.

Table 10. Number of significant SNP at two significance levels.

	pj_wt		pj_cond	
	intercept	slope	intercept	slope
	P < 0.001			
nlb	53	48*	NA	NA
mean_lambwt	68	80*	64	63*
	P < 0.0001			
nlb	10	11	NA	NA
mean_lambwt	4	6	9	9

*genes investigated for known function

Between 48 and 80 SNPs were significant at P < 0.001 across nlb and mean_lambwt (Table 10). At a more stringent significance level only between 4 and 11 SNPs have significant associations with production traits. One hundred and ninety one SNPs were significant for the sire effect on the slope across traits and environmental descriptors (marked with * in Table 9) and potentially underlying genes identified by comparison to Ovis aries 3.1 Ensembl gene annotation (version 1.74). Existing data bases such as Genecards (www.genecards.org), NCBI (www.ncbi.nlm.nih.gov) were searched in addition to google searches for published research on the involvement of these genes in reproductive processes, such as ovulation, fertility, etc. The search was not exhaustive and all findings are speculative considering that the power to find outstanding candidate genes was low. Nineteen genes across the traits and environments were involved in follicle and placenta development, embryo implantation or interactions with hormone release (Appendix 2-4). Of these 19 genes 7 were actually at the same position as the significant SNP (Appendix 2-4, Distance from SNP = 0). The most interesting gene was found on chromosome 4 for mean lambwt born with pre-joining condition as environmental descriptor. The gene SEMA3A has very recently been identified to be involved in fertility (Gacobini et al. 2014) by promoting the growth of cells that secret gonadotropin-releasing hormone.

5. Discussion

Studies for weight and meat traits in the INF indicated that GxE interactions exist between the different sites (Hagan, 2012). In this current study all models showed little evidence of GxE interaction for reproduction traits. The traditional approach tested potential GxE interactions defining the environment as physical location, in this study “INF site”. Good genetic linkage existed between sites through sires being used at two or more sites. This enabled fitting a model that included sire x site variance component. Sire x site variance component only accounted for small proportions of the phenotypic variation in any of the reproduction traits. This would indicate that a GxE interaction was present, but of little effect. Experimental findings for GxE interactions in reproduction traits are variable (Amores et al. 1999, Notter et al. 2000a and b, Fogarty et al. 2001), because varying breeds and environments were under consideration. It was not surprising that GxE interactions were low in this study, because additive genetic variances for reproduction traits were overall low. Unless, the interaction component would have been able to partition off some of the error variance, it was unlikely that the inclusion of the GxE variance component in the model would have been significant.

It was hypothesised that “INF site” might not be a sufficiently detailed descriptor of the environment, which might hinder the ability to detect GxE interactions. The biological descriptors of either condition score or weight of the ewe at time of joining was chosen, based on the assumption that these are important drivers of reproduction traits and might reflect environmental conditions more appropriately than INF site. Despite the management strategy to manage ewes across various sites to the same condition score, means in body condition score differed between sites, demonstrating that it makes a suitable environmental descriptor for the purpose of this study. The correlation between reproduction traits expressed at high and low body weights and condition scores were all below 1, which might indicate the existence of GxE interactions, but high standard error demonstrated that more records are required to obtain a conclusive result, indicating clear evidence for the existence or absence of GxE interactions for reproduction traits.

Heritabilities were low for all models and are in agreement with previously published results (Safari et al., 2005; Newton et al, 2014). The bivariate analysis yielded slightly higher heritabilities than the other approaches, due to higher genetic variance. The reason could be due to the environmental descriptor being another biological expression and even though it was adjusted for genetic effects of the sire, other effects, such as the genetic effect of the dam and effects from Mendelian sampling could still be unaccounted for and inflate the additive genetic variance.

The random regression approach relied on repeated daughter expressions of sires across the environmental descriptors, which was basically INF site. Sorting the INF site based on their deviation of pj_cond from the overall mean of all sites formed the trajectory for the repeat measures. Ideally an environmental descriptor would have been based on repeated measures of condition score on a time trajectory, which then would allow the regression of reproduction traits on condition score. This would remove the current confounding of the biological descriptor, such as pj_cond in this study with physical location, which was INF site in this study, and would also allow the estimation of residual covariances. The random regression analysis found only small variances associated with the sire slope and intercept, which was expected due to the nature of the traits. However, the sire solutions indicated that some sires breeding values changed with increasing or decreasing environmental descriptor whereas others stayed constant. Small variances for the sire effect of the slope indicated little variability in sire performance across environments. It was unlikely that whole genome studies would detect SNP with significant effect. Therefore, WGAS were only conducted for the traits that showed the highest variance for the sire effect of the slope.

The power of the whole genome association study was low due to the small variances and a small number of sire genotypes. Some significant associations were found, but the findings are speculative due to the low number of genotypes and the low power with small variances to explore in the WGAS. There was little overlap of genes that were associated with significant SNP between the different traits and more importantly for mean lamb weight born across two environments. An overlap of genes would have been interpreted as a confirmation of associations, which this study did not demonstrate.

Despite the lack of significant results from the random regression approach and the GWAS, the approach certainly provides a useful method to analyse GxE interactions. Data structures and connectedness of environments are key aspects to resources for the exploration of GxE interactions. Aspects of this were explored in the attached manuscript that was submitted to the 10th World Congress in Genetics Applied to Livestock Production (Appendix 5).

In this study it was hypothesised that the definition of the environment might limit detection of GxE interactions. However, it is likely that the traditionally measured phenotypes, such as number of lambs born, might be insufficiently detailed and encompasses such a wide range of complex processes, including ovulation, conception, embryo implantation, that it is difficult to partition the genetic variance that is underlying this broadly defined phenotype. Novel measurement techniques for reproduction traits and the development of new phenotypes might help to disentangle the complex phenotype and describe the underlying genetic processes more precisely than can currently be done with the traditional phenotypes.

Anecdotal evidence from industry exists for GxE interaction for various sheep production and reproduction traits. Research approaches for the characterisation (Mather and Jones, 1958) and detection of GxE interactions have been developed (Falconer, 1952), but it has not been possible to develop practical methodologies to consider GxE interaction in breeding programs. Genomic approaches certainly offer a wider range of approaches to explore GxE interactions and to provide potentially tangible solutions for industry, such as marker tests or genomic breeding values for robustness of production. As this study demonstrated GxE interaction for reproduction traits remains a difficult area. However, for production traits where GxE interactions have been shown to be of significant effect, the random regression approach coupled with a GWAS could potentially be a useful methodology to determine genes underlying robustness.

6. Conclusions

Genomic approaches certainly offer new approaches to explore GxE interactions and to provide potentially tangible solution approaches, such as marker tests or genomic breeding values for robustness. As this study demonstrated GxE interaction for reproduction traits remains a difficult area. However, for production traits where GxE interactions have been shown to be of significant effect, the random regression approach coupled with a GWAS could potentially be a useful methodology to determine genes underlying robustness. Also, novel measurement techniques for reproduction traits might disentangle the complex phenotype and describe the underlying genetic processes more precisely than can currently be done with the traditional phenotypes.

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8. Appendices

Appendix 1 Records for number of lambs born (NLB), weaned (NLW), pregnancy scanning result (pregscan) in maiden (m) and adult (a) Merino ewes

mnlb	**INF01**	**INF02**	**INF03**	**INF04**	**INF05**	**INF06**	**INF07**	**INF08**
0	162	85	60	104	133	135	101	223
1	290	73	87	104	105	63	177	278
2	20	99	119	65	19	35	69	47
3	0	4	2	2	1	1	1	0
4	0	0	0	0	0	0	1	0
anlb	**INF01**	**INF02**	**INF03**	**INF04**	**INF05**	**INF06**	**INF07**	**INF08**
0	149	49	48	119	107	97	89	113
1	186	41	49	49	57	39	86	183
2	30	69	90	56	18	15	84	76
3	0	14	18	6	0	1	5	1
4	0	0	2	1	0	0	0	0
mnlw	**IN01**	**IN02**	**IN03**	**IN04**	**IN05**	**IN06**	**IN07**	**IN08**
0	207	128	100	136	151	154	126	256
1	246	62	91	102	95	56	167	251
2	19	67	77	37	12	23	54	41
3	0	4	0	0	0	1	2	0
anlw	**IN01**	**IN02**	**IN03**	**IN04**	**IN05**	**IN06**	**IN07**	**IN08**
0	168	79	86	131	121	111	103	126
1	178	41	58	56	53	30	89	183
2	19	46	54	44	8	11	69	63
3	0	7	8	0	0	0	3	1
4	0	0	1	0	0	0	0	0
mpregscan	**IN01**	**IN02**	**IN03**	**IN04**	**IN05**	**IN06**	**IN07**	**IN08**
0	35	73	54	70	64	71	31	76
1	401	66	87	117	149	101	214	388
2	33	114	123	84	42	54	103	70
3	0	5	3	1	0	0	0	0
apregscan	**IN01**	**IN02**	**IN03**	**IN04**	**IN05**	**IN06**	**IN07**	**IN08**
0	22	42	42	30	27	41	13	22
1	264	38	49	76	105	31	125	215
2	74	73	95	118	45	32	126	128
3	0	16	19	6	1	0	0	0
4	0	0	2	0	0	0	0	0

Appendix 2 Chromosome, SNP position, underlying gene and distance of gene from SNP position for number of lambs born with pre-joining weight as environmental variable.

Chr	**SNP Name**	**SNP Position**	**Distance from SNP**	**Gene**	**Gene name**	**Known involvement in reproductive**
1	OAR1_273716249.1	253263691	5621	RYK	Receptor like tyrosine kinase	Effect on embryonic telecephalon uterine stroma
2	OAR2_59050366.1	54886112	81315	TLE1	Transducin-like enhancer of split 1	Ranks 12th in maternal placenta development
2	OAR2_209720831.1	198155571	0	PGAP1	Post-GPI attachment to protein 1	Follicle epithelium
4	OAR4_27443364.1	26061873	133588	AHR	Aryl hydrocarbon receptor	Ovarian follicle growth in various
4	OAR4_114740920.1	107282076	0	TPK1	Thiamine pyrophosphokinase 1	Association with birth weight in human genome scan
6	OAR6_63152491.1	57283478	0	TBC1D1	Tbc1 domain family/TBC1D1	Stimulates cell differentiation in follicle development
10	DU410344_269.1	2499392	0	DIAPH3	Diaphanous-related formin 3	Follicle reserves - menopause
10	s14742.1	83964085	46734	IRS2	Insulin receptor substrate 2	Gestational diabetes
16	OAR16_42312325.1	38943467	25806	PRLR	Prolactin receptor	Embryo implantation and early pregnancy

Appendix 3 Chromosome, SNP position, underlying gene and distance of gene from SNP position for mean lamb weight born with pre-joining condition as environmental variable.

Chr	SNP Name	SNP Position	Distance from SNP	Gene	Gene name	Known involvement in reproductive
2	OAR2_143658091.1	135084325	112489	SP3	Sp3 transcription factor	Differentiation of ganulosa cells and interaction with follicle-stimulating
2	OAR2_219083907.1	206886750	20509	NRP2	Neuropilin 2	Candidate gene for litter size in pigs
2	OAR2_221698208.1	209401403	10866	PTH2R	Parathyroid hormone 2 receptor	Oozyte maturation
4	OAR4_38383717.1	36395312	0	SEMA3A	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	Controls ovarian-steroid dependent release
6	s42746.1	102729149	6618	JAKMIP1	Janus kinase and microtubule interacting protein 1	Activated in small follicles compared to large ones

Appendix 4 Chromosome, SNP position, underlying gene and distance of gene from SNP position for mean lamb weight born with pre-joining weight as environmental variable.

Chr	SNP Name	SNP Position	Distance from SNP	Gene	Gene name	Known involvement in reproductive
2	s19463.1	91180532	179915	ELAVL2	ELAV like neuron-specific RNA binding protein 2	Oocyte specific protein isoform
3	OAR3_30948262.1	28715652	4920	APOB	Apolipoprotein B	Male and female fertility
3	OAR3_63601465.1	60022882	632	IL1B	Interleukin 1, beta	Codes protein that affects embryo implantation rate
7	s40127.1	13340668	0	SMAD6	SMAD family member 6	Ovulation
12	s55747.1	40645808	0	MTOR	Mechanistic target of rapamycin (serine/threonine kinase)	Embryo implantation

Appendix 5 Manuscript submitted to the 10th World Congress of Genetics Applied to Livestock Production

Exploring Genotype x Environment Interaction and Heritabilities for a Reproduction Trait in Merino Sheep Using Three Approaches

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ABSTRACT: Three approaches were used to detect genotype x environment (GxE) interaction and estimate heritabilities for "number of lambs born" (NLB) in Merino progeny of the Sheep CRC Information Nucleus Flock. Approaches included a univariate model, fitting site x sire interaction as a random effect and a bivariate model that treated NLB expressed at high and low pre-joining condition score as two genetically separate traits. The third approach was a random regression sire model that regressed NLB on mean deviation in pre-joining condition score at each experimental site from the overall mean of all sites. All approaches indicated that there was no significant GxE interaction for NLB, irrespective of the model used. The consistently low heritabilities across all approaches also reflected that neither approach differed in the partitioning of the variance components.

Keywords: Merino sheep, Genotype x environment interaction, Reproduction, Random regression

Introduction

Reproduction is one of the most important profit drivers in the Merino industry, but genetic progress is generally difficult to achieve due to low heritabilities for reproduction traits (Safari et al. (2005)). Low heritabilities could be due to several reasons, one reason being that traditionally measured traits, such as number of lambs born and weaned, might describe the complex processes underlying such traits insufficiently to capture the genetic variation. An alternative reason could be that other sources of variance such as non-additive genetic effects or variation due to genotype x environment (GxE) interaction are important. Results of the few studies that have investigated GxE interaction for reproduction traits in sheep are variable and detected effects have undetermined effect on breeding programs (e.g. Fogarty et al. (2001), Notter et al. (2000 a,b)). Large data sets are required to explore the existence of GxE interactions comprehensively and possibly the definition of the environment as a physical entity, such as "flock", might not be the most appropriate categorization to distinguish between environments in a relevant manner. The Australian Sheep CRC Information Nucleus Flock (INF) constitutes a large data set that could be used to investigate GxE interaction for reproduction traits in sheep, as common sires were used across a wide range of environments. Here we explore, whether the definition of the environmental descriptor influences the ability to detect and extract variation associated with GxE interaction and whether it affects the heritability for the trait of number of lambs born (NLB) in Merino sheep. We will compare different definitions of 'environment' and different models to explore the interaction between environment and genotype for NLB.

Materials and Methods

Data. The data set originated from the Australian Sheep CRC INF and comprised records from eight sites (INF01-INF08) collected between 2007 and 2011 (van der Werf et al. (2010)). The reproduction trait analysed was "number of lambs born" (NLB) and we used records from purebred Merino ewes across two parities. The phenotypic data comprised a total of 4,592 records for NLB. A summary of the number of records is provided in Table 1., Out of 184 sires 173 were used in two or more of the eight INF sites, with each sire having an average of 4-11 female reproduction records per site. The mean total number of records per sire across INF sites was 52 with a minimum number of 4 and a maximum of 212 records.

The analysis required an environmental descriptor that reflects a combination of climatic and local environmental conditions that affect reproduction. In one approach "INF site" was used as the environmental descriptor. However, INF site might not be a sufficient descriptor, because prevailing pasture conditions at each site might not reflect feeding status appropriately with supplementary feeding occurring during feed shortages. Therefore, pre-joining condition score (pj_cond) of the ewe, recorded as a score ranging from 1 (low) to 5 (high), was also used as an environmental descriptor in a different approach. After adjusting the pj_cond phenotype for fixed effects and effect of sire, the data set was split based on residual pj_cond into ewes with residual pj_cond below (low pj_cond) or above (high pj_cond) the mean. Five percent of records immediately above and below the mean were discarded, to clearly distinguish the high and low pj_cond categories. A total of 3,242 ewes with records on both, NLB and pj_cond, were included in the analysis. Six hundred and sixty two ewes were represented in the high and low pj_cond environments due to multiple parities and 174 sires were represented across the two environments. On average each sire had 13 and 12 records in low and high pj_cond environments, respectively, with number of records per sire ranging between 2 and 30.

Models. Three different models were used to investigate GxE interaction and estimate heritabilities for NLB. Model 1, defined detectable GxE interaction as the variance associated with the sire x INF site interaction, fitting sire and sire x INF site as random effects in a univariate model for NLB. In Model 2, pj_cond of the ewe was defined as the "environment" and NLB expressed at high and low condition scores (NLB_{low} and NLB_{high}) was treated as two genetically different traits (Falconer, 1952). Model 2 then fitted a bivariate animal model to NLB_{low} and NLB_{high} and the genetic correlation (r_G) between the two traits reflects the existence of a GxE interaction. If $r_G < 1$ between NLB_{high} and NLB_{low}, it would indicate the existences of a GxE interaction. Model 3 was a random regression sire model. It also incorporated pj_cond in the definition of the environment by regressing NLB on the mean deviation in pj_cond score of individual INF sites from the overall mean of all sites (mean INF site deviation). Sites were sorted by increasing mean deviation in pj_cond from all INF sites. A similar approach was used successfully in the context of GxE interaction on dairy data (Lillehammer et al., 2009). The model included a random regression of sire on mean INF site deviation in pj_cond by fitting the interaction of sire and the first order Legendre polynomial on mean INF site deviation in pj_cond. A permanent environmental effect was fitted as a constant across parities and environments. Individual residual diagonal variance structures were specified for each INF flock.

All models fitted drop (2007 to 2011), birth-rearing type (three classes: born single/raised as single, born twin/raised as twin, born as twin/raised as single), sire breed (Merino or Poll Merino) as fixed effects and pre-joining date as covariate. INF site (Site 1 to 8) was fitted as fixed effect in Models 2 and 3. (Co)variance components, heritabilities and correlations for Models 1 and 2 and the coefficients of covariance function (matrix K) for model 3 were estimated using ASReml (Gilmour et al (2002)). For the random regression model, the genetic (co)variances and heritabilities were estimated from matrix K as summarized by van der Werf (1998). Correlations were calculated as functions of the variances and co-variances of IN08, the site with the lowest INF site deviation in pj_cond, with all other sites.

Results and Discussion

All models showed little evidence of GxE interaction for NLB across three different environmental descriptor and models. In the first model, the traditional approach was used to test potential GxE interactions by defining the environment as physical location, in this study “INF site”. The INF design allowed for good genetic linkage between sites through sires being used at two or more sites, which enabled fitting a sire x site variance component, as was done in Model 1. The sire x site variance component only accounted for 4.5% of the phenotypic variation. This would indicate that a GxE interaction was present, but it accounted for little variation.

Model 2 treated NLB under low and high condition scores as two separate traits and did not show conclusive evidence of a GxE interaction, yielding a genetic correlation of r_G=0.66$\pm$0.20. The biological descriptor of condition score at time of joining was chosen, based on the assumption that it is an important driver of number of lambs born and reflects environmental conditions more appropriately than INF site. However, the high standard error indicated that more records are required to obtain a conclusive result with this approach.

Model 3, is a combination of the previous two descriptors of the environment as the deviation of pj_cond at each INF site from the average pj_cond of all sites. The genetic variance was low, as in Models 1 and 2, but a slight increase in genetic variance was observed with increasing mean site deviation in pj_cond (Figure 1). Residual variances differed along the trajectory with no clear trend (Figure 2), although they appeared to be lower with increasing mean INF site deviation in pj_cond. A reason for this could be that the environmental descriptor of the random regression approach was based on INF sites and residual variances are independent without residual covariance between INF sites. The model allowed for this by defining individual residual variance structures for each environment. The genetic correlations between NLB at low pj_cond (IN08) and NLB expressed at all the other INF sites ranged from r_G = 0.80-0.96 with the majority being r_G > 0.95. Again, this provides little evidence for a significant GxE interaction on NLB in Merino ewes in the INF.

Heritabilities were low for all models and the consistent results would indicate that variances were partitioned very similarly in the different analysis approaches, independent of the definition of the environmental descriptor (Table 2). The bivariate analysis yielded slightly higher hertiabilities than the other approaches, due to higher genetic variance. The reason could be due to the environmental descriptor being another biological expression and even though it was adjusted for genetic effects of the sire, other effects, such as the genetic effect of the dam and effects from Mendelian sampling could still be unaccounted for and inflate the additive genetic variance. The difference in residual variances in the random regression approach (Figure 2) did not affect the heritabilities across the different environments, because the genetic variances were generally low.

The random regression approach worked unexpectedly well on number of lambs born considering the moderate number of records, estimating the genetic variances in a probable range that agrees with reported estimates (Safari et al (2005)). The advantage of the random regression analysis is that all co-variances can be estimated from a single analysis. The random regression approach relied on repeated daughter expressions of sires across the environmental descriptors, which was basically INF site. Sorting the INF site based on their deviation of pj_cond from the overall mean of all sites formed the trajectory for the repeat measures. Ideally an environmental descriptor would have been based on repeated measures of condition score on a time trajectory, which then would allow the regression of NLB on condition score. This would remove the current confounding of the biological descriptor, such as pj_cond in this study with physical location, which was INF site in this study, and would also allow the estimation of residual covariances.

Conclusion

The study compared different ways to characterize the environmental descriptor in GxE interaction studies. Besides the traditional approach of “sire x flock” we used condition score as a more biological meaningful ‘proxy’ for environment. Generally, no significant GxE interactions were found for number of lambs born in Australian Merino sheep and heritabilities were low. The random regression model was useful even for a trait of low heritability, such as number of lambs born, and a moderate number of records compared to a bivariate model, because co-variances can be estimated between all environments with one model using all data.

Acknowledgements

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Table 1. Number of NLB records per sire and number of ewes in each environment.

Environment	No of records per sire	No of ewe records	pj_cond
IN01	1882	833	0.64[\$]
IN02	802	432	0.10[\$]
IN03	1030	473	0.31[\$]
IN04	876	506	-0.19[\$]
IN05	942	440	-0.22[\$]
IN06	868	379	-0.13[\$]
IN07	1104	613	-0.10[\$]
IN08	1938	916	-0.41[\$]
NLB_{low}	2355	1650	< 0.028[&]
NLB_{high}	2114	1592	> 0.028[&]

[\$] mean site deviation from overall mean

[&] residual pj_cond

Table 2 Heritabilities for NLB, NLB within high and low pj_cond (NLB_{low} and NLB_{high}) from Models 1 to 3.

Model	Trait	h^2
1	NLB	0.09 ± 0.03
2	NLB_{low}	0.17± 0.04
2	NLB_{high}	0.14 ±0.05
3	NLB	0.02 – 0.12

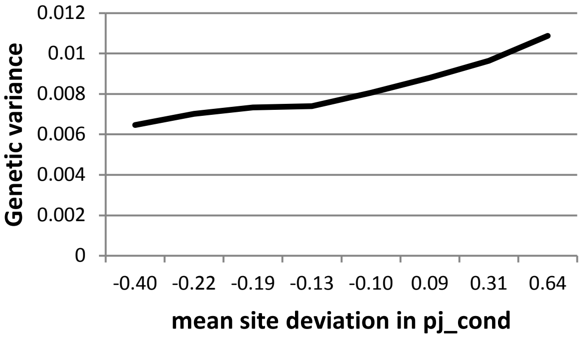

Figure 1: Genetic variances across the environmental trajectory (mean individual site deviation in pj_cond from all site mean)

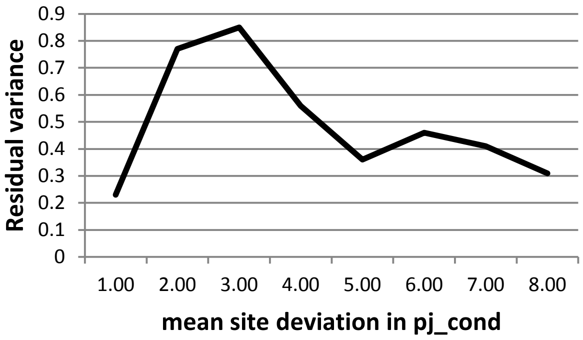

Figure 2: Residual variances across the environmental trajectory (mean individual site deviation in pj_cond from all site mean)