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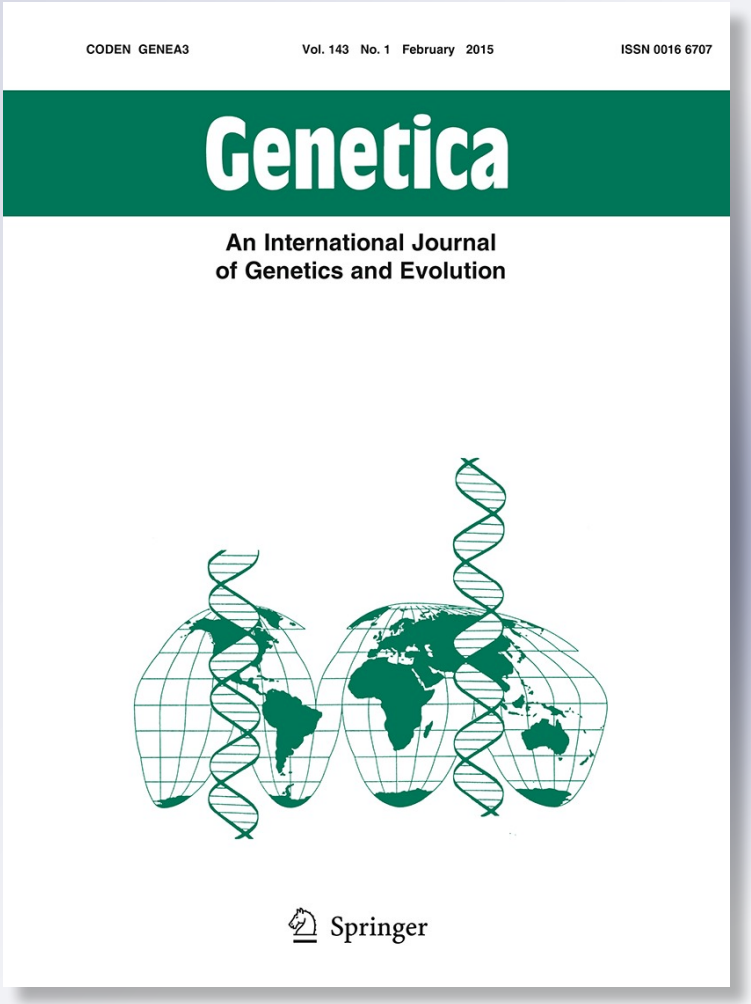

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Evidences of local adaptation in quantitative traits in *Prosopis alba* (Leguminosae)

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Abstract Signals of selection on quantitative traits can be detected by the comparison between the genetic differentiation of molecular (neutral) markers and quantitative traits, by multivariate extensions of the same model and by the observation of the additive covariance among relatives. We studied, by three different tests, signals of occurrence of selection in *Prosopis alba* populations over 15 quantitative traits: three economically important life history traits: height, basal diameter and biomass, 11 leaf morphology traits that may be related with heat-tolerance and physiological responses and spine length that is very important from silvicultural purposes. We analyzed 172 G1-generation trees growing in a common garden belonging to 32 open pollinated families from eight sampling sites in Argentina. The multivariate phenotypes differ significantly among origins, and the highest differentiation corresponded to foliar traits. Molecular genetic markers (SSR) exhibited significant differentiation and allowed us to provide convincing evidence that natural selection is responsible for the patterns of morphological differentiation. The heterogeneous selection over phenotypic traits observed suggested different optima in each population and has important implications for gene resource management. The results suggest that the adaptive significance of traits should be considered together with population provenance in breeding program as a crucial point prior to any selecting program, especially in *Prosopis* where the first steps are under development.

Keywords Adaptation · Neutrality test · Selection · Progeny trials · Quantitative genetics

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Introduction

Local adaptation in plants is of fundamental importance in evolutionary, population, conservation and global-change biology. Among the principal facts that are altering the environments are the climate change (Howe et al. 2003; Chown et al. 2010; Jump and Penuelas 2005) and environment degradation (Adamoli et al. 2011) both driven by natural or human forcing. In developing countries, like Argentina, arid and semiarid ecosystems are undergoing severe environmental degradation and biodiversity loss as a consequence of unsustainable use of natural resources. Human activities, including extractive exploitation of native forests and the expansion of stock breeding and agricultural areas over natural ecosystems, generate an alarming reduction of biodiversity, desertification, overgrazing, soil erosion, loss of soil fertility, and a predisposition to periodic drought (Secretaría de Ambiente y Desarrollo Sustentable de la Nación 2004; FAO 2007). *Prosopis* (Leguminosae, Mimosoideae), is a genus well represented in Argentina that includes woody species adapted to arid and semiarid regions. Several members of this genus represent both ecologically and economically ideal multipurpose trees that may be included in programs of reforestation and afforestation as they provide fuel wood, stabilize sand dunes and are valuable to combat

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desertification. These trees have become an essential source for fuelwood and a provider of several other products and services in areas where it has become established (Roig 1993; Verdes 2007; Walter 2011). However, despite the numerous benefits these trees provide to rural people, in several regions of the world Genus *Prosopis* has become a noxious weed with a negative impact on the environment and a bad-influence to the economy of farmers and landowners. The invasive *Prosopis* strategy impacts in a negative way on croplands, in its occupation of the banks of irrigation canals and other water sources. When they grow tightly along the sides of rural road they restrict the path for animals, and in some cases the stiff thorns results in wounds and subsequently septic conditions and even the death of the animals (Walter 2011; FAO 2013). An important challenge to the forestry sector is finding a balance between benefits, namely production, and forest protection; this goal is hard to achieve due to the strong pressure on wood resources and their low potential for wood production. *Prosopis* planting programs are currently based on plus trees selected from natural stands mainly on phenotypical basis (Verga 2000). However, the success of phenotypic based selection depends on the genetic component of the phenotypic variance, which has not been sufficiently evaluated.

The study of the genetic parameters of quantitative traits of economic and adaptive importance may be interpreted in terms of evolutionary forces acting, such as selection and genetic drift. The response to natural selection can be different in demes (Wade 2000), and can maintains the genotypes locally adapted. Understanding the association of quantitative traits to particular environmental conditions is key to evaluate the probability of success of selected phenotypes to grow in different regions. The identification of such association usually requires the comparison of patterns of phenotypic variation with the expectation under a neutral differentiation scenario. In a forest management practice aimed to obtain healthy and productive forests, is relevant to plant a stock that is well adapted to local growing conditions. Using a stand that lacks important adaptive traits may lead to immediate or long term problems. Trees that are stressed because of mal-adaptation have suboptimal productivity and tend to be vulnerable to insect pests and diseases and if this condition is not considered at the initial steps, it can cause an important economic loss as the damage can occur in the entire plantation.

Microsatellite markers can be used to evaluate the hypothesis of neutrality of phenotypic diversity; they are assumed to be neutral and may be used to infer genetic relationships (O'Hanlon et al. 2000). The molecular differentiation between populations or sample sites, quantified by the F_{ST}, is expected to reflect the selectively neutral component of the genetic variation resulting from interaction between limited migration and drift (Latta 2004; Reed and Frankham 2001). The degree to which the pattern of genetic divergence in neutral molecular markers parallels that of quantitative traits, as measured by the Q_{ST}, depends mainly on the extent to which genetic variation is maintained through selection (interacting with gene flow and drift) and also on the selection mechanism (diversifying, stabilizing).

The comparison of the levels of phenotypic and molecular differentiation can contribute to discriminate selection from genetic drift effects over a group of quantitative traits (Leinonen et al. 2008; Merilä and Crnokrak 2001), however, several restrictions and problems have been noticed (Beaumont 2008; O'Hara and Merilä 2005; Whitlock 2008; Ovaskainen et al. 2011). The classical interpretation is that if $F_{ST} = Q_{ST}$, there is no need to invoke natural selection and differentiation is explained by simply by gene flow–genetic drift interaction, if $Q_{ST} > F_{ST}$ positive directional natural selection favoring different phenotypes in different populations is suspected; and $Q_{ST} < F_{ST}$ suggests stabilizing selection favoring the same phenotype in different populations (Merilä and Crnokrak 2001). However, the comparison of the coefficients of quantitative and genetic differentiation is overshadowed when a small number of populations are locally adapted to their environments, because reliable estimates require data from more than ten populations in controlled environmental conditions (Martin et al. 2008; O'Hara and Merilä 2005).

As alternative, multivariate extensions of F_{ST}–Q_{ST} comparisons are somewhat more powerful due to the integration of information across multiple traits but criticized by some shortcomings. A technical difficulty in conducting a multivariate F_{ST}–Q_{ST} neutrality test is that one of the quantities (Q_{ST}) is a matrix, while the other one (F_{ST}) is a scalar as pointed by Ovaskainen et al. (2011). The Martin et al. (2008) multivariate neutrality test approach provides more accurate evolutionary predictions and allows studying adaptation on several traits simultaneously (Chapuis et al. 2008; Martin et al. 2008). This approach addresses more complex questions about the specific phenotypic effects of different evolutionary process. It compares the among-population (D) and within-population (G) genetic covariance matrices and tests the neutral pattern of $D = 2F_{ST}/(1 - F_{ST})G$. The test proposed by Martin et al. (2008) is twofold: (1) testing for equality between an estimate of proportionality coefficient ρ between the G and D covariance matrices and its expectation, $2F_{ST}/(1 - F_{ST})$, from neutral markers for diploid non inbred model; and (2) testing the proportionality itself between D and G. The first approach (1) is very close to de classic Q_{ST}–F_{ST} comparison but in a multivariate framework avoiding the problem of comparing a matrix (Q_{ST}) and a scalar (F_{ST}), while test

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(2) is similar to the approaches proposed in the studies of G matrix evolution (Schluter 1996). Although the multivariate extensions of F_{ST}–Q_{ST} comparisons suffer from the same principal shortcomings, they are somewhat more powerful due to integration of information across multiple traits (Chenoweth and Blows 2008; Martin et al. 2008).

A new recent approach for testing the role of directional or stabilizing selection behind population divergence in interconnected local populations was proposed by Ovaskainen et al. (2011) that is based on the simple observation of the additive covariance among relatives. In comparison to earlier methods, Ovaskainen et al. (2011) claim that the method (1) accounts for the inherent randomness in the evolutionary process, (2) utilizes in the multivariate case the information on all traits without an averaging procedure, (3) accounts for population-to-population level coancestry coefficients and thus omits the averaging procedure used to compute F_{ST}, and (4) accounts for population-to-population level information on similarity in trait values and thus omits the averaging procedure used to compute Q_{ST}. The novel approximation uses a Bayesian framework and can also be used to reject the null hypothesis of divergence by random genetic drift even in cases where F_{ST} and Q_{ST} are identical. The S neutrality test proposed by Ovaskainen et al. (2011) can be interpreted as follows: $S = 0.5$ implies perfect match at random genetic drift, whereas $S < 0.05$ implies stabilizing natural selection at 95 % credibility level, and $S > 0.95$ implies divergent natural selection at this credibility level. In the last year, Karhunen et al. (2013a, b) extended the method proposed by Ovaskainen et al. (2011) to detect footprints of natural selection in quantitative traits by the incorporation of a new statistical (H) that helps in the determination whether populations found in similar habitats are similar to each other more than expected by chance, based on their shared evolutionary history and genetic correlations among traits. The H test ask whether the mean genotypes correlates with the environmental covariates more than expected on the basis of the pattern of coancestry among populations.

The success of management programs oriented to conservation and improvement of multipurpose species depends strongly on the information about the amount of genetic variability of quantitative traits as well as the nature of such variation. Predicting how a population will respond to selection or whether different populations will respond similarly are aims in forest genetics, and it contributes to set the basis for decision-making in conservation management. The parallel analysis of molecular and quantitative divergence is meaningful to properly define management units (MUs) and evolutionarily significant units (ESUs) following Moritz (1994). The *Prosopis* common garden experiments were primary constructed to identify optimal seed sources for reforestation within regions (Cony 1993, 1996; Felker et al. 2001) as they yields high quality phenotypic data for among and within population variation studies. They constitute a very expensive long-term research and can be exploited by considering the discrepancy between differentiation at the phenotypic and molecular levels to analyze the adaptative variation as it reflects the population's phenotype response to evolutionary processes. The largest discrepancy is obtained under strong divergent local selection under moderate to high levels of gene flow. It is a complex interplay between the genetic architectures of quantitative traits and the selection–migration balance (Savolainen et al. 2013). The approaches based on quantitative trait and molecular markers tools are complementary and their integration provides robust inferences about evolutionary mechanisms and adaptive strategies.

Attempts to identify, understand, and detect selection pressures for local adaptation are vigorous areas for research willing to detect potential for evolutionary responses to environmental changes, in particular in tree populations (Leimu and Fisher 2008; Fang et al. 2013; Sork et al. 2013). The study of a provenance trials results critical to assess the relative contribution of genetic and environmental factors on adaptively and economically important traits under a common garden; this information is crucial because the response in any management program is based in the additive genetic variance component and the selection pressure that is going to be applied. In 1991 a *Prosopis alba* stand was established at San Carlos, Santiago del Estero, Argentina, with material from eight provenances representing the range of this species. This trial offers the opportunity to test the hypotheses that (1) the phenotypic differentiation among provenances is the result of local adaptation to their particular environmental conditions and (2) the selection on the multivariate phenotype is heterogeneous yielding different optima in each provenance. The strategy to achieve these goals was comparing the distribution of genetic variation of quantitative traits with that of neutral molecular markers. For this analysis we chose 3 economically important life history traits: height, basal diameter and biomass, 11 leaf morphology traits that may be related with heat-tolerance and physiological responses and spine length that is very important from silvicultural purposes by avoiding the damage to cattle/animals. We searched for signals of selection using three different approaches: the classical Q_{ST}–F_{ST} test, the multivariate approach of Martin et al. (2008), and the Ovaskainen et al. (2011) neutrality test including habitat information (Karhunen et al. 2013a, b). The results from these approaches were compared, and were discussed in terms of the convenience of considering local adaptation in defining the strategies in conservation and breeding programs.

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Materials and methods

Study species and populations

A total of 142 G1-generation trees were sampled in an orchard established in 1991 in San Carlos, Santiago del Estero. The orchard was initiated from seeds collected from individual trees (half sib families) belonging to 8 sampling sites of northern Argentina: Añatuya, Castelli, Gato Colorado, Ibarreta, Pinto, Quimili, Rio Dulce Irrigation Zone, and Sumampa (Fig. 1). The experimental design was a randomized complete block comprising 57 families, seven replicates (blocks), and four trees per replicate (with a 4 × 4 m spacing). The total planting material was 1,596 individual trees, of which 1,289 still survived in 1999. For the last 10 years, this stand was affected by natural conditions without any silvicultural care. For this study, we sampled 142 individuals belonging to 32 different families that had kept their original identification label. The number of trees per family varied between 3 and 12.

Morphological and molecular data

The traits analyzed included: three life history, 11 leaf morphology and spine length from Bessega et al. (2009). Height (HEI) and trunk diameter (DB) (basal diameter at 20 cm above the ground) were scored in the field and Biomass (BMS) of each tree was estimated using the regression equation $BMS = \log_{wt} = 2.7027 \times \log DB - 1.1085$ proposed by Felker et al.(1989), where $\log_{wt}$ is the logarithm of fresh weight (kg) and log DB is the logarithm of trunk diameter (cm). We included spine length because for silvicultural programs the reduction of spine size is a desirable condition. We also considered 11 traits that characterize the foliar anatomy because the importance of leaf size, morphology, structure, and orientation in influencing tree productivity has been demonstrated in numerous physiological studies and they were also found to be related with heat-tolerance (Das 2014; Dunlap et al. 1995; Wu et al. 1997; Godoy and Gianoli 2013). To measure leaf morphology traits samples from each tree were first mounted on specimen boards. The morphological measured traits were petiole length (PEL), number of pairs of leaflets per pinna (NLP), pinna length (PIL), spine length (SPL), number of pinnae (NPI), leaflet length (LEL), leaflet length/width (LEL/LEW), leaflet falcate (LEF), leaflet apex (LEX), and leaflet apex/total area (LEX/LEA) (Fig. 2). In each individual, nine repeats of PEL, NLP, PIL, SPL, and NPI were obtained involving boards from three different canopy regions. Ninety repeats were obtained of LEL, LEL/LEW, LEF, LEX, LEX/LEA, involving 10 leaflets from nine pinnae sampled from three different regions of the canopy.

To obtain estimates of neutral genetic differentiation the genetic variability of 6 unlinked microsatellites markers was assessed. The data are those from Bessega et al. (2009) and comprise the genotypes of 142 individuals in the following loci (Mo05, Mo07; Mo08, Mo09, Mo13 and Mo16) (Mottuora et al. 2005). Data on genetic variability, characterization of microsatellite, heritability estimates can be found in Bessega et al. (2009).

Statistical methods

The analysis was performed by three different approaches; the first implies the comparison of phenotypic differentiation (Q_{ST}) and the neutral marker genetic based differentiation (F_{ST}), the second by a multivariate neutrality test

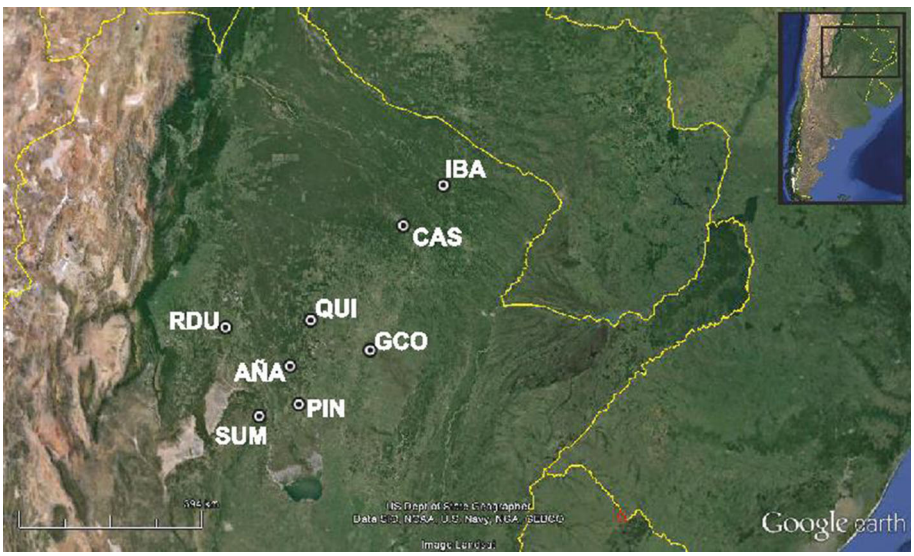

Fig. 1 Map showing the location of the collection sites of *Prosopis alba*. The trees used as parental generation in the orchard study originated from the following sites: *ANA* Añatuya, *CAS* Castelli, *GCO* Gato Colorado, *IBA* Ibarreta, *PIN* Pinto, *QUI* Quimili, *RDI* Rio Dulce Irrigation Zone, *SUM* Sumampa

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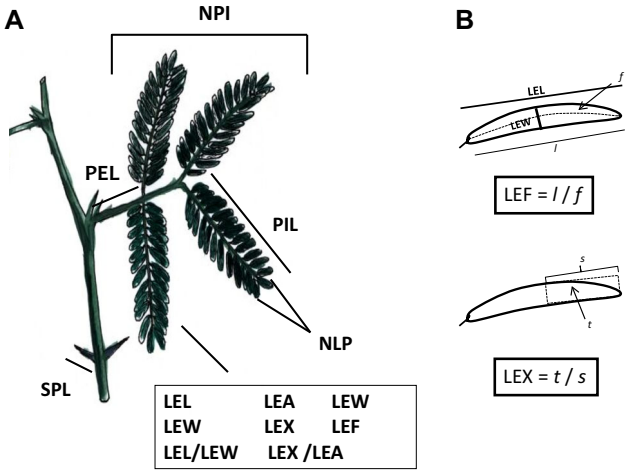

Fig. 2 Morphological traits measured over the herbarium specimens of *P. alba*. **A** General aspect of leaves and spines. **B** Details of measurements used to estimate leaflet falcate (*top*) and leaflet apex (*bottom*). *Notes A* SPL spine length, PEL petiole length, NLP pairs of leaflets per pinna, PIL pinna length, NPI number of pinnae, LEL leaflet length, LEW leaflet width, LEL/LEW length/width, LEF leaflet falcate, LEA leaflet area, LEX leaflet apex, LEX/LEA leaflet apex/total area, LEX leaflet apex, *B l* denotes distance from the base to the tip of the leaflet, *f* the length from the base to the tip of the leaflet following a curved line running along the middle of the leaflet, *t* the area of the upper leaflet third and *s* the area of a rectangle with the same dimensions as *t*

(Martin et al. 2008) and the third based on the recent developed neutrality test proposed by Ovaskainen et al. (2011) including habitat information (Karhunen et al. 2013a, b).

Q_{ST} and F_{ST} estimations

An analysis of phenotypic variance component was conducted applying the unbalanced generalized linear mixed model:

$$y_{ijk} = \mu + o_i + f(o)_{ij} + e_{ijk}$$

where y_{ijk} is an observation of the trait for an individual tree belonging to the family j, sampled in the origin i, in the environment k, and μ is the overall mean o_i represents random origin effects, $f(o)_{ij}$ is the random family nested within origin effect, and ε_{ijk} is the random residual error. Variance components as well as their confidence intervals were estimated using the package lme4 (Bates et al. 2013) of the software R (ver.2.13.1) (R Development Core Team 2013). The package used for all traits assumes a Gaussian distribution, using the identity link function.

The coefficient Q_{ST} (Spitze 1993) for each trait can be estimated assuming from the expression:

$$Q_{ST} = \frac{\sigma_O^2}{(2/r)\sigma_F^2 + \sigma_o^2}$$

where σ_O^2 is the variance between origin and σ_F^2 is the variance between families, and r is the within family relatedness coefficient.

For a half-sib model the expression is (Pressoir and Berthaud 2004):

$$Q_{ST} = \frac{\sigma_O^2}{8\sigma_F^2 + \sigma_o^2}.$$

Taking into account that evidence from Bessega et al. (2009) indicates that the *P. alba* plantation differ from a half-sib model, in the current study we modified the estimate according to Gauzere et al. (2013). In a fine scale mating study in *P. alba* Bessega et al. (2000) found that within the offspring of individual mother plants about 17 % correspond to full-sibs and 83 % to half-sibs. According to this estimate the average relationship (r) within a family group would be approximately 0.29, and accordingly the factor in the denominator of the equation should be approximately 6.8 instead of 8. Standard errors of these coefficients were estimated from 1,000 pseudo replicates obtained by bootstrapping the original dataset using the command *sample* of R.

Based on the microsatellites marker information, the coefficient F_{ST} and its confidence interval were estimated with the package *hierfstat* (Goudet 2005) of the software R (R Development Core Team 2013). A matrix of pair-wise differentiation among origins was obtained from pairwise F_{ST} estimated with the same package.

Multivariate Q_{ST}–F_{ST} comparison

The comparison between F_{ST} and multivariate Q_{ST} estimates was conducted following Martin et al. (2008). The method involves a dual test; the first one evaluates whether the MANOVA estimates of D and G covariance matrices are proportional, and the second one evaluates if the coefficient of proportionality between such matrices (ρ) is equal to the expectation under neutrality, that is:

$$\rho = \frac{D}{G} = \frac{2F_{ST}}{1 - F_{ST}}, \quad \rho = \frac{2Q_{ST}}{1 - Q_{ST}}$$

MANOVA was conducted with the software R (R Development Core Team 2013), and the estimates of 95 % confidence intervals for ρ were obtained with the script for R available at http://www.isem.cnrs.fr/spip.php?article934 (Martin et al. 2008). As this analysis is dependent on the assumption of normality distribution of morphological traits, all the data were transformed with the Box–Cox method (Box and Cox 1964), using the package MASS of the program R (Venables and Ripley 2002). Furthermore, normalized data were rescaled to the mean to have in all cases mean = 1.

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Ovaskainen et al. (2011) approach

The signals of natural selection were also estimated using the neutrality test proposed by Ovaskainen et al. (2011). The method is based on data from quantitative traits and genotypes of neutral molecular markers. The marker data are first analyzed using the R package *RAFM* (Karhunen 2013) resulting in a posterior distribution of the coancestry matrix θ_ρ. The differential contribution of genetic drift and gene flow from different lineages to the populations was calculated by alpha and kappa estimation with the package *RAFM*. The pattern of neutral genetic differentiation inferred from this matrix was produced using the function viz. *theta* of the package *driftsel* which calculates expected differences of the populations' means of a neutral trait in units of ancestral standard deviation. The function performs a multidimensional scaling for the distances and plots the results in a 2D plane.

This posterior is then used as prior by package *driftsel* (Karhunen et al. 2013a) which yields the posterior distribution of all parameters needed for the neutrality test. Convergence for both analyses was checked and the options chosen to run were: number of Monte Carlo iterations = 1,000, burnin = 500, thining interval = 2. Signatures of selection were evaluated by the tests *S* and *H* (Karhunen et al. 2013b).

Environmental variables

To evaluate correlation between genotypes and habitats by means of *H* test, 10 environmental climatic variables reflecting the differences among provenance (Table 1) were considered: mean annual temperature, average minimum and maximum temperature, mean annual rainfall per month, evapo-transpiration potential, water vapor press, wind speed, sunshine fraction, dryness and aridity. Data were obtained using the software NewLocClim ver. 1.10 (Grieser et al. 2014).

Correlations

The correlations between the matrices of geographical distances, pairwise F_{ST}, and euclidean phenotypic distances were evaluated by Mantel (1967) test (with 2,000 permutations) using the command *mantel.randtest* of the *ade4* package of R program (R Development Core Team 2013).

Results

Morphological differentiation

Average phenotypic values of the 15 analyzed traits were compared among provenances (Table 2). Expressed in proportion of total variance (R^2), the trait with the highest differentiation among provenances was LEL/LEW, for which R^2 was higher than 43 %. In contrast, R^2 was near 0 for SPL, NLP, and LEX/LEA, indicating virtually no differences among populations. The proportion of variance explained by provenance for traits directly related to production varied from approximately 8 (BMS and DB) to 11 % (HEI). In average, the proportion of variance attributed to provenance for morphological traits was 15.7 %. According to individual ANOVAs the differences among origins were significant or highly significant for 7 traits

Table 1 Geographic and climatic information of the *P. alba* collection sites

	Latitude (S)	Longitude (W)	T° mean (°C)	T° max (°C)	T° min (°C)	Precipitation (mm/month)	PET (mm)	WVP (hPa)	WS (km/hs)	SF (%)	Dryness index	Aridity index
ANA	28°27′56.09″	62°50′0.82″	20.42	28.58	13.53	51.42	121.57	14.94	9.48	50.42	2.206	0.42
CAS	25°56′58.96″	60°37′1.42″	21.15	28.78	15.37	89	117.01	18.17	7.95	49.92	1.356	0.76
GCO	28° 9′16.00″	61°12′60.00″	20.43	29.28	14.47	93.67	12.57	16.62	7.11	50.42	1.236	0.77
IBA	25°12′54.19″	59°51′26.46″	21.03	28.04	14.33	136.5	97.3	20.36	9.9	41.83	0.821	1.44
PIN	29° 8′33.07″	62°39′5.00″	20.42	28.58	13.53	51.42	121.57	14.94	9.48	50.42	2.18	0.42
QUI	27°38′39.06″	62°24′56.03″	20.42	28.58	13.53	51.42	12.57	14.94	9.48	50.42	2.225	0.42
RDU	27°46′58.56″	64° 8′59.84″	20.35	27.78	13.62	50.73	131.8	14.98	11.31	58	2.387	0.38
SUM	29°21′58.95″	63°28′4.56″	18.28	25.68	10.9	72.1	94.07	13.3	4.89	44.92	1.474	0.77

T° mean, mean annual temperature; T° max, average maximum temperature; T° min, average minimum temperature; Precipitation, mean annual rainfall per month; PET, evapotranspiration potential; WVP, Water vapor pressure; WS, Wind speed; SF, sunshine fraction; Dryness index, Budyko's radiative index (local radiation balance/energy needed to evaporate all precipitation); Aridity index, Annual precipitation/PET)

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Table 2 Summary of quantitative traits according to the sampling site

Trait	ANA	COS	GCO	IBA	PIN	QUI	RDU	SUM	R^2	P
Height (HEI) (m)	4.82 (0.72)	5.36 (1.65)	5.80 (1.04)	5.57 (1.63)	4.07 (1.15)	5.17 (1.07)	5.06 (0.70)	4.87 (0.98)	10.85	0.0610
Biomass (BMS) (kg)	2,698.33 (1,283.03)	5,606.72 (3,341.13)	4,980.23 (2,189.30)	17,016.64 (28,695.80)	3,513.44 (2,344.01)	4,709.73 (2,649.28)	6,821.87 (8,257.09)	7,482.03 (6,948.10)	7.78	0.1257
Basal Diameter (DB) (cm)	46.67 (8.38)	58.57 (19.67)	59.00 (9.63)	78.00 (41.38)	49.36 (15.47)	56.82 (11.84)	60.96 (21.66)	64.55 (20.39)	8.19	0.1325
Petiole length (PEL) (mm)	20.843 (6.537)	23.540 (5.071)	18.583 (1.470)	23.453 (7.783)	22.632 (5.129)	22.507 (6.097)	33.455 (8.039)	26.069 (6.792)	26.80	0.0003***
No. of leaflet per pinna (NLP)	35.780 (3.792)	35.619 (14.137)	37.472 (2.871)	37.606 (5.037)	32.638 (5.447)	36.486 (4.049)	35.213 (4.360)	33.130 (3.960)	0	0.5105
Pinna length (PIL) (mm)	85.854 (14.393)	90.429 (22.712)	82.694 (19.010)	89.808 (18.855)	87.607 (19.293)	86.764 (14.623)	107.645 (17.869)	96.176 (12.161)	14.70	0.0411*
Spine lenth (SPL) (mm)	2.940 (4.053)	4.349 (8.518)	4.903 (3.418)	2.925 (2.956)	4.571 (7.777)	1.672 (2.703)	2.001 (4.738)	2.893 (5.027)	0	0.9382
No. of pinnae (NPI)	2.981 (0.556)	2.151 (0.580)	3.194 (0.699)	2.861 (0.453)	2.344 (0.520)	2.809 (0.775)	2.173 (0.321)	2.116 (0.237)	39.11	0.0001***
Leaflet Length (LEL) (mm)	0.598 (0.147)	0.783 (0.365)	0.595 (0.076)	0.614 (0.104)	0.701 (0.170)	0.669 (0.150)	0.940 (0.163)	0.882 (0.133)	36.47	3.01×10^{-5}***
Leaflet Width (LEW) (mm)	0.144 (0.029)	0.192 (0.083)	0.151 (0.010)	0.154 (0.017)	0.146 (0.031)	0.148 (0.033)	0.169 (0.024)	0.165 (0.022)	10.67	0.1045
Length/ Width (LEL/ LEW)	4.401 (0.965)	4.097 (0.206)	3.970 (0.242)	4.008 (0.403)	4.847 (0.484)	4.603 (0.471)	5.589 (0.644)	5.372 (0.546)	43.36	4.85×10^{-5}***
Leaflet Falcate (LEF)	0.951 (0.014)	0.943 (0.011)	0.939 (0.003)	0.913 (0.047)	0.935 (0.031)	0.945 (0.005)	0.936 (0.029)	0.921 (0.045)	1.36	0.5515
Leaflet Total Area (LEA)	0.076 (0.033)	0.145 (0.140)	0.076 (0.014)	0.079 (0.019)	0.089 (0.034)	0.088 (0.045)	0.135 (0.039)	0.122 (0.035)	20.12	0.0064**
Leaflet Apex/ Total area (LEX/ LEA)	0.213 (0.009)	0.209 (0.015)	0.219 (0.007)	0.222 (0.013)	0.207 (0.018)	0.213 (0.017)	0.205 (0.017)	0.207 (0.025)	0.004	0.5738
Leaflet Apex (LEX)	1.016 (0.077)	0.980 (0.075)	0.998 (0.010)	0.985 (0.056)	0.971 (0.067)	0.991 (0.048)	0.931 (0.034)	0.938 (0.041)	20.26	0.0078*

The table exhibits the mean (SD) phenotypic values in the offspring (G1) generation of the common-garden study. The R^2 column indicates the percent of the phenotypic variation explained by the component population obtained by ANOVA (data from Bessega et al. 2009). P = significance of differences among origins

ANA Añatuya, *CAS* Castelli, *GCO* Gato Colorado, *IBA* Ibarreta, *PIN* Pinto, *QUI* Quimili, *RDI* Rio Dulce irrigation zone, *SUM* Sumampa

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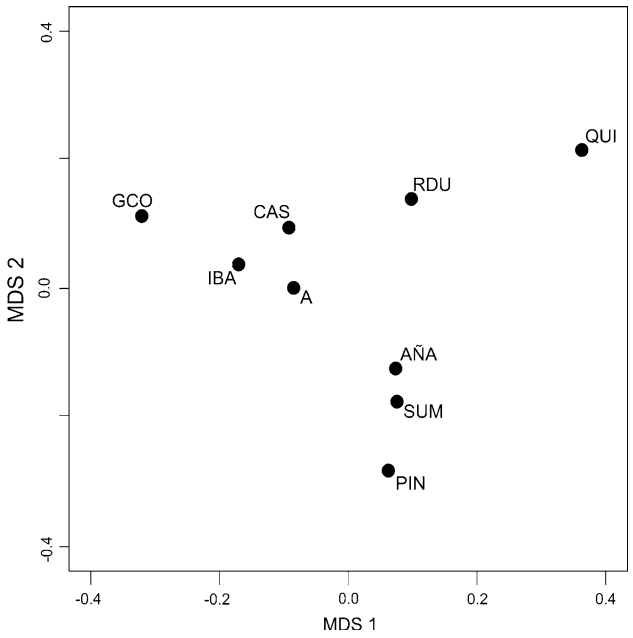

Fig. 3 Pattern of genetic differentiation inferred from neutral genetic markers. The populations were mapped into a two dimensional system coordinate system by using multidimensional scaling. The pattern of genetic differentiation is expected to be similar under random genetic drift. The figure was conducted with *vis.theta* in *driftsel* (Karhunen 2013). *Note*: *AÑA* Añatuya, *CAS* Castelli, *GCO* Gato Colorado, *IBA* Ibarreta, *PIN* Pinto, *QUI* Quimili, *RDI* Rio Dulce Irrigation Zone, *SUM* Sumampa, *A* refers to the unobserved ancestral population

(PEL PIL, NPI, LEL, LEL/LEW, LEA, LEX), all of them related with leaf size or shape. A multivariate analysis shows highly significant differences among origins (Wilk's lambda = 0.093, $p = 2 \times 10^{-16}$).

Pair wise Euclidean phenotypic distances between populations are not significantly correlated to geographical distances ($r = 0.323$, $P = 0.068$ according to Mantel test based on 2,000 permutations).

Microsatellites differentiation

The overall level of neutral genetic differentiation among the eight origins estimated by the package *hierfstat* was highly significant ($F_{ST} = 0.069$ 95 % CI 0.022–0.114, $p < 10^{-4}$). The genetic differentiation among origins is not correlated with the geographical distances as demonstrated by the mantel test ($r = -0.075$, $P = 0.575$) between pair wise F_{ST} and geographical distance matrices.

The pattern of neutral genetic differentiation estimated according the method of Karhunen et al. (2013a, b) is shown in Fig. 3. The populations Añatuya, Sumampa and Pinto, appear very similar to each other, Ibarreta and Castelli, both from the sub humid Chaco, are similar to each other and close to the unobserved ancestral (A) population, whereas Gato Colorado and Quimili are the most differentiated ones.

Table 3 Q_{ST} and Signals of Selection in quantitative traits in populations of *P. alba* by Q_{ST}–F_{ST} test, *S* test (Ovaskainen et al. 2011) and *H* test (Karhunen et al. 2013a, b)

Trait	Q_{ST}	Q_{ST}–F_{ST}	*S* test	*H* test
Height (HEI)	0.169 (0.004)	0.079^{D}	0.66	0.64
Biomass (BMS)	0.125 (0.005)	0.040	1^{D}	1
Basal diameter (DB)	0.083 (0.001)	0.003	0.96^{D}	1
Petiole length (PEL)	0.249 (0.002)	0.151^{D}	0.97^{D}	0.80
No. of leaflet per pinna (NLP)	5.660×10^{-09} (0.001)	-0.069^{S}	0.54	0.48
Pinnae length (PIL)	0.073 (<0.001)	−0.006	0.99^{D}	0.84
Spine length (SPL)	1.820×10^{-09} (<0.001)	-0.069^{S}	0.28	0.32
No. of pinnae (NPI)	0.362 (0.004)	0.257^{D}	0.95^{D}	0.76
Leaflet length (LEL)	0.348 (0.004)	0.243^{D}	0.96^{D}	0.96
Leaflet width (LEW)	0.107 (0.003)	0.024	0.51	0.48
Length/width (LEL/LEW)	0.210 (0.002)	0.116^{D}	0.99^{D}	0.96
Leaflet falcate (LEF)	0.005 (<0.001)	-0.064^{S}	0.42	0.52
Leaflet total area (LEA)	0.234 (0.003)	0.138^{D}	0.78	0.64
Apex/total area (LEX/LEA)	0.006 (<0.001)	-0.063^{S}	0.53	0.56
Leaflet apex (LEX)	0.116 (0.003)	0.032	0.86	0.72
	Ave: 0.139	Ave: 0.054	All: 1	All: 1

Test *S* takes into account the ancestral genetic correlations and the pattern of relatedness among local populations. *H* test incorporates data on the environmental variables: mean maximum T° (°C), precipitation (mm/month), wind speed (km/h) and sunshine fraction (%)

Standard errors of Q_{ST} (in brackets) were estimated from 1,000 bootrstaped peudoreplicates

^{D} Denotes directional selection and ^{S} denotes stabilizing selection

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Table 4 *H* neutrality test (Karhunen et al. 2013a, b) in populations of *P. alba* considering one environmental variable at a time

Trait	T° mean (°C)	T° max (°C)	T° min (°C)	Precipitation (mm/month)	PET (mm)	WVP	WS (km/h)	SF (%)	Dryness	Aridity
Height (HEI)	0.56	0.56	0.48	0.48	0.56	0.60	0.40	0.48	0.56	0.56
Biomass (BMS)	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Basal diameter (DB)	0.76	0.68	0.56	0.84	0.84	0.72	0.76	0.88	**0.92**	**0.92**
Petiole length (PEL)	0.84	0.88	0.64	0.64	0.80	0.72	**0.92**	**1.00**	0.80	0.76
No. of leaflet per pinna (NLP)	0.24	0.64	0.48	0.44	0.40	0.48	0.52	0.64	0.28	0.40
Pinna length (PIL)	0.80	0.88	0.88	**0.92**	0.80	0.80	**0.96**	**1.00**	0.88	0.80
Spine length (SPL)	0.28	0.28	0.32	0.24	0.32	0.40	0.40	0.24	0.32	0.28
No. of pinnae (NPI)	**0.92**	**0.92**	0.88	0.88	0.84	0.82	**0.92**	**1.00**	0.88	0.88
Leaflet length (LEL)	0.88	**0.96**	**0.92**	**0.96**	**0.92**	**1.00**	**0.96**	**0.96**	0.88	**0.96**
Leaflet width (LEW)	0.48	0.52	0.56	0.44	0.44	0.40	0.52	0.44	0.56	0.40
Length/width (LEL/LEW)	**1.00**	**1.00**	**0.92**	**0.92**	**0.92**	**0.96**	**1.00**	**1.00**	**0.92**	0.88
Leaflet falcate (LEF)	0.48	0.24	0.32	0.56	0.20	0.48	0.32	0.56	0.32	0.64
Leaflet total area (LEA)	0.68	0.56	0.68	0.64	0.76	0.72	0.72	0.80	0.48	0.52
Apex/total area (LEX/LEA)	0.40	0.44	0.68	0.68	0.64	0.68	0.48	0.52	0.64	0.60
Leaflet apex (LEX)	0.88	0.76	0.68	0.60	0.48	0.52	0.88	**0.96**	0.48	0.64
No. of traits with H $\geq$ 0.90	3	**4**	3	**4**	3	3	**6**	**7**	3	3

Bold numbers denotes H $\geq$ 0.90

T° mean, mean annual temperature; T° max, average maximum temperature; T° min, average minimum temperature; Precipitation, mean annual rainfall per month; PET, evapotranspiration potential; WVP, water vapor pressure; WS, wind speed; SF, sunshine fraction; Dryness index, Budyko's radiative index (local radiation balance/energy needed to evaporate all precipitation); Aridity index, annual precipitation/PET

Neutrality tests

The phenotypic differentiation (Q_{ST}) ranged from near cero for spine length (SPL) and number of leaflets per pinna (NLP) to 0.362 for the number of pinnae (NPI) (Table 3 column 1), with an average over traits of 0.139. For the three life history traits (HEI, BMS, and DB) and 7 foliar traits (PEL, NPI, LEL, LEW, LEL/LEW, LEA, LEX) $Q_{ST} > F_{ST}$ whereas for the remaining five traits (NLP, PIL, SPL, LEF, LEX/LEA) Q_{ST}–$F_{ST} < 0$. Cases where Q_{ST} was higher than the upper limit of F_{ST} confidence interval were considered as suggestive of directional selection while the cases where Q_{ST} was lower than the lower F_{ST} confidence limit are indicative of stabilizing selection (Table 3, col. 2).

The multivariate neutrality test (Martin et al. 2008) revealed signals of selection over the quantitative phenotypic traits from two evidence sources. The multivariate ρ estimate for quantitative traits ($\rho = 0.32$, 95 % CI 0.25–0.47) was higher than that for molecular markers ($\rho = 0.148$, 95 % CI 0.05–0.26) with a minimal overlapping of confidence intervals. The comparison between *G* and *D* matrices rejected the hypothesis of proportionality ($\rho \approx 0.32$, $P = 0$).

Signal of selection was also tested by *S* test (Ovaskainen et al. 2011) for all the quantitative traits. A very strong signal of diversifying natural selection was found ($S = 1$) in the multivariate analysis with all the traits. Regarding each trait, in seven out of the 15 cases *S* was higher than 0.95, indicating divergent natural selection (Table 3, column 3). In the remaining cases the hypothesis of neutral differentiation cannot be ruled out, although for spine length (SPL) the low *S* value might be considered as a weak signal of stabilizing selection.

The inclusion of habitat information, using environmental variables in the *H* neutrality test was also performed (Table 4). We found signals of selection (H $\geq$ 0.90) for all variables on at least 3 traits. For further analysis we selected only those environmental variables that affected 4 or more traits: mean maximum temperature, precipitation, wind speed, sunshine fraction. The global ($H = 1$) and single traits results of *H* and *S* tests were rather consistent, revealing selection over the same phenotypic traits. However in 3 cases (PL, PIL and NPI) the signal of directional selection was weaker for the *H* than for the *S* test. To determine if these discrepancies are due to the inclusion a particular environmental variable the *H* test was also performed considering one environmental variable at the time. The results (Table 5) suggest that the lower *H* values (in comparison to *S*) may be explained by the inclusion of environmental variables that do not contribute to phenotypic differentiation. That is the case of mean maximum temperature and precipitation in the case of petiole length

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Table 5 Effect of environmental variables on phenotypic differentiation in *P. alba* for those traits where the signal of directional selection was weaker for the *H* than for the *S* test

Trait	*S* test	*H* test (all)	*H* test (mean maximum T°)	*H* test (precipitation)	*H* test (WS)	*H* test (SF)
Petiole length (PEL)	0.97	0.80	0.88	0.64	0.92	1
Pinna length (PIL)	0.99	0.84	0.88	0.92	0.96	1
No. of pinnae (NPI)	0.95	0.76	0.92	0.88	0.92	1

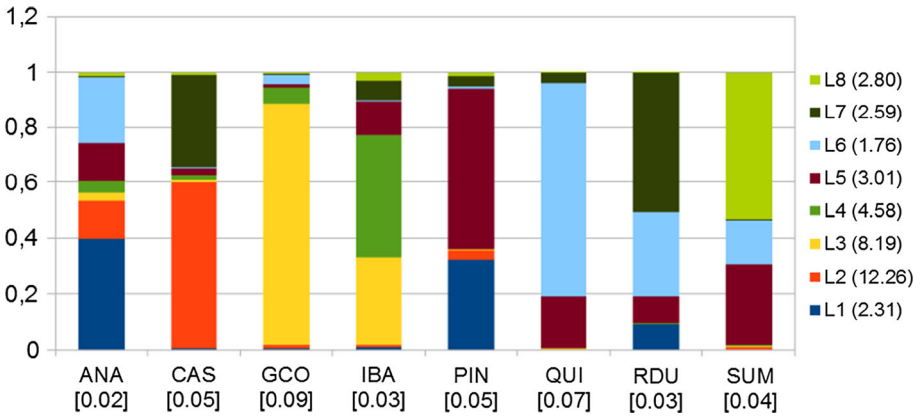

Fig. 4 Relative contribution of each lineage (L1–L8) on each origin estimated by kappa parameter with *RAFM*. Drift contribution estimated by alpha values for each lineage is presented in parenthesis on the right of lineage name. Variance of lineage contribution to each provenance is indicated in *brackets* below provenance acronym. *ANA* Añatuya, *CAS* Castelli, *GCO* Gato Colorado, *IBA* Ibarreta, *PIN* Pinto, *QUI* Quimili, *RDI* Rio Dulce Irrigation Zone, *SUM* Sumampa, *A* refers to the unobserved ancestral population

(PEL), mean maximum temperature for pinna length (PIL), and precipitation for number of pinnae (NPI). Apparently the most important environmental variables are sunshine fraction and wind speed.

Contribution of genetic drift and gene flow to molecular differentiation among provenances

The analysis of molecular diversity by RAFM identified 8 lineages (L1–L8) from the hypothetical ancestral population contributing to the 8 sampled origins (Fig. 4). According to alpha parameter estimates the amount of drift effect differed strongly among lineages (1.76–12.26). Also the kappa parameters indicated that the composition of each origin in terms of the relative contribution of each lineage was quite variable. The lineage that underwent higher drift effect (L2) has its highest contribution to Castelli, whereas Quimili is mostly integrated by individuals derived from other lineage (L6) with the minimum drift effect. The most diverse origins are Añatuya and Ibarreta, where the variances in the contribution of different lineages were 0.02 and 0.03, respectively. By contrast Gato Colorado and Quimili are the origins with the highest asymmetry as most individuals (0.87 and 0.77, respectively) derived from a single lineage and the variances of lineage contribution were 0.09 and 0.07, respectively (Fig. 4).

Discussion

The *Prosopis* breeding programs in Argentina are still in the initial progeny trial installation phase from material collected in wild populations. Only a few progeny trials have been so far installed in Argentina in the Provinces of Cordoba, Santiago del Estero, Chaco, Salta, and Formosa, each of them involving the first generation of families collected in the wild, including different numbers of provenances and families per provenance (Lopez et al. 2001; Delvalle et al. 2003; Verga 2009a). With the hope of getting improved material from the very beginning, these progeny trials were established from seeds representing wild open pollinated families from mother plants with good phenotypic characteristics, taking into account vigor, proportion between trunk and crown, un-branched condition, long and straight trunk, and healthy aspect. Furthermore, in order to capture genetic variation, all stands included several origins. These trials are just being under genetic

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evaluation to select candidates for establishing seed orchards or clonal seed orchards, a process that is expected to take several years.

The success of the seeds from different provenances in a new environment depends on the responses to the changing condition. The phenotypic plasticity, the local adaptation or the combination of both are the responses expected to survive (Aitken et al. 2008). Testing plasticity and possible genotype x environment interactions has not been yet possible in species of *Prosopis*, because replicates of provenance tests are only under the installation stage, but they will be available in the near future. Taking into account these limitations, for this work we analyzed a G1 trees' trial of *P. alba* established in Santiago del Estero in 1991 that was started from seeds belonging to eight different provenances in Argentina. The genetic differentiation estimated by Molecular genetic markers (SSR) markers among origins was highly significant but not correlated with the geographical distances. Possible explanations for the result are the recent fragmentation of the populations and/or the lack of equilibrium between migration and genetic drift forces. Using the Ovaskainen et al. (2011) and Karhunen et al.'s (2013a, b) approach we estimated the differential contribution of genetic drift and gene flow from different lineages to the populations. According to our results most provenances are variable and include individuals from different lineages, but the relative contribution of each lineage is variable among sampling sites. This result is consistent with the significant genetic differentiation among provenances and stress the importance consider both among and within provenance contribution in both conservation and breeding programs.

The distribution of genetic variation within and among populations is the cause and effect of the pollen flow dynamics defined by the mating system (Holsinger 2000; Petit and Hampe 2006; Marchelli et al. 2012), and it is of considerable relevance to understand the evolutionary process. Pollen and seed dispersal in *P. alba* would be limited and can explain the low differentiation among regions in Santiago del Estero previously studied (Bessega et al. 2011). Pollen in *Prosopis* is dispersed by insects (Genisse et al. 1990), mainly the honey bee *Apis mellifera* and the average distance of pollination estimated for *P. alba* was from 5 to 30 m depending of the method used (Bessega et al. 2011). *Prosopis* species have an endozoic seed dispersal system mediated by native small herbivorous and livestock (Mares et al. 1977; Hafez 1962). Although livestock are able to disperse seeds over larger distances (up to 4–6 km) in comparison with small rodents (approximately 50 m; Reynolds 1954, Keys 1993; Keys and Smith 1994), in all cases herbivorous tend to eat as many pods as they can from each single tree, and scarified seeds after passing through the animal gut are deposited in dungs forming groups of related individuals (Keys 1993; Bessega et al. 2000). Moreover, previous works on natural populations of *P. alba* geographically distant (Ferreyra et al. 2004, 2007, 2010) have also shown that most genetic variation occurs within populations and that the differentiation among populations and regions for molecular markers is rather low. The ability to maintain high genetic coherence in spite of limited dispersal ability may be associated with the long life span of *Prosopis* species together with original large populations' sizes and continuous distribution that characterized forests in the Chaqueña Region before the habitat fragmentation produced by human activities.

Our result is consistent with several studies on morphological variation of quantitative traits in *P. alba* that allow the assumption of the existence morphological ecotypes or even subspecies in *P. alba* (Morello et al. 1971; Burghardt et al. 2004; Verga et al. 2009b, 2014). Significant quantitative differentiation were detected in fruits and foliar traits among the *P. alba* sampled and three ecotypes had been described: "Santiagueño", "Chaco Norte" and "Chaco Sur" (Verga et al. 2009b, 2014). Bioclimatic differences between the areas of Santiagueño and Chaco Norte morphological groups of species were recorded and depend in part on the type of soil (Verga et al. 2014). Taking into account the sampling areas associated with these ecotypes in the paper by Verga et al. (2009b, 2014) the populations Rio Dulce Irrigation Zone (RDU), Sumampa (SUM), Pinto (PIN) and Añatuya (ANA) should correspond to "Santiagueño", Castelli (CAS) and Ibarreta (IBA) to Chaco Norte, and Quimili (QUI) and Gato Colorado (GCO) to Chaco Sur. In the present work we were not able to find significant differences between the origins corresponding to Santiagueño and Chaco Sur ecotypes. The probable cause for the inconsistency with our results and those of Verga et al. (2009b, 2014) is that fruit traits were not included in our analysis. However a clear differentiation can be seen between Chaco Norte (Castelli, Ibarreta) and the remaining populations. Castelli and Ibarreta are grouped near the ancestor in the multidimensional scale (MDS) analysis based on genetic neutral differentiation and they are grouped in the MDS based on Euclidean morphological distances (result not shown). Based on the results of the present work, the occurrences of the ecotypes in *P. alba* can be partially explained by divergent selection favoring different ecotypes in different habitat conditions. Habitat information by including environmental variables in our analysis is relevant to identify the factor with the highest influence on population differentiation. The *H* neutrality test detected that, among the variables considered, the sunshine fraction and wind speed are the variables with the highest contribution to phenotypic variation of petiole and pinna length and number of pinnas.

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This study demonstrates the power of population studies for identifying genetic signatures of directional selection for breed-defining provenances and phenotypic traits to include in future programs. The inclusion of genes underlying adaptive phenotypes will be also necessary in conservation genetics (Nichols and Neale 2010) and a balance among economic costs and beneficial should be carried on in order to avoid the selection based only in phenotypes when planning translation experiments either for seed or wood production. Although some discrepancies were observed between the results of Q_{ST}–F_{ST} and S tests in terms of the significance, the general trends are similar for 12 out of 15 traits. In all 10 cases where Q_{ST} was higher than F_{ST}, S was higher than 0.5, as expected for local adaptation by directional selection. Inconsistencies were limited to only 3 cases where Q_{ST}–$F_{ST} < 0$, but S was higher than 0.5 (NLP, PIL, and LEX/LEA). Taking into account the consistency between these tests, signals of directional selection are suspected for life history traits (height, biomass and basal diameter), and seven foliar traits (leaflet total area, leaflet width, petiole length, number of pinnae, leaflet length, leaflet apex, and leaflet length/width ratio). For spine length and leaflet falcate the results suggest stabilizing selection. The discrepancies among the methods to estimate signals of selection can be analyzed considering the limitations of each methodology. The novel Ovaskainen et al. (2011) method seems to be more conservative and robust to evaluate balance among selection and drift. In sum, applying F_{ST}–Q_{ST}, Multivariate (Martin et al. 2008) and S and H neutrality (Ovaskainen et al. 2011; Karhunen et al. 2013a, b) tests we detected signatures of past natural selection on both life history and morphological traits. Taken as a whole, the univariate and multivariate tests suggest heterogeneous selection over phenotypic traits with different optima in different populations. This result is in agreement with several authors that described the occurrence of ecotypes adapted to particular areas in several species of the genus *Prosopis* (Morello et al. 1971; Burghardt et al. 2004; Verga et al. 2009b, 2014). As a consequence of the local adaptation each provenance should be considered as a management unit (MU) with particular properties to be preserved in conservation programs.

Some characteristics, which are not measured by economic criteria, but are adaptively important for the species, should be taken into account in breeding programs. Adaptive and profitable characteristics described in species of this genus include high net photosynthetic rate, ability to fix atmospheric nitrogen, ability to grow in harsh climatic conditions, and ability to survive under salinity conditions equivalent to seawater supply. However, these properties are variable among species, among populations within species and among individuals within populations, and most probable they are under selection for local adaptation. Breeding programs should take advantage of this variability to get the best genotypes according to the environmental conditions in the areas to be afforested of reforested. As an example, it has been suggested that strong and long thorns and multiple stems, which are undesirable traits in breeding programs, are properties favored by natural selection pressure mediated by herbivorous and fuel wood harvesting by humans (Felker 2009). In fact *P. juliflora*, with large spines and non-palatable leaves (which are seldom eaten by animals) is one of the most invasive *Prosopis* species in tropical countries outside its native range. By contrast, it has been suggested that *P. alba* is not able to survive outside its native range in Argentina because of its tiny thorns and its highly palatable foliage that is browsed on by livestock (Felker 2009). However, the genetic variance of spine length within population is high (Bessega et al. 2009) and inappropriate use of this species might drive an increase of spine length by negative selection.

We conclude that the comparison of molecular and phenotypic differentiation allowed us to provide convincing evidence that natural selection is responsible for the patterns of differentiation. The heterogeneous selection over phenotypic traits observed, suggest different optima in each population and have important implications for gene resource management. The results suggest that population source and traits with adaptive significance should be considered in breeding program as a crucial point prior to any selecting program, especially in *Prosopis* where the first steps are just taken.

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Conflict of interest The authors declare no conflict of interest.

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