March 2022

Project Report No. PR640-05

Genomics of sheep resilience to climate change

Georgios Banos[1] and Bingjie Li[1]

[1]Scotland's Rural College (SRUC), RIB, Easter Bush, EH25 9RG Midlothian

CONTENTS

1. Abstract

Background and facts

- Climate change is occurring worldwide and manifested in changing air temperatures and increasing weather volatility.
- Climate change affects farm animal performance and poses a threat to the livestock production sector.
- Different animals are known to react differently to changing climatic and weather conditions, and part of these differences is known to be genetic.
- Animal resilience to climate has been defined as the capacity of individual animals to produce and perform unaffected by climate and weather changes.
- Breeding livestock for enhanced resilience to climate change has been proposed as a mitigating practice.
- Genomic data can be used to facilitate and optimise livestock breeding

This project, aims and results

- The present study set out to examine the genomic background of resilience of growing lambs to climate change.
- Specific objectives were to i) estimate genetic parameters of climate resilience in Scottish Blackface sheep; (ii) identify genomic markers and regions associated with climate resilience, and (iii) derive and examine genomic breeding values of individual animals for climate resilience.
- Live body weight data of individual Scottish Blackface sheep were analysed jointly with air temperature data collected from the nearest weather station to the farm, to derive sheep resilience phenotypes.
- Resilience phenotypes were analysed jointly with animal genotypes and pedigree data to examine the genomic background of the trait.
- Sheep resilience to air temperature changes was found to be a heritable trait manifested in notable genetic differences among individual sheep regarding their capacity to cope with weather change.
- The feasibility of deriving genomic breeding values for resilience was demonstrated. Estimated genomic breeding values were of reasonably high accuracy and could be used to distinguish between animals with inherently low and high resilience.
- Certain genomic markers and regions associated with resilience were identified. Although neither markers nor regions would be individually responsible for substantial proportions of the trait variance, they may be used to improve the accuracy of genomic breeding values.

Conclusions

- It is possible to enhance sheep resilience to climate change with selective breeding.
- Development of resilience phenotypes on individual animals is a straightforward process based on the combination of data already available in the genetic evaluation and improvement programme with public weather records.
- Genomic breeding values can be calculated as for any other trait and underpin genetic selection decisions. This would benefit sheep breeders and farmers.
- In addition to production and growth, improving sheep resilience to climate change may also enhance animal health and welfare.

2. Introduction

Climate change is occurring worldwide and is associated with increasing air temperature and seasonal weather variability (Agovino, et al, 2019). Several studies have assessed the impact of these changes on both plants and livestock, predicting a possible compromise in product quality and quantity (Powell, 2005; Thornton et al, 2009). A relatively recent climate change risk assessment commissioned by the UK government recognised the need to address the effects of environmental degradation on animal and plant production, and to develop mitigation strategies (UK Government, 2017). In livestock, it would be desirable if individual animals were capable of coping with climate change and expressed their genetic potential regardless of weather.

Previous work on small ruminants resulted in the development of novel animal phenotypes reflecting changes in their performance in response to weather volatility (Sánchez-Molano et al, 2019; Sánchez-Molano et al, 2020). These phenotypes exhibited both phenotypic and genetic variation. Therefore, selective breeding for enhanced animal resilience was suggested as a potential strategy to mitigate the effect of climate change on small ruminant production. Knowledge of the genomic architecture of the trait would cast insight into and inform optimal selective breeding strategies. Such genomic studies on sheep are missing from the literature.

In the present study, phenotypes and genotypes of Scottish Blackface sheep were available for the genomic study of climate resilience, defined as the capacity of individual animals to maintain their performance level in the face of weather volatility.

The overarching aim of the project was to assess the genomic background of sheep resilience to climate change. Specific objectives set to achieve this aim were to (i) estimate genetic parameters of climate resilience in Scottish Blackface sheep; (ii) identify genomic markers and regions associated with climate resilience, and (iii) derive and examine genomic breeding values of individual animals for climate resilience.

3. Materials and methods

3.1. Data

Climate resilience phenotypes were available on 5,814 Scottish Blackface lambs born on the research hill farm of Scotland's Rural College and raised extensively in the period 1993-2017. These phenotypes had been previously derived as described by Sánchez-Molano et al (2020). Briefly, live body weight records were collected on these lambs at four time-points during the growth phase: at birth (within 24 hours), pre-weaning (average 50 days), weaning (average 116 days) and post-weaning (average 237 days) stage (Sánchez-Molano et al, 2020). Weather data including daily ambient (air) temperature and relative humidity were obtained from the nearest weather station for

the same time period and matched to body weight records. To account for possible cumulative effects of temperature, each body weight record was matched to the average weather variable in the 10-day period preceding the recording date (Sánchez-Molano et al, 2020). Weather and body weight data were then jointly analysed by fitting reaction norm functions to random regression models. These analyses resulted in individual animal slopes across the weather variable trajectory, reflecting changes in animal growth in response to weather fluctuations. After further examination, average ambient temperature was revealed as the weather event eliciting the most variable reaction amongst animals. Therefore, the rest of this report focuses on animal resilience to air temperature change. Two traits were defined to this effect, one reflecting resilience to changes in average air temperature in the 10 days leading to the body weight recording date, and another reflecting resilience to changes in the standard deviation of air temperature in the same period.

Furthermore, genome-wide genotypes were available on 1,802 lambs. These genotypes had been derived under a previous project (iSAGE, 2016-2020) based on three different commercial DNA arrays: a low-density array of approximately 15,000 Single Nucleotide Polymorphisms (SNP), a conventional 50,000 SNP array, and a high density (ca. 700,000 SNP) array. All genotypes were subsequently imputed to the conventional scale (50K) using the *findhap* software (VanRaden, 2011). Following standard quality control (min. 90% call rates for animal and SNP, min. 0.05 minor allelic frequency, and Hardy-Weinberg equilibrium test) 1,766 lamb genotypes and 45,827 SNPs were retained for further analysis.

In addition to animal phenotypic and genotypic data, pedigree records on a total of 20,082 animals were available for the analyses.

3.2. Methods of analysis

3.2.1. Variance component and genetic parameter estimation

In the first instance, animal phenotypic and pedigree records were jointly analysed with the following mixed effect model for statistical analysis:

$$Y = mu + BY + S + LN + BR + D + GC + Animal + e \qquad [1]$$

Where *Y* was the climate resilience phenotype of each animal; *mu* was the overall population mean for the trait; *BY* was the fixed effect of the birth year of lamb; *S* was the fixed effect of the sex of the lamb; *LN* was the fixed effect of the genetic line of the lamb (three genetic lines were defined, emanating from previous and other ongoing studies on this population); *BR* was the fixed effect of the birth-rearing type of the lamb (coded 11, 21, 22 etc. if born single and reared single, born twin and reared single, born twin and reared twin etc., respectively); *D* was the fixed effect of the age of the lamb's dam (in years); *GC* was the fixed effect of the grazing code of each lamb based on the

corresponding grazing area or heft; *Animal* was the random additive genetic effect of each lamb; *e* was the random residual effect capturing anything that the model did not account for.

Variance components were derived for the random effects of Model 1. Heritability of climate resilience was then estimated as the ratio of the animal genetic to total phenotypic variance. The ASREML software was used for these analyses (Butler et al, 2018).

3.2.2. Genomic association of single markers

In this step, animal phenotypic and genotypic data were jointly considered. A principal component analysis was first conducted to examine animal genotypes for population structure. For this purpose, the genomic relationship matrix and Eigen vectors were derived using the Gemma software (Zhou and Stephens, 2012) and principal components were estimated and visualised in RStudio (2020). All fixed effects listed in Model 1 above were considered in these analyses. At the same time, the linkage disequilibrium status in the population of study was also examined using the Plink software (Purcell et al, 2007) and RStudio (2020).

Subsequently, a genome-wide association analysis of animal phenotypes and genotypes took place to assess the effect of individual SNP markers on climate resilience. The same fixed effects as in Model 1 were considered and the Gemma software (Zhou and Stephens, 2012) was used for this purpose. A Bonferroni correction was applied for multiple testing to determine a genome-wide ($P < 0.05$) and suggestive (one false positive per genome scan) significance thresholds.

Afterwards, candidate genes located within 0.25Mb upstream and downstream of SNP with the largest effects were identified using the ISGC Oar_v3.1/oviAri3 sheep genome assembly (Archibald et al, 2010), which is the genome assembly that our sheep genotypes were based on.

3.2.3. Genomic breeding value derivation and examination

In the final set of analyses, all data available i.e., animal phenotypes, genotypes and pedigree, were jointly analysed in a process known as single-step genomic best linear unbiased prediction using the BLUPf90 software (Misztal et al, 2002). This approach allows the effective combination of all sources of information available.

The same model of analysis as Model 1 was used in this case. Genomic breeding values (GEBV) were derived for all animals and their accuracy was estimated with the following formula:

$$Acc = \sqrt{1 - \frac{PEV}{Varg}} \qquad [2]$$

Where *Acc* was the accuracy of the estimated genomic breeding value of an individual animal; *PEV* was the prediction error variance associated with the estimate; *Varg* was the estimated genomic variance of the trait.

After GEBV were derived and examined, they were back solved to estimate the effects of individual SNP markers. The latter were examined in sliding windows representing genomic regions of 1 Mb in length; additional testing of windows of different size in that scale did not produce markedly different results. The proportion of the genomic variance accounted for by each genomic region (window) was also estimated in this step.

4. Results

4.1. Descriptive statistics, variance components and genetic parameters

The number of lambs with phenotypic data by year of birth is illustrated in **Figure 1**. Data were distributed relatively evenly across 15 years, thereby capturing substantial seasonal and inter-annual weather variation.
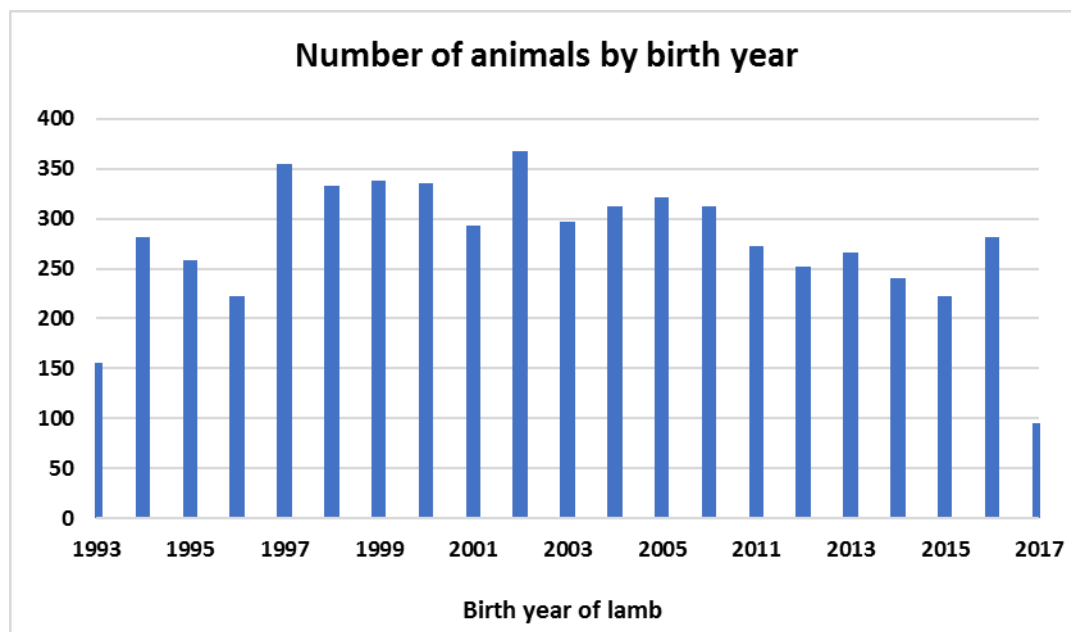

Figure 1. Number of lambs with resilience phenotypes by birth year.

Two animal resilience traits were analysed at first. One reflecting the lamb growth change in response to average air temperature in the 10-days preceding the live body weight record and the other in response to the standard deviation of the air temperature in the same period. Descriptive statistics, and estimates of variance components and heritability for the two traits are summarised in **Table 1**.

Trait variation was much more evident for the trait reflecting resilience to the average air temperature change, manifested in a coefficient of variation of nearly 22% (**Table 1**), which is approximately similar to estimates for many production traits. For the trait reflecting resilience to the standard deviation of air temperature change, this statistic was very low, close to zero, suggesting that there is no meaningful variation among individual animal phenotypes.

Table 1. Descriptive statistics, variance components and heritability estimates of two sheep resilience traits*

	Resilience_ave	Resilience_std
Mean (kg/^{o}C)	0.5051	-0.8905
Standard deviation (kg/^{o}C)	0.1098	0.0002
Coefficient of variation (%)	21.74%	0.02%
Genetic variance estimate	0.00096	4.32765E-10
Residual variance estimate	0.00809	2.83002E-08
Heritability estimate (standard error)	0.106 (0.023)	0.015 (0.013)

*Resilience_ave: sheep resilience to changes in average air temperature; Resilience_std: sheep resilience to changes in the standard deviation of air temperature.

Consistently with the observation on the coefficient of variation, the heritability of resilience to average temperature change was significantly greater than zero ($P < 0.05$) and close to 0.11, suggesting the presence of significant genetic variation among animals, thereby rendering the trait amenable to improvement with selective breeding. On the contrary, heritability of the other trait was very low and not statistically different from zero.

In further analyses, when animal genotypes were added to animal pedigree and phenotypic data in single-step genomic analyses, similar trait heritability estimates were derived, namely 0.11 and zero for resilience to average and standard deviation of air temperature change, respectively.

Given that genetic variation was present only for the one trait, the rest of the reported results pertain to resilience to average air temperature change.

4.2.   Genomic associations of genomic markers and regions

The principal component analysis of animal genotypes revealed the presence of population structure due to the genetic line (**Figure 2**.), which was expected given that the sheep belonged to a resource population where different selection lines had been previously developed within research programmes. To capture this effect in the present analyses, the first three variance components were fitted in Model 1 and tested for impact. However, in all cases, the effect was not significant, probably because the genetic line was already fitted as a fixed effect in the model.

The linkage disequilibrium analysis revealed the expected decay as distance between neighbouring SNPs increased, especially within the first 250Kb. This is illustrated in **Figure 3** and the value of 250

kb was used as input in the search of candidate genes linked with significant genomic markers and regions identified in the ensuing genomic analyses.
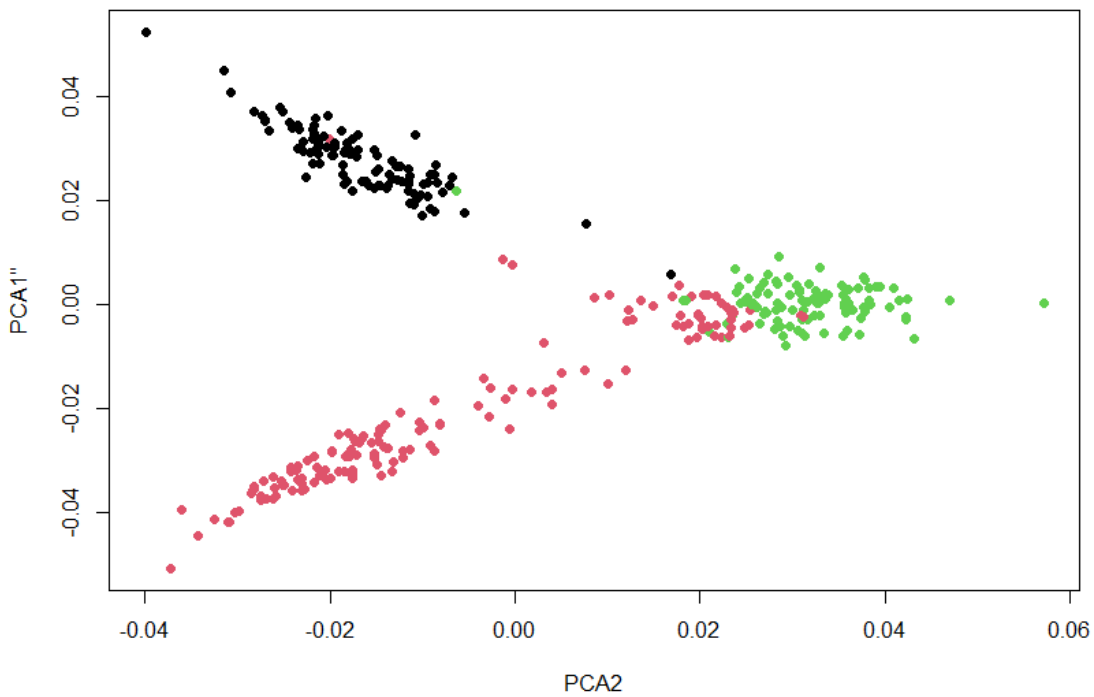

Figure 2. Population structure by genetic line (back, red and green dots) demonstrated with the first two components in a principal component analysis (PCA).
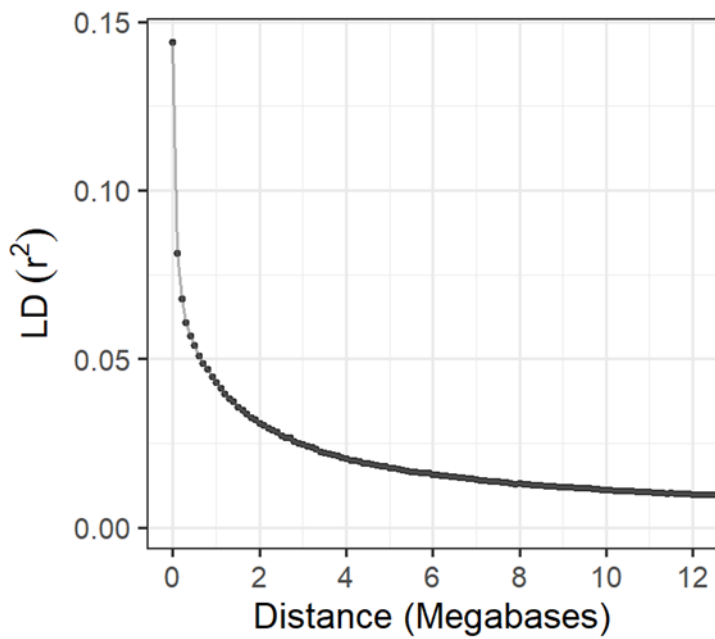

Figure 3. Linkage Disequilibrium (LD) decay with increasing distance between markers.

Genome-wide association study revealed one SNP on chromosome 5 exceeding the genome-wide significance level (**Figure 4**) suggesting that the SNP may be linked with one or more genes whose function relate with animal resilience. Indeed, according to the sheep genome assembly, gene ENSOARG00000025290 encompasses the identified SNP on chromosome 5. This gene encodes long intergenic non-coding RNAs (lincRNAs) for gene expression regulation, suggesting a possible regulatory role in biological functions associated with growth response to weather changes. However, a closer inspection of this chromosome (**Figure 5**), did not reveal any trailing SNPs in the area, suggesting that caution must be exercised with this result.
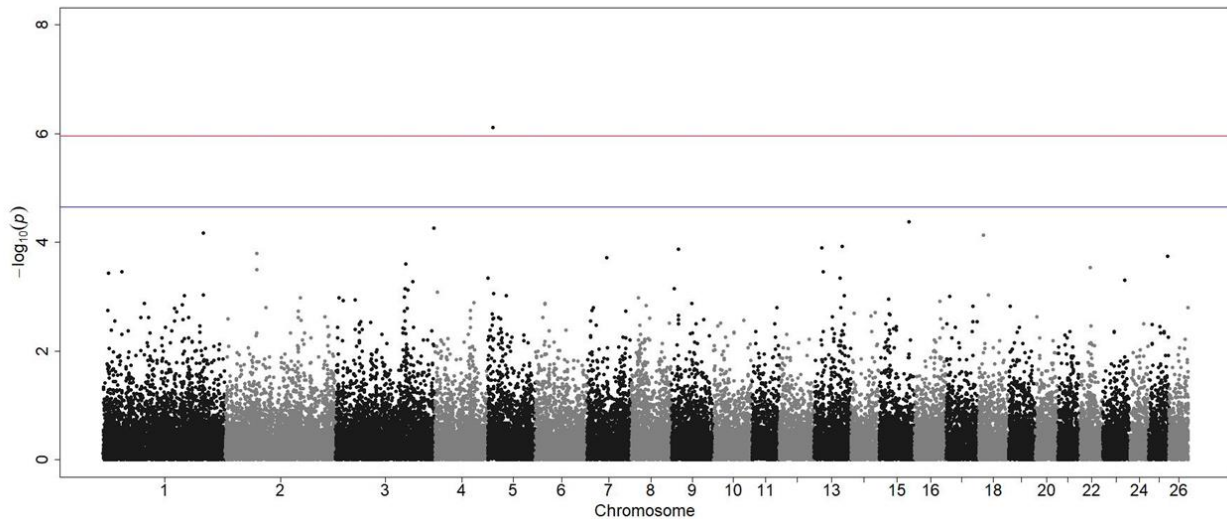

Figure 4. Manhattan plot of individual SNP markers associated with sheep resilience to air temperature change by chromosome; a single SNP on chromosome 5 was significant at genome-wide level (top horizontal line).
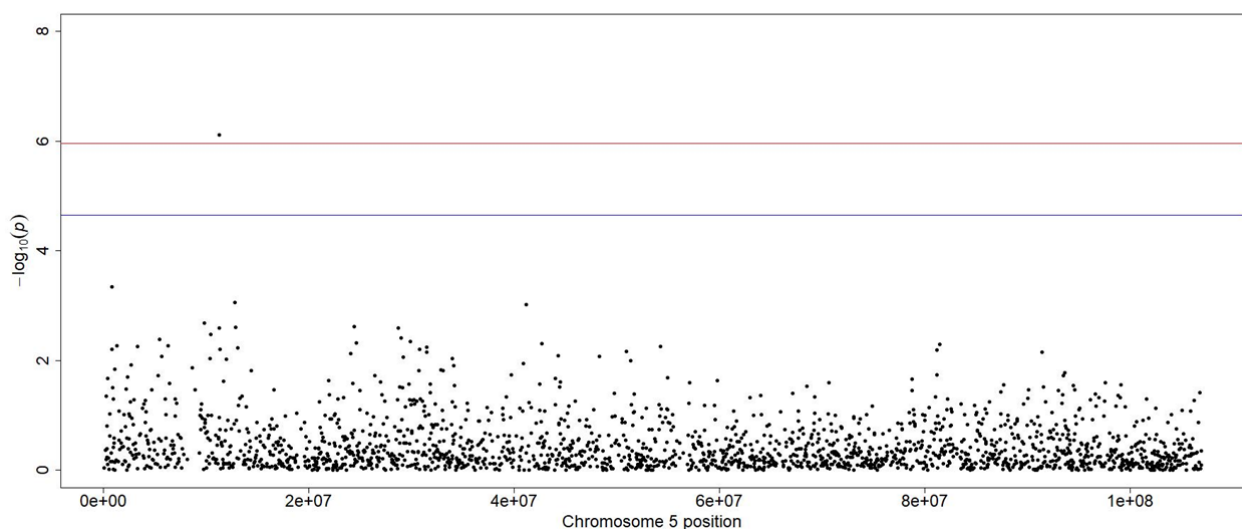

Figure 5. Manhattan plot of individual SNP markers on chromosome 5 associated with sheep resilience to air temperature change.

In addition to studying the association of individual SNP markers with resilience, genomic regions defined by sliding windows of SNP across the genome were also examined for possible associations. Results are illustrated in **Figure 6**. The highest proportion of genetic variance of resilience was accounted for by a region on chromosome 4 and amounted to 0.5% of the genetic variance of the trait. This observation combined with results from the single-marker analysis suggest that sheep resilience to air temperature change is a largely polygenic trait under the control of multiple genes. There is not a single genomic marker or region with a massive effect on the trait, whereas many genes seem to be acting together in establishing the significant genetic variation found among animals. Nevertheless, some other regions on chromosomes 1, 2, 3, 9, 12 and 13 were noted in **Figure 6,** each explaining at least 0.3% of the genetic variance of resilience.
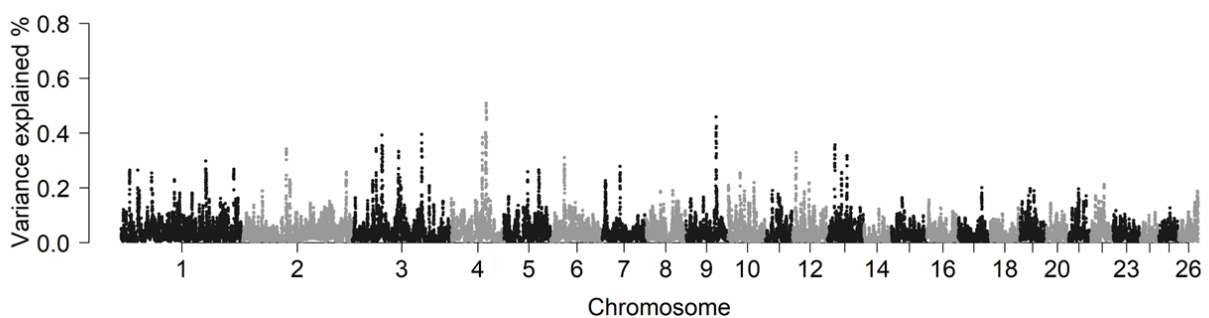

Figure 6. Manhattan plot of 1 Mb genomic regions associated with sheep resilience to air temperature change by chromosome; proportion of the genetic variance accounted for by each region is on the vertical axis.

Examination of the sheep genome assembly revealed the presence of gene SUGCT (succinyl-CoA:glutarate-CoA transferase) within the genomic region on chromosome 4 (**Figure 6**). This is annotated as a protein coding gene suggesting a possible direct control of biological functions associated with resilience. Three more protein coding genes annotated in the sheep genome assembly are located within the genomic region identified on chromosome 9 (**Figure 6**). These are the EIF3E (eukaryotic translation initiation factor 3 subunit E), RSPO2 (R-spondin 2) and ANGPT1 (angiopoietin 1) genes, whose potential functional role in controlling sheep resilience may warrant further investigation.

4.3. Genomic breeding values

Using single-step methodology, it was possible to combine all phenotypic, genotypic and pedigree data available, and derive estimated genomic breeding values (GEBV) on all 20,082 animals in the study, regardless of the type of information available on each individual sheep.

Average GEBV by year of birth were calculated for all lambs with resilience phenotypic records (**Figure 7**). There seems to be no real genetic trend for this trait across the 15 years our data

spanned. A relatively visible increase in year 2012 followed by a new plateau warrants notice but more recent data will need to be analysed in order to draw conclusions. The overall stability in the genetic trend observed may imply a lack of correlation between resilience and the different selection practices applied to the studied population. Fitting genetic line in the model of analysis would account for these differences but a separately conducted analysis excluding genetic line from the model resulted in the same trend. In fact, the genetic trend shown in **Figure 7** reflected largely the phenotypic trend in the data.
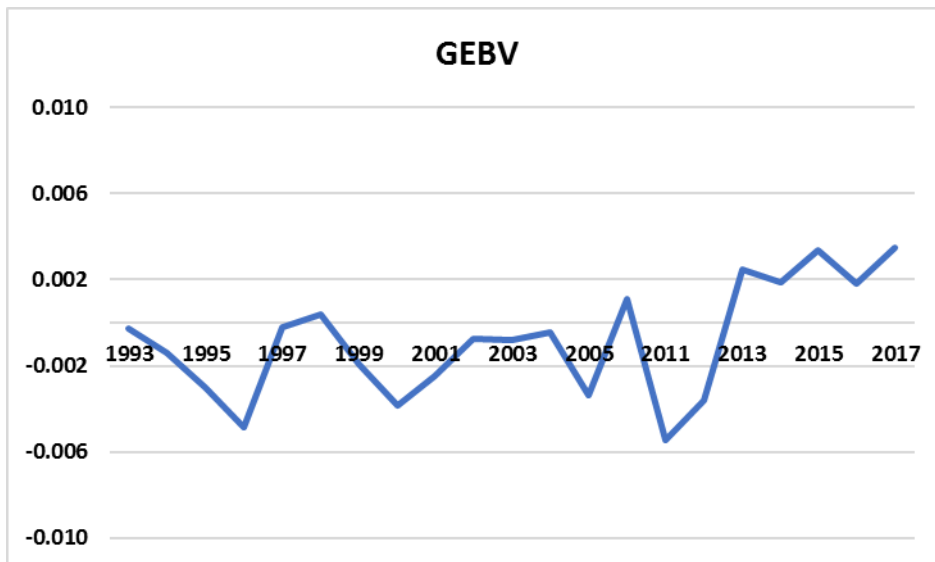

Figure 7. Average genomic breeding value (GEBV; vertical axis) of lambs with resilience phenotypes by birth year (horizontal axis).

Estimated GEBV of individual animals exhibited considerable variation, consistently with the statistically significant estimated genetic variance and heritability of the trait reported in **Table 1**. The distribution of GEBV is illustrated in **Figure 8** for lambs with resilience phenotypes and their sires. Presence of variation in GEBV may underpin genomic selection aiming to enhance climate resilience. The exact direction of selection will still have to be validated but a value close to zero, implying growth stability in the face of climate change, is probably a desirable target.

Estimates of the accuracy of the GEBV of individual animals are summarised in **Table 2**. Accuracy of the genetic evaluation affects the accuracy of selection and the success of the breeding programme. Estimates derived in the present study are encouraging in this regard, especially for the animals (lambs) with resilience phenotypes. Sires of these lambs also had GEBV with reasonably high accuracy, which, expectedly, was highest for sires with multiple offspring. In fact, these sires had on average 21 progeny with resilience phenotypes, the number ranging from 1 to 83. The latter was the sire with the highest accuracy (0.82) in the present study (**Table 2)**.
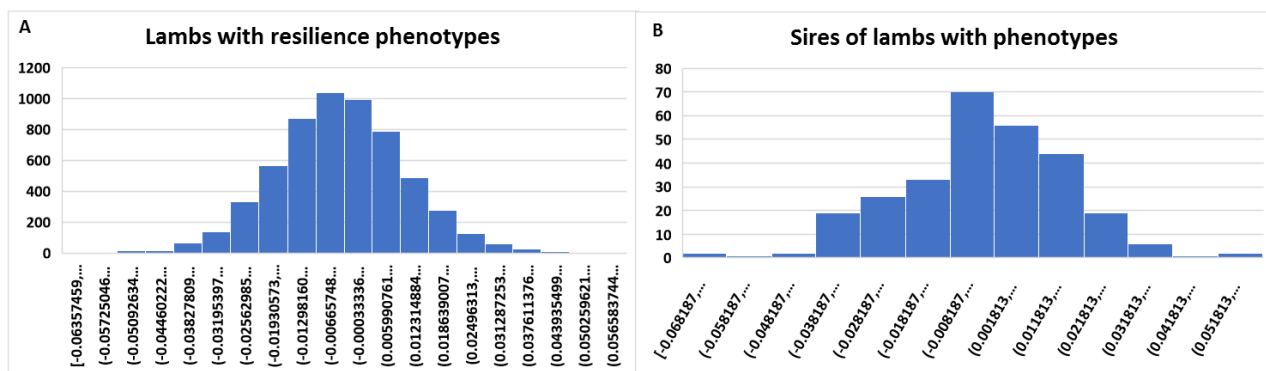
Figure 8. Histograms of estimated genomic breeding values of lambs with resilience phenotypes (A) and their sires (B); spread of values across the trajectory (horizontal axes) reflects presence of genetic variation and underpin the feasibility of genomic selection.

Table 2. Average accuracy of genomic breeding values from single-step analysis for different groups of animals

	Number of animals	Average accuracy	Maximum accuracy
All animals in the data	20,082	0.36	0.82
All phenotyped lambs	5,814	0.47	0.74
All genotyped lambs	1,766	0.31	0.64
Phenotyped and genotyped lambs	355	0.44	0.59
Phenotyped but not genotyped lambs	5,459	0.47	0.74
Genotyped but not phenotyped lambs	1,411	0.27	0.64
Pedigree animals (neither phenotyped nor genotyped)	12,857	0.33	0.82
Sires of lambs with phenotypes	281	0.62	0.82

5. Discussion

The present study set out to assess the genomic background of sheep resilience to climate change, when the trait was defined as the capacity of individual animals to maintain their performance level in the face of weather volatility. Animal phenotypes on Scottish Blackface sheep that were available from previous projects were analysed with existing genotypic and pedigree data for this purpose.

Results from the present study revealed a strongly polygenic genomic architecture of climate resilience, suggesting the presence of multiple genes affecting the trait, each with a small or modest effect. Collectively, genes controlling climate resilience led to a substantial level of genetic differences among sheep, manifested in a statistically significant estimated genetic variance and heritability, and notable variation in the GEBV of individual animals. The heritability estimate was about 011, which is within a range of low to moderate values expected for a fitness related trait such as resilience (Kruuk et al, 2000). Despite the modest magnitude of heritability, presence of significant genetic variance suggests that the trait is amenable to improvement with selective breeding.

The present study also demonstrated the feasibility of implementing a single-step procedure to combine all possible sources of information (animal phenotypes, genotypes and pedigree) in order to derive genomic breeding values for resilience with reasonably high accuracy. This is especially interesting when different pieces of information pertain to different animals. Importantly, estimated genomic breeding values varied substantially among individual animals suggesting that desirable genotypic profiles may be inferred and used to underpin genetic selection for enhanced resilience.

The direction of selection to render animals more resilient to climate change needs to be validated and refined. Quite possibly a target value of zero for the trait is desirable, implying no fluctuation in animal performance in response to weather volatility. This is consistent with the very few previous genetic studies of climate resilience in small ruminants (Sánchez-Molano et al, 2020; Ramón et al, 2021). Based on simulations, the latter studies suggested that climate resilience as defined here should be included in the breeding goal and receive a relative emphasis of 10-20%. Those studies also addressed the relationship of resilience with other traits in the breeding goal. Pedigree-based analyses revealed an antagonistic genetic correlation between resilience and weaning weight, implying that animals with the genetic propensity to grow faster before weaning would exhibit more variable response to air temperature changes (Sánchez-Molano et al, 2020). Nevertheless, the possibility to combine such antagonistic traits in a selection index leading to overall improvement was also demonstrated (Sánchez-Molano et al, 2020). Estimation of these correlations at the genomic level were beyond the scope of the present study. Future studies may assess the genomic correlation of resilience and other traits and provide new input to the development of a relevant genomic selection index.

The present study focused on climate resilience of lamb growth, which is an animal production trait. Similar studies on other traits associated with animal health, reproduction and product quality would be needed for a more comprehensive view of resilience to climate change.

Changing weather is not the only environmental stressor that challenges livestock. Variability in pasture quality, the nutrient content of feed, and parasitic challenge constitute additional stressors that grazing animals face. Climate change may actually influence the severity of the impact of these factors, in addition to directly affecting animal performance and function. Weather variables such as air temperature are probably easier to record than many of the other environmental conditions. Nevertheless, relevant data on the latter would allow studies on animal resilience to other sources of environmental variability to be conducted.

In conclusion, lamb growth resilience to climate change is a heritable trait and can be improved with selective breeding. Phenotypes reflecting performance changes in response to weather events can

be readily developed based on existing performance records and publicly available meteorological data.

6.   References

Agovino M, M Casaccia, M Ciommi, M Ferrara and K Marchesano. 2019 "Agriculture, climate change and sustainability: the case of EU-28" Ecol. Indic.105:525–43.

Archibald AL, NE Cockett, BP Dalrymple, T Faraut, JW Kijas, et al. 2010 "The sheep genome reference sequence: a work in progress" Anim. Genet. 41:449–453.

Butler DG, BR Cullis, AR Gilmour, BJ Gogel and R Thompson. 2017 "ASReml-R Reference Manual Version 4" VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Innovation for Sustainable Sheep and Goat Production in Europe (iSAGE) project. 2019-2020 www.isage.eu.

Kruuk LE, TH Clutton-Brock, J Slate, JM Pemberton, S Brotherstone and FE Guinness. 2000 "Heritability of fitness in a wild mammal population" Proc. Natl. Acad. Sci. USA. 97(2):698–703.

Misztal I, S Tsuruta, T Strabel, B Auvray, T Druet and DH Lee. 2002 "BLUPF90 and related programs (BGF90)" Proceedings of the 7th World Congress on Genetics Applied to Livestock Production: 19–23 August 2002; Montpellier, France.

Rowell DP. 2005 "A scenario of European climate change for the late twenty-first century: seasonal means and interannual variability" Clim. Dyn. 25(7):837–49.

Purcell S, B Neale, K Todd-Brown, L Thomas, MA Ferreira, D Bender, et al. 2007 "PLINK: a tool set for whole-genome association and population-based linkage analyses" Am. J Hum. Genet. 81:559–75.

Ramón M, MJ Carabaño, C Díaz, VV Kapsona, G Banos and E Sánchez-Molano. 2021 "Breeding strategies for weather resilience in small ruminants in Atlantic and Mediterranean climates" Front. Genet. 12:692121. doi: 10.3389/fgene.2021.692121.

RStudio Team. RStudio: Integrated Development for R. Boston: RStudio, Inc.; 2020. http://www.rstudio.com/.

Sánchez-Molano E, VV Kapsona, JJ Ilska, S Desire, J Conington, S Mucha and G Banos. 2019 "Genetic analysis of novel phenotypes for farm animal resilience to weather variability" BMC Genet. 20(1):84.

Sánchez-Molano E, VV Kapsona, S Oikonomou, A McLaren, N Lambe, J Conington and G Banos. 2020 "Breeding strategies for animal resilience to weather variation in meat sheep" BMC Genet. 21, 116 https://doi.org/10.1186/s12863-020-00924-5.

Thornton PK, J van de Steeg, A Notenbaert and M Herrero. 2009 "The impacts of climate change on livestock and livestock systems in developing countries: a review of what we know and what we need to know" Agric. Syst. 101(3):113–27.

UK Government. 2017 https://www.gov.uk/government/publications/uk-climate-change-risk-assessment-2017.

VanRaden, P. 2011 findhap.f90. https://aipl.arsusda.gov/software/findhap/.

Zhou X and M Stephens. 2012 "Genome-wide efficient mixed-model analysis for association studies" Nat. Genet. 44:821–4.