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Genotype-by-environment interaction and genomic basis of adaptation to altitude in local cattle breeds

E. Rulli[1*], E. Mancin[1], D. López-Carbonell[2], G. Gomez Proto[1], A. Oian[1], L. Varona[2], R. Mantovani[1], and C. Sartori[1]

[1]University of Padua, Agripolis, Legnaro (PD), Italy; [2]Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain; [*]Corresponding author: eugenio.rulli@studenti.unipd.it

Introduction

Alpine livestock systems provide essential ecosystem services while exposing animals to multiple environmental stressors, including hypoxia, steep terrain and variable forage quality (Battaglini *et al*., 2014). In the Aosta Valley (Italy), altitude represents an indicator of these environmental and management conditions, with higher elevations typically associated with low-input, grazing-based systems (Bassignana *et al*., 2011). Local dual-purpose breeds Aosta Red Pied (ARP) and Aosta Black Pied-Chestnut (ABPC) are traditionally reared across this altitudinal gradient, making their resilience and adaptability crucial for the sustainability of Alpine production systems. Genotype-by-environment interactions (G×E) may bias genetic evaluations and reduce selection response if ignored (Mulder, 2016). In this context, random regression models (RRMs) provide a suitable framework to investigate G×E by modelling phenotypes as functions of continuous environmental descriptors (Schaeffer, 2004). While linear reaction norms are commonly applied, environmental responses may be non-linear along complex gradients. The inclusion of first and second order polynomials allows the joint estimation of genetic intercepts and linear and quadratic components of environmental sensitivity, offering greater flexibility in describing G×E when non-linear relationships occur (Streit *et al*., 2012). Integrating RRMs with single-step genome-wide association studies (ssGWAS) enables the identification of genomic regions associated with both average performance and environmental sensitivity, using information from genotyped and non-genotyped animals (Wang *et al*., 2012; Misztal *et al*., 2009). In this context, investigating altitude-related G×E and the underlying genomic architecture in Aosta cattle provides insight into the biological mechanisms supporting functional adaptation and efficient use of marginal Alpine environments. Therefore, this study aimed to analyse G×E related to altitude in ARP and ABPC cattle, and to identify genomic regions associated with baseline performance and environmental sensitivity through ssGWAS.

Materials & Methods

Data were provided by National Breeders' Association and were collected from herds located between 314 and 2,005 meters above sea level in 34 years. Given the data editing, the ARP dataset comprised 997,883 test day records from 57,253 cows, whereas the ABPC dataset comprised 534,479 observations from 35,950 cows. Five milk production traits were analysed: test day milk yield (MY), protein yield (PY), fat yield (FY), protein percentage (PP), and fat percentage (FP). The general structure of RRM used to investigate G×E interaction was consistent with the repeatability test day model currently adopted in routine genetic evaluations for productive traits. The model included: the fixed effect of herd, gestation class, number of lactations, age at parity class within lactation, and month at parity class within lactation, with the latter two covaried by the days in milk expressed as third-order Legendre polynomials; the random effects of herd-test day, permanent environment, and additive genetic component. This

baseline model was extended by including altitude as a continuous environmental descriptor. The genetic intercept represents (l_0) the average genetic performance, whereas the first order (l_1) and second order (l_2) Legendre polynomials describe linear and non-linear components of genetic sensitivity. Variance components were estimated under a Bayesian framework using GIBBSF90+ (Lourenco *et al*., 2022). Following the RRM analyses without genomic data, genomic information was subsequently incorporated into the single-step approach. To achieve this, single-step genomic BLUP (ssGBLUP) and ssGWAS were performed using the same RRMs and variance estimates obtained before. Genotype quality control was performed by excluding single nucleotide polymorphisms (SNPs) with a minor allele frequency < 0.05, a call rate < 0.95, or an absolute difference between observed and expected heterozygosity > 0.05. Additionally, SNPs and individuals were removed if they showed more than 2% parent-progeny incompatibilities. After quality control, genomic data from 7,391 genotyped ARP animals and 5,066 genotyped ABPC animals were retained, with 79,931 and 81,485 imputed SNPs available for ARP and ABPC, respectively. The ssGWAS was then performed using non-overlapping windows of 25 adjacent SNPs, and the top 0.5% of windows were selected based on the proportion of additive genetic variance explained. Candidate genes within selected windows were annotated using the R package GALLO (Fonseca *et al*., 2020) based on the ARS-UCD2.0.

Results & Discussion

In ABPC, mean MY was 11.1±3.9 kg, followed by FY of 0.38±0.14 kg and PY of 0.37±0.12 kg; mean FP and PP were 3.41±0.60 % and 3.40±0.32 %, respectively. In ARP, MY averaged 14.8±4.4 kg, with FY and PY of 0.51±0.17 and 0.48±0.14 kg, and FP and PP of 3.46±0.58 % and 3.28±0.34 %, respectively (data not shown). These differences reflect the higher production level of ARP compared with ABPC, consistent with their distinct breeding objectives. Genetic variances components for l_0, l_1 and l_2 estimated from the RRMs are reported in Table 1.

Table 1. Posterior means (95% HPD) of additive variances of random regression coefficients describing the genetic intercept (l_0) and first and second order polynomials (l_1, l_2).

		Variances		
Trait	Breed	l_0	l_1	l_2
MY	ABPC	3.933 (3.596; 4.280)	0.969 (0.690; 1.275)	0.769 (0.548; 1.027)
	ARP	3.769 (3.516; 4.033)	0.587 (0.409; 0.812)	0.757 (0.595; 0.933)
FY[1]	ABPC	4.882 (4.447; 5.335)	1.015 (0.693; 1.392)	0.776 (0.540; 1.063)
	ARP	4.425 (4.115; 4.776)	0.391 (0.208; 0.593)	0.426 (0.298; 0.577)
PY[1]	ABPC	3.801 (3.477; 4.151)	0.951 (0.670; 1.254)	0.752 (0.538; 1.001)
	ARP	3.310 (3.073; 3.560)	0.614 (0.446; 0.808)	0.647 (0.502; 0.802)
FP[1]	ABPC	98.190 (93.190; 103.400)	2.770 (1.418; 4.468)	0.822 (0.442; 1.271)
	ARP	79.809 (75.980; 84.140)	1.902 (1.055; 3.178)	2.542 (1.415; 3.703)
PP[1]	ABPC	42.369 (40.290; 44.590)	2.438 (1.519; 3.415)	0.381 (0.252; 0.523)
	ARP	40.301 (38.610; 42.070)	1.324 (0.808; 2.030)	0.833 (0.434; 1.283)

[1] Variances have been multiplied by 10^3.

Genetic correlations were evaluated between representative low and high altitudes, defined as approximately the 10th and 95th percentiles of the altitude distribution within breeds. In ABPC, the mean genetic correlation between extreme altitudes across traits was 0.79±0.09, with substantial variability among traits: correlations ranged from 0.70 to 0.77 for yield traits (MY, FY, PY) and from 0.86 to 0.92 for composition traits (FP, PP; data not shown). This pattern indicates mild but trait-dependent G×E, with yield traits showing greater sensitivity to altitude. In ARP, genetic correlations were consistently higher and less variable, with a mean correlation

of 0.95±0.03 across traits; yield traits showed correlations between 0.93 and 0.94, while composition traits approached unity, suggesting a more stable genetic ranking along the altitudinal gradient (data not shown). Across traits, the proportion of additive genetic variance associated with regression coefficients l_1 and l_2 averaged 14.5±9.1% in ABPC and 9.4±6.3% in ARP, bearing out a limited but consistent genetic component underlying environmental sensitivity. In both breeds this proportion was markedly higher for yield traits (ABPC: 19.0–22.3%; ARP: 10.2–16.0%) than for composition traits, for which it remained consistently low (≤6.0% in ABPC and ≤3.1% in ARP). Together, these results indicate that G×E effects along the altitude gradient are modest but non-negligible, predominantly affecting yield traits and being more pronounced in ABPC than in the more intensively selected ARP, consistent with a greater genetic sensitivity to environmental variation. Percentages of additive genetic variance for MY, FP and PP explained by genomic windows for l_0, l_1 and l_2, are shown in the Miami plots in Figure 1.

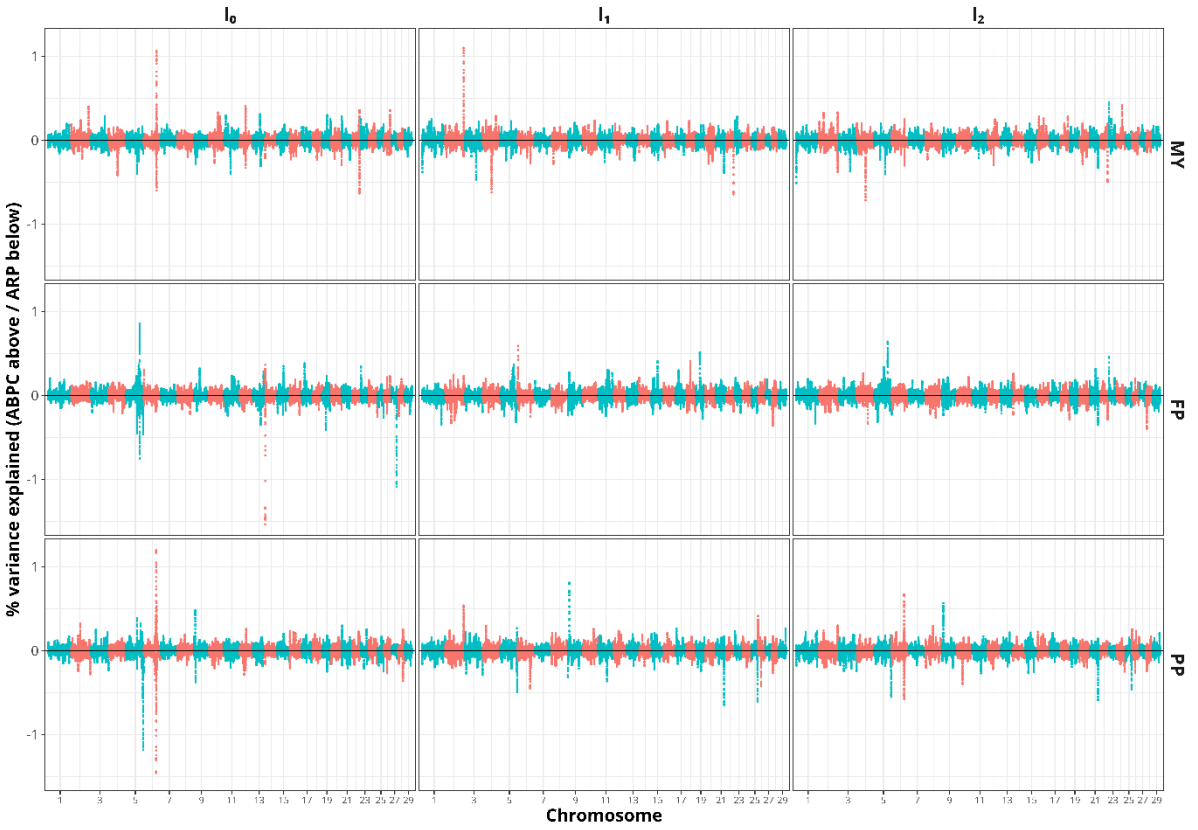

Figure 1. Miami plots for MY, FP and PP showing the percentage of additive genetic variance explained by windows of 25 adjacent SNPs for the genetic intercept (l_0) and first and second order polynomials (l_1, l_2); ABPC is shown above and ARP below.

The ssGWAS revealed more pronounced genomic signals for l_0, identifying regions explaining larger proportion of additive genetic variance compared with l_1 and l_2. The regions related to the average genetic performance (l_0) were largely consistent with those previously reported for milk traits in same breeds (Bernini *et al*., 2024). In contrast, genomic regions associated with l_1 and l_2 explained smaller fractions of variance and showed a more diffuse genomic architecture, consistent with the limited magnitude of G×E detected. Despite this, a limited set of genes associated with l_1 and l_2 effects was shared between ABPC and ARP, including *EXTL1*, *PAFAH2*, *SLC30A2*, *AFM*, *AFP*, *ALB*, *ANKRD17*, and *COX18*. These genes are mainly involved in metabolic regulation, vascular and cellular stress-related processes, oxidative and

inflammatory stress response, and nutrient and energy metabolism, suggesting conserved biological mechanisms underlying adaptation to altitude across both breeds. In addition, a partial overlap between genes associated with l_0 and those associated with l_1 and l_2 was observed, particularly in ARP, indicating a tighter coupling between average productivity and environmental sensitivity in the breed showing overall higher milk yield. Conversely, in ABPC a lower overlap was found between intercept- and slope-associated regions and several genes uniquely associated with l_1 and l_2, including *STMN1*, *CSRNP3*, *GALNT3*, *TREM1*, and *TREML2*. These genes are primarily involved in cellular plasticity, transcriptional regulation, metabolic modulation, and innate immune response, supporting a stronger role of stress-responsive and immuno-metabolic pathways in the genetic sensitivity of ABPC to altitude.

Conclusions

Overall, G×E interactions related to altitude were modest but biologically consistent and were a bit more evident in ABPC than in ARP. Genetic sensitivity to altitude was mainly expressed in yield traits and in ABPC, whereas ARP showed a largely stable genetic response along the gradient. Genomic analyses supported a polygenic and diffuse architecture for altitude-related effects, involving a limited set of genes linked to metabolic, vascular, and stress-response functions. Accounting for these findings should help in better designing breeding programs and matings.

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