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FORESTRY SCIENCE

Signatures of natural selection in morphological quantitative traits in Argentinean populations of *Senegalia gilliesii* (Fabaceae)

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Abstract: In order to elucidate the role of evolutionary forces in shaping the variation of quantitative traits in *Senegalia gilliesii* we evaluate seven phenotypic traits in three Argentinean populations, two of them sharing environmental and vegetation type conditions, and a third one ecologically differentiated from the former. The phenotypic traits were compared with molecular markers. Here, we search for signatures of selection by means of the comparison P_{ST}-F_{ST}. We assessed if the averages of the seven phenotypic traits were different among populations by means of ANOVA and we performed discriminant analysis of principal components (DAPC) for both morphological and molecular data. The ANOVA showed significant results only for two traits. For all foliar traits and two spine traits, the P_{ST}-F_{ST} comparison suggested the occurrence of stabilizing selection. The DAPC obtained from AFLP data showed three well defined groups of populations; when the same analysis was conducted with morphological data the scatterplot showed high overlapping among individuals and could not separate the populations. Overall, our findings suggest a prominent role of stabilizing selection in all foliar traits and stipular spine length. These results could be extrapolated to other tropical and subtropical acacias. Further studies are needed to analyse the mechanisms underlying genetic differentiation in natural populations of *S. gilliesii*, find its relationship with eco-geographical variables.

Key words: *Senegalia gilliesii*, AFLP, conservation and reforestation programmes, natural selection, phenotypic traits.

INTRODUCTION

Biological populations of plant and animal species do not constitute uniform units but are usually subdivided, represented by many partially isolated subpopulations. The extent of both genetically and phenotypic differentiation among these sub-units over time depends on the relative contribution of interacting evolutionary processes (natural selection, genetic drift, migration and mutation) (Wright 1931, Holsinger & Weir 2009). Unveiling the causes and consequences of this differentiation may represent a significant contribution to the theoretical evolutionary biology and ecology fields as well as to applied realms (for example, forestry and conservation biology). One key question in conservation biology and domestication of profitable resources is determining to what degree population differentiation is caused by selective versus neutral processes (Leinonen et al. 2013).

Between-population differentiation in neutral alleles, quantified by F_{ST}, shows the level of expected differentiation across populations caused by stochastic processes (genetic drift, gene exchange) (Wright 1943). Q_{ST} is a quantitative genetic analogue of F_{ST} that measures the

amount of genetic variance among populations relative to the total genetic variance for each trait (Merilä & Crnokrak 2001, Whitlock 2008, Leinonen et al. 2013). In wild populations, the phenotypic differentiation between them is approximated by the surrogate of Q_{ST}, P_{ST} (Leinonen et al. 2006, 2008, Saether et al. 2007, Wojcieszek & Simmons 2012). Quantification of P_{ST} is based on phenotypic measures of a trait in the wild in several individuals across a number of populations (Brommer 2011, Pujol et al. 2008, Ojeda et al. 2016, Antoniazza et al. 2010, Brommer et al. 2014, Pometti et al. 2019). The precise estimation of Q_{ST} requires the foundation of provenance trails in order to correct for environmental factors, however in wild populations, the phenotypic differentiation, quantified by the P_{ST} may be used, with care, as a proxy to Q_{ST} (Leinonen et al. 2008). The evolutionary inferences, taking care of the pitfalls, are quite similar to those for Q_{ST}. In the cases in which Q_{ST}=F_{ST} the differentiation could be explained by genetic drift alone. If Q_{ST}> F_{ST} the he differentiation can be attributed to directional selection. If Q_{ST} < F_{ST} the occurrence of stabilising selection can be assumed.

Using P_{ST} as an approximation of Q_{ST} involves bias due to nonadditive genetic variances or environmental factors and genotype-environment interactions (Pujol et al. 2008). Although it is thus not generally recommended to simplify Q_{ST} by its phenotypic analogue P_{ST}, rearing individuals from different populations in a common environment may not be feasible (especially when working with long-living wild species) (Brommer 2011).

Morphological variation within plant species that occupy different habitats could be due to genetic differentiation among populations or to environmental effects (Yucedag & Gailing 2013). Therefore, measurement, description and analysis of morphological variation are fundamental steps to answer questions of biological adaptability (Ge & Hong 1995). Genetic variation underlying phenotypic traits and phenotypic plasticity are particularly important when the long-term stability of forest ecosystems is increasingly threatened by environmental stress and mismanagement. Yucedag & Gailing (2013) studied morphological traits relative to cones and seeds in seven populations of *Juniperus excelsa*. They found differences for all traits and observed in several analyses that northern populations were more similar than southern ones, in spite of the absence of a correlation between morphological and geographical distances between populations. Then, this could be explained by different environmental conditions in northern and southern populations (Yucedag & Gailing 2013). The case of *Acacia karroo* is quite similar, Mboumba & Ward (2008) studied two populations: one from the semi-desert Karoo in central South Africa and other from the eastern coast of South Africa, in a subtropical forest. They analysed several morphological traits like number of spines, spines length, stem diameter, etc. Their results showed that the most plastic traits were above- and below-ground biomass and spine length. Arid trees showed to have longer spines and greater stem diameter than forest trees. As the water level increased, stem diameter and above-ground biomass increased. Finally, local adaptation was observed for stem diameter and spine number (Mboumba & Ward 2008). Thus, the genetic characterization of natural forest resources is an essential step for a better understanding of genetic resources for the implementation of *in-situ* and *ex-situ* conservation activities (Turna et al. 2001). In this context, the characterization of local genetic resources is often based on the knowledge of variation in morphological characters (Delgado et al. 2001). The previous examples of *Juniperus excelsa* and *Acacia karroo* reveal the importance

of assessing the relation between morphological and ecological variation contributing to design the correct management strategy for forest species.

Because selection occurs on the whole organism and not on single traits independently (Lande & Arnold 1983), a complete characterization of adaptive variation in polygenic traits is required. A multivariate approach provides an alternative to evolutionary predictions and allows studying adaptation on several traits simultaneously (Chapuis et al. 2008, Martin et al. 2008). This approach involves a multivariate neutrality test, which addresses more complex questions about specific phenotypic effects of different evolutionary process. The idea is to compare the among-population (D) and within-population (G) covariance matrices and to test the neutral pattern of $D=2F_{ST}/(1-F_{ST})$ G (e.g. Bertram et al. (2011) compare both matrices in crickets looking for evidences of selection; Costa e Silva et al. (2020) compare D and G matrices in *Eucalyptus* also tracking for signals of selection from a multivariate phenotype) . For the multivariate quantitative phenotype, the equivalent to the genetic variance is the within population genetic covariance matrix G, whereas the multivariate equivalent to the total among population phenotypic variance is represented by the phenotypic covariance matrix (D). G provides a powerful tool to move beyond retrospective analysis and to address more complex questions about phenotypic effects of different evolutionary process. Furthermore, G can identify evolutionary constraints and differences among populations in their potential to evolve and specifically predict the direction and rate of phenotypic divergence (adaptive or neutral) (McGuigan 2006).

The genus *Senegalia* that belongs to the Fabaceae family, Caesalpinodeae subfamily (Azani et al. 2017) has 12 species represented in Argentina (Rico-Arce 2007). *Senegalia gilliesii* (Steud.) Seigler & Ebinger (2006) (*Acacia gilliesii* Steud.; *Acacia furcatispina* Burkart) is distributed in South America, in Argentina, Bolivia and Paraguay, where it is commonly known as "garabato blanco", "garabato macho", "mochuelo", "teatin", "brea" or "tinticaco", among others (Rico-Arce 2007). The particular form of its spines, makes unmistakable its identification in the field. For comparisons with other species formerly comprised in a unique genus called *Acacia*, we would have referred to *Acacia s. l.*

The *Acacia s. l.* species generally, have the ability of being nitrogen fixers, they provide wood for fuel, medicinal extracts, tannins, gums, wood, fibres, shadow and food for wild and domestic animals (Pometti et al. 2012). Although this is not a threatened species, some previous works recommend *S. gilliesii*, among other species of the genus, for reforestation programmes; moreover, its wood presents characteristics considered desirable for the forest exploitation (Bravo et al. 2006). The natural regeneration of *S. gilliesii* is by means of seeds and its most important dispersion agent is the domestic livestock (bovine, ovine and equine. The seed germination is accelerated due to the process of scarification received in the herbivorous intestine (Abedini et al. 2000). Studies on this species from the genetic point of view although very important for conservation and rational use are still very scarce.

Up to now, only one American species of *Acacia s.l.* (*A. aroma* Gillies ex Hook. & Arn. = *Vachellia aroma* Seigler and Ebinger) was studied in order to detect signs of natural selection by the P_{ST}-F_{ST} comparison (Pometti et al. 2019). As pointed out by Pometti et al. (2019) in the case *A. aroma*, the length of stipular spines and leaf size and shape are among the main quantitative traits of taxonomical importance for the genus and

used in silvicultural management programmes (Mahmood et al. 2005). The remarkable variation observed for these traits in natural populations suggests the possibility of a genetic basis which might be subjected to selection programmes. The feasibility of such programmes depends greatly on the knowledge of the distribution and possible adaptive effect of these traits. In order to elucidate the role of evolutionary forces in shaping the variation of quantitative traits in *S. gilliesii*, we first studied the relationships of genetic and phenotypic variation by means of the P_{ST}-F_{ST} comparison, analysing seven phenotypic traits and AFLP markers; second, we assessed if the averages of the seven phenotypic traits were significantly different among populations by means of non-paramentric ANOVA and MANOVA; third, in order to study adaptation on several traits simultaneously, we compared the among-population (D) and within-population (G) covariance matrices and evaluated if the coefficient of proportionality between such matrices (ρ) is equal to the expectation under neutrality; and finally we performed discriminant analysis of principal components (DAPC) for both morphological and molecular data sets and compared the results obtained in order to recognize if the phenotypic traits discriminate the three populations with the same power as the AFLP data.

MATERIALS AND METHODS

In this work, two sample sites of *Senegalia gilliesii* belonging to two different eco-regions were studied in Argentina. Cerro de la Gloria (CG: -32.885; -68.892) (Mendoza province) belongs to the Monte eco-region and Pasaje Pozo Zuni (PP: -27.965933; -63.9242) (Santiago del Estero province) is in the Chaco eco-region. In each locality 20 adult trees were found and sampled. Previous structure analysis by means of AFLP markers showed the occurrence of three genetic populations (CG1, CG2 and PP) (Cerdeira et al. 2019). In the present work, we reanalysed the genotypic data matrix obtained previously (Cerdeira et al. 2019), based on this result.

Vouchers of representative individuals of each population were collected and one of them was deposited at the herbarium SI, Instituto de Botánica Darwinion, San Isidro, Buenos Aires, Argentina.

Data scoring and AFLP analysis

Each AFLP band was considered for presence (scored as 1) or absence (scored as 0). Non-hierarchical Wright's (1978) F_{ST}, its signficance (based on G-test) as well as its 95% confidence intervals (based on 5000 bootstraps) were estimated with the package hierfstat (Goudet 2005) of the R software 4.0.2 (R Core Team 2020). This analysis was conducted in order to compare F_{ST} with the P_{ST} coefficient as described below.

Discriminant Analysis of Principal Components (DAPC)

DAPC was applied to AFLP data matrix using the *adegenet* package (Jombart 2008) (function *dapc,* Jombart et al. 2010) for the software R (R Development Core Team 2020). This analysis was performed with prior information on individual populations found in Cerdeira et al. (2019).

Phenotypic analysis

We measured seven morphological traits on the 40 individuals sampled (10 for CG1, 10 for CG2 and 20 for PP). The morphological traits were measured on two replicates of the herbarium material and the average of these measurements was considered. The measurements were performed with a millimetric ruler and, when necessary, were made under a magnifying glass. All the measurements were made by the

same person (CP). The morphological traits were chosen based on their use in silvicultural activities and as forage. Besides, leaf and spines are important from the taxonomic point of view. Fruit morphology was not taken into account since it is considered as a uniform character for the entire genus *Senegalia*. The following morphological characters were measured: rachis length (cm) (RAL), pairs of leaflets on the apical pinna (PLA), pairs of leaflets on the basal pinna (PLB), left stipular spine length (cm) (SSLl), right stipular spine length (cm) (SSLr), minimum base of stipular spine length (cm) (BSL min), maximum base of stipular spine length (cm) (BSL max) (Fig. 1). The original data matrix is available upon request from the corresponding author.

The differences among populations for each trait were evaluated by a nonparametric Kruskal & Wallis (1952) ANOVA test. We choose this test because it is a distribution-free method that does not require any assumption about trait distribution. In the cases when the test was significant, Dunn's post-hoc multiple comparisons were conducted for each trait in order to identify which population (s) was different from each other. These analyses were carried out with the software Statistica 5.5 (StatSoft Inc. 2000).

Moreover, MANOVA test was performed in order to cover more than one dependent variable that cannot be combined in a simple way. This test, tries to identify the degree of association of a multivariate response with the independent variable. This analysis was also performed with the software Statistica 5.5 (StatSoft Inc. 2000).

The variance in phenotypic values within and between populations was assessed via P_{ST} coefficient (Brommer 2011, Pujol et al. 2008). The surrogate of Q_{ST} is defined by the expression:

$$P_{\mathrm{ST}} = \frac{\sigma_B^2}{\sigma_B^2 + 2\left(\sigma_W^2\right)} \quad (1)$$

where σ_B^2 is the phenotypic variance between groups and σ_W^2 is the phenotypic variance within groups.

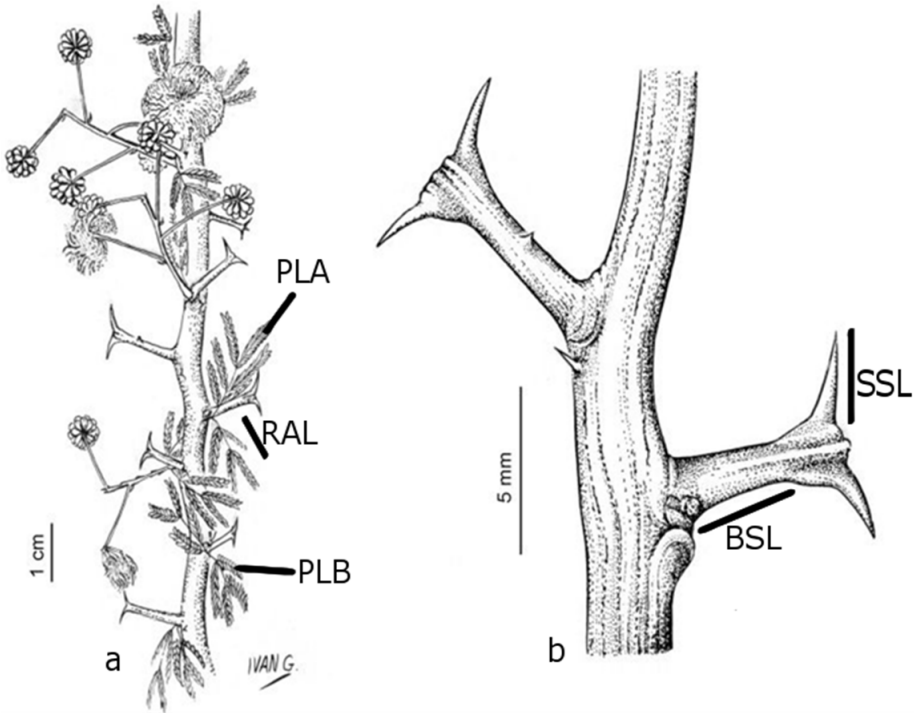

Figure 1. ***Senegalia gilliesii*** **specimen exemplifying the studied morphological traits. a- Branch with flowers, leafs and stipular spines; b- amplification of the stipular spines. RAL: rachis length (cm), PLA: pairs of leaflets on the apical pinna, PLB: pairs of leaflets on the basal pinna, SSLl: left stipular spine length (cm), SSLr: right stipular spine length (cm), BSL min: minimum base of stipular spine length (cm), BSL max: maximum base of stipular spine length (cm). Image modified from the "Flora de Jujuy, de la Flora del Conosur, Catalogo de plantas Vasculares" Instituto de Botánica Darwinion, darwin.edu.ar.**

The variance components for this statistic were estimated from the following linear random model:

$$y_{ijk} = \mu + p_i + l_j + e_{ijk} \quad (2)$$

where y_{ijk} is the phenotypic measure of the repeat *k* in individual *j* from population *i*, μ is the general mean, *p* represent the random effect of population, *l* is the random individual effect, and *e* is the residual error. These components were estimated with the function *lmer* of the package *lme4* (Bates et al. 2015) of R software 3.4.3 (R Core Team 2020). The inferences of directional selection were limited to traits for which the confidence intervals of P_{ST} do not overlap with those of F_{ST} and $P_{ST} >> F_{ST}$. Stabilizing selection inference, in time, was limited to traits for which $P_{ST} << F_{ST}$ with no overlapping confidence intervals (Brommer 2011).

The complete formula to calculate P_{ST} is (Brommer 2011):

$$P_{\mathrm{ST}} = \frac{\left(c/h^2\right)\sigma_B^2}{\left(c/h^2\right)\sigma_B^2 + 2\left(\sigma_W^2\right)} \quad (3)$$

where the ratio c/h^2 is the proportion of additive variance across populations relative to the within-population heritability. When compared with the neutral expectation based on the differentiation of AFLP bands in the same populations (e.g., P_{ST}-F_{ST}), an approximation of the extent to which selection drives the differentiation was obtained by estimating P_{ST} under the assumption of c/h^2=1. More details about this comparison and its inferences could be read in Brommer et al. (2014) and Pometti et al. (2019).

In this work, the covariance matrices D and G were calculated and was evaluated if the coefficient of proportionality between such matrices (ρ) was equal to the expectation under neutrality, that is:

$$\rho = D/G = 2F_{ST}/1\text{-}F_{ST}$$

The comparison between expected and observed ρ was made by means of a *t* test: $t = (\rho_{exp} - \rho_{obs})/Se$

where Se is the standard error of the ρ_{obs} estimated.

The covariance matrices D and G were obtained using the package MCMCglmm (Hadfield 2009) of R. The running conditions were: n° iterations=130000, thinning=100, burnin=30000, retaining the 1000 final iterations.

Discriminant Analysis of Principal Components (DAPC) was applied to phenotypic data matrix using the *adegenet* package (Jombart 2008) (function *dapc*, Jombart et al. 2010) for the software R (R Development Core Team 2020). As in the case of AFLP, this analysis was performed with prior information on individual populations.

RESULTS

The AFLP analysis previously reported (Cerdeira et al. 2019) showed a total of 121 discernible bands in the 40 individuals belonging to the three populations studied (CG1, CG2 and PP), ranging from 90 to 400bp with three combinations of selective primers.

The estimate of the non-hierarchical F_{ST} was 0.27 ($CI_{95\%}$= 0.22-0.32), indicating highly significant genetic differences between the populations.

Analysis of variance

The comparison of morphological differences among populations (Table I) showed significant differences only for BSL max and BSL min (Fig. 2). Dunn's contrasts showed significant differences between PP and CG2 for BSL max, and that PP differs from CG1 and CG2 for BSL min.

According to the multivariate analysis of variance (MANOVA) differences among populations were highly significant (*P*= 0.003).

Table I. Basic statistics of the three populations of *S. gilliesii* studied. Mean, SD: standard deviation of each trait, N= 40. K-W ANOVA: Non parametric Kruskal-Wallis ANOVA, H: values of test statistic, P: statistical significance. RAL: rachis length, PLA: pairs of leaflets on the apical pinna, PLB: pairs of leaflets on the basal pinna, SSLl: left stipular spine length, SSLr: right stipular spine length, (BSL min) minimum base of stipular spine length, (BSL max) maximum base of stipular spine length. **P≤0.01

			K-W ANOVA	
Trait	**Mean**	**SD**	**H**	***P***
RAL	1.32	0.43	1.04	0.59
PLB	10.08	2.35	3.13	0.21
PLA	12.59	2.72	0.18	0.92
SSLl	0.24	0.06	1.47	0.48
SSLr	0.24	0.06	0.13	0.93
BSL min	0.48	0.23	9.34	0.01**
BSL max	1.02	0.33	10.53	0.00**

Phenotypic variation

The phenotypic differentiation (P_{ST}) ranged from 3.73 x 10^{-10} for right stipular spine length (SSLr) to 0.15 for maximum basal spine length (BSL max). These estimates were compared with those of the molecular differentiation between populations assessed from the 121AFLP loci. In all cases P_{ST} were lower than F_{ST}, although for BSL max and BSL min, the 95% CI overlapped (Fig. 3), indicating that the Ho of neutrality cannot be rejected. For the remaining traits (RAL, PLB, PLA, SSLl and SSLr) the differences between P_{ST} and F_{ST} were significant (95% CI did not overlap, Fig. 3). The critical c/h^2 values for SSLr and PLB are 0.24 and 0.73, respectively (Table II), meaning that the upper 95% confidence interval of P_{ST} would not overlap the lower 95% confidence interval of F_{ST}.

The comparison between D and G matrices did not reject the hypothesis of proportionality (ρ= 1.011, P= 4x 10^{-9}). This observed value for ρ was compared with the expected one calculated as $2F_{ST}/(1-F_{ST})$= 0.74. The difference between the observed and expected ρ, was significant (*t*= 1.92, *P*= 0.03, df=48).

Discriminant Analysis of Principal Components (DAPC)

DAPC was first made for the AFLP data set. The clusters were defined a priori, according to the genetic population. In this case, 2 axes were retained for DAPC, explaining the 83.38% and the 16.62% respectively. The scatterplot showed three well defined groups of populations (Fig. 4a). When the same analysis was conducted with morphological data, also 2 axes were retained for the DAPC, explaining the 90.7% and the 9.3% respectively, but the scatterplot showed high overlapping among individuals and could not separate the populations by neither the two axes (Fig. 4b).

DISCUSSION

Little is known about how selective forces are acting in tropical and subtropical *Acacia* species in general. The first genetic study in *S. gilliesii*, showed a high percentage of polymorphic loci, high mean heterozygosity estimated with AFLP markers, showed also that most part of variation resides within populations and SGS was detected at short and middle distances (Cerdeira et al. 2019). All these results are in accordance with those obtained for other American and African species of the genera *Senegalia* and *Vachellia*. In the present work we study for the first time a species of the genus *Senegalia* using the P_{ST}-F_{ST} comparison to track for signals of natural selection in *S. gilliesii*. We assessed seven quantitative traits and the existence of differences between populations

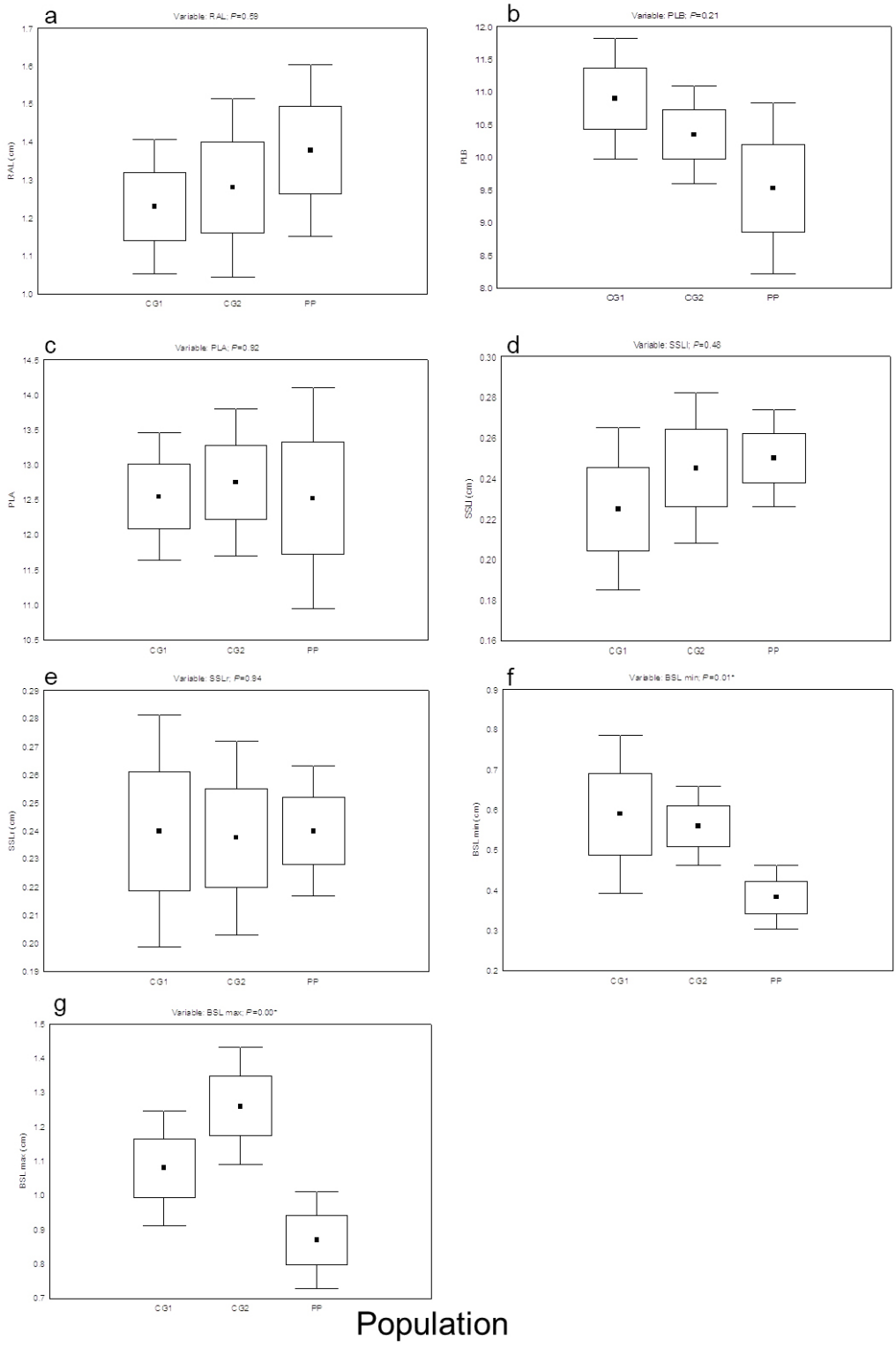

Figure 2. Box-plot comparisons of phenotypic traits measured among three populations of *S. gilliesii*. A) RAL, B) PLB, C) PLA, D) SSLl, E) SSLr, F) BSL min, G) BSL max. Statistical significance at 0.05 level for non- parametric ANOVA. Full squares represent the mean of the trait for each population; empty squares represent the mean ± SD; and vertical lines represent the mean ± SEM.

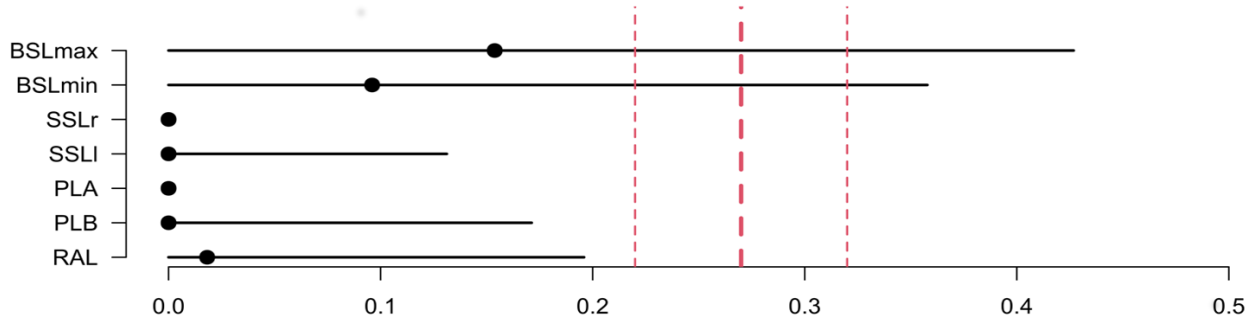

Figure 3. Pairwise phenotypic differentiation (PST) values (±95% CI) for the seven traits in the three populations of *S. gilliesii*. Vertical dashed lines denote the upper and lower 95% confidence intervals of pairwise FST obtained from AFLP data. FST value estimated with the total (121) AFLP data set.

and analysed the results obtained aiming to determinate the evolutionary forces modulating differentiation.

When applying the P_{ST}- F_{ST} comparison it is important to not confound the effects in additive genetic variance of the environment (Merilä & Crnokrak 2001, Pujol et al. 2008). To patch this issue, the approach of Brommer (2011) incorporates the environment effects between populations by evaluating the effect of between-population additive genetic variance (c) to within-population additive variance relative to total variance (h^2). Therefore is paramount to see the point when the ratio c/h^2 is smaller and the P_{ST} exceeds F_{ST}. In that point, the support for local adaptation for a particular trait is stronger (Brommer 2011). In this sense, the evaluation of the c/h^2 ratio allows a more rigorous test of natural selection making more robust the estimate of $P_{ST.}$ However, in the case of the CG vs. PP, the differences between populations could be attributed at least partially to phenotypic plasticity as a response to variation. In order to confirm the trends observed in this paper a larger sampling involving a higher number of populations and provenance tests under uniform conditions would be needed. However, plasticity in phenotypic responses would probably produce an overestimation rather than an underestimation of genetic variation quantified by P_{ST} what suggest that our conclusion of stabilising selection is well supported.

In this work, five of the seven traits measured showed evidence of stabilizing selection. The critical c/h^2 values obtained here for the P_{ST}'s, could be considered moderately high, supporting the robustness of the results (Brommer 2011). Three of them are foliar traits (RAL, PLB and PLA) and the other two refer to the length of the stipular spines. The same traits measured in seven populations of *A. aroma (=V. aroma)* gave similar results, all showing signs of stabilizing selection (Pometti et al. 2019) evidencing a similar trend for these traits in American *Acacia s. l.* species. Other American woody Caesalpinoideae species showed similar results, for example in *Prosopis flexuosa*, four leaf traits evidenced stabilizing selection (Darquier et al.

Table II. P_{ST} values and c/h^2 for the seven traits analysed.

Trait	P_{ST}	c/h^2
RAL	0.02	0.57
PLB	0.00	0.73
PLA	0.00	0.56
SSLl	0.00	0.37
SSLr	0.00	0.24
BSLmin	0.10	0.29
BSLmax	0.15	0.51

2013); the same trend was observed in *P. alba* for three foliar traits and spine length (Bessega et al. 2015). Finally, in *P. chilensis* stabilizing selection was described for spine length based on F_{ST}-Q_{ST} test and D_{JOST} and $\delta_{GREGORIUS}$ alternative coefficients of differentiation (Chequer Charan et al. 2020). In summary, Argentinean related woody legume species showed the same trend to uniform selection in at least some foliar traits.

When plants grow or are adapted to a resource-rich environment, they generally invest more energy in growth, but when the environment have poor resources they invest more in defence, like spine length (Coley et al. 1985). In this work, there was no evidence of differences in stipular spines length among populations, suggesting there is no difference in resources among environments. Similar results for spine length were found by Mboumba & Ward (2008), assessing two populations of *Acacia karroo* of contrasting environments.

According to some authors (Martin et al. 2008, Chapuis et al. 2008) a multivariate analysis provides a more accurate picture of the impact of selection versus drift on the system as a whole. In this work, G and D matrices are proportional. Taken as whole the results suggest that different regimes may be acting on different traits: stabilizing selection and neutrality (Fig. 3). Moreover, the MANOVA was significant due to the influence of two traits: BSL max and BSLmin, however these traits resulted selectively neutral in accordance to the P_{ST} value. In future projects more populations could be included if possible in order to recover to the maximum the existing morphological variation in this species for these traits and be able to solve this question.

In this study, the multivariate DAPC analysis showed a better discrimination of the populations in clear clusters when analysing the molecular data matrix rather than the morphological one. This result showed a consistency when those results obtained with Canonical Discriminant Analysis in the species under the name of *Acacia aroma* (Pometti et al. 2019). This is not the first time that molecular markers discriminate better groups that morphology; in previous work in other Acacias this was also observed (Pometti et al. 2010, Pometti et al. 2019). These results were explained based on the neutrality of the molecular markers (Strauss et al. 1992) and the large coverage along

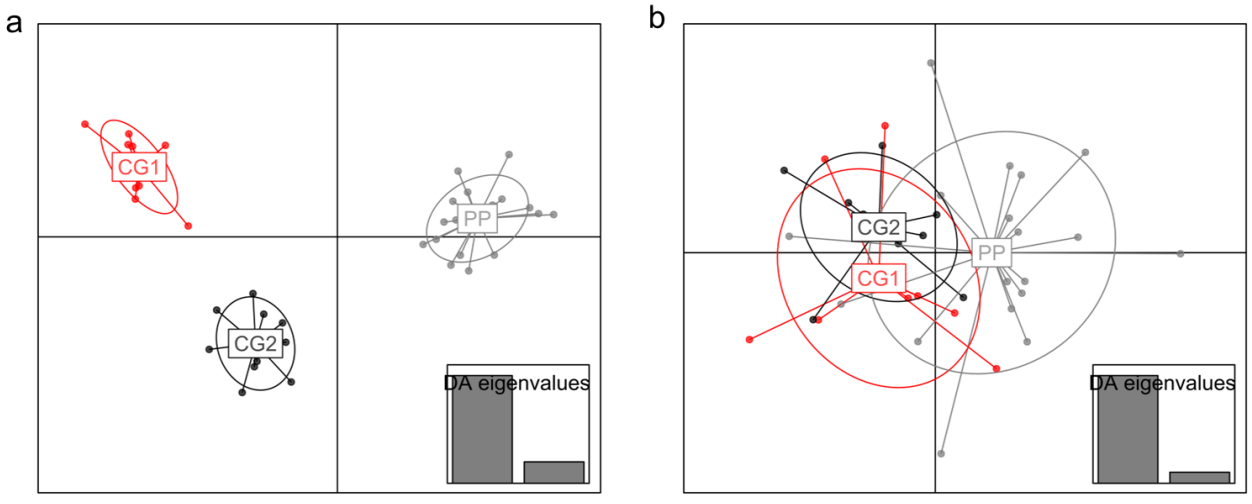

Figure 4. Plot of Discriminant Analysis of Principal Components (DAPC)1 and 2 of *S. gilliesii* populations. a- from AFLP data; b- from morphological data.

all the genome (Stammers et al. 1995). So, both data should be complemented, that obtained by molecular markers and the morphological characterization (Artyukova et al. 2000, Li et al. 2002, 2008). For this reason, in all these cases, molecular markers discriminate populations better than phenotypic traits which may be affected by selection.

Overall, our findings suggest a prominent role of stabilizing selection in all foliar traits and stipular spine length although there was no evidence of significant differentiation among populations for these characters. These results could be extrapolated to other tropical and subtropical acacias belonging to the genus *Senegalia* in particular, although also are consistent with the findings for *A. aroma* (= *V. aroma*). Further study is needed to analyse the mechanisms underlying genetic differentiation in natural populations of *S. gilliesii*, and try to find its relationship with eco-geographical variables.

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