

Fine-scale spatial genetic structure analysis in two Argentine populations of *Prosopis alba* (Mimosoideae) with different levels of ecological disturbance

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Abstract Spatial genetic structure (SGS) in plants is primarily determined by the interaction between pollen and seed dispersal, but it is strongly affected by both evolutionary and ecological processes. SGS studies in forest species also allow evaluating the consequences of human-mediated disturbance on pollen and seed movement and designing strategies of sustainable use of native forest resources. The present paper compares fine-scale SGS between two populations of *Prosopis alba*: Fernandez-Forres (FF) and Campo Duran (CD), based on the variation of 12 SSR markers. Populations show different history, management, and levels of disturbance. FF is highly disturbed and fragmented by agricultural activity and stockbreeding. Although no significant differences were observed in genetic variability parameters, highly significant genetic differentiation was detected with virtually no admixture between populations. SGS was positive and significant at short distances only in the non-disturbed population of CD. Accordingly, estimated neighborhood size and effective gene dispersal are higher in FF than in CD. This result might be explained by the higher incidence of livestock in seed dispersal and the patchy structure favoring longer pollen movement and artificial thinning

and selection in FF. The results are relevant to conservation and breeding programs, suggesting that the distance between seed trees to be sampled should be larger than 22 m, the estimated distance of significant SGS in *P. alba*.

Keywords Autocorrelation analysis · Isolation by distance · Neighborhood size · Pollen dispersal · Spatial genetic structure

Introduction

In plants, gene flow is primarily determined by pollen and seed dispersal, which affect allele distribution patterns (Bradshaw 1972; Levin and Kerster 1974). As the average dispersion distance is usually small compared to the area covered by plant populations, fine-scale spatial genetic structure (SGS) is detected in most plant populations (Vekeman and Hardy 2004; Lopez-Gallego and O’Neil 2010). Some tree species are extremely dependent on animals for pollination and seed dispersal, and these processes are strongly affected by anthropogenic effects on the landscape. Human-induced activities might produce habitat degradation and promote large impacts on the SGS of forest populations (Paffetti et al. 2012; Rajendra et al. 2014; Sjölund and Jump 2015).

In open-pollinated populations, the extent of SGS is the result of interaction between pollen and seed dispersal. If both are limited, this will result in inbreeding and an increase in SGS (Chybicki et al. 2011; Forti et al. 2014). When long-distance pollen movement occurs (as in wind pollen dispersal), SGS is determined by restricted seed dispersal, which might result in the grouping of siblings near to the maternal plant (Bittencourt and Sebbenn 2007; Moran and Clark 2012). Localized dispersal of half- and

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full-sibling progeny from singly sired fruits could result in strong local structuring of genetic diversity within populations (Pardini and Hamrick 2008). In contrast, even with short-distance pollen dispersal, wide and independent seed dispersal will ensure the distribution of genetic variation, leading to weak or near-random structuring (Geburek 1993; Schroeder et al. 2014). Other factors such as plant breeding system, life form, population density, successional status, and regeneration mode are also important in SGS determination (Vekeman and Hardy 2004; Premoli and Kitzberger 2005; Zeng et al. 2011).

The knowledge of the causes of SGS in tree species is helpful for conservation and management strategy design that ensure the maintenance of genetic diversity, especially in the face of rapid habitat degradation (Lowe et al. 2005). This is particularly relevant to the white mesquite *Prosopis alba* (Leguminosae, Mimosoideae), an ecologically and economically important species in arid and semi-arid regions of South America. This is a native forest tree widely distributed in Argentina, Uruguay, Paraguay, and from south of Bolivia to Peru. The wood exhibits high quality (Pometti et al. 2009), it is used for carpentry and floors, and the fruits are considered a good resource as forage and for human use (Roig 1993) resulting very valuable for regional economies in Argentina. It is considered the most important species of genus from an economic perspective. The widely spread distribution in a great variety of environments has been related to the occurrence of genetic differentiation that might be used in forest tree breeding programs (Salto 2011). Overexploitation and subsequent land conversion into crop plantations in some areas of the country have led to considerable loss of forest habitat. In *P. alba* insect pollination and endozoic seed dispersal are expected to produce localized distribution of family groups. An additional factor that contributes to SGS is the fact that about 64 % of the progenies from single fruits are full sibs (Bessegga et al. 2011) and tend to be moved by herbivores and deposited together in dung. The processes of seed and pollen dispersal in *P. alba* are strongly affected by anthropogenic activities. Understanding how species differ in their genetic structure is relevant to developing scientifically informed sustainable management and restoration strategies. In the last years, the application of SGS studies in forest tree species proved to be valuable as a tool to monitor indirectly pollen and seed movement in the wild (Born et al. 2008; Dick et al. 2008). Comparing SGS among sites may provide insights into the range of variation of gene flow within species.

Our study aims to characterize and compare fine-scale SGS in two populations of *P. alba* with different history, management, and levels of disturbance. The underlying hypothesis was that landscape differences between these populations should be reflected in population genetic and

dispersal parameters. As family structure is usually reduced by managing practices (Rajendra et al. 2014), our prediction was that homozygote excess and SGS should be reduced in the population with highest anthropogenic influence. The results are expected to contribute to conservation, domestication, and breeding strategies in this promissory resource.

Materials and methods

Sample locations and material collection

Two *P. alba* populations (“algarrobo blanco” and white mesquite) were sampled from different geographical regions of Argentina in December 2009 and January 2012, respectively (Fig. 1). The first population, Fernandez-Forres (FF), is located following road 34 near Santiago del Estero, from 27°54'53"S, 63°53'22"W to 27°50'46"S, 64°0'53"W. It is immersed into a complex agro-forest landscape where the region has become highly fragmented mainly by overexploitation and the expansion of the agricultural frontier. The area originally involved a very dense natural forest where dominant species were hardwood trees (*Schinopsis* sp., *P. alba*, *P. nigra*, *P. kuntzei*). The population extends on both sides of a road constructed in the 1960s. Along both sides of the road to a width of about 70 m all vegetation was cut down during the 1970s. After this period the population recovered from preexisting seeds or reintroductions favored by livestock drive. By the end of the 1980s most of the road sides were colonized by “algarrobos” and other shrubs. Since the 1990s, road sides have been managed and small shrubs and trees have been removed, keeping bigger and healthier trees. The sampled trees were adults with basal diameters at 20 cm above the ground (DAB) ranging from 0.35 to 2.55 m. Historical information supports the assumption that most of them are of about the same age (Bessegga et al. 2015).

The second sample, Campo Duran (CD) (Fig. 1), corresponds to a population constituted mainly by “algarrobo blanco” trees from an aboriginal community Chané located in north of Salta Province near Bolivia border (from 22°10'23"S, 63°42'59"W–22°10'57"S, 63°43'11"W–22°12'5"S, 63°38'7"W–22°11'8"S, 63°37'37"W). It is located in the ecotone between the Yungas and the Chaqueña Biogeographical Ecoregions. In this site, tree densities within the sampled area are variable. Some trees are located on both sides of roads. In these areas population is not managed, the density is relatively high, and younger trees coexist with older ones. Some other trees are present in semi-urban areas. Local people must have protected the best and old trees that are more dispersed and usually located in sites near houses, school, and habited areas. All

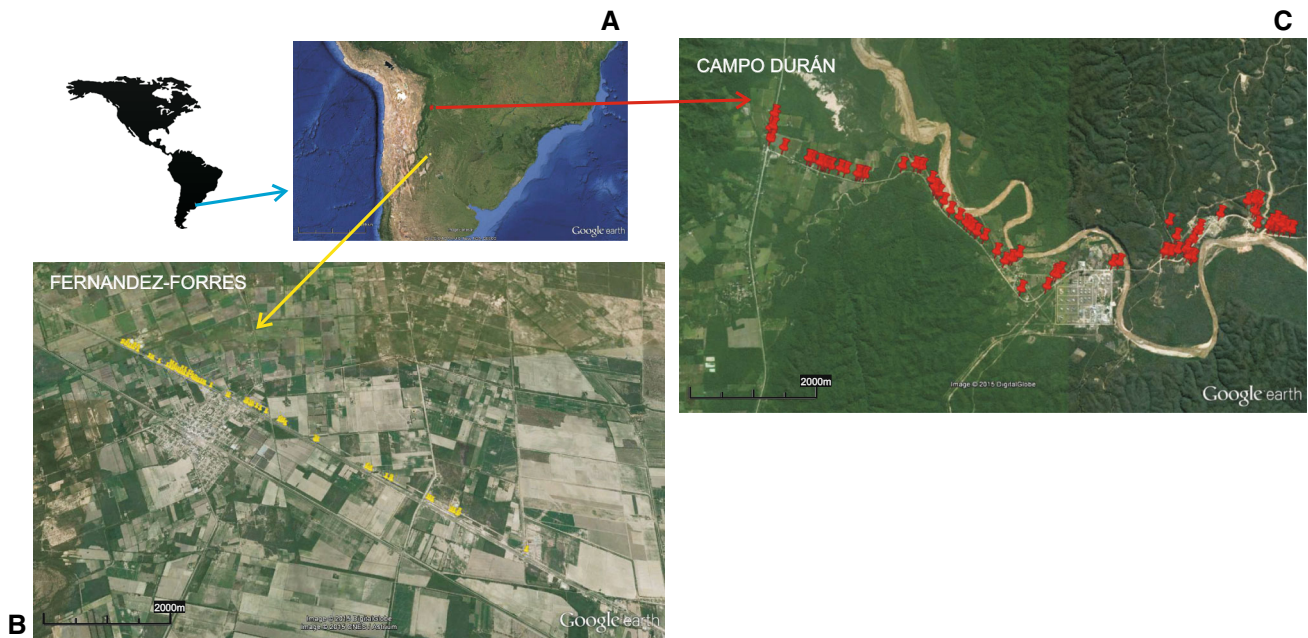


Fig. 1 Geographical location of the sampled populations of *Prosopis alba* in Argentina (A) and enlarged maps showing sampled trees in Fernández-Forres, Santiago del Estero (B), and in Campo Durán, Salta (C)

sampled trees were adult with DAB ranging from 0.6 to 4.80 m.

Previous studies had demonstrated that pollen and seed dispersal are limited in *P. alba* and it was recommended for conservation purposes and genetic variation estimation to sample trees separated more than 50 m from each other in order to avoid sampling of genetically related material (Bessega et al. 2000a, 2011). The application of the recommended strategy for *Prosopis* collection implied sampling 87 mother trees separated from each other by more than 50 m in Fernández-Forres and 96 individuals in Campo Durán. GPS coordinates were recorded for each sample tree in both populations. Although FF was sampled about 2 years before CD, no significant population genetics changes have occurred in FF during this period and the sampled trees from both populations are still on foot by now.

Microsatellite genotyping

Leaf material was collected and stored in silica gel until DNA extraction. Total genomic DNA was extracted using the Quiagen DNeasy Plant kit (Quiagen, Valencia, CA) following the manufacturer's instructions.

Both populations were characterized at 12 microsatellite markers including six described by Mottura et al. (2005) and six by Bessega et al. (2013). PCR amplifications are described in Bessega et al. (2009, 2015). PCR products were electrophoresed in an ABI313XL (HITACHI)

automated DNA sequencer and automatically sized using GENEMARKER version 1.91 (SoftGenetics LLCTM www.softGenetics.com).

In order to determine the power of the loci analyzed to discriminate between unique individuals, we constructed the genotype accumulation curves using the command *genotype_curve* with the *poppr* package (Kamvar et al. 2014, 2015) from R program (R Core Team 2013), setting the number of resampling of loci to 1000.

Genetic variability was quantified in each population through the number of alleles (N_A), effective number of alleles (N_{Ae}) (Nielsen et al. 2003), allelic richness (A_r), and unbiased Nei's gene diversity (H_e) (Nei 1978). Homozygote excess was quantified by the individual inbreeding coefficient (F_{IS}). Diversity measures and F_{IS} were compared between populations by Wilcoxon tests. The possibility that some deficiency of heterozygotes may be due to null alleles was tested by Monte Carlo with 1000 randomizations as described by Guo and Thompson (1992) and the U test statistic described by Rousset and Raymond (1995) with 10,000 using the software ML-Null (Kalinowski and Taper 2006).

Linkage disequilibrium was tested by the index of association (I_A) and a slightly modified statistic which is independent of the number of loci (r_d) (Agapow and Burt 2001). These coefficients are based on the comparison between the observed variances of the "distance" between all pairs of individuals with the expected under linkage equilibrium. Both coefficients were also estimated with the

package *poppr*. Significance of I_A and r_d was estimated by permutation test with 1000 replicates.

Identification of single gene pools

In order to identify single gene pools and genetic structure, three different approaches were applied. The first one estimates the relationships among individuals and populations by minimum spanning networks (MSN). This method is efficient to visualize relationships among individuals in the data and allow the inclusion of reticulation condition (Kamvar et al. 2015). Two measures of genetic distance were tested: the Prevosti (Prevosti 1974) and Bruvo (Bruvo et al. 2004) distances. The first one is based in absolute differences in allele presence, whereas the second incorporates information on microsatellite repeat number. MSN were obtained using *poppr* package (Kamvar et al. 2015).

The second analysis was based on an admixture F-model (Karhunen 2013) conducted with the R package *RAFM* (Karhunen 2013). This approach allows estimating the differential contribution of genetic drift and gene flow from different lineages to the populations measured through the alpha and kappa coefficients, respectively.

Finally, in order to detect historical events that may have produced spatial discontinuities in the genetic structure within each population, we used a Bayesian analysis of population structure that was carried out with the software *STRUCTURE* 2.2 (Pritchard et al. 2000, 2009). This analysis is based on a clustering method for inferring population structure using genotype data consisting of unlinked markers to identify the presence of population structure and distinct genetic populations (Pritchard et al. 2000). We explored which value of K maximized the likelihood of the data. Ten replications were performed for each value of K from 1 to 3 with the following settings: admixture model (individuals may have mixed ancestry), burn-in period of 25,000 generations, and MCMC repetitions of 50,000. The approach adopted to select the most reliable K was the lower $\ln P(K)$ criteria.

Spatial genetic structure analysis

Fine-scale spatial genetic structure, SGS, was analyzed through spatial autocorrelation with the software *SPAGeDi* version 1.4 (Hardy and Vekemans 2002). The spatial autocorrelation method analyzes the degree of dependency among the genetic variation and the geographical space. Kinship single-locus and multilocus coefficients were computed for all pairs of individuals in each population using the statistics of Loiselle et al. (1995). In order to test for isolation by distance, the multilocus kinship coefficient (F_{ij}) for each pair of individuals was plotted against the logarithm of the geographical distance separating them

(kinship–distance plot). To estimate standard errors, we used the jackknife method in the regression analysis of kinship coefficients on geographical distances. The pairwise Loiselle et al. (1995) kinship matrix was also compared with the corresponding geographical distance matrix in both populations by Mantel test.

Additionally, the Sp statistics (Vekeman and Hardy 2004) was calculated to allow quantitative comparison of the extent of spatial genetic structure among populations.

The Sp statistics is calculated based on the regression slope of F_{ij} on geographical distance, such that:

$$Sp = \frac{b_f}{1-F_1}$$

where F_1 is the mean pairwise kinship coefficient over all loci between individuals belonging to the first distance class and b_f is the linear regression slope of average F_{ij} on the spatial distance. Statistical significance of F_1 and b_f was determined under a 95 % confidence interval of F_{ij} created by 10,000 permutations of individuals among distance classes. The size of the distance class was determined by *SPAGeDi* (Hardy and Vekemans 2002) to ensure an equal number of comparisons within each distance class. Here we computed 10 distance classes spanning from 0 to 90 m.

Estimation of dispersal distance and neighborhood size

Wright's neighborhood size, N_b , is defined as (Wright 1943):

$$N_b = 4\pi d_e \sigma_g^2$$

where d_e is the effective density of reproductive individuals and σ_g is the mean-squared parent–offspring distance and can be estimated as the inverse of Sp (Rousset 2000; Vekeman and Hardy 2004) yielding:

$$N_b = -\frac{1-F_1}{b_f}$$

Here we estimated N_b and σ_g for Fernandez-Forres and Campo Duran populations considering alternative values for effective density (d_e) of 6, 25, and 36 trees per ha (corresponding to 1 tree separated from the other by 40, 20, and 10 m, respectively).

Results

Microsatellite statistics

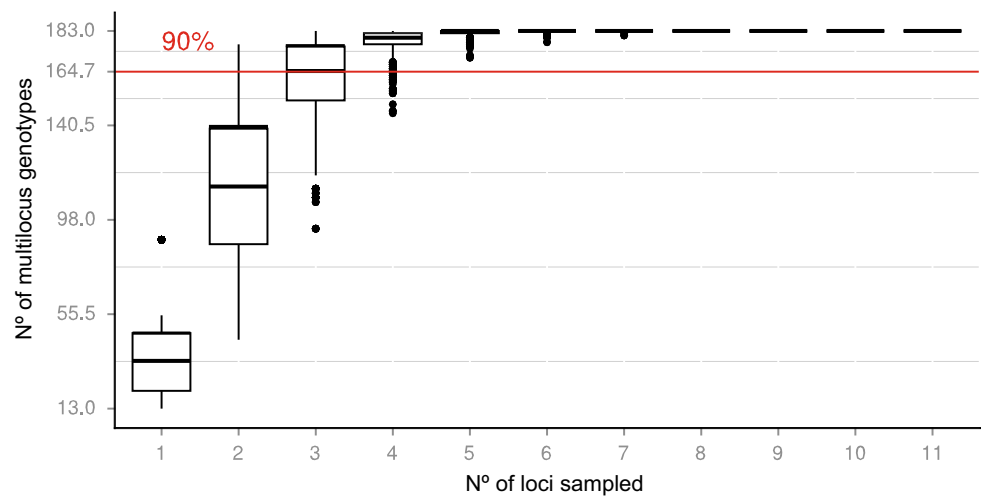
The SSR loci analyzed in both populations were highly variable showing from 5 to 23 alleles in FF population and 5–17 alleles in CD (Table 1). Variability measures did not

Table 1 Genetic variability and structure parameters in Fernandez-Forres (FF) and Campo Duran (CD) *Prosopis alba* populations

Locus	Population	N	MG	N _A	N _{Ae}	A _r	H _E	F _{IS}	P (null)
Mo08	FF	87	5 (5.7 %)	6	1.94	5.91	0.484	0.145	0.066
	CD	96	1 (1.0 %)	7	2.18	6.94	0.541	-0.070	0.244
Mo09	FF	87	11 (12.6 %)	5	1.26	4.99	0.206	0.170	0.011
	CD	96	2 (2.1 %)	7	1.35	6.91	0.259	0.139	0.068
GL18	FF	87	3 (3.4 %)	11	2.8	10.66	0.642	0.26	0.031
	CD	96	1 (1.0 %)	17	2.72	16.84	0.632	0.335	0.004
GL8	FF	87	4 (4.6 %)	17	4.84	16.52	0.794	-0.002	0.202
	CD	96	1 (1.0 %)	16	4.51	15.89	0.778	0.013	0.166
Mo16	FF	87	4 (4.6 %)	10	3.62	9.99	0.724	0.051	0.033
	CD	96	4 (4.2 %)	14	7.38	14	0.865	0.184	0
Mo05	FF	87	2 (2.3 %)	6	1.96	5.99	0.491	0.426	0
	CD	96	4 (4.2 %)	5	2.33	5	0.571	0.716	0
GL6	FF	87	2 (2.3 %)	10	2.69	9.65	0.628	-0.238	1
	CD	96	1 (1.0 %)	12	2.73	11.89	0.634	0.121	0.024
GL12	FF	87	3 (3.4 %)	9	4.15	8.77	0.759	-0.099	0.949
	CD	96	1 (1.0 %)	13	4.27	12.73	0.766	0.011	0.005
Mo13	FF	87	1 (1.1 %)	10	3.14	9.73	0.681	-0.144	0.982
	CD	96	2 (2.1 %)	10	4.03	9.91	0.752	0.038	0.004
Mo07	FF	87	12 (13.8 %)	11	2.51	11	0.601	0.624	0
	CD	96	6 (6.3 %)	9	3.98	9	0.749	0.438	0
GL15	FF	87	12 (13.8 %)	16	5.4	16	0.815	-0.080	0.493
	CD	96	0 (0.0 %)	13	5.8	12.87	0.828	-0.032	0.924
GL24	FF	87	3 (3.4 %)	23	12.15	22.44	0.918	-0.064	0.746
	CD	96	2 (2.1 %)	11	8.2	10.96	0.878	0.189	0
Average	FF	87	5.2 (5.9 %)	11.17	3.87	10.97	0.645	0.058	-
	CD	96	2.1 (2.2 %)	11.17	4.12	11.08	0.688	0.166	-

N, sample size, N_A, number of alleles, N_{Ae}, effective number of alleles (Nielsen et al. 2003), A_r (k = 150), allelic richness (expected number of alleles among 150 gene copies), H_E, Nei's (1978) unbiased gene diversity, F_{IS} = individual inbreeding coefficient, P(null): probability of heterozygote deficiency due to null alleles tested by Monte Carlo (1000 replicates)

Fig. 2 Genotype accumulation curve. Box plots constructed after randomly sampling the loci without replacement (1000 samples) and counting the number of multilocus genotypes observed for both populations of *P. alba*



differ significantly between populations according to Wilcoxon test. The genotype accumulation curve (Fig. 2) indicated that 4 loci are enough to recognize 90 % of the multilocus genotypes and the utilization of at least 5 loci allows the recognition of single individuals.

The measures of genetic diversity retrieved were similar to estimates based on SSR in other forest trees (Degen et al. 1999; Assoumane et al. 2009; Rajendra et al. 2014; Curtu et al. 2015) and, as expected, much higher than isozyme variability recorded in *P. alba* (Ferreira et al. 2007). The average F_{IS} was positive and significant in both populations ($P < 0.005$) although the ratio between F_{IS} in CD and FF was 3:1 (Table 1). Positive F_{IS} values may be indicative of excess of homozygotes, compatible with population substructure and/or low rates of selfing as previously described for *P. alba* (Bessegga et al. 2011). However, the presence of undetected null alleles may also account for an overestimate of F_{IS} . Table 1 suggests high excess of homozygosity in both populations in *loci* Mo09, GL18, Mo16, Mo05, and Mo07 that might be the consequence of null alleles rather than population structure. The test conducted with the software ML-Null confirmed a deficiency of heterozygotes at GL18, Mo16, Mo05, and Mo07 loci in both populations which may be caused by one or more null alleles. After excluding these loci, average F_{IS} in CD is positive ($F_{IS} = 0.066$), whereas in FF average F_{IS} is negative ($F_{IS} = -0.029$). The ratio between positive and negative F_{IS} estimates differs significantly between populations according to an independent Chi-square test ($P = 0.045$, $DF = 1$), suggesting that genetic structure differs between populations.

In both populations, linkage disequilibrium differs significantly from zero ($P < 0.05$), rejecting the hypothesis of independence between loci. However, both I_A and r_d estimates in Fernandez-Forres are almost three times the value in Campo Duran (Table 2).

Detection of genetic discontinuities within populations and differentiation

Minimum spanning networks revealed two clear differentiated groups (Fig. 3), each of them integrated mostly by individuals from the same population. The network obtained with Prevosti's distances suggests that CD

Table 2 Linkage disequilibrium evaluation by index of association I_A and r_d in two populations of *Prosopis alba*

	I_A	r_d	P
Campo Duran	0.122	0.011	0.024
Fernandez-Forres	0.309	0.029	0.045
Total	0.320	0.030	0.001

P : significance of r_d estimated by 1000 permutation

population exhibits a higher proportion of reticulate events. Genetic distance among individuals seems similar among individuals within population and between both populations studied (according to edge thickness).

Genetic differentiation between populations is consistent with the significant F_{ST} coefficient ($F_{ST} = 0.09$, $P < 0.0001$). The analysis of molecular diversity by *RAFM* identified 2 lineages (L1–L2) from the hypothetical ancestral population contributing to the 2 sampled populations. According to alpha parameter estimates the amount of drift effect differed slightly between lineages (alpha = 9.65 and 7.50 for L1 and L2, respectively). Also the kappa parameters indicated that the composition of each population in terms of the relative contribution of each lineage was different. The lineage L1 has contributed a 99 % to Campo Duran population, whereas Fernandez-Forres is 99 % integrated by individuals derived from L2. According to kappa estimates, the admixture between these populations is negligible (1 %).

Bayesian analyses performed with STRUCTURE showed population structuring neither for Campo Duran nor for Fernandez-Forres ($K = 1$, $\ln P_{(X/K)} = 3725$ and 2979, respectively). The lack of evidence of genetic discontinuities suggests no influence of historical events within each population.

Spatial analysis

Mantel tests between Loiselle et al. (1995) kinship coefficient (F_{ij}) and geographical distance matrices were significant for both populations ($r_{FF} = -0.0282996$, $P = 0.03195$; $r_{CD} = -0.0325$, $P = 0.01998$). However, significant SGS was detected via spatial autocorrelation only in CD (Fig. 4).

In CD, SGS is positive and significant for the first distance class (0–4.5 m) with an average F value of 0.0112 (Fig. 4a). For the distances ranging from 4.5 to 86 m the kinship coefficients decrease and the P value became non-significant, being negative for classes higher than 22 m. The decrease in kinship with distance was highly significant ($P = 0.0007$) for both log-linear (b_{\log}) and linear (b_l) models, although the first model was the best fitted ($R_{\log}^2 > R_l^2$) (Table 2), as expected for an SGS pattern resulting from isolation by distance in a two-dimensional space (Rousset 2000). In FF although the kinship decreases with the geographical distance and both b_{\log} and b_l were significant ($P = 0.039$), SGS is not significant for any distance class. Kinship gets negative values from 15 m (Fig. 4b; Table 3).

The statistics Sp assessed at a scale <100 m allows for comparison of the extent of SGS between both populations (Table 3). Sp estimate for FF was less than a half of that of CD. As a consequence, the Wright neighborhood size (N_b) in FF is more than twice as the CD value (Table 3).

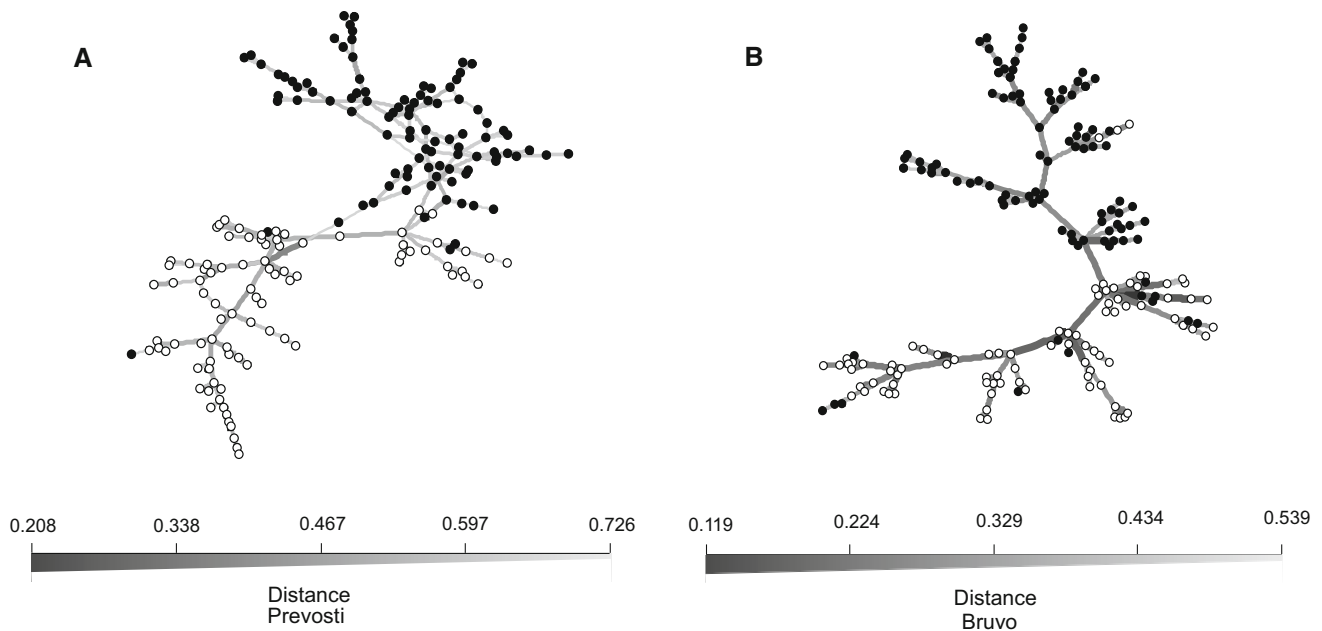


Fig. 3 Minimum spanning network based on Prevosti (A) and Bruvo’s (B) distances. *Filled circles* represent multilocus genotypes (MLG) from Campo Duran and *open circles* those from Fernandez-Forres. *Edge thickness* represents genetic distance between the connected MLG

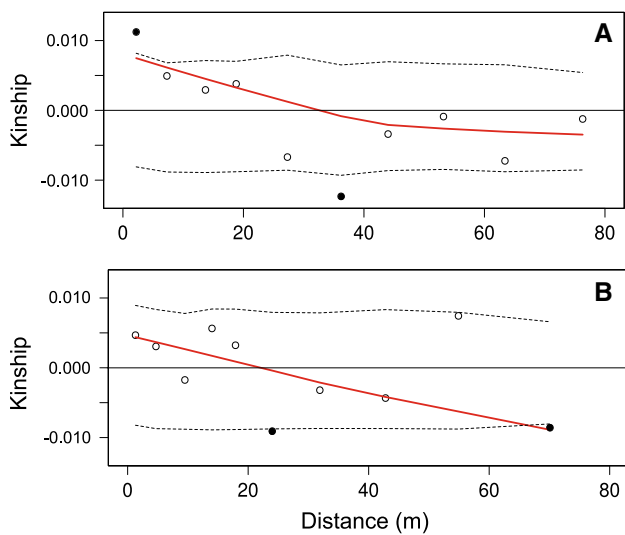


Fig. 4 Kinship coefficients (Loiselle et al. 1995) versus geographical distance for Campo Duran (A) and Fernandez-Forres (B) populations of *Prosopis alba*. Each point represents the average comparisons ending at 80-m class. The *filled circles* indicate significant *Fij* values. The *dotted lines* indicate the 95 % confidence intervals (10,000 permutations)

As density is not uniform throughout the sampling area, gene dispersal distance (σ_g) was estimated considering three different effective densities. Dispersal distance ranged between 102 and 250 m in FF, and 66 and 162 in CD, extreme values corresponding, respectively, to the highest and lowest density estimates.

Discussion

The impact of human activities such as deforestation activity without any reforestation strategy, the establishment of different crop plantations, and natural fires become a very important fact in the forest in the last years (Fernández and Busso 1997; Secretaría de Ambiente y Desarrollo Sustentable de la Nación 2004; FAO 2007). In order to assess the effects of human activities on genetic diversity and spatial genetic structure, two populations widely separated were chosen to give recommendations concerning the future management of *Prosopis alba* forests. We considered populations with contrasting human activities influences: one situated in Santiago del Estero (Fernandez-Forres) representing a fragmented forest and one isolated population in Salta (Campo Duran) as a relatively low disturbed forest.

We used 12 microsatellites markers to characterize the populations. According to Cavers et al. (2005), 10 microsatellite loci are usually adequate to characterize fine-scale SGS. In the present study we were able to determine that 5 loci yield unique multilocus genotypes that allowed us to identify all the individuals studied, indicating that the number of loci analyzed provides information suitable for genetic and SGS analysis. The genetic diversity estimates were high, and values do not exhibit significant variation between populations. This result is in agreement with works on *P. alba* and other related species of section Algarobia of genus *Prosopis* (Bessega et al. 2000b, 2011;

Table 3 Fine-scale spatial genetic structure parameters estimated from 12 microsatellite markers in two populations of *Prosopis alba*

	Fernandez-Forres	Campo Duran
F_1	0.0047 NS (SE = 0.0028)	0.0112*** (SE = 0.0056)
$bf(l)$	-0.1194 (R ² = 0.0008)	-0.1609 (R ² = 0.0017)
$bf(\log)$	-0.0021 (R ² = 0.0008)	-0.0049 (R ² = 0.0038)
Sp	0.0021	0.0050
Nb	471.21	198.51
σ_g^2 ($D = 0.0036$)	102.08	66.26
σ_g^2 ($D = 0.0025$)	122.50	79.51
σ_g^2 ($D = 0.0006$)	250.05	162.30
Max. distance of SGS (m)	0–93	0–86

D , density range considered (individuals per hectare); F_1 , multilocus kinship coefficient (Loiselle et al. 1995) between individuals of the first distance class; $bf(l)$, regression slope of F on distance; $bf(\log)$, regression slope of F on natural log distance; Sp , quantification of the SGS; Nb , Wright's (1943) neighborhood size; σ_g^2 , mean-squared parent-offspring distance estimated for different densities

NS $P > 0.05$; *** $P < 0.001$

Ferreira et al. 2007, 2010). The high genetic diversity is a good basis for adaptation processes (Hamrick et al. 1992), and this condition can be partially related to the extreme conditions where this species lives. Particularly, Campo Duran is located in a region with higher temperatures ($T_{\text{mean}} = 22.5$ °C), humidity (PET = 127.18), and percent sun fraction (Sfr = 67.92 %) than Fernandez-Forres ($T_{\text{mean}} = 18.0$ °C, PET = 94.07, and Sfr = 44.92 %). These climatic differences apparently are not reflected in differences in genetic variability parameters between these populations. When all loci are included in the analysis both populations exhibited significant homozygote excess, but F_{IS} estimate in FF is a third of that in CD. If loci with significant evidence of null alleles are excluded, average F_{IS} in CD remains positive, whereas in FF the estimate becomes negative. This result indicates that population structure differs between populations. Homozygote excess was previously recorded analyzing isozyme markers in populations of *P. alba* and other related species (Bessega et al. 2000a) which might be explained by population substructure and/or low rates of selfing. However, a more recent analysis of the mating system of *P. alba* based on SSR (Bessega et al. 2011) indicates that selfing in this species is virtually absent, but biparental inbreeding may take place as a consequence of mating between related individuals. The positive F_{IS} estimate in CD would be better explained by population substructure. The comparison between the fragmented (FF) and not disturbed (CD) population suggested that fragmentation has not yet affected genetic diversity of the former, probably due to large effective population sizes, efficient gene dispersal in *P. alba*, or relatively short time since the human disturbance onset in terms of generation interval of this species which is known to live up to 300 years. The higher linkage disequilibrium in FF is also consistent with the hypothesis

of a relatively recent establishment of FF population. The reduction in homozygote excess in this population in time is reflecting possible effects of artificial thinning and selection on population structure. This conclusion is consistent with a previous work (Bessega et al. 2011) where the spatial genetic structure was analyzed at short distances in the FF area. In that study the differentiation among patches was relatively low. The lack of substructure within populations could not be explained by dispersal ability because insect pollination and endozooic seed dispersal are usually limited in terms of distances. The most plausible conclusion is that fragmentation in FF is a relatively recent process and artificial non-random thinning also contributed to erase population structure.

The lack of genetic discontinuities within and significant differentiation between populations was indicated by the F_{ST} estimate, the minimum spanning networks recovered, the Bayesian approach (STRUCTURE), and the lineage composition of each population analysis (retrieved with RAFM). The differentiation between FF and CD suggests that these populations were founded by two well-differentiated lineages from a hypothetical ancestral population. As more than 90 % of each population is constituted by a single lineage, the evidence suggests that very low if any admixture between populations has occurred.

Our study revealed that population CD of *P. alba* possesses significant SGS in the smaller distance classes. In this population, mostly non-disturbed by human activities, positive SGS extended up to ~22 m. By contrast, in the disturbed population of Fernandez-Forres, although a drop-off in the F values was observed, SGS was not significant at any distance class. The differences in the SGS between populations could be the result of differences in densities of individuals and/or consequences of management of FF. Campo Duran has a bidimensional distribution, its landscape

is almost pristine, and *P. alba* trees are intermixed with several woody species including *Schinopsis sp* (“quebracho”), *Bulnesia sarmientoi* (“palosanto”), *Anadenanthera colubrina* (“cebil”), *Geoffroea decorticans* (“chañar”), *Caesalpinia paraguariensis* (“guayacán”), *Saccolium lanceolatum* (“guayabil”), *Celtis tala* (“tala”), *Acacia aroma* (“tusca”), and several Cactaceae. Higher density in this locality might promote mating among neighboring trees probably belonging to the same family promoting a SGS fitting an isolation-by-distance model. FF, by contrast, is virtually unidimensional following route 34 in Santiago del Estero, which represents a remain of an ancient wide forest currently replaced by commercial crops, and trees are here semi-isolated. Management of this population involved thinning and phenotypic selection based on tree size and health, reducing possible family structure. The lack of SGS in this population is consistent with the conclusions from F_{IS} estimates. In FF the semi-isolated trees might serve as stepping stones among groups of individuals which could result in a critical role in determining gene movement within fragmented landscapes (Hamrick 1994; Fuchs and Hamrick 2011). This view is consistent with evidence that gene dispersal is higher in populations with low densities (Vekeman and Hardy 2004) and some species are capable of compensating at least partially the effect of density on SGS (Pandey et al. 2004). In the present work we detected that dispersal distance in Campo Duran ranges from 66 to 162 m, whereas in Fernandez-Forres the range is 102–250. Considering that pollen dispersal distance was previously reported for a population of *P. alba* close to FF between 5.36 and 30.92 m (Bessega et al. 2011), seed dispersal should be invoked to explain total dispersion. Differences in dispersal distances between populations are not unexpected because the most frequent seed dispersal of *P. alba* is herbivores, which in Campo Duran are mainly represented by native small mammals (rodents, armadillo, etc.), whereas in Fernandez-Forres the role of livestock (mainly goats) with higher roaming ability would be more important. Different dispersal patterns between protected and unprotected forest stands have been observed in other species (Pacheco and Simonetti 2000; Wang et al. 2007; Dick et al. 2008).

Considering that the microsatellites markers used in this study are presumed to be neutral, guidelines that may be inferred from this work can be relevant to any area irrespective of environmental variations. The present analysis of population structure in *P. alba* was based on a higher number of genetic markers with high variability than previous surveys. The evidence retrieved indicating that each population involves different lineages is very important for management programs because it shows that the gene pool is quite different between regions. In previous works we obtained evidence of local adaptation for quantitative traits in *P. flexuosa* (Darquier et al. 2013) and *P. alba* (Bessega

et al. 2014). As a consequence, experimental stands founded with breeding or ex situ conservation purposes should take into account that gene pool differences may be the result of local adaptation. Replicated provenance trials established in different regions with a wide genetic basis would be advisable to select the best genotypes for particular environments. The analysis of SGS in the population CD of *P. alba* confirmed the existence of small-scale family structures. The results are relevant to sampling trees avoiding the collection of seeds from individuals with similar genetic characteristics. The present results suggest that the distance between seed trees should be larger than the distance of significant SGS, which in this case is about 22 m.

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