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ORIGINAL PAPER

Evidence of heterogeneous selection on quantitative traits of *Prosopis flexuosa* (Leguminosae) from multivariate Q_{ST}–F_{ST} test

M. Rosario Darquier · Cecilia F. Bessega · Mariano Cony · Juan C. Vilardi · Beatriz O. Saidman

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Abstract *Prosopis flexuosa* is an arboreal Leguminosae that grows in arid and semiarid temperate zones of Argentina, in the Monte eco-region. It is a promising native forest species for recovering arid and semiarid regions because it plays an important role in erosion control as well as in soil fertility. Furthermore, it provides diverse economical resources. The main challenge to the forestry sector is finding a balance between production and forest protection. For this purpose, it is necessary to gather information about genetic parameters. In this study, we measured the distribution of the variation of 14 quantitative traits in an experimental half-sib stand, where families are representative of hierarchically structured populations. We applied a multivariate extension of the classical Q_{ST}–F_{ST} neutrality test to determine the relative importance of drift versus selection in the distribution of genetic variability. We found strong evidence that different selective regimes act on different traits and that selection favors different optima in each sampling site. The selection to different optima is much stronger among than within provenances. This result helps explain the possible causes for the regional variation observed in *P. flexuosa* and to define the management units and the evolutionarily significant units for this species.

Keywords Multivariate Q_{ST} · Quantitative traits · Molecular markers · F_{ST} · *Prosopis flexuosa* · Forest trees · Selection

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M. R. Darquier (✉) · C. F. Bessega · J. C. Vilardi · B. O. Saidman
Departamento de Ecología, Genética y Evolución. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1458 Buenos Aires, Argentina
e-mail: darquiermrosario@ege.fcen.uba.ar

M. R. Darquier · C. F. Bessega · M. Cony · J. C. Vilardi · B. O. Saidman
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),
Buenos Aires, Argentina

M. Cony
Instituto Argentino de investigaciones de las zonas Áridas (IADIZA), Centro científico tecnológico (CCT),
Mendoza, Argentina

Introduction

Forests are the single most important repositories of terrestrial biological diversity. Historically, people have used a wide range of products and services obtained from natural and planted forests. Many other organisms are dependent on forest trees and have developed complex mechanisms to maintain high levels of genetic diversity associated with natural forest ecosystems.

Forest resources in arid and semi-arid areas are overexploited because of economics needs and inadequate information for appropriate management. Extractive exploitation produces negative selection, as the best trees are harvested, leading to the impoverishment of natural ecosystem and loss of short-term recovery ability. The indiscriminate exploitation generates also an alarming loss of biodiversity of other resources in the arid zones causing desertification, which must be stopped and reversed (FAO, FLD, IPGRI 2004). In highly degraded arid ecosystems, such as some areas of the Monte eco-region (Burkart et al. 1999), the general goal of reforestation is to restore the plant cover as rapidly as possible.

The main challenge to the forestry sector is finding a balance between production and forest protection; this goal is hard to achieve in arid zones due to the strong pressure on wood resources and their low potential for wood production. Management of promising species requires information about genetic parameters of quantitative economics traits.

The genus *Prosopis* has been studied with growing interest during the last few years because it includes shrub and tree species promising for recovering of arid and semiarid regions and production of diverse economically important resources such as wood, charcoal, fodder, and human food (Roig 1993; Saidman et al. 1996). They also play an important ecological role controlling soil erosion and improving soil fertility by fixing atmospheric nitrogen (Acosta et al. 1994). *Prosopis flexuosa* represents a promising multipurpose resource for reforestation of degraded arid ecosystems, very much appreciated in the Monte eco-region because of the excellent timber and non-timber products it provides (Alvarez and Villagra 2009).

In order to assess the relative contribution of genetic and environmental factors on adaptively and economically important traits, a provenance trial of this species was established at El Sauce, Mendoza, Argentina in 1991. For many years, this orchard was the only one available to assess the native germplasm of this species (Cony 1996) under constant monitoring, until the relatively recent establishment of two new orchards at Chamical (La Rioja Province) and San Rafael (Cordoba Province) (Verga et al. 2005).

The analysis of variance components of quantitative traits is a traditional method to estimate important genetic parameters, mainly the heritability and the proportion of genetic variance occurring within and between populations and between geographical or ecological regions (Lynch and Walsh 1998).

Neutral molecular markers provide valuable insight into historical and contemporary population dynamics. However, variation at marker loci is unlikely to be an accurate predictor of genetic variation at loci contributing to phenotype, that is, the adaptive variation (Reed and Frankham 2001; Mc Guigan 2006). Analysis of adaptive variation is of interest because it reflects historical evolution and determines the population's phenotype response to evolutionary processes. The approaches based on quantitative trait and molecular markers tools are then complementary, and their integration provides robust inferences about evolutionary mechanisms and adaptive strategies.

A number of studies in different species (Hamrick 2004) have compared the degree of differentiation at different hierarchical levels estimated from molecular markers using the Wright's (1951) fixation index F_{ST} and Spitze's (1993) quantitative index of population divergence Q_{ST}.

The total genetic variation in neutral marker loci can be partitioned into within (V_w) and between population (V_b) components, where V_b is equivalent to the expected gene diversity (heterozygosity assuming random mating) between populations that is in excess of that within populations (Nei 1987). From this, a standardized measure of the degree of between population genetic differentiations is obtained as (Wright 1951; Nei 1987):

$$F_{ST} = \frac{V_b}{V_b + V_w}$$

which scales from 0 to 1. Under the assumption of similar and low mutation pressure in different populations (Nagylaki 1998), the F_{ST} of neutral marker loci is primarily determined by the balance between random genetic drift and migration (Kimura 1983; Hartl and Clark 1989). Consequently, the degree of among-population differentiation at neutral marker loci, as estimated by the F_{ST} index, indicates the expected degree of population differentiation as a result of the combined effect of genetic drift and gene flow (Wright 1951; Rogers 1986; Lande 1992).

For quantitative traits with an additive genetic basis, Wright (1951) showed that the neutral expectation for mean additive genetic variation within populations is $\sigma_{GW}{}^2=(1-F_{ST})\ \sigma_o{}^2$, and that for between population variance $\sigma_{GB}{}^2=2F_{ST}\ \sigma_o{}^2$, where $\sigma_o{}^2$ is the expected additive genetic variance that would exist if all the populations under study formed a panmictic unit (Lande 1992). As the expectation for total genetic variance ($\sigma_T{}^2$) in a trait equals $(1+F_{ST})\ \sigma_o{}^2$, it follows that an estimate of population differentiation for a quantitative trait (termed Q_{ST} by Spitze 1993) analogous to that for single locus F_{ST} estimate can be obtained as:

$$Q_{ST} = \frac{\sigma^2_{GB}}{2\sigma^2_{GW} + \sigma^2_{GB}}$$

The comparison between Wright's F_{ST} and Spitze's Q_{ST} is widely used to assess the relative importance of selection and drift as determinants of genetic differentiation for quantitative traits (Lopez-Fanjul et al. 2007; Leinonen et al. 2008). There are three possible outcomes from the comparisons of FST and QST indices:

$F_{ST}=Q_{ST}$	No need to evoke natural selection; differentiation may be explained by genetic drift alone.
$Q_{ST}>F_{ST}$	Positive directional natural selection must be involved favoring different phenotypes in different populations.
$Q_{ST}<F_{ST}$	Stabilizing selection favoring same phenotype in different populations (Merilä and Crnokrak 2001).

Nevertheless, as selection occurs on whole organism and not on single traits independently (Lande and Arnold 1983), a complete characterization of adaptive variation in polygenic traits is required. A multivariate approach provides more accurate evolutionary predictions and allows studying adaptation on several traits simultaneously (Chapuis et al. 2008; Martin et al. 2008).

Besides the classical comparison Q_{ST}–F_{ST}, this approach involves a multivariate neutrality test, which

addresses more complex questions about the 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 $D=2F_{ST}/(1-F_{ST})\ G$.

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 testing to among-population covariance (*D*) 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 test (1) is very close to de classic Q_{ST}–F_{ST} comparison but in a multivariate framework, while test (2) is similar to the approaches proposed in the studies of *G* matrix evolution (Schluter 1996). In spite of some shortcomings inherent to F_{ST}-Q_{ST} comparisons (see Ovaskainen et al. 2011), the method avoids the problem of comparing a matrix (Q_{ST}) and a scalar (F_{ST}).

In the present paper, we conducted parallel analyses of population structure using molecular markers (inter simple sequence repeat (ISSR)) and 14 quantitative traits on the provenance trial of *P. flexuosa* at El Sauce. The relative contribution of genetic and environmental factors to total morphometric variation was estimated, and the parameters of population structure obtained from molecular and quantitative traits were compared to test the neutrality of morphological differentiation among sampling sites and provenances. Quantitative characters analyzed include life history traits (height and trunk diameter), foliar traits (size and shape), and spine length. Life history traits are very important in the context of potential use of this resource. Similarly, reduction in number and spine size is a goal in silvicultural management programs. Foliar and spine traits are considered of adaptive relevance, as different ecotypes are describe in related species of the section Algarobia that are differentiated by these characteristics (Morello et al. 1971; Burghardt et al. 2004; Verga et al. 2009). The consequences of the estimated distribution of genetic variability are discussed in terms of the strategies for conservation and management of this resource as well as to properly define in this species the management units (MUs) and evolutionarily significant units (ESUs).

Materials and methods

Material of study

P. flexuosa is distributed in the Monte eco-region and some areas of the Chaco, Espinal, and Patagonia eco-regions. It is an important multipurpose resource, providing pods used as forage for livestock and human food, flowers for the production of honey, wood for fences, gates, and furniture, etc. It also plays an important role in erosion control as well as in soil fertility, due to its ability to fix atmospheric nitrogen.

Plantation

The *P. flexuosa* trial was established in December 1991, at El Sauce, Mendoza (32°54′S, 68°50′W). Eighty-six half-sib families from 14 provenances of the Monte eco-region (Fig. 1) were arranged in a completely randomized block design with five replications. The families were planted in plots consisting of one row of three trees. Spacing in the trial was 4×4 m, with a single buffer row surrounding the study area (Cony 1996).

Morphometric data

In the present study, 400 trees from the trial representing all 86 families were measured. The number of individuals per family ranged from three to six (Table 1). Individuals were chosen among survivors at the age of 16 years (February 2007), trying to have at least one individual per family and block. The provenances and the sampling sites (populations) from which families were sampled are indicated in Table 1 and represented in Fig. 1.

A total of 14 morphometric traits were analyzed: Two life history traits, height (HEI), and trunk diameter (TDI) were measured in the field. These traits were chosen because of their importance for selection programs; their measurement is relatively simple and non-destructive. TDI was measured at 20 cm over ground level to obtain data comparable to previous measurements, because this trait was recorded yearly since the stand was established.

The same day when TDI and H were measured, vouchers from the same individuals were obtained to measure the remaining 12 morphology traits in the laboratory (Fig. 2). Spine length (SPL) and 11 foliar traits were measured over herbarium specimens. Foliar traits were petiole length (PEL), number of pairs of leaflets per pinna (NLP), pinna length (PIL), number of pinnae (NPI), leaflet length (LEL), leaflet width (LEW), leaflet length/width (LEL/LEW), leaflet falcate (LEF), leaflet area (LEA), leaflet apex (LEX), and leaflet apex/total area (LEX/LEA). Falcate is defined as the ratio *l*/*f*, where *l* is the length of a right segment from the base to the tip of the leaflet and *f* is the length from the same points but following the curve line that runs following the middle of the leaflet (Fig. 3a). LEX is the ratio *t*/*s*, where *t* is the area of the upper leaflet third and *s* is the area of a rectangle with the same dimensions (width and length) of the upper leaflet (Fig. 3b). In each individual, PEL, NLP, PIL, SPL, and NPI were measured on vouchers collected from three different canopy regions, and three repeats were

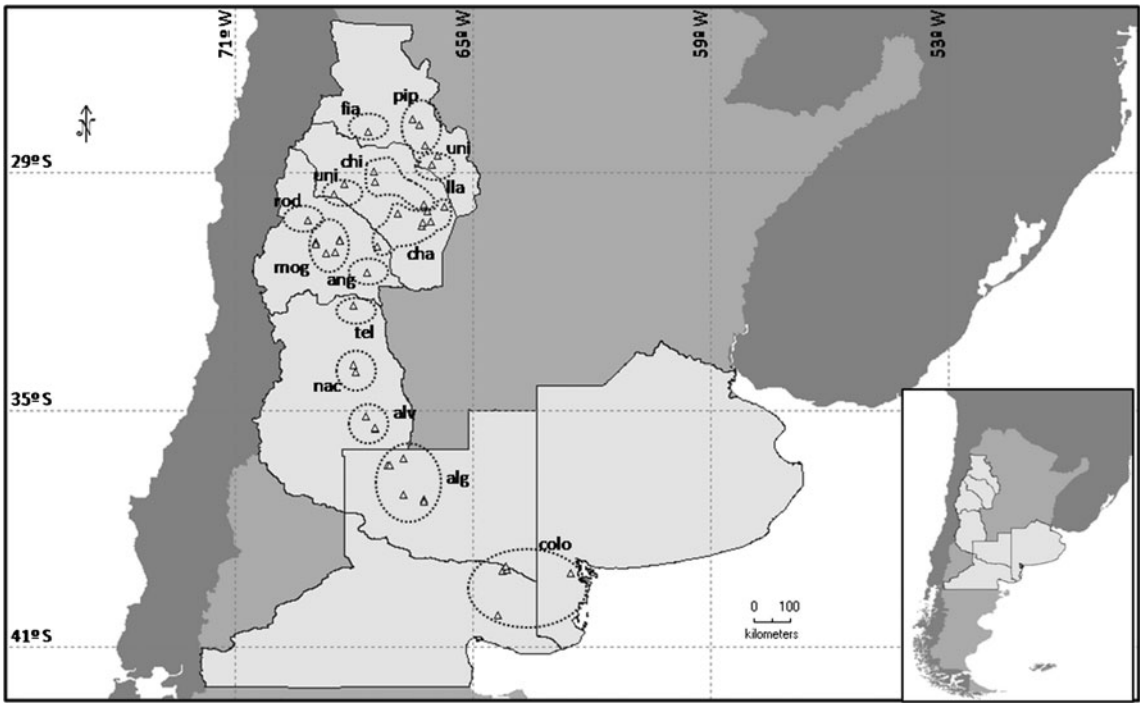

Fig. 1 Map of Argentina indicating the 14 provenances studied for *P. flexuosa*. *Triangles within dotted lines* show the number of sampling sites per provenances. The corresponding population *acronyms* are indicated in Table 1

measured from each canopy region, making a total of nine repeats per individual. Spine and foliar traits are assumed as important adaptively as the exhibit regional variation associated to environmental conditions within species of the section Algarobia (Morello et al. 1971; Burghardt et al. 2004; Verga et al. 2009) and are important for diagnosing species (Burkart 1976), an essential step in rational management. All leaflet measures were obtained with the software HOJA1.1 (available from the author upon request: A. Verga, INTA-IFFIVE, arverga@yahoo.com.ar). This software allows easily obtaining accurate simultaneous measures of many leaflet size and shape traits from binary (black and white 100 dpi) scanned images. The images of the traits analyzed (LEL, LEW, LEL/LEW, LEF, LEA, LEX, and LEX/LEA) were obtained with a HP F4480 multifunction device. Each trait was measured in two pinnae from each of three canopy regions. Ten leaflets from each pinna were measured. Individual data corresponded to the average of the 60 leaflets measured (3 canopy regions×2 pinnae×10 leaflets).

Molecular markers analysis

Plant material

ISSR analysis was based on 257 individuals representing 60 families randomly chosen from 31 sample sites and 14 provenances (Table 1).

Leaves were collected from each tree in February 2007 and were silica-gel preserved. DNA was extracted using DNA easy Plant mini kit (QIAGEN Inc., Valencia, CA, USA), and samples were placed in a –20° freezer until analysis.

PCR amplifications

ISSRs were carried out following Zietkiewicz et al. (1994). Nine primers were initially screened to identify well amplified, polymorphic bands. Two primers from the initial screening process, ISSR2 and AE2 which had a higher number of bands per individual and the best readability were used for polymerase chain reaction. Their sequences are respectively $[AC]_8G$, $[CA]_8G$

Polymerase chain reaction (PCR) amplifications were performed in 30 μL of reaction mixture containing the following reagents—30 ng of template DNA, 1 μM of primer (Invitrogen), 0.5 Unit of Taq DNA polymerase (Invitrogen), 0.20 mM of each dNTP (Biodynamics), and 1× reaction buffer supplied with the enzyme. The PCR program used for amplification in a MyCycler Thermal Cycler System (Bio-Rad) programmed as follows: an initial heating at 94 °C for 90 s, 30 cycles of denaturing at 94 °C for 30 s, annealing at 46 °C for 60 s, extension at 72 °C for 90 s, and a final extension of 5 min at 72 °C. The capacity of the program to generate reliable and reproducible patterns was confirmed.

Table 1 List of provenance, geographic coordinates of sampling sites (Cony 1993), number of families, and number of offspring studied per family for morphological traits and molecular markers studied in *P. flexuosa*

Provenance	Sampling site	Latitude (S)	Longitude (W)	# Families	# offspring per family	
					Morph	Molec
Angaco (ang)	ang	31.53	67.68	4	20	19
Chamical (cham)	pat	30.05	66.90	1	5	5
	tum	30.88	67.40	1	3	4
	r79	30.37	66.28	1	5	5
	s79	30.28	66.27	2	10	10
	r79a	29.98	66.15	1	4	4
	cam	30.25	66.07	3	16	13
Chilecito (chi)	r74	29.82	66.23	5	24	24
	fam	28.98	67.50	1	6	6
Fiambalá (fiam)	epu	27.97	67.63	6	30	30
Pipanaco (pip)	bel	27.67	66.53	3	15	15
	pip	27.80	66.33	2	9	9
	rbl	28.33	66.20	3	14	15
Llanos de Catamarca (lla)	cpi	29.88	65.72	1	3	4
	nca	33.85	68.02	1	4	1
	hui	34.05	67.97	1	4	1
Ñacuñan (nac)	csa	34.05	67.97	1	4	4
	rnc	34.05	67.97	4	15	16
Telteca (tel)	pte	32.37	68.02	1	5	5
	tel	30.23	69.15	2	10	10
Rodeo (rod)	rod	30.76	68.95	2	9	1
Mogna (mog)	hua	30.83	68.95	2	10	3
	cie	30.70	68.35	1	5	5
	tal	30.70	68.35	1	5	0
	mog	30.70	68.35	8	39	10
Villa Unión (uni)	gua	29.30	68.23	1	5	5
	uni	29.30	68.23	4	20	10
Alvear (alv)	alv	35.16	67.70	1	3	0
	coc	35.48	67.46	1	3	4
	parc	35.48	67.46	2	7	0
	rn143	35.43	67.46	1	4	0
Algarrobo del Aguila (alg)	sisa	36.23	66.76	1	4	0
	alg	36.40	67.15	1	4	0
	alg	36.40	67.10	4	18	0
	lim	37.15	66.75	1	5	0
	chac	37.25	66.23	3	14	2
Río colorado (colo)	ade	39.06	64.25	4	19	14
	colo	40.18	64.38	2	10	1
	vil	39.13	62.53	2	10	2
Total				86	400	257

The acronyms used in further tables and figures are indicated in parentheses

Morph morphological traits, *Molec* molecular markers

Electrophoresis of PCR products

PCR amplification products were run for 3.5 h on 6 % denaturing polyacrylamide gels with 7.5 M urea at constant 60 W power. A 100-bp ladder (Invitrogen) was included to estimate band sizes. Bands were visualized by silver nitrate staining using the method of Bassam et al. (1991). Only those bands that showed consistent amplification were scored. Smeared and weak bands were excluded. ISSR bands were scored as present (1) or absent (0) for each sample.

To test the reliability of PCR products, we routinely used several controls, one without primer, a second maintaining no Taq DNA polymerase, and the third with no genomic DNA. No amplifications occurred in any of these controls.

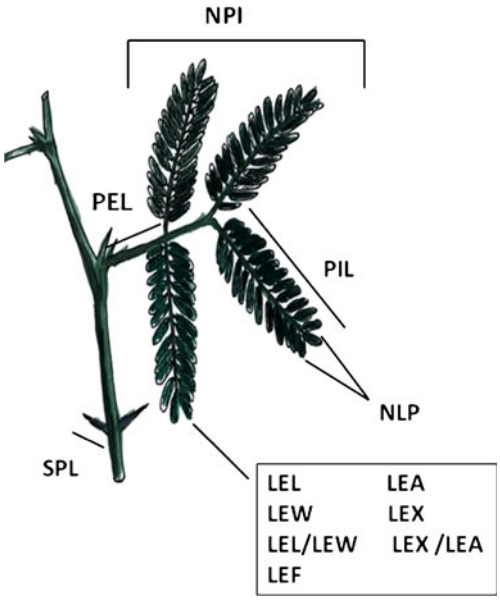

Fig. 2 Morphological traits measured over the herbarium specimens. *SPL* spine length, *PEL* petiole length, *NLP* pairs of leaflets per pinna, *PIL* pinna length, *NPI* number of pinnae, *LEL* leaflet length, *LEW* leaflet width, *LEL/LEW* length/width, *LEF* leaflet falcate, *LEA* leaflet area , *LEX* leaflet apex, *LEX/LEA* leaflet apex/total area, *LEX* leaflet apex

Linkage analysis between molecular markers

Possible gametic disequilibrium was evaluated using Otha's (1982) method. This approach distinguished gametic disequilibrium due to limited migration from that attributed to epistatic natural selection. Estimates were obtained with the software RAPDLD (Black 1996).

Data analysis

Morphometric traits

Heritability of quantitative traits in the orchard was estimated from an analysis of variance component, applying an unbalanced generalized linear mixed model:

$$y_{ijkl} = \mu + f_i + I_{(f)ij} + b_k + \varepsilon_{ijkl}$$

where y_{ijkl} is an observation of the trait for a repeat in the individual tree j of family i in the block k, and environment l, μ is the overall mean, f_i represents random family effects, $I_{(f)ij}$ is the random effect of individual j nested in family i, b_k is the (fixed) block effect, and ε_{ijkl} is the random residual error. In the case of HEI and DAB, where a single observation is obtained from each individual tree, the component $I_{(f)ij}$ is absent as it actually represent the residual error. Variance components were estimated by restricted maximum likelihood; this is a relatively flexible method in which within-group errors are allowed to be correlated and/or have unequal variances (Pinheiro et al. 2011). The package used for all traits assumes a Gaussian distribution, using the identity link function.

For a half design, heritabilities were estimated as:

$$h^2 = \frac{4\sigma_b^2}{\left(\sigma_b^2 + \sigma_w^2\right)}$$

where ${\sigma_b}^2$ denotes the estimated variance between families and ${\sigma_w}^2$ is the within-family variance component. Heritabilities were considered significant whenever the ANOVA yielded significant differences among families. Significance was corrected for multiple comparisons by the method of Benjamini and Hochberg (1995).

In order to evaluate variance distribution at different regional levels and to estimate Q_{ST} statistics for all traits, we also applied a model that takes into account the effect of provenance, sampling site within provenance, and family within sampling site as follows:

$$y_{ijkl} = \mu + p_i + s_{(p)ij} + f(ps)ijk + \varepsilon_{ijkl}$$

Where y_{ijkl} is an observation of the trait for an individual tree belonging to the family k, sampled in the site j, belonging to the provenance i, in the environment l, and μ is the overall mean; p_i represents random provenance effects, $s(p)_{ij}$ is the random sampling site nested within provenance effect, $f(ps)_{ijk}$ is the family nested within sampling site effect, and ε_{ijkl} is the random residual error. Variance components as well as their confidence intervals were estimated using the package *nlme* (Pinheiro et al. 2011) of the software R (ver. 2.13.1) (R Development Core Team 2011).

The coefficient Q_{ST} for each trait was estimated assuming a half-sib model as:

$$Q_{ST} = \frac{\sigma_{ST}^2}{8\sigma_f^2 + \sigma_{ST}^2}$$

where ${\sigma_{ST}}^2$ is the variance between sampling sites, equivalent to ${\sigma_{GB}}^2$ and ${\sigma_f}^2$ is the variance between families, which is equivalent to 1/4 ${\sigma_{GW}}^2$.

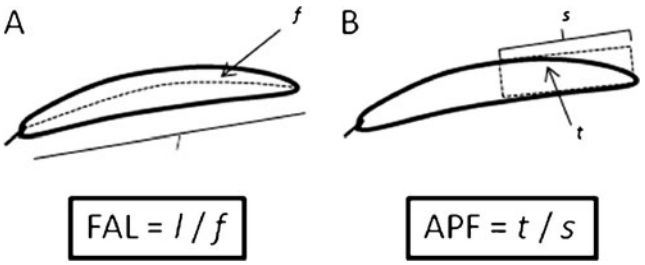

Fig. 3 Description of measurements to estimate **a** leaflet falcate and **b** leaflet apex for *P. flexuosa. l:* distance from the base to the tip of the leaflet, *f* : length from the base to the tip of the leaflet following a curved line running along the middle of the leaflet. *t:* area of the upper leaflet third; *s*: area of a rectangle with the same dimensions as *t*

This coefficient was partitioned into two components: among provenances (Q_{PT}) and among sampling sites within provenances (Q_{SP}), according to the expressions:

$$Q_{PT} = \frac{\sigma^2_{PT}}{8\sigma^2_f + \sigma^2_{SP} + \sigma^2_{PT}}$$

$$Q_{SP} = \frac{\sigma^2_{SP}}{8\sigma^2_f + \sigma^2_{SP}}$$

where $\sigma_{SP}{}^2$ is the variance between sampling sites within provenances and $\sigma_{PT}{}^2$ is the variance among provenances. Standard errors of these coefficients were estimated from 1,000 pseudoreplicates obtained by bootstrapping the original dataset using the command *sample* of R.

Molecular markers

Genetic diversity was quantified by the percent of polymorphic loci (*P*), unbiased expected heterozygosity (*H*), and effective number of alleles per locus (*ne*) at three levels: global (whole sample), within provenances, and within sampling sites. *H* was estimated by a Bayesian method with uniform prior distribution of allele frequencies (Zhivotovsky 1999) using the software AFLPsurv (Vekemans 2002). Effective number of alleles was calculated from averaged *H* values according to the expression $ne = 1/(1-H)$ (Hedrick 2000).

The distribution of molecular variance at different hierarchical levels (provenance, sampling sites within provenance, and individuals within sampling site) was evaluated by two methods. The first one was a hierarchical *F* statistics analysis conducted with the package *hierfstat* (Goudet 2005) of the software R (R Development Core Team 2011). The second method was analysis of molecular variance (AMOVA Φ statistics) (Excoffier et al. 1992) conducted with the package *ade4* (Chessel et al. 2004; Dray and Dufour 2007; Dray et al. 2007) of R. In both cases, the among-sampling site differentiation (F_{ST} and Φ_{ST}) was partitioned into among-sampling-sites within provenances (F_{SP} and Φ_{SP}) and among provenances with respect to total population (F_{PT} and Φ_{PT}) components.

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

Table 2 Basic statistics, components of phenotypic variance, and heritability estimates in *P. flexuosa*

Trait (unit)	Acronym	Mean (SD)	Variance components			h^2	*P* value	*P* adj
			Family	Individual	Repeat			
Height (m)	HEI	3.25 (1.29)	0.01	1.56	–	2.75×10^{-2} (7.30×10^{-7}–1)	0.41	0.45
Basal diameter (cm)	TDI	17.15 (9.70)	1.02×10^{-6}	92.50	–	4.43×10^{-8} (2.2×10^{-52}–1)	0.51	0.54
Petiole length (mm)	PEL	21.75 (12.86)	8.39×10^{-6}	60.01	103.90	2.05×10^{-7} ($5,81\times10^{-39}$–4)	0.63	0.65
Pair of leaflets/Pinna	NLP	17.56 (6.70)	0.49	11.42	32.95	0.04 (6.77 10^{-3}–0.27)	0.21	0.25
Pinna length (mm)	PIL	69.62 (23.51)	10.14	276.77	255.068	0.07 (6.92×10^{-3}–0.69)	0.14	0.19
Spine length (cm)	SPL	12.41 (12.93)	1.08×10^{-5}	94.66	73.15	2.58×10^{-7} (8.31×10^{-15}–4)	0.86	0.87
Number of pinnae	NPI	1.26 (0.50)	0.09	0.38	0.32	0.14* (0.03–0.58)	0.03	0.04
Leaflet length (cm)	LEL	0.79 (0.42)	8.76×10^{-3}	0.14	0.025	0.20* (5.30×10^{-2}–0.69)	0.03	0.05
Leaflet width (cm)	LEW	0.13 (0.06)	2.46×10^{-4}	3.18×10^{-3}	7.46×10^{-4}	0.23* (0.07–0.65)	0.02	0.04
Leaflet length/width	LEL/LEW	5.90 (1.81)	0.133	2.22	0.89	0.16 (0.04–0.52)	0.07	0.10
Leaflet falcate	LEF	0.93 (0.06)	3.80×10^{-4}	9.81×10^{-4}	2.27×10^{-3}	0.41** (0.28–0.60)	1.35×10^{-10}	4.20×10^{-10}
Leaflet area (cm^2)	LEA	0.10 (0.15)	9.77×10^{-4}	0.02	3.36×10^{-3}	0.16 (0.03–0.71)	0.07	0.11
Leaflet apex	LEX	0.95 (0.21)	5.04×10^{-4}	3.40×10^{-3}	4.09×10^{-2}	0.04** (0.01–0.10)	2.83×10^{-3}	5.90×10^{-3}
Leaflet apex/total area	LEX/LEA	0.21 (0.04)	2.24×10^{-5}	1.35×10^{-4}	1.88×10^{-3}	0.04** (0.02–0.09)	1.41×10^{-3}	3.30×10^{-3}

Confidences intervals (95 %) are indicated in parentheses

P and *P adj* significance of heritability estimates before and after correction for multiple tests

Table 3 Analysis of variance components of 14 quantitative traits of *P. flexuosa*

Trait	Variance components (%)						Total Var
	S (Tot)	*P* value	*S* within *P*	Fam within S	Ind within Fam	Within Ind	
HEI	3.94	2.59	1.34	7.08×10^{-7}	96.06	–	1.57
TDI	0.72	0.72	1.04×10^{-7}	7.18×10^{-7}	99.28	–	92.56
PEL	0.59	0.59	0.00	2.05×10^{-7}	36.02	63.39	163.90
NLP	1.46×10^{-6}	2.00×10^{-7}	0.00	1.10	25.45	73.44	44.87
PIL	2.36	0.40	1.96	3.97×10^{-5}	50.68	46.96	543.20
SPL	1.50	0.68	0.81	2.81×10^{-6}	54.83	43.67	167.51
NPI	0.95	0.95	0.00	2.65	56.65	39.75	0.26
LEL	3.09	0.00	3.09	2.29	79.80	14.82	0.17
LEW	1.02	0.00	1.02	4.96	76.14	17.88	4.17×10^{-3}
LEL/LEW	4.03	3.13	0.90	0.31	68.27	27.40	3.25
LEF	10.19	8.89	1.29	6.60×10^{-6}	26.47	63.35	3.59×10^{-3}
LEA	1.66×10^{-4}	9.33×10^{-6}	0.00	4.12	81.72	14.16	0.02
LEX	1.45	0.94	0.50	0.00	7.41	91.14	0.04
LEX/LEA	1.24	0.21	1.03	0.00	6.49	92.27	2.03×10^{-3}
Average	2.22	1.37	0.85	1.10	54.66	40.48	

S sampling sites, *P* provenances, *Fam* families, *Ind* individuals, *Total Var* total variance

expectation under neutrality, that is:

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

MANOVA was conducted with the software R (R Development Core Team 2011), 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.

Results

Quantitative trait analysis

The analysis of variance components of quantitative traits considering the variance among and within families (Table 2) showed that, out of 14 traits, three exhibit highly significant heritability ($P< 0.01$) (LEF, LEX, and LEX/LEA) and other three (NPI, LEL, LEW) have significant ($P< 0.05$) heritability. When significance was adjusted for multiple comparisons, the significance level remains the same for five of these traits: NPI, LEW, LEF, LEX, and LEX/LEA (Table 2). All these traits are related with leaflet size and shape. By contrast, neither of the life history traits analyzed showed significant heritability.

Table 4 Coefficients for population structure from quantitatives traits

Trait	Q_{ST}	Q_{SP}	Q_{PT}	Q_{ST}–F_{ST} (AMOVA)
HEI	1.00 (2.1×10^{-3})	1.00 (2.0×10^{-3})	0.66 (9.0×10^{-4})	0.930
TDI	1.00 (3.5×10^{-3})	0.02 (3.2×10^{-3})	1.00 (1.3×10^{-3})	0.930
PEL	1.00 (3.2×10^{-3})	0.74 (0.01)	1.00 (6.2×10^{-3})	0.930
NLP	1.65×10^{-7} (6.2×10^{-3})	1.43×10^{-7} (5.6×10^{-3})	2.26×10^{-8} (2.4×10^{-3})	−0.070
PIL	1.00 (0.01)	1.00 (0.01)	0.17 (6.1×10^{-3})	0.930
SPL	1.00 (6.2×10^{-6})	1.00 (6.2×10^{-4})	0.46 (7.9×10^{-3})	0.930
NPI	0.04 (2.6×10^{-3})	0.00 (1.0×10^{-3})	0.04 (2.5×10^{-3})	−0.030
LEL	0.14 (0.002)	0.14 (0.002)	4.80×10^{-7} (1.8×10^{-8})	0.070
LEW	0.03 (5.6×10^{-4})	0.03 (5.6×10^{-4})	1.70×10^{-7} (5.6×10^{-4})	−0.040
LEL/LEW	0.62 (0.02)	0.27 (0.04)	0.48 (0.02)	0.550
LEF	1.00 (2.4×10^{-7})	1.00 (1.9×10^{-6})	0.87 (2.8×10^{-3})	0.930
LEA	5.03×10^{-6} (2.4×10^{-4})	4.74×10^{-6} (2.4×10^{-4})	2.83×10^{-7} (1.8×10^{-7})	−0.070
LEX	1.00 (0.01)	1.00 (0.02)	0.65 (0.02)	0.930
LEX/LEA	1.00 (0.02)	1.00 (0.02)	0.17 (0.02)	0.930

Q_{ST} among sampling sites, Q_{SR} among sampling sites within provenances, Q_{PT} among provenances

Standard errors (SE) in parenthesis. * $0.01\leq P< 0.05$; ** $P< 0.01$

When the analysis of variance component considered regional levels (Table 3), the highest components correspond to among-individuals and residual effects. Wide variation was observed among traits in the proportion of variance represented at provenance (ranging from 2×10^{-7} to 9 %), sampling site (ranging from 10^{-7} to 3 %), and family (ranging from 7×10^{-7} to 5 %) levels. The highest proportion of variance among all sampling sites (10 %) and among provenances (9 %) corresponded to LEF, whereas the minimum values for the same components were recorded for NLP (1.5×10^{-6}, and 2×10^{-7}%, respectively). For among-sites within provenance, the maximum and minimum values corresponded respectively to LEL (3.1 %) and TDI (10^{-7}%).

The different traits showed different behaviors in reference to the relative Q coefficient estimates (Table 4, columns 1–3). For eight traits, Q_{ST} was virtually equal to 1, with very low standard error, indicating strong differentiation among sampling sites. For six of these traits (HEI, PIL, SPL, LEF, LEX, and LEX/LEA), the differentiation among sampling sites within provenances (Q_{SP}) was also close to 1 with low standard errors. By contrast, for the two remaining traits (TDI and PEL), the main source of differentiation among sampling sites is the provenance component, as Q_{PT} is close to 1 (with standard errors<0.01). For all components, LEL and LEW showed moderate to low Q coefficient values, however, the ratio LEL/LEW exhibit high Q_{ST} estimate (0.62 +/− 0.04), with the highest component being among provenances (Q_{PT}= 0.48 +/− 0.04). This result suggests that the leaflet shape, rather than size, shows among-site significant differentiation. Finally, NPI also showed low but significant heterogeneity among provenances (Q_{ST}=0.04 +/− 0.005).

Molecular diversity

Of 2,415 pairwise combinations of 70 loci, none showed significant or highly significant gametic disequilibrium. Molecular diversity was estimated at global, average within provenance, and average within-sampling-site levels (Table 5). Estimated values are intermediate between those recorded for isozymal and microsatellite loci used in Q_{ST}–F_{ST} comparisons (see Fig. 3 in Edelaar et al. 2011). Polymorphism within sampling sites is reduced to about 40 % in comparison with global estimates, whereas heterozygosity decreases about 25 %. By contrast, diversity within provenances is quite similar to global values, indicating low genetic differentiation among provenances.

Table 5 Molecular diversity estimations indicated as percentage of polymorphic loci (*P*), unbiased heterosigosity (*He*), and effective number of alleles (*ne*)

	P	*He*	*ne*
Global	98.6 (0.014)	0.437 (0.015)	1.78
Within provenance	98.07 (0.016)	0.413 (0.012)	1.70
Within sampling site	60.28 (0.058)	0.329 (0.007)	1.49

Standard deviations are indicated in parenthesis

The distribution of molecular variance at different hierarchical levels was measured by AMOVA and hierarchical F statistics. The variance among sampling sites (σ_{ST}^2) was partitioned into the differentiation among sampling sites within provenances (σ_{SP}^2) and among provenances (σ_{PT}^2). The variance among sampling sites within provenances (SP) was higher than the among provenance (PT) component (Table 6). The Φ and F values were consistent showing highly significant Φ_{SP} and F_{SP} estimates.

It might be argued that the presence of related individuals (half-sibs) in the sample analyzed produced a bias in Φ and F estimates. However, considering the family structure in the orchard, each individual in average is related with only three of the remaining 256 studied individuals (that is about 1 %). If only one individual per family is included in the analysis, the trend observed after pruning the data was the same as that described for the whole sample (data not shown).

Neutrality tests for diversity of morphometric traits

Estimates of quantitative genetic divergence of quantitative traits (Q_{ST}) were compared with that obtained from molecular markers (Table 4, column 4). The differences shown in Table 4 are based on Φ_{ST} of AMOVA, as this estimate takes into account quantitative differences among band patterns and may be considered an appropriate quantitative measure of relative neutral genetic divergence among populations with which to compare Q_{ST} (Edelaar et al. 2011). In both life history traits (HEI and TDI), 8 out of 11 foliar traits and SPL showed estimated Q_{ST}–F_{ST}>0 values, suggesting the occurrence of divergent selection. By contrast, for the remaining four leaf traits, Q_{ST}–F_{ST}<0, compatible with stabilizing selection.

The comparison of phenotypic with molecular divergence measured by the rho (ρ) coefficient (Table 7)

Table 6 Hierarchical analysis of population genetic structure estimated by AMOVA and *F* coefficients

	AMOVA		*F* hierarchical	
	Var (%)	Φ	Var (%)	F
Among all sampling sites (ST)	6.74	0.07	11.86	0.12
Among sampling sites within provenances (SP)	5.07	0.05 (5×10^{-4})	7.47	0.08 (1.5×10^{-3})
Among provenances (PT)	1.67	0.02 (0.03)	4.39	0.04 (0.28)
Within sampling sites	93.26	–	88.14	–

P values of Φ and F coefficients are indicated in parentheses

Table 7 Proportionality multivariate and molecular rho coefficients (ρ) among sampling sites, among provenances, and among sampling site within provenances

	Df	ρ molecular	ρ multivariate
One-level test			
Among sample site (uncorrected for provenance)	39	0.21 (0.18–0.24)	0.28 (0.24–0.34)
Two-level hierarchical test			
Among provenances	13	0.08 (0.05–0.12)	22 (18–30)
Among sampling sites within provenance	26	0.14 (0.10–0.19)	0.22 (0.19–0.27)

Confidence intervals (95 %) are indicated in parentheses

Df degrees of freedom

indicated significant departures of quantitative traits from the expectation for a neutral model. In all cases, multivariate ρ estimates for quantitative traits were higher than ρ estimate for molecular markers, and confidence intervals of ρ estimates from the two datasets show virtually no overlapping (the overlapping is reduced to a single point between the highest possible value of molecular estimated ρ and the lowest multivariate ρ CI in rows 1 and 3 of Table 7). The most remarkable difference between molecular and multivariate ρ estimates is observed for relative contribution of the among-provenance and among-sampling-sites within provenances. The highest component for molecular dataset estimates is among sampling sites, whereas, by far, the highest component corresponds to among provenance (100 times bigger than the among-site component). The comparisons between G and D matrices rejected the hypothesis of proportionality for all levels, among all sampling sites (ρ=0.28, $P=3\times10^{-5}$), among provenances (ρ=22, $P\approx0$), and among sampling within provenances (ρ=0.22, $P=4\times10^{-6}$)

Discussion

The success of management programs oriented to conservation and improvement of multipurpose species such as *P. flexuosa* 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 genetic basis of phenotypic variation is usually described by means of variance component analysis to decompose the total phenotypic variance into genetic and non-genetic (environmental) components (Lynch and Walsh 1998). For the multivariate quantitative phenotype, the equivalent to the genetic variance is the genetic covariance matrix G, whereas the multivariate equivalent to the total 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 specific 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) (Mc Guigan 2006). However, a limitation in many studies when estimating G is the occurrence of related individuals in common environments. The consequence is an overestimation of G because it will include environmental covariance components. When common environmental effects are prevalent, they can substantially inflate G. But on the other hand, it is not clear how G can be accurately estimated in wild population where environment cannot be manipulated (Kruuk 2004).

In the present study, we took advantage of the availability of an experimental orchard where possible genotype–environment association is eliminated within the stand by means of a block design. Family arrays in all cases include individuals grown in different blocks in order to account for possible genotype–environment interaction effects. This strategy allows obtaining of good estimates of genetic components of phenotypic variance both in univariate (V_A) and multivariate analyses (G matrix).

Univariate analyses indicated that five out of 14 traits exhibited significant heritability after correction for multiple tests. The traits with highest heritability values are those related to leaflet size and shape, whereas traits as height and basal diameter showed non-significant h^2 values. These results are consistent with the general view that life history traits tend to have lower heritability than morphological traits (Trevor and Schluter 1991; Falconer and Mackay 1989). The estimates obtained in this study were much lower than those recorded by Bessega et al. (2009, 2010) in an experimental orchard of *Prosopis alba*, a species of the same section and closely related to *P. flexuosa*. Heritability estimates in *P. alba* were, in many cases, higher than 1, what was partially explained by the authors (Bessega et al. 2009, 2010) by the possible occurrence of selves and full sibs within family groups assumed as half-sib families. Thus, difference in the rate of inbreeding and the number of pollen donors per mother plant (Bessega et al. 2000a, 2011) may affect the estimate of heritability. Furthermore, different adaptive strategies between *P. alba* and *P. flexuosa* cannot be ruled out as they are adapted to different eco-regions.

Quantitative and molecular tools have been coupled, increasing the scope of relative rates to infer the action of drift or selection (Merilä and Crnokrak 2001). The comparison of patterns of genetic variation distribution between neutral traits (usually represented by molecular markers) and quantitative traits contributes to evaluate possible

adaptive value of phenotypic variation. Specifically, the combination of quantitative and molecular tools and the comparison of the relative estimates of population structure parameters provide a test to infer the action of drift and selection (Q_{ST} vs. F_{ST}). The Q_{ST}–F_{ST} comparison has become an increasingly common method for inferring adaptive quantitative trait divergence among populations (Chenoweth and Blows 2008).

Q_{ST} is best measured in a randomized "common garden" experimental design (as the one studied in this paper) because the effect on the trait of environmental differences among populations is precluded, allowing to estimate the among population proportion of the total additive genetic variance of quantitative traits. The appropriate design proportions the within-population variance and allows removing non-additive effects (Sæther et al. 2007).

The analysis of variance components considering among and within sampling site phenotypic variance showed, in average, high differentiation among sampling sites, with a Q_{ST} averaged over traits of 0.63. This value was much higher than the genetic differentiation at the same level as evaluated by Φ_{ST} (0.07) or F_{ST} (0.12). A high heterogeneity in Q_{ST} estimates were observed among traits, ranging from almost 0 to 1.

Our results are consistent with those reviewed by Hamrick (2004) in other forest species. The comparison of F_{ST} and Q_{ST} in six tree species of different genera showed that, in all cases, average Q_{ST} was higher than F_{ST}. Furthermore, high variation among species were observed in Q_{ST} estimates, ranging from 0.11 to 0.49, contrasting with F_{ST} estimates which were less variable and ranged only from 0.02 to 0.09.

The different behaviors of phenotypic traits and molecular markers may be discussed in the light of possible selective pressure differences over each of them (Merilä and Crnokrak 2001; Stean et al. 2006; Lopez-Fanjul et al. 2007). Hamrick (2004) observed that, in the white fir (*Abies concolor*), growth-related traits have in average higher Q_{ST} values (0.37) than needle characteristics (0.08) and claimed that these results would be the consequence of higher selection pressures acting on growth traits. In our case, growth traits, five leaf traits, and spine length exhibit $Q_{ST}>F_{ST}$, a result interpreted as evidence of diversifying selection on those traits. For the remaining four leaf traits, $Q_{ST}<F_{ST}$, suggesting stabilizing selection (against phenotypic divergence). According to the present results, none of the traits analyzed seems to be selectively neutral.

The hierarchical analysis of variance components considering regional levels indicated that the relative levels of variation among-provenance and among-sampling-sites within provenance differed among traits, a fact evidenced by the relative estimates of Q_{PT} and Q_{SP}. This result suggests that selective mechanisms might be operating in different ways on different traits. The difference between Q_{PT} and Q_{SP} does not follow any clear pattern to be associated with the nature of the trait considered. Univariate analyses are likely to paint a simplified scenario of adaptive divergence, and the power of the test with single traits Q_{ST} is very low with the sampling designs typically possible in empirical studies (O'Hara and Merilä 2005; Martin et al. 2008).

Most Q_{ST}–F_{ST} comparisons use mean Q_{ST} values among a set of quantitative traits, which are compared to F_{ST} estimates from several marker loci (Chapuis et al. 2008). The confidence intervals of averaged Q_{ST} are reduced, but information on individual traits is lost, and this method implicitly assumes independence among traits, what usually is not tenable (Chapuis et al. 2008; Martin et al. 2008).

A multivariate analysis provides a more accurate picture of the impact of selection versus drift on the system (Martin et al. 2008; Chapuis et al. 2008). Recently, Ovaskainen et al. (2011) proposed a new promising approach based on the application of the animal model to a population level for which the software is not yet implemented for public access. It accounts for the inherence randomness in the evolutionary process, and in the multivariate case, it uses the information of all traits without an averaging procedure. It also accounts for population-to-population level coancestry coefficients and similarity in trait values omitting the averaging procedure to compute F_{ST} and Q_{ST}, respectively. The multivariate test here applied, although ignores the process error and does not account for the full matrix structure in a single test, avoids the problem of comparing a matrix (Q_{ST}) with a scalar (F_{ST}), and it is publicly available by means of an R language script.

One important methodological constraint when analyzing a set of quantitative traits is the need of an appropriate scaling to interpret G matrix (Chapuis et al. 2008). In the present study, we scaled the dataset to the trait mean and transformed the data to get normal distribution. Another methodological implication is the choice of biologically coherent sets of traits to provide meaningful interpretations. We tried to do that by including growth traits, foliar traits, and spine size.

The multivariate statistical dual test (Chapuis et al. 2008; Martin et al. 2008) applied confirmed the observed trend in the univariate comparisons. The first test showed that the multivariate ρ estimate was significantly higher than the molecular-based ρ estimate, both without and with correction considering hierarchical population structure. This result indicates that multivariate Q_{ST} is higher than F_{ST}, supporting the hypothesis of selection for different optima among populations (Merilä and Crnokrak 2001). A remarkable fact is that multivariate ρ is much higher for the provenance level than both multivariate ρ estimated for sampling site level and the corresponding molecular-based ρ estimate. This result indicates a strong divergent selection at

provenance level. Consistently, the proportionality of the matrices *G* and *D* (second test) is strongly rejected both without and with correction considering hierarchical population structure. This result confirms that different selective regime is acting on different traits and that selection favors different optima in each sampling site. The selection to different optima is much stronger among provenances.

Our result is consistent with several studies on morphological variation of quantitative traits in *P. flexuosa* that have demonstrated significant differentiation among provenances (Cony 1996; Brizuela et al. 2000; Mantován 2002). Consistency between results obtained from field observations and measurements on experimental orchards supports the genetic basis of at least part of the morphological variation observed among geographical regions (Brizuela et al. 2000). In order to understand the forces that determine such differences and develop programs for rational use, improvement, and conservation, the parallel analysis of morphological and molecular genetic structure is very important. The present study demonstrates the occurrence of heterogeneous selection on different traits and suggests local adaptation of quantitative traits. This result contrasts with the relatively lower genetic diversity among provenances in comparison with the local diversity within provenances detected by neutral molecular markers. This information contributes to explain the possible causes for the regional variation observed in *P. flexuosa* and suggests similar trends in other related species of *Prosopis* where different morphological ecotypes have been described (Morello et al. 1971; Burghardt et al. 2004; Verga et al. 2009).

The parallel analysis of molecular and quantitative divergence is meaningful to conservation programs to properly define MUs and ESUs. According to Moritz (1994), MUs are recognized as populations with significant divergence of allele frequencies at nuclear or mitochondrial (or chloroplast in plants) loci, regardless of the phylogenetic distinctiveness of the alleles. According to this definition, the sampling sites within provenances analyzed in this paper correspond to MUs, as they show the highest contribution to molecular variance components. Univariate analysis of quantitative trial divergence showed high differentiation among sampling sites, but the highest component of variance differed among traits. The multivariate analysis of quantitative traits indicated that the highest differentiation component of variance corresponded to provenances, although the contribution of sampling sites within provenance was also significant. These results indicate that molecular markers might underestimate the actual regional genetic divergence. The joint information from both datasets suggests that many sampling sites from several regions (provenances) along the entire range are required to avoid variability loss in proper conservation programs. With respect to ESUs, they are defined as a historically isolated set of populations that leads to a qualitative criterion based on the distribution of alleles in relation to their phylogeny (Moritz 1994). ESUs will usually complement rather than replace "species" defined under traditional, predominantly morphological criteria. In the case of *P. flexuosa* and other related species of section Algarobia, interspecific hybridization events are frequent, and the group of hybridizing species has been largely referred as a syngameon (Palacios and Bravo 1981). However, more recent molecular studies have supported the biological meaning of the specific limits within this group (Saidman and Vilardi 1987; Bessega et al. 2000b) and indicate the possible existence of isolating mechanisms that restrict the introgression among its species.

Therefore, the entities involved in this syngameon might be considered species under the Templeton (1989) cohesive concept assuming demographically and/or ecologically rather than genetically cohesive mechanisms (Saidman et al 1998a, b). According to these, *P. flexuosa*, as defined under morphological criteria, should be considered the evolutionarily significant units.

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Ethical standards In order to fulfill the objectives proposed, the experiments conducted comply with the current Argentine laws, and all the trials were made without disturbing the natural ecosystem taking into consideration the current legislation in the country. For the experimental laboratory practice, done at *Facultad de Ciencias Exactas y Naturales* (Universidad de Buenos Aires), we followed the recommendations from *Servicio de Higiene y Seguridad* (SHyS, FCEyN, UBA).

Conflict of interest The authors declare that they have no conflict of interests.

References

Acosta M, Oliva L, Abril A (1994) Colección de Rhizobios de *Prosopis* arbóreos en la zona semiárida de la provincia de Córdoba (Argentina). Ciencia del Suelo 12:38–40

Alvarez JA, Villagra PE (2009) *Prosopis flexuosa* D.C (Fabeaceae, mimosoideade). Kurtziana 35(1):47–61

Bassam BJ, Caetano-Anollés G, Greshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:81–84

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300

Bessega C, Ferreyra L, Julio N, Montoya S, Saidman BO, Vilardi JC (2000a) Mating system parameters in species of genus *Prosopis* (Leguminosae). Hereditas 132(1):19–27

Bessega C, Ferreyra L, Vilardi JC, Saidman BO (2000b) Unexpected low genetic differentiation among allopatric species of section Algarobia of *Prosopis* (Leguminosae). Genetica 109:255–266

Bessega C, Saidman BO, Darquier MR, Ewens M, Sánchez L, Rozenberg P, Vilardi JC (2009) Consistency between marker

and genealogy-based heritability estimates in an experimental stand of *Prosopis alba* (Leguminosae). Am J Bot 96:458–465

Bessega C, Saidman BO, Darquier MR, Ewens M, Felker P, Vilardi JC (2010) Accuracy of dominant markers for estimation of relatedness and heritability in an experimental stand of *Prosopis alba* (Leguminosae). Tree Genet Genome 7:103–115

Bessega C, Pometti CL, Ewens M, Saidman BO, Vilardi JC (2011) Strategies for conservation for disturbed *Prosopis alba* (Leguminosae, Mimosoidae) forests based on mating system and pollen dispersal parameters. Tree Genet Genome. doi:10.1007/s11295-011-0439-6

Black WC IV (1996) Fortran programs for the analysis of RAPD-PCR markers in populations. Colorado State University, Ft. Collins, CO

Box GEP, Cox DR (1964) An analysis of transformations (with discussion). J R Stat Soc Ser B 26:211–252

Brizuela MM, Burghardt AD, Tanoni D, Palacios RA (2000) Estudio de la variación morfológica de *Prosopis flexuosa* en tres procedencias y su manifestación en cultivos bajo condiciones uniformes. Multequina 9:07–15

Burghardt AD, Espert SM, Braun-Wilke SH (2004) Variabilidad genética en *Prosopis ferox* (Mimosaceae). Darviniana 42(1–4):31–36

Burkart A (1976) A monograph of the genus *Prosopis*. J Arn Arb 57: 3–4

Burkart R, BarbaroN, Sánchez RO, Gómez DA (1999) Ecorregiones de la Argentina. Administración de Parques Nacionales PRODIA

Chapuis E, Martin G, Goudet J (2008) Effects of selection and drift on *G* matrix evolution in a heterogeneous environment: a multivariate Q_{st}–F_{st} test with the freshwater snail *Galba truncatula*. Genetics 180:2151–2161

Chenoweth SF, Blows MW (2008) *Qst* meets the *G* matrix: the dimensionality of adaptive divergence in multiple correlated quantitative traits. Evolution 62:1437–1449

Chessel D, Dufour AB, Thioulouse J (2004) The ade4 package-I—one-table methods. R News 4:5–10

Cony MA (1996) Genetic variability in *Prosopis flexuosa* D.C. a native tree of Monte phytogeography province, Argentina. For Ecol Manage 87:41–49

Cony MA (1993) Programa de consetvaci6n y mejoramiento de especies de1 genera Prosopis en la Provincia Fitogeografica del Monte, Argentina. In: Contibuciones Mendocinas a la Quinta Reunion Regional para America Latina y el Caribe, de la Red de Forestaci0n de1 CIID. Unidades de Botanica y Fisiologia Vegetal, IADIZA, pp. 37–72

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria. ISBN 3-900051-07-0. Available from: http://www.R-project.org

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. J Stat Softw 22(4):1–20

Dray S, Dufour AB, Chessel D (2007) The ade4 package-II: two-table and K-table methods. R News 7(2):47–52

Edelaar P, Burraco P, Gomez-Mestre I (2011) Comparison between Q_{ST} and F_{ST}—how wrong have we been? Mol Ecol 2:4830–4839

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Falconer DS, Mackay TFC (1989) Introduction to quantitative genetics, 3rd edn. Longman, Harlow, UK

FAO, FLD, IPGRI (2004) Forest genetic resources conservation and management vol. 1: overview, concepts and some systematic approaches. International Plant Genetic Resources Institute, Rome, Italy

Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical F statistics. Mol Ecol Notes 5:184–186. http://www.unil.ch/popgen/softwares/hierfstat.htm

Hamrick JL (2004) Response of forest trees to global environmental changes. For Ecol Manage 197:323–335

Hartl DL, Clark AG (1989) Principles of population genetics. Sinauer, Sunderland, MA

Hedrick PW (2000) Genetics of populations (2nd edn). Jones and Bartlett Publishers, Sudbury, MA

Kimura M (1983) The neutral theory of molecular evolution. Evolution of Genes and Proteins Sinauer, Sunderland, MA

Kruuk LEB (2004) Estimating genetic parameters in natural populations using the 'animal model'. Philos Trans R Soc Lond B Biol Sci 359:873–890

Lande R (1992) Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. Evolution 46:381–389

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Leinonen T, O'Hara RB, Cano JM, Merilä J (2008) Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. J Evol Biol 21:1–17

Lopez-Fanjul C, Fernandez A, Toro MA (2007) The effect of dominance on the use of the Q_{ST}–F_{ST} contrasts to detect natural selection on quantitative traits. Genetics 176:725–727

Lynch M, Walsh B (1998) Genetics and analyses of quantitative traits. Sinauer, Sunderland, MA

Mantován NG (2002) Early growth differentiation among *Prosopis flexuosa* D.C provenances from the Monte phytogeographic province, Argentina. New For 23:19–30

Martin G, Chapuis E, Goudet J (2008) Multivariate Q_{ST}–F_{ST} comparisons: a neutrality test for the evolution of the *G* matrix in structured populations. Genetics 180:2135–2149

Mc Guigan K (2006) Studying phenotypic evolution using multivariate quantitative genetics. Mol Ecol 15:883–896

Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. J Evol Biol 14:892–903

Morello J, Crudelli N, Saraceno M (1971) Los vinalares de Formosa, República Argentina. Serie Fitogeográfica Nº 11. INTA. Pp. 111

Moritz C (1994) Defining 'evolutionarily significant units' for conservation. Tree 9:373–375

Nagylaki T (1998) Fixation indices in subdivided populations. Genetics 148:1325–1332

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

O'Hara RB, Merilä J (2005) Bias and precision in QST estimates: problems and some solutions. Genetics 171:1331–1339

Otha T (1982) Linkage disequilibrium due to random genetic drift in finite subdivided populations. Proc Natl Acad Sci 79:1940–1944

Ovaskainen O, Karhunrn M, Zheng CH, Cano Arias JM, Merilä J (2011) A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. Genetics 189:621–632

Palacios RA, Bravo LD (1981) Hibridación natural en *Prosopis* (Leguminosae) en la Región Chaqueña Argentina. Evidencias morfológicas y cromatográficas. Darwiniana 23:3–35

Pinheiro J, Bates D, DebRoy S, Sarkar D, the R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models. R Package

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55(6):1095–1103

Rogers AR (1986) Population differences in quantitative characters as opposed to gene frequencies. Am Nat 127:729–730

Roig FA (1993) Aportes a la Etnobotánica del Género Prosopis. In: Unidades de Botánica y Fisiología vegetal, IADIZA (eds) Contribuciones Mendocinas a la quinta Reunión Regional para América Latina y el Caribe de la Red de Forestación del CIID. pp. 99–121

Sæther SA, Fiske P, Kalas JA, Kuresoo A, Luiguoe L, Piertney SA, Sahlaman T, Höglund J (2007) Inferring local adaptation from

Q_{ST}–F_{ST} comparisons: neutral genetic and quantitative trait variation in European populations of great snipe. J Evol Biol 20:1563–1576

Saidman BO, Vilardi JC (1987) Analysis of the genetic similarities among seven species of *Prosopis* (Leguminosae: Mimosoideae). Theoretical App Genet 75:109–116

Saidman BO, Vilardi JC, Pocovi MI, Acreche N (1996) Genetic divergence among species of the section *Strombocarpa* genus *Prosopis* (Leguminosae). J Genet 75:139–149

Saidman BO, Vilardi JC, Montoya S, Dieguez MJ, Hopp HE (1998a) Molecular markers: a tool for the understanding of the relationships among species of *Prosopis* (Leguminosae, Mimosoidae). In: Puri S (ed) Tree improvement: applied research and technology transfer. Science Publishers Inc, U. S. A, pp 311–324, Ch 21

Saidman BO, Bessega C, Ferreyra L, Vilardi JC (1998b) Random amplified polymorphic DNA (RAPDS) variation in hybrid swarms and pure populations of genus *Prosopis*. In: Bruns S, Mantell S, Tragärdh C, Viana AV (eds) Recent advances in biotechnology for tree conservation and management. International Foundation for Sciences, Stockholm, pp 122–134, ISBN: 91 85798 460

Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774

Spitze K (1993) Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135:367–374

Stean DA, Conod N, Jones RC, Vaillancourt RE, Potts BM (2006) A comparative analysis of population structure of forest tree, *Eucaliptus globules* (Myrtaceae), using microsatellite markers and quantitative traits. Tree Genet Genome 2:30–38

Templeton AR (1989) The meaning of species and speciation: a genetic perspective. In: Otte D, Endler JA (eds) Speciation and its consequences. Sinauer Associates Inc, Sunderland, Massachusetts, pp 3–27

Trevor P, Schluter D (1991) On the low heritability of life-history traits. Evolution 45(4):853–861

Vekemans X (2002) AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium

Venables WN, Ripley BD (2002) Modern applied statistics with S, Fourthth edn. Springer, New York, ISBN 0-387-95457-0

Verga A, Mottura M, Melchiorre M, Jouseau J, Carranza C, Ledesma M, Recalde D, Tomalino L, Mendoza S, Vega R (2005) El Proyecto Algarrobo del INTA IDIA XXI(8):195–200

Verga A, López Lauenstein D, López C, Navall M, Joseau J, Gómez C, Royo O, Degano W, Marcó M (2009) Caracterización morfológica de los algarrobos (*Prosopis sp.*) en las regiones fitogeográficas Chaqueña y Espinal norte de Argentina. Quebracho 17(1,2):31–40

Wright S (1951) The genetic structure of populations. Annu Eugenics 15:323–354

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. Mol Ecol 8(6):907–913

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183