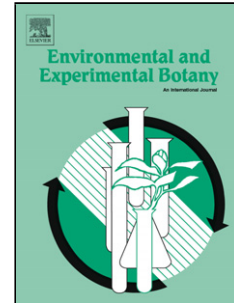


# Journal Pre-proof

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## Local adaptation along a sharp rainfall gradient occurs in a native Patagonian grass, *Festuca pallescens*, regardless of extensive gene flow

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### Highlights

- Climate change might alter adaptation patterns in North Patagonian forage grasses
- *F. pallescens* populations exhibited a weak genetic structure along this region
- Plant architectures entails growth patterns associated to their home environments
- Increasing aridity might favour a more compact cespitose architecture

### ABSTRACT

Increasing global aridity combined with changing environmental conditions might cause irreparable alterations in arid and semiarid ecosystems. Knowledge of within-species genetic diversity and adaptive responses, especially along climatic gradients, becomes critical to inform management, conservation and restoration efforts. We combine the analysis of neutral (microsatellites) and adaptive divergence (morphological traits in common garden experiments) between populations along a sharp precipitation gradient in the native Patagonian grass *Festuca pallescens*, to disentangle patterns of local adaptation and genetic diversity. The identification of genetically based traits under divergent selection denoted the existence of local adaptation in spite of extensive gene flow. Two plant growth patterns were detected associated with two ecological regions. In arid environments, a trade-off between not resigning foliage production, and reducing the exposure to drying agents resulted in a compacted plant architecture; while in dry-sub humid environments, populations exhibited a wide architecture to cope with rainfall and radiation interception. Increasing aridity and grazing might favor a compact plant architecture, and fragmentation might reduce genetic diversity across these environments. Being a widely distributed species, *F. pallescens* is an excellent model species to study adaptive responses across environmental gradients facing climate change predictions, especially in dryland rangelands that maintain multiple ecosystem functions.

**Keywords:** Drylands - Environmental gradients - *Festuca pallescens* - Local adaptation – Neutral genetic diversity - Phenotypic variation

## INTRODUCTION

Facing an increase of global aridity, many rangelands from arid, semi-arid and dry subhumid ecosystems over the world might undergo irreparable alterations. Natural rangelands cover about 41% of the Earth's land area (MEA, 2005) and their species provide different ecosystem services like climate regulation, forage production, participation on processes such as nutrient cycling, infiltration and energy flow (Aguir and Sala, 1999; Franklin et al., 2016). Plant populations in these ecosystems are predicted to decline due to high vulnerability to increasing aridity (Golodets et al., 2015). Moreover, climate change is expected to promote a reduction in annual precipitation deepening alterations in those environments where the precipitation gradients are sharp, having detrimental effects on biodiversity and species' geographical distributions (Aguirre-Liguori et al., 2019). Therefore, species might experience alterations in their natural environments, to which they may adapt or evolve (Holderegger and Wagner, 2008). In this context, it becomes necessary to increase our knowledge on the level of local adaptation in dominant grasses that are the main forage resource for livestock grassing (Johnson et al., 2015). Climate change might alter patterns of local adaptation (Aguirre-Liguori et al., 2019), therefore knowledge of within-species genetic diversity and adaptive response, especially along climatic gradients, becomes critical to inform management, conservation and restoration efforts (Harris et al., 2006).

Rangelands are characterized by high environmental heterogeneity, which results from the spatial and temporal variation of abiotic factors and has a strong incidence in the distribution patterns and abundance of species (Stewart et al., 2000). The spatial-temporal variability of temperature, precipitation, soil water availability and / or evapotranspiration, favors the formation of gradients (Chapin III, 1993; Jump et al., 2009), lending support for the existence of natural variation and local adaptation, mostly for plant species. Populations across gradients have the potential to undergo local adaptation to a specific environment (Conner and Hartl, 2004) or, alternatively, phenotypic plasticity could promote changes in the phenotypic expression of physiological and morphological traits to match environmental cues (Tienderen, 1997). Hence, phenotypic plasticity with genetically based adaptive variation is also possible. Understanding local adaptation requires the identification of genetically based traits under divergent selection.

The maintenance of high levels of genetic variation provides populations with the potential to adapt to changes in their environment, whether these changes are relatively slow (e.g. climate change) or rapid (e.g. inter-annual climate cycles), which determine alterations in multidimensional variables such as temperature and

precipitation (Jump et al., 2008). Habitats from heterogeneous environments maintain ecologically important genetic variation among populations (McKay et al., 2001), which can be neutral or adaptive. Nevertheless, both within-species genetic diversity and adaptive responses provide populations with the possibility to inhabit changing and heterogeneous environments (Lowry et al., 2014). The study of genetic diversity across these environments give insight into the role of environmental drivers in adaptive differentiation (Johnson et al., 2015). Neutral genetic variation using molecular markers, such as microsatellites, allows the evaluation of intraspecific patterns of diversity among populations (Zhan et al., 2005), and the inference of the relative influence of gene flow and genetic drift in promoting differentiation between populations. On the other hand, plant morphological, physiological and phenological traits might reflect possible adaptations to their ecological niche (Khasanova et al., 2019a). Common garden experiments have been extensively used to determine the relative contribution of genetics and environment to phenotypic variation (Kawecki and Ebert, 2004) and are a powerful approach to investigate local adaptation among populations in plant communities (de Villemereuil et al., 2016).

The Patagonia region is the largest dryland in South America (Villalba et al., 2003), characterized by a large environmental heterogeneity, resulting from a complex geomorphologic history and the occurrence of sharp environmental gradients. Several geomorphological past events, such as glaciations, marine transgressions, the existence of a continuous permafrost and an elevated ancient and isolated basaltic plateau have shaped the current topography and probably left an imprint in the vegetation (Flint and Fidalgo, 1964; Malumian and Nanez, 2011; Rabassa et al., 2011). Particularly in North Patagonia, a narrow rainfall gradient accompanied by a highly complex geomorphology influences the distribution of its vegetation. North Patagonian rangelands are the most important forage resource for domestic livestock, the main agricultural activity of the region (Villagra, 2005), being currently threatened by unsustainable land use, climate change, desertification and overgrazing (Gaitán et al., 2018). Perennial grasses and shrubs dominate these environments. Climatic models predict an increase in aridity for Patagonian drylands, which could accentuate the negative effects of overgrazing, increase the fragmentation of suitable habitat for perennial grasses and reduce the capability of Patagonian ecosystems to provide forage production (Gaitán et al., 2019). Moreover, being wide distributed species, grasses might exhibit an increase of genetic differentiation among populations generally from the center to the periphery of their distribution (Eckert et al., 2008), tending to be smaller and more fragmented towards the limits (Bridle and Vines, 2007; Thomas and Kunin, 1999).

Widely distributed species give an excellent opportunity to study within-species genetic diversity and adaptive responses across changing and heterogeneous environments (Eckert et al. 2008). *Festuca pallescens* (St. Yves) Parodi is a key native forage grass with high participation in small ruminants diet (Bonvissuto et al., 2008) and an extended distribution in Patagonia. This species inhabits diverse environments, type of soils and ecological areas (Bertiller et al., 1990; Catalán and Müller, 2012) covering a sharp rainfall gradient in North Patagonia, from

2000 mm of average annual precipitation up to 200 mm in less than 300 km (León et al., 1998). In addition, it is an indicator of ecosystem regression since its elimination is one of the most evident steps of degradation and loss of wildlife diversity (Bertiller et al. 1990). The genetic connection between populations of *F. palleescens* might decrease towards the edges of its distribution along the rainfall and topographical gradient due to the reduction of favourable environments for the species (i.e. wetlands). In general, the inability of peripheral populations to adapt to environments beyond their current range might be due to inadequate environmental conditions for their development (Holt and Keitt, 2000; Pfennig, 2016). This could mean a reduction of genetic diversity, highlighting the effects of genetic drift and inbreeding.

Past demo-stochastic processes, such as glaciations, might have modeled the distribution of genetic variation among populations of *F. palleescens*, as in other species of this region (rev. in Sercic et al. 2011). More recently, the rainfall gradient could have a strong incidence in the genetic structure of these populations. Hence, the isolated and small populations located at the extreme of the rainfall gradient in the periphery of its distribution in north Patagonia, might exhibit less diversity and greater genetic structuring than the rest of the populations. On the other hand, the variety of environments in which *F. palleescens* develops, might have promoted local adaptation processes evidenced in morphological characters of adaptive importance related to plant growth. We hypothesized that precipitation differences across the environmental gradient would exert selection pressures strong enough to overcome gene flow, resulting in local adaptation. The comparison between patterns of both phenotypic and neutral genetic variation among populations would help revealing the factors driving population differentiation, and the existence (or not) of local adaptation. Here we combine the analysis of neutral (microsatellites) and adaptive divergence (morphological traits in common garden experiments) from different environments along a precipitation gradient in a native Patagonian grass, *Festuca palleescens* to disentangle the following questions: *i*) is the neutral genetic variation structured along the rainfall and topographical gradient, *ii*) do populations exhibit genetic differences related to main environmental factors (such as precipitation and temperature) characteristic of their home environments, *iii*) are populations of *F. palleescens* locally adapted?

## MATERIALS AND METHODS

### *Plant material and Sampling*

Populations of *F. palleescens* were collected in five main sampling sites situated along the west-east decreasing rainfall gradient of about 500 km in North Patagonia (Argentina) (Table 1; Fig. 1). At each sampling site, between one to four populations from highland steppes and wet meadows were sampled, gathering 12 populations belonging to different altitudes (Table 1), and covering three ecological regions that include diverse vegetal communities (Bran et al., 2000; Gaitan et al., 2019): *Cordillera*, *Precordillera* and the *Hills and Plateaus*.

The *Cordillera* ecological region covers the Andes mountain range, with cold and humid weather, an annual mean temperature lower than 10 °C and a wide rainfall regime (700 - 3000 mm mostly concentrated in the autumn-winter). The vegetation is characterized by forests and thickets that occur discontinuously (with patches of natural grasslands and wetlands), and are dominated by the tree species: *Nothofagus pumilio*, *Nothofagus dombeyi*, *Nothofagus antarctica* and *Austrocedrus chilensis*. *Precordillera* ecological region (Bran et al., 2000) represents an ecological gradient between the *Cordillera* and *Hills and Plateaus*. Climate is dry and cool, the annual mean temperature is lower than 10 °C and rainfall is concentrated in autumn-winter seasons (700-300 mm). The populations of *Festuca pallescens* are located in grasslands steppes at the Andes foothills, in the form of patches or mosaics with the deciduous forest (*Nothofagus* spp. and *Austrocedrus chilensis*) or in meadows that could be located at high plateaus (Bran et al., 2000). The *Hills and Plateaus* ecological region is characterized by saw relief, basaltic plateaus above 900 m.a.s.l., an annual mean temperature of around 8 - 10°C, a low precipitation regime (300-150 mm), a strong spring-summer water deficit and the vegetal community is the Occidental District of the Patagonia phytogeographic province (León et al., 1998). *Festuca pallescens* populations are located in grasslands steppes and meadows above 1200 m.a.s.l or in meadows in lower sectors. With this sampling strategy we covered the different environments where *F. pallescens* is distributed. At each sampling site, we selected similar sized rangelands.

For molecular analysis we collected leaves from at least 30 individuals in each population (a tussock is considered an individual); while for common garden experiments, we sampled a pool of seeds (10-20gr; 1000-seed weight is 1-1,3gr) of each population. The leaves were conserved in paper bags and stored at -20 °C until processing for genetic analysis. Eight of the twelve populations were selected for common garden experiments based on the availability of seeds and the representativeness of the diverse environments that the species inhabits along the entire rainfall gradient. Seedlings were obtained after germination at 15, 5°C in a germination chamber with saturated moisture.

#### *Molecular analyses: DNA extraction, PCR amplification, genotyping and microsatellite analyses*

Leaf tissue was frozen in liquid nitrogen and grounded to fine powder using an automatic mixer mill (Resch, Germany) to extract DNA following a modified protocol of Doyle & Doyle (1987) according to Gonzalo-Turpin & Hazard (2009). We used a set of eight microsatellite loci (SSR) (HVM4, B1B6, M4-213, Fo74, CML127, B1C9, KSUM157, F037) developed for *Lolium perenne*, *Festuca-Lolium* complex of grasses and *Festuca* spp (Fu et al., 2006; Jensen et al., 2007; Lauvergeat et al., 2005), that proved to be polymorphic in *F. pallescens* (López et al., 2018). PCR amplifications were carried out according to Lauvergeat et al. (2005), as described in Lopez et al. (2018) (see Supplementary material for detailed protocols). Microsatellite primers were labelled with different fluorophores and then pooled in three groups for analysis in an ABI 3700 sequencer at

Genomics Unit, Biotechnology Institute, CICVyA, INTA, Hurlingham, Argentina. Fragments visualization and allele scoring was performed with Genemarker Version 1.97 (SoftGenetics, State College, PA, USA).

*Statistical analysis of microsatellite genetic data: Genetic diversity, differentiation and structure.*

*Festuca pallescens* is an allohexaploid forage grass (Dubcovsky and Martínez, 1991). The estimation of genetic parameters such as heterozygosity or null allele detection is difficult in polyploid organisms, because of dosage uncertainty, the problem of inferring allele frequencies and assumptions regarding inheritance (Dufresne et al., 2014). Therefore, there is a degree of ambiguity in the determination of the correct number of alleles of an individual. To avoid the assignment of an allele to a doubtful peak, we considered an allele based on a fluorescence intensity greater than 400 UFR (Relative Fluorescence Units) nm and the absence of "stuttering". Alleles from polymorphic loci were autoscored on GeneMarker V2.6.3 (SoftGenetics, demo version), manually edited and analyzed as dominant markers. Although some of the genetic information is lost when treating the markers as dominant, this method is still more robust than using dominant markers (i.e. AFLPs) in genetic analysis (Moscoe and Emshwiller, 2015) and widely used in polyploid species (Rouger and Jump, 2014; Sampson and Byrne, 2012).

The genetic diversity in each population was estimated with the percentage of polymorphic loci ( $P$ ), the number of alleles per locus ( $A$ ), number of alleles with a frequency greater than 5% ( $A_{5\%}$ ), number of exclusive alleles ( $A_E$ ) and Nei's unbiased genetic diversity (Nei, 1987) ( $uh$ ). Nei's diversity measure is equivalent to the Simpson index (Simpson, 1949) and is calculated with the following equation  $uh = n / (n-1) (1 - \sum p_i^2)$  where  $p$  = frequency of the present band. All these parameters were estimated with GenAlEx v. 6.5 (Smouse and Peakall, 2012). In addition, the genetic distance between populations was evaluated with the distance of Bruvo (Bruvo et al., 2004) which is recommended for polyploid organisms (Dufresne et al., 2014) using *polysat* package (Clark and Jasieniuk, 2011) in R (R Development Core Team 2013). This distance takes into account the mutational distance between alleles (Clark and Jasieniuk, 2011) and it is independent of the level of ploidy and the allele dosage. Using the estimated Bruvo's distance a Principal Coordinate Analyses (PCoA) was also calculated in *polysat*.

Genetic structure of the populations of *F. pallescens* was estimated with a hierarchical Analysis of the Molecular Variance (AMOVA) based on the three ecological regions (Table 1) using the  $\Phi_{PT}$  fixation index recommended for polyploid organisms in GenAlex 6.5 (Smouse and Peakall, 2012). The level of significance was evaluated by performing 999 permutations. On the other hand, to establish the number of genetic groups and evaluate signals of admixture, we used STRUCTURE v. 2.3.4. (Pritchard et al., 2000). The SSRs were analyzed as a binary database (presence/absence of alleles). We used the admixture model with correlated allele frequencies because it is more conservative and provides greater power to detect populations that are closely related (Porrás-

Hurtado et al., 2013). We run Structure 10 times for each K; K varied from 1 to 12, using a burn-in period length of 100,000 iterations and then 300,000 additional iterations of random Markov Chains with a Monte Carlo procedure (MCMC). The optimal number of groups was determined through the method of Evanno et al. (2000) by estimating the  $\Delta K$  in the online platform STRUCTURE HARVESTER (Earl and von Holdt, 2012). Then, we used Clumpak (Kopelman et al., 2015) to combine the files of the 10 repetitions of the optimum K and prepare the graphic representation.

STRUCTURE assumes that populations are in Hardy-Weinberg equilibrium and that linkage disequilibrium exists between the loci of a population (Pritchard et al., 2000). These assumptions do not generally apply for polyploid data, therefore we additionally implemented the approach by Arrigo et al. (2010) based on a non-hierarchical K-means clustering (KMNS). This method assigns individuals to a genetic group (k) defined a priori to maximize the intergroup variance (Legendre and Legendre, 1998). The analysis was carried out using *stats* package in R (Development Core Team 2013) based on Arrigo et al. (2010). A set of 100,000 independent runs were performed for each K value (K varied from 1 to 7).

Finally, the degree of gene flow between populations was estimated indirectly through the differentiation ( $\Phi_{PT}$ ) between pairs of populations using GenAlex 6.5 (Smouse and Peakall, 2012) and its significance by performing 999 permutations. Isolation by distance was estimated through a Mantel test, correlating the matrix of the natural logarithm of the geographical distances with the matrix of genetic Bruvo's distances. The significance of the test was evaluated with 999 permutations in GenALEX v 6.5. Likewise, correlations were made between the genetic diversity, expressed as the total number of alleles (A) and environmental and geographical variables (precipitation and temperature of the driest and warmest months, altitude, longitude and latitude) obtained from Worldclim ([www.worldclim.org](http://www.worldclim.org)) for each sample site using the program GraphPad Prism demo version 5.00 for Windows, GraphPad Software, San Diego California USA, [www.graphpad.com](http://www.graphpad.com).

#### *Common garden experiments:*

Eight populations out of the twelve were selected for common garden experiments (see *Plant material and Sampling*). Twenty plants per population were obtained from germination of seeds at 15.5°C and saturated moisture, in 200-cavity plugs with inert substrate. When the seedlings developed three leaves, they were transplanted into 3 liter plastic pots with a substrate containing a 1:1 ratio of volcanic ash and black earth soil, and 1:2 turf (0.15 mS electrical conductance (EC) and pH = 6.97). In order to favor the initial development, the seedlings were fertilized once a week. Before starting the experiments, plants of *F. palleescens* were grown for three months (from January to March) under the same conditions of light, temperature and humidity in a common environment (greenhouse). The plants were maintained at pot capacity (volumetric water content in the pot close



to 15%) measuring the pot volumetric water content (% v/v) with a TDR (“Time domain reflectometer”, Trime FM, Eijelkamp) every four days along the entire essay. Twenty plants per population were distributed in four replicates of 5 plants each, totalizing 160 plants. The experimental design consisted in a complete randomized design (CRD) with four repetitions per population:  $Y_{ij} = \mu + P_i + b_j + e_{ij}$  where P is the fixed effect (*i-iv*) of the population ( $i = 1 \dots 8$ ), b is the repetition ( $j = 1..4$ ) and  $e_{ij}$  the experimental error ( $b_j \sim N(0, \sigma_b^2)$ ;  $P_i \sim N(0, \sigma_p^2)$ ,  $e_{ij} \sim N(0, \sigma_\epsilon^2)$ ). Normality assumptions and heteroscedasticity of variance were considered. Differences between populations were evaluated with a posteriori LSD Fisher test applying the Bonferroni correction for multiple comparisons.

We marked four tillers per plant with metal hoops and measured genetic differences in morphological characters in the following traits: *i) Tillering* (the average difference in number of tillers produced by a marked tiller per plant at the end and at the beginning of the experiment), *ii) Green leaf production* (the average difference in the number of leaves produced by a marked tiller per plant at the end and at the beginning), *iii) Leaf elongation* (the average difference in the sum of the total length of the green leaves produced by a marked tiller per plant relativized to the number of leaves produced by each marked tiller), *iv) Production of biomass* and *v) Distribution of biomass* (the difference between the total biomass and the root/shoot ratio at the beginning and at the end of the trial) and *vi) Tiller weight*. At the beginning and at the end of the experiment, 3 plants were selected at random per population to determine the dry weight (total biomass). Star Plot graphs were used to plot morphological variables related to the architecture of the plant (tillering, green leaf production and leaf elongation) allowing a visual representation of the relative magnitudes of the selected variables (Di Rienzo et al., 2008). In these graphs, each variable is represented as a radius of a star. If several observations are represented in a single star, the radius is a function of the average value of each variable. The magnitude of the radius is given by the value of the variable represented by the star observation. Therefore, different shapes of stars indicate which variables make most difference between the observations.

#### *Testing for local adaptation*

Local adaptation due to natural selection can be inferred when the additive genetic divergence in a quantitative trait across populations ( $Q_{ST}$ ), exceeds the neutral expectation based on the between-population differentiation in alleles that are not under selection (e.g.  $F_{ST}$ ,  $\Phi_{PT}$ ). In the absence of familiar relations that allows estimating the heritability of each trait,  $Q_{ST}$  can be approximated by the phenotypic divergence in a trait across populations ( $P_{ST}$ ) (Brommer, 2011). Hence, to assess the patterns of local adaptation among the populations of *F. pallescens*, we compared neutral genetic differentiation ( $\Phi_{PT}$  calculated by the AMOVA between all the populations) with phenotypic differentiation ( $P_{ST}$ ) quantified for each morphological trait measured in the common garden test (Bertrand et al., 2016; Leinonen et al., 2008). If  $P_{ST} - \Phi_{PT} = 0$ , the level of differentiation

displayed by phenotypic traits equals neutral genetic differentiation, indicating that the main mechanisms accounting for the divergence among the spatially structured populations are neutral processes. On the contrary, if  $P_{ST} - \Phi_{PT} > 0$ , the level of genetic differentiation displayed by phenotypic traits is greater than expected in the absence of any selective pressure, suggesting directional or divergent selection as the mechanism promoting phenotypic divergence among populations. Finally, if  $P_{ST} - \Phi_{PT} < 0$  the level of genetic differentiation displayed by phenotypic traits is smaller than expected under the assumption of neutrality. Under this scenario, stabilizing selection might restrict population phenotypic divergence.

$P_{ST}$  was calculated following the equation suggested by Brommer (2011).

$$P_{ST} = \frac{\frac{c}{h^2} \sigma_B^2}{\frac{c}{h^2} \sigma_B^2 + 2\sigma_w^2} \quad \text{eqn 1}$$

where  $\sigma_B^2$  and  $\sigma_w^2$  are the phenotypic variance between and within populations, respectively.  $h^2$  is the heritability of the morphological trait (i.e. the proportion of the phenotypic variance due to additive genetic effects), and the scalar  $c$  indicates the proportion of the between-population variance according to genetic effects across populations. Under controlled conditions, such as our common garden experiment, it is expected that the phenotypic differentiation is due to additive genetic effects ( $c/h^2=1$ ) and  $P_{ST}$  is equivalent to  $Q_{ST}$ . As both  $h^2$  and  $c$  are difficult to calculate in wild populations, we followed the approach described by Brommer (2011) and also applied by Bertrand et al. (2016). We quantified the variations of  $P_{ST}$  calculated for each morphological trait with the variations in the  $c/h^2$  ratio. We did not evaluate the heritability in these populations.

$P_{ST}$  values were calculated with “Assessing  $P_{ST}$  Statistics” in *Pstat* package version 1.2 (Da Silva & Da Silva 2017) in R 3.5.2. This package estimates  $P_{ST}$  values with the function “ $P_{ST}$ ” to assess differentiation among populations from a set of quantitative traits. The bootstrap method provides confidence intervals (upper and lower 95% CI) and distribution histograms of  $P_{ST}$  using the “*BootPst*” function. Variations of  $P_{ST}$  in function of the parameter  $c/h^2$  are studied with the function “*TracePst*”. To compare  $P_{ST}$  with  $\Phi_{PT}$ , we followed Brommer (2011) comparisons between neutral differentiation and the estimate of  $P_{ST}$  (according to eqn 1) depending on the  $c/h^2$  ratio. The robustness of the comparisons of the  $P_{ST}/\Phi_{PT}$  relies on the overlap between the upper confidence estimate of the neutral divergence estimated as  $\Phi_{PT}$  (horizontal line in Fig. 3) and the lower 95 % confidence estimate of  $P_{ST}$  (lower dashed line in Fig. 3). If the lower 95% confidence interval for  $P_{ST}$  is smaller than  $\Phi_{PT}$  over a mayor part of  $c/h^2 < 1$ ,  $P_{ST}$  do not differ from  $\Phi_{PT}$  then the level of differentiation displayed by phenotypic traits equals neutral genetic differentiation. However, if the opposite pattern is observed, then the difference in  $P_{ST}$  and  $\Phi_{PT}$  is fairly robust indicating a trait with a strong phenotypic divergence.

## RESULTS

### Genetic characterization of the populations of *Festuca palleescens*

A total of 128 alleles was found and the number of alleles per locus varied from 43 (PHB) to 63 (PB) with a mean of  $51.7 \pm 7.02$ . In addition, the percentage of polymorphic loci was different among populations, ranging from 32.8% (PHB) to 48.4% (PB). Genetic diversity was moderate, varying from  $uh = 0.063$  to  $uh = 0.085$  (Table 1; Fig. 1). In general, low levels of genetic diversity were found in the populations from the *Cordillera* ecological region (corresponding to populations PHA and PHB) since they present lower percentage of polymorphic loci and number of alleles (both total and with frequencies above 5%) (Table 1). Moreover, an interesting fact is the absence of exclusive alleles in PHA, while the populations with the highest number of exclusive alleles are two from *Precordillera* ecological region (SRA-1 and SRB). The highest value of polymorphism was found in the center of the distribution in PB (Table 1).

### Genetic structure between and within populations

Molecular variance analysis (AMOVA) showed that the variation between the ecological regions was 5% while 9 % of the variation is explained as differentiation between populations within regions ( $\Phi_{PT}$ , percentage of differentiation between populations,  $p < 0.001$ ), with most of the variation found within the populations (86%) (Table S1). When the hierarchical analyses was not considered, the genetic differentiation between populations was 12%. Interestingly, the values of pairwise among populations are very high between PHB, and the rest of the populations (0.367-0.655), whereas the values of  $\Phi_{PT}$  varied between 0.006 and 0.120 in the rest of the populations (Table S2).

On the other hand, the analysis of principal coordinates (PCoA) showed that one of the westernmost populations (PHB) is separated from the rest (Fig. 1): while most populations are genetically similar to each other, PHB is very different. Also the Mantel test indicated that there is not isolation by distance when all populations of *Festuca palleescens* ( $R^2 = 0.0006$ ;  $p > 0.1$ ) are evaluated; but, when PHB is removed from the analyses, the Mantel test indicates a significant but low correlation value of isolation by distance ( $R^2 = 0.22$   $p = 0.009$ ) (Supplementary material). Clustering results (both STRUCTURE as well as the non-hierarchical K-means clustering) suggested the presence of two distinct genetic groups ( $K = 2$ ), showing a clear genetic differentiation between PHB and the rest of the populations. The majority of the populations were grouped in the first cluster (orange) with values of cluster membership coefficient above 0.85 ( $Q \geq 0.85$ ). The second genetic group (violet) comprised only PHB. The individuals of this population showed also high cluster membership coefficient ( $Q \geq 0.9$ ) (Fig. 1). Therefore, most populations of *F. palleescens* were represented by the same genetic group and showed lower values of pairwise  $\Phi_{PT}$ , between them.

Finally, the correlations between genetic diversity and environmental variables were only significant for precipitation of the driest months and precipitation of the warmest months, showing an inverse relation between the genetic diversity (A) and the aforementioned variables. This indicated that the number of alleles increases in the populations from *Hills and Plateaus* ecological region (Fig. 2).

#### Phenotypic differentiation between populations of *Festuca palleescens*:

The populations of *F. palleescens* showed differences in morphological traits of adaptive importance related to their natural environment across the west-east rainfall gradient. The main differences were found in the growth components such as tillering or biomass production, but no differences were found in the biomass distribution (root/aerial). The highest production of tillers was registered in the populations from the middle of the gradient (PA and PB), and the lowest production was registered in the westernmost population (PHA) from *Cordillera*, whereas the rest of the populations presented similar values ( $F = 3.61$ ,  $p = 0.0038$ ). The highest value of green leaf production was registered in population from the center of the gradient (PA and SRA-3;  $F = 2.94$ ,  $p = 0.012$ ) (Table 2). The westernmost populations (PHA) showed the highest value of leaf elongation while SRB, PA, and JA exhibited the lowest values ( $F = 3.59$ ,  $p = 0.0047$ ).

Likewise, populations of *F. palleescens* presented differences in biomass production (total,  $F = 7.44$ ,  $p = 0.0001$ , aerial biomass,  $F = 6.86$ ,  $p = 0.0002$ , radical biomass,  $F = 7.14$ ,  $p = 0.0002$ ). The highest biomass production was registered in PHA and SRB, while the smallest production was found in SRA-3. This pattern was also observed in both aerial and root biomass. As mentioned above, no differences were found in the biomass allocation ( $F = 0.61$ ,  $p = 0.77$ ) (Table 2).

The plant architecture differs between populations. The Star plot showed different star shapes between populations, highlighting two main groups (Fig. 4). The easternmost populations (*Hills and Plateaus* ecological region) presented similar star shapes while the westernmost populations (*Cordillera and Precordillera* ecological region) were more variable; all the morphological variables were similar between these populations. In the westernmost populations, leaf elongation (LE) was the variable that made the main difference between the observations.

#### Local adaptation: $P_{ST}$ vs $\Phi_{PT}$

Phenotypic differentiation ( $P_{ST}$ ) was significantly higher than neutral genetic differentiation  $\Phi_{PT}$  for three of the six morphological traits based on the  $P_{ST}/\Phi_{PT}$  comparisons: tillering, green leaf production and leaf elongation, with  $P_{ST} = 0.67$ ,  $0.63$  and  $0.65$  respectively (Table 3, Figure 3 a,b,c). For these traits, the lower limit of  $P_{ST}$  95% confidence interval was significantly higher than the fixation index between all populations ( $\Phi_{PT} = 0.12$ ). On the other hand, the lower limit of the 95% confidence interval was moderately higher than the fixation index

for the three remaining traits (biomass production, biomass allocation, and tiller weight) in comparison to the aforementioned traits. The robustness of these conclusion were obtained by plotting  $P_{ST}$  and its 95% confidence interval (CI) as a function of  $c/h^2$  on top of the neutral expectation,  $\Phi_{PT}$  and its 95% confidence interval for  $c \leq h^2$  (Fig. 3).

Therefore, these results suggest that tillering, green leaf production and leaf elongation are more divergent than expected under neutrality (i.e. under genetic drift-driven evolution).  $P_{ST}$  distribution histograms confirm that the first three traits might be under divergent selection (skewed distributions; Fig. 3). However, the level of differentiation displayed by biomass production and its distribution between roots and aerial parts might equal neutral genetic differentiation as the lower 95% CI of  $P_{ST}$  is almost the same to the  $\Phi_{PT}$  value over a major part of  $c/h^2 < 1$ . In addition,  $P_{ST}$  distribution histograms for these traits indicate that they do not show evidence of selection acting on them (normal distribution; Fig. 3).

## DISCUSSION

Widely distributed species usually show an increase of genetic differentiation among those populations coming from less suitable environments along their distribution (Bridle and Vines, 2007; Eckert et al., 2008). However, along the rainfall and topographical gradient evaluated in populations of *F. pallescens* from North Patagonia, a weak genetic structure was detected suggesting extensive gene flow. Nevertheless, local adaptation to their native environments occurred despite extensive gene flow highlighted by the detection of two different plant architectures in common garden experiments related to two ecological regions of this austral area.

### *Genetic diversity, differentiation and structure.*

Our results showed that past demographic events might have modeled the neutral genetic diversity of the populations of *F. pallescens* but the environmental heterogeneity that characterizes this region is not playing an important role. Despite inhabiting diverse environments along the rainfall gradient in north Patagonia, the populations showed a weak genetic structure. Although genetic differentiation was significant (AMOVA), it was mostly due to a single divergent population. The lack of genetic structure as well as the low pairwise  $\Phi_{PT}$  values between the populations indicate high genetic connectivity, suggesting the existence of extensive gene flow between most of the studied populations. Being an anemophilous species, gene flow could be favored by the strong Patagonian west-east oriented winds, suggesting a concomitant gene flow direction between the populations. Accordingly, the genetic diversity increased moderately towards the most arid environments located in the *Hill and Plateaus* ecological region (Fig. 2). Hence, these populations are connected with populations from the *Precordillera* ecological region despite differences in altitude. The correlations between genetic diversity and

environmental variables related to precipitation indicated that populations from arid environments are more genetically diverse than populations from wetter environments (*Cordillera* and *Precordillera*). Therefore, populations from environments with greater environmental variation in warmer and drier climatic conditions exhibit greater genetic diversity allowing local adaptation (Fitzgerald et al., 2011).

Nevertheless, gene flow is apparently limited towards population PHB, the only different population, even though only 200 m in altitude separate PHB from population PHA. Limitation to gene flow between nearby populations located at the edges of the distribution range was also registered in other species of *Festuca* (Gonzalo-Turpin and Hazard, 2009; Zhang et al., 2017). Since average temperature decreases with elevation, this might exert a direct effect on physiological processes (Carey and Alexander, 2003). The difference in altitude between PHA and PHB might promote non-overlapping phenological phases due to temperature decrease with elevation, reducing the effective window of time for gene flow. In addition, phenological phases might be outdated due to differences in the local environmental conditions of each population: PHA is a cold and humid habitat, while PHB is relatively more arid and warm. Since most populations are strongly connected despite the differences in altitude, this might not be the only explanation for PHB being genetically distinctive.

The distinction of PHB population is one of the most remarkable results. This population showed higher pairwise  $\Phi_{PT}$  values and comprised a different genetic cluster, being highly differentiated from the geographically close PHA population. This genetic differentiation could be expected with greater distances between populations (Galloway and Fenster, 2000; Vergeer and Kunin, 2013), or to restricted gene flow. Alternatively, past geomorphological events in North Patagonia might have shaped the genetic diversity generating the particular genetic structure of PHB population. During the Oligocene and the Miocene, the region between 38° and 43° S was invaded by marine transgressions from the Atlantic Ocean (Premoli et al., 2012; Ramos, 1983) and the Pacific Ocean (the latter continued from the Oligocene to the Miocene; Bechis et al., 2011). Then, during the Pleistocene, glaciers covered much of Patagonia and the area where the PHB and PHA populations are today was covered by ice according to the limits of the Last Glacial Maximum (Hollin and Schilling, 1981) (Fig. 1). The great genetic differences found at this location might suggest that PHB population would be the result of a post-glacial recolonization, with the consequent reduction in genetic diversity after a founder event (Hewitt, 2000). The difference with PHA might be related to a different origin, i.e. migration from two different refugia. Alternatively, PHA might have been an ice-free location above the ice shield (nunatak refugium; Holderegger and Thiel-Egenter, 2009) as observed in alpine plants (Kosiński et al., 2019; Stehlik, 2000); although to our knowledge nunatak refugia were not reported in North Patagonia. The rest of the populations laid outside the ice limits (Hollin and Schilling, 1981) (Fig. 1), and could have been connected and unperturbed for long periods of time. Additional research with more adequate markers (chloroplast DNA) is necessary to unravel this question.

### ***Local adaptation despite extensive gene flow***

Environmental heterogeneity can trigger the variation in functional traits, leading to the formation of locally adapted ecotypes (Khasanova et al., 2019). The rainfall and topographical gradient constitute heterogeneous environments for the evaluated populations of *Festuca pallescens* and might also have triggered local adaptation. The plant architectures based on the three morphological traits (*tillering*, *green leaf production* and *leaf elongation*) indicate that *F. pallescens* populations exhibited differences in growth patterns under common garden conditions. Therefore, this phenotypic divergence has a genetic bases. The significant  $P_{ST}/\Phi_{ST}$  comparisons for these three traits are consistent with the hypothesis of local adaptation driving phenotypic differentiation along the gradient. Towards the west, populations from the *Precordillera* region (PHA, PHB, SRA-1, SRA-2, SRA-3 and SRB) occur in the most favorable environments for the species (Godagnone and Bran, 2009) in terms of temperature and hydric balance (i.e center of ecological niche sensu Maciel-Mata et al., (2015)). Plants from these populations exhibited a wide architecture (long leaves/low tillering), while plants from *Hills and Plateaus* region are more compacted (Fig. 4). Since the surrounding vegetation might have positive or negative effects in the shape of vegetation (Grassein et al., 2014), plants from population PHA that inhabit a shrub-grass steppe within a forest, might exhibit a plant architecture adapted to cope with rainfall and radiation interception caused by trees (Fernández et al., 2006). Therefore, these plants could have certain adaptation to growth under trees and high bushes (i.e. *Berberis* sp; *Nothofagus pumilio*), a response related to growing in shaded microenvironments adapted to capture more light (Fernández et al., 2004).

On the other hand, populations from *Hills and Plateaus* ecological region presented a large production of tillers, but with smaller leaves than those from *Cordillera* and *Precordillera* (Fig. 4). In arid environments, this growth pattern could denote a trade-off between not resigning foliage production (photosynthetically active area) but reducing the exposure to drying agents (small leaves and compact plants). Higher production of tillers but with shorter leaves could represent an adaptive advantage, since compact tussock morphotypes would have less surface exposure (Grime, 2006; López, 2011). All these morphological features would also be related to sun/wind exposure, water availability, temperature or altitude (Godagnone and Bran, 2009). Particularly, altitude could play an important role in plant development. For example, two populations from *Hills and Plateaus* ecological region located at high altitudes (PA and JA) showed smaller leaves than populations of the same sites but inhabiting lower locations. High rangelands might be exposed to freezing/desiccation due to cold winters, and might acquire adaptations that are commonly related to drought and herbivory (Golluscio et al., 2011; Moreno and Bertiller, 2015). Leaves are mainly responsible for the central metabolism processes (Schwinning and Ehleringer, 2001). Therefore, short leaves and a more compact cespitose architecture may avoid water loss due to frosts or hydrological deficit and generates certain protection against herbivores.

The  $P_{ST}/\Phi_{ST}$  comparisons for biomass production and allocation as well as for tiller weight suggest that selection is playing a moderate to low role on these traits. There could be a morphological trade-off between tillering and leaf size; while in the west populations exhibited larger leaves/lower tiller density, the east showed shorter leaves/higher tiller density, attenuating differences in biomass production. Therefore, plants may compensate biomass production and biomass allocation by changing their architecture and morphology due to growth regulatory mechanisms (Hecht et al. 2016). The biomass allocation, on the other hand, showed no differences between populations, all having a low R/S ratio ( $<1$ ), indicating a greater assignment to aerial biomass under non-limiting water conditions. The biomass distribution (R/S) is a functional trait strongly related to drought tolerance; in this case, all populations exhibited an acquisitive root strategy with greater above-ground productivity (Balachowski et al., 2016).

*Festuca pallelescens* has been described as an intermediate xeromorphic species, with high production of light tillers and low relative maximum growth rate under common garden experiments (Couso and Fernández, 2012). However, our results indicate that there are intraspecific differences reflecting local adaptation to their natural environments, highlighting that the environmental heterogeneity plays an outstanding role in the formation of locally adapted populations. The differences in the neutral and adaptive genetic variation among the populations would be persistent even in the presence of high levels of gene flow like in other species (Andrew et al., 2012; Gonzalo-Turpin and Hazard, 2009).

Climate change predictions for Patagonia describe increases in mean annual temperature and higher frequency of stochastic events (drought and humid years) as well as alterations in precipitation patterns (IPCC 2014) (Pachauri et al., 2014). Therefore, a modification in the rainfall gradient of North Patagonia could favor the maintenance of populations with short-leaf plants and a more compact cespitose architecture, which are also those that hold the highest genetic variability. Nevertheless, patterns of local adaptation might change as a result of selective impact of climate change in heterogeneous environments for wild species (Aguirre-Liguori et al., 2019). On the other hand, grazing might favour a community shift from tall perennial grasses towards short grasses (Watson et al., 2019), which would determine a synergy between arid conditions and grazing as modulators of ecotypes of *F. pallelescens*. In this context, it remains uncertain if the populations of these native forage species would maintain this large level of gene flow and genetic diversity as unsustainable land use, desertification, overgrazing and periods of drought could be more intense. Nonetheless, a decrease in annual precipitation (combined with an increase in summer temperature) emphasizes the need to adapt grazing management in arid and semi-arid ecosystems to secure their long-term viability as sustainable rangelands. Without appropriate ecological and agricultural management of grazing intensity on these extensively managed rangelands, plant diversity will be continuously reduced. Therefore, rangeland management activities should aim to enhance species richness, the coverage of palatable grasses and maintain the vegetation structure (Gaitan et al. 2018). For this, it is



essential to generate knowledge and technologies that allow the species to adapt to new environmental conditions and management. In this sense, the results of this work provide relevant knowledge to initiate programs of genetic improvement of one of the key species of North Patagonian for its adaptation to future changes.

### **AUTHOR CONTRIBUTIONS**

ASL, DRL GLS and PM conceived and designed the experiments. ASL performed both lab and common garden experiments. GC helped with the design and analyses of common garden tests and provided technical support. ASL, PM and GC analysed the data. PM provided instruments, lab space and consumables. DRL and PM supervised the experiments, provided administrative and logistic support and obtained the funding. All the authors wrote, read and approved the manuscript.

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### **CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

### **AUTHOR STATEMENT**

ASL, DRL GLS and PM conceived and designed the experiments. ASL performed both lab and common garden experiments. GC helped with the design and analyses of common garden tests and provided technical support. ASL, PM and GC analysed the data. PM provided instruments, lab space and consumables. DRL and PM supervised the experiments, provided administrative and logistic support and obtained the funding. All the authors wrote, read and approved the manuscript.

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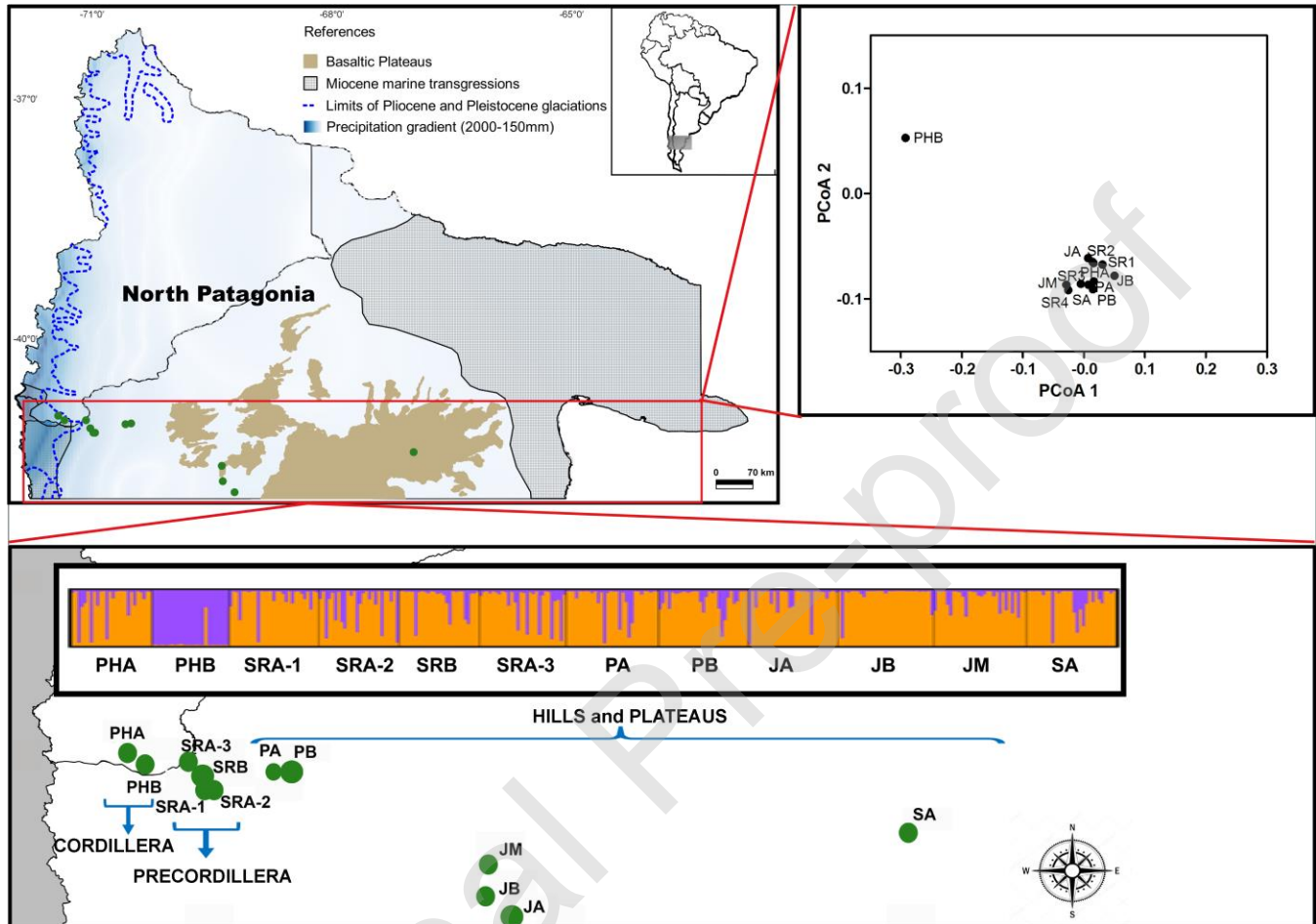
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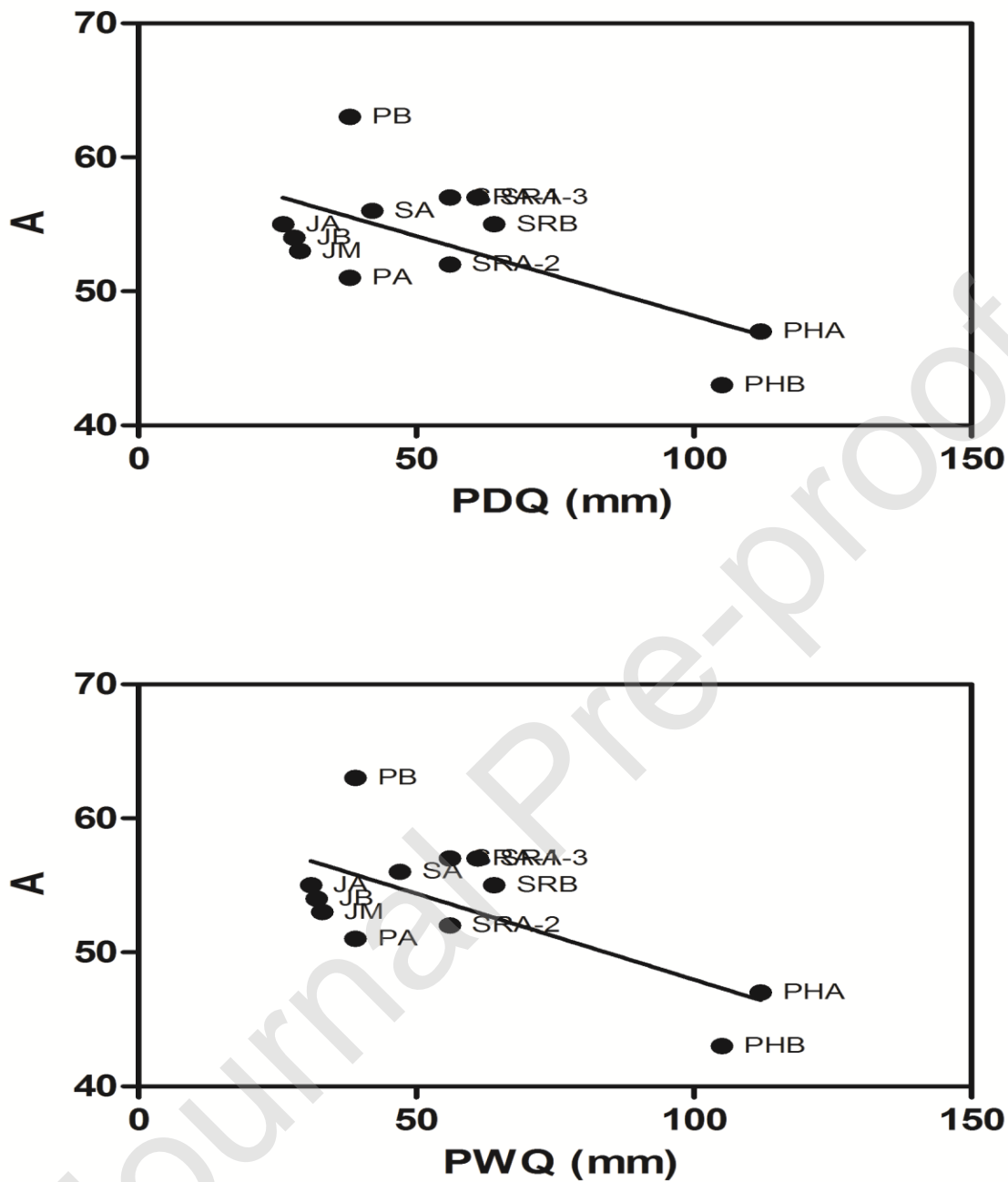
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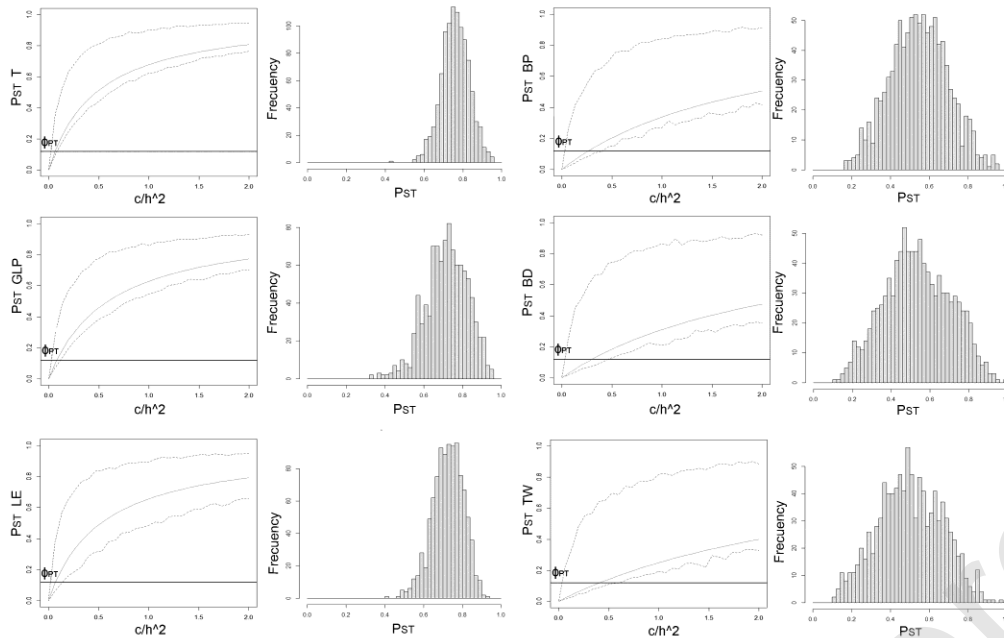
## FIGURES CAPTIONS



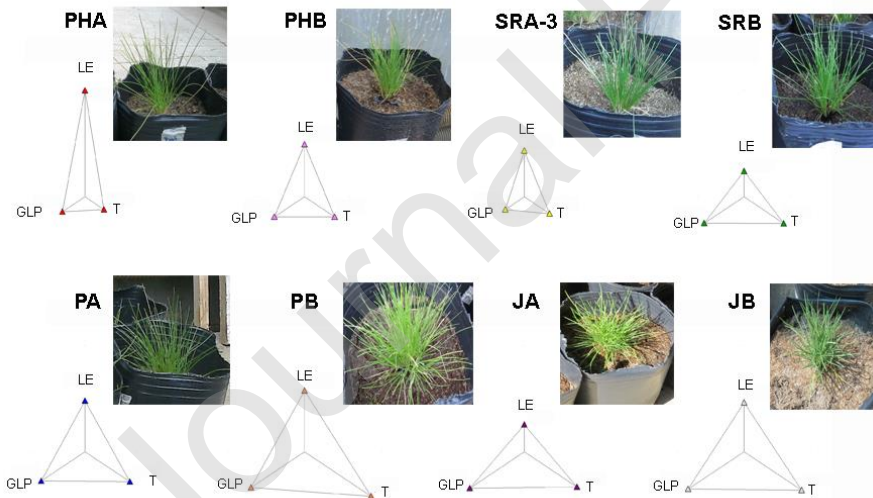
**Figure 1:** Distribution and genetic structure of populations of *Festuca pallescens* located across the west-east transect that covers the whole longitudinal distribution range of the species in North Patagonia. Sample Sites: PHA, PHB: Península Huemul, SRA-1, SRA-2, SRA-3 and SRB: San Ramon, PA, PB: Pilcaniyeu Experimental Field; JA, JB and JM: Ingeniero Jacobacci and SA: Somuncura Plateau. Principal Coordinates Analysis (PCoA) based on Bruvo genetic distance is shown on the top-right of the figure, while genetic structure based on STRUCTURE results is shown at the bottom of the figure. Circles representing the populations are proportional in size to the number of alleles.



**Figure 2:** Correlations between the precipitation variables and A (allele richness). PDQ: Precipitation of the driest months; PWQ: Precipitation of the warmest months. All correlations were significant ( $p < 0.05$ ).



**Figure 3:** Evolution of  $P_{ST}$  for different values of  $c/h^2$  at six morphological traits. Horizontal black lines represent the  $\Phi_{PT}$  value. Dashed lines represent the 95% confidence interval for  $P_{ST}$  values. The panels on the right show the  $P_{ST}$  distribution histogram of the corresponding evaluated trait.



**Figure 4:** Star Plot graph with architecture morphological variables for each population. Each variable is represented as a radius of a star: LE: Leaf elongation, T: Tillering, GLP: Green leaf production. The magnitude of the radius is given by the value of the variable represented by the star observation.



## TABLES

**Table 1:** Environmental and genetic characterization of *Festuca pallescens* sampled populations. Floristic physiognomic type (FPT) was characterized following Bran et al. (2000). *Pop*: population; *Pp*: Precipitation (mm); *Lat*: Latitude; *Long*: Longitude; *Alt*: Altitude (m); *P*(%): percentage of polymorphic loci, *A*: Number of alleles per locus; *A*<sub>5%</sub>: Number of alleles frequently greater than 5%; *A<sub>E</sub>*: Number of exclusive alleles; *Uh*: Nei's unbiased genetic diversity and its standard deviation (ds). The populations are listed from west to east. References: *NQN*: Neuquén, Argentina. *RN*: Río Negro, Argentina.

<i>Pop</i>	<i>Ecological region</i>	<i>Sampling site</i>	<i>Pp</i>	<i>Lat</i>	<i>Long</i>	<i>Alt</i>	<i>FPT</i>	<i>P</i> (%)	<i>A</i>	<i>A</i> <sub>5%</sub>	<i>A<sub>E</sub></i>	<i>Uh</i>	<i>ds</i>
<i>PHA</i>	<i>Cordillera</i>	Península Huemul (NQN)	831	40°57'	71°25'	1220	Shrub-grass steppe	35,2	47	32	0	0,071	0,011
<i>PHB</i>				41°1'	71°20'	845	Shrub-grass steppe	32,8	43	29	3	0,067	0,011
<i>SRA1</i>	<i>Precordillera</i>	San Ramon (RN)	584	41°10'	70°59'	1140	Exposed Shrub-grass steppe	42,2	57	38	4	0,072	0,010
<i>SRA2</i>				41°10'	70°57'	1100	Meadow	39,1	52	34	1	0,079	0,012
<i>SRB</i>				41°7'	71°1'	902	Meadow	42,2	57	40	2	0,085	0,012
<i>SRA3</i>				41°1'	71°4'	1139	Grass steppe	43,0	55	35	4	0,080	0,012
<i>PA</i>	<i>Hills and Plateaus</i>	Pilcaniyeu Experimental Field (RN)	264	41°4'	70°34'	1260	Shrub-grass steppe	36,7	51	33	0	0,063	0,010
<i>PB</i>				41°3'	70°30'	970	Meadow	48,4	63	35	2	0,078	0,011
<i>JA</i>		Ingeniero Jacobacci (RN)	170	41°55'	69°12'	1400	Grass steppe	42,2	55	38	1	0,081	0,012
<i>JB</i>				41°46'	69°21'	970	Salty Meadow	38,3	54	37	1	0,065	0,011
<i>JM</i>				41°35'	69°22'	1135	Salty meadow	39,1	53	39	0	0,071	0,011
<i>SA</i>		Somuncura plateau (RN)	150	41°25'	66°58'	1430	Meadow	42,2	56	34	3	0,075	0,011

**Table 2:** Morphological variables of *Festuca pallescens* populations evaluated in a common garden assay. The mean of each variable is presented with the standard error. The letters indicate the significant differences with a posteriori LSD Fisher test with the Bonferroni correction. The abbreviations of the populations correspond to Table 1. Leaf elongation (mm), Biomass production (gr), Biomass distribution (R/S), Tiller weight (gr).

Population Variable	PHA	PHB	SRA-3	SRB	PA	PB	JA	JB
Tillering	2,2 ± 1 <b>b</b>	3,7 ± 0,7 <b>ab</b>	3,04 ± 0,7 <b>ab</b>	5 ± 1,4 <b>ab</b>	8,8 ± 2 <b>a</b>	8,6 ± 0,6 <b>a</b>	6,75 ± 1 <b>ab</b>	7,04 ± 1,5 <b>ab</b>
Green leaf production	11,5 ± 2,4 <b>ab</b>	14,3 ± 4,1 <b>ab</b>	10,2 ± 2,9 <b>b</b>	17,8 ± 5 <b>ab</b>	30,4 ± 5,6 <b>a</b>	22,9 ± 2,6 <b>ab</b>	23,25 ± 2 <b>ab</b>	23,75 ± 4 <b>ab</b>
Leaf elongation	12,4 ± 2,3 <b>a</b>	7,04 ± 1,2 <b>ab</b>	6,4 ± 1,2 <b>ab</b>	4,35 ± 0,8 <b>b</b>	5,65 ± 0,3 <b>b</b>	7,9 ± 1,6 <b>ab</b>	4,5 ± 0,8 <b>b</b>	6,7 ± 1 <b>ab</b>
Biomass production	2,12 ± 0,4 <b>ab</b>	2,05 ± 0,8 <b>ab</b>	1,17 ± 0,2 <b>ab</b>	2,6 ± 0,3 <b>a</b>	1,1 ± 0,3 <b>b</b>	1,9 ± 0,8 <b>ab</b>	1,2 ± 0,04 <b>ab</b>	1,7 ± 0,3 <b>ab</b>
Biomass distribution	0,7 ± 0,05	0,9 ± 0, 1	0,8 0,12	0,88 ± 0,1	0,7 ± 0,16	0,8 ± 0,05	0,68 ± 0,03	0,7 ± 0,08
Tiller weight	0.05 ± 0.02	0.05 ± 3.10 <sup>-3</sup>	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 4.10 <sup>-3</sup>	0.05 ± 0.02	0.03 ± 4.10 <sup>-3</sup>

**Table 3:** P<sub>ST</sub> values for each of the six morphological traits and the associated credible interval at 95%.

Phenotypic trait	P <sub>ST</sub> (95% CI)
Tillering	0.67 (0.62-0.89)
Green leaf production	0.63 (0.54-0.85)
Leaf elongation	0.65 (0.48-0.91)
Biomass production	0.34 (0.27-0.82)
Biomass distribution	0.31 (0.24-0.85)
Tiller weight	0.25 (0.19-0.81)