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Strategies for conservation for disturbed *Prosopis alba* (Leguminosae, Mimosoidae) forests based on mating system and pollen dispersal parameters

C. Bessega · C. L. Pometti · M. Ewens · B. O. Saidman · J. C. Vilardi

Abstract *Prosopis* species forests in Argentina are increasingly fragmented in the last years mainly by the deforestation activity without any reforestation strategy, the establishment of different crop plantations, and natural fires. The consequence of habitat fragmentation on the genetic potential of *Prosopis alba* requires a fine-scale analysis of population structure, in particular mating system and pollen dispersal. By means of short sequences repeats, we analyzed a fragmented population of this species in Santiago del Estero (Argentina). Most genetic variation was observed among families within zones (65.5%), whereas the lowest proportion corresponded to the differentiation among zones (2.8%). The fine analysis of structure at family level suggests that this population is complete outcrosser and there is a low but significant biparental inbreeding. Outcrossing rates differ among mother plants and the proportion of full sibs within mother plants ranged from 64% for seeds proceeding from the same pod to 10% for seeds from different pods. The average pollen dispersal distance was estimated to be among 5.36 and 30.92 m by using the KinDist or TwoGener approach. About seven pollen donors are siring each progeny array and the number of seed trees necessary for seed collection aiming to retain an effective population size of 100 was estimated in 16–39 individuals depending on the relatedness estimator used. Pollen and seed dispersal would be limited, what determines the need of conserving short distant patches to avoid the effects of inbreeding and drift within populations as a consequence of intensive use resource for agriculture.

Keywords Pollen dispersal · Microsatellites · Conservation · Native forest

Introduction

Information on the patterns and the distance of pollen dispersal is fundamental to the development of strategies for conservation in situ and ex situ. *Prosopis* species (algarrobos) forests in Argentina are increasingly fragmented in the last years. This is especially notorious in the Chaco region, where most of the original *Prosopis* forests have been already lost (Fernández and Busso 1997; Secretaría de Ambiente y Desarrollo Sustentable de la Nación 2004; FAO 2007). The main causes that should be taken into account are the deforestation activity without any reforestation strategy, the establishment of different crop plantations, and natural fires. The most important damage is caused by irrational deforestation stimulated by the multiple uses of "algarrobo" wood (Roig 1993; Verdes 2007) and the extension of agricultural areas. The consequence of these activities is the desertification of wide areas, endangering these tree species.

Habitat fragmentation reduces areas of continuous forest to small, separate remnants and may decrease the effective

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size of tree populations (Cascante et al. 2002), disrupt the mating system, and interrupt gene flow (Jump and Penuelas 2006). When populations become genetically isolated, they are at risk of losing the genetic diversity that is critical to their long-term survival (Sork and Smouse 2006). According to the classical view, as a consequence of the isolation, the immediate loss of alleles happens due to the reduction of the population, with the consequence of inbreeding, population divergence increase, and genetic diversity reduction within population patches (Young et al. 1996; Lowe et al. 2005). Relatively recent reviews (Aguilar et al. 2008; Eckert et al. 2008) describe a significant reduction in genetic variability in remnant fragmented populations. However, the longevity of the trees and effective seed and pollen dispersal can enhance their resistance to the negative effect of the forest fragmentation (Hamrick 2004; Jump and Penuelas 2006). Indeed, Kramer et al. (2008) claim that population genetics theory may be misapplied in the case of forest trees and that the actual impact of forest fragmentation on genetic structure depends on a number of ecological factors including the abilities and behavioral characteristics of pollen and seed dispersal vectors.

In Argentina, two groups of "algarrobos" are differentiated by local people in terms of their uses. They are commonly recognized by pod color and shape as white (*Prosopis alba* and *Prosopis chilensis*) and black algarrobos (*Prosopis flexuosa* and *Prosopis nigra*). Issues relative to uses, conservation status, and actual geographic distribution are different among these groups. Black algarrobos are widely distributed, whereas white algarrobos are found in more humid areas or behave as phreatophyte. The first group is prone to be attacked by borer insects and therefore, they are discarded for furniture, restricting their use to firewood, poles, and stakes. The second group, preferred by the furniture industry because of the highest wood quality (Pometti et al. 2009; 2010), represents an almost exhausted resource, being reduced to a few forests in different provinces of Argentina. In particular, the area Machagai–Quitilipi, Chaco province, which used to be an important timberland center is going to stop sawmill activity in a near future as a consequence of resource extinction. In Santiago del Estero province, forests able to be used for furniture industry are almost absent by now. White algarrobos are restricted to small areas (protected between rivers), road sides, or maintained as small patches in ranches to provide shade to livestock.

The effects of habitat disturbance on the genetic properties of populations are highly dependent on the mating and dispersal mechanism. The distance of pollen movement is a significant determinant of the effective pollination neighborhood for a plant population (Austerlitz and Smouse 2002), which is especially important in calculating the number of seed trees for seed collection as

part of ex situ conservation and reforestation programs. Choosing populations that carry most of the species genetic variation is essential in in situ conservation programs, and this choice should be based on population structure analyses. Furthermore, the effective population size can be quiet difficult to predict because it depends not only on the census number but also on the inbreeding coefficient, variation in fertility, nonrandom mating, and fluctuation population sizes (Crow and Kimura 1970).

Available information on population structure of *P. alba* and other related species (Ferreyra et al. 2007, 2010) indicated that most of genetic variability occurs within populations and regional differentiation is limited. A previous study on the mating system in seven species of *Prosopis* (Bessega et al. 2000) indicated that they are mostly outcrossers, but information about the genetic structure at a fine scale and pollen dispersal is lacking. Seeds are dispersed endozoically by small mammals (Reynolds 1954; Mares et al. 1977), and pollen is dispersed by insects (Genisse et al. 1990), both conditions usually associated to limited dispersal. However, dispersal patterns may have been highly modified after the European colonization by the introduction of livestock and the honey bee *Apis mellifera* which might have increased dispersal range. In this paper, we sampled a population of *P. alba* in Santiago del Estero (Argentina) that represents a remnant of an ancient extended forest in order to evaluate population structure, pollen dispersal distance, and mating system parameters. The hypotheses to be contrasted are (1) the fragmentation prevents gene flow between remnant population patches and the long-term consequence would be a reduction of population diversity and an increase of inbreeding within populations and differentiation among populations; or (2) gene flow might still occur following a stepping stone model with scattered trees or small patches acting as bridges, contributing to the metapopulation viability; this condition added to the long life cycle of *P. alba* trees would delay the genetic effects of fragmentation.

Material and methods

Study species

P. alba is included in the section Algarobia (Burkart 1976), which comprises ca. 28 species very important economically and ecologically. It is a native tree that grows naturally as secondary tree species in the semiarid region called Chaco, in northern Argentina. It may reach up to 18 m tall and 70–150 cm of trunk diameter. It is an important nitrogen-fixing tree adapted to the semiarid regions. The tree provides fuelwood and dimensionally stable lumber for flooring and furniture; its pods that have high sugar content are consumed by livestock and humans.

P. alba has been one of the most heavily harvested species. In the last years, the high use of *P. alba* wood by the industry yielded a qualitative and quantitative diminution of the populations; the best trees are harvested and the deforestation rate is higher than the grow rate and regeneration.

Flowers are protogynous and are grouped in inflorescences consisting of 7–11 cm long spikes. Pollination in *Prosopis* is made by insects (Genisse et al. 1990) and seed dispersal is endozoic (Reynolds 1954, Mares et al. 1977). The leaves are pinnately compound with 35 leaflet pairs per pinnae in average (Bessega et al. 2009). The ripe fruits are indehiscent and usually range 12–25 cm long, 12–18 mm wide, and yellow colored. The dried fruits fall from the tree at maturity and are readily eaten by nearly all herbivorous.

The studied population

The sampled population of *P. alba* is located near Fernandez, Santiago del Estero, Argentina (Fig. 1). This population has been a fairly continuous forest of several square kilometers that now has a patchy distribution embedded in an agricultural matrix. Forest cover in this area has been reduced by deforestation and developing of soybean, alfalfa,

onion, oat, and cotton, or invaded by Bermuda grass (*Cynodon dactylon*) as shown in Fig. 1. Taking into account habitat fragmentation, the sampling area was divided into three zones: Ruta 34, Tomey, and Rojas.

Sixteen mother plants were sampled. In all cases, the trees were separated at least 50 m. from each other. About 50 pods were collected from each mother plant. All seeds from each pod were stored in single bags. From each of these bags, seeds were randomly sampled for the short sequence repeats (SSR) analysis of each progeny array. The number of seeds analyzed per mother plant (family array size) was 11.

DNA extraction and genotyping

Leaf material collected in the wild population was dried immediately using silica gel and stored at room temperature until DNA extractions were completed. Genomic DNA was extracted both from the leaves of adult individuals and from 2- to 5-day-old seedling cotyledons using DNA easy Plant mini kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer's instructions.

Six SSR that had been developed in *P. chilensis* exhibits cross-species amplification (Mottura et al. 2005) and shown to be amplified successfully in *P. alba* (Bessega et al. 2009) were analyzed. Forward primers (website,
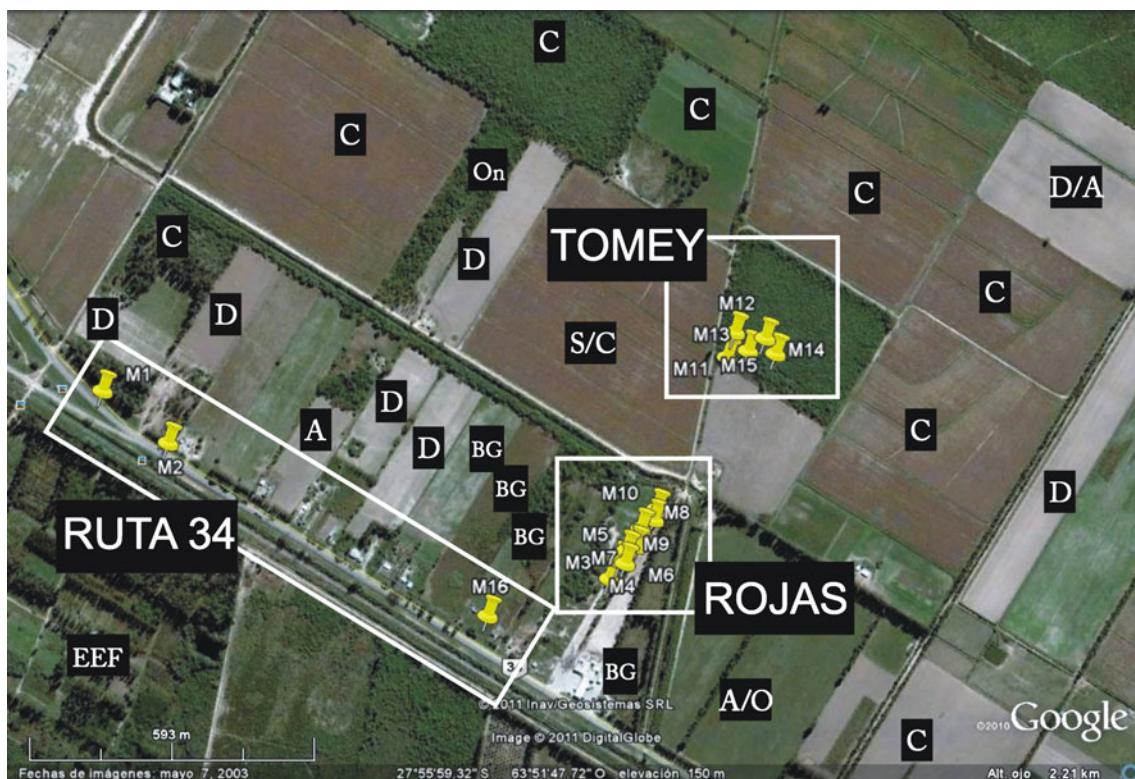
Fig. 1 Spatial distribution of the sampled *Prosopis alba* trees (*M1–M16*). *M1*, *M2*, and *M16* belong to *RUTA 34*; *M3–M10* belong to *ROJAS*; and *M11–M15* are from *TOMEY*. *D* Dismounted, *S* soy, *C* cotton, *A* alfalfa, *O* oat, *On* onnion, *BG Cynodon dactilon*=bermuda grass, *EEF* Estación Experimental Fernandez (*Eucaliptus* sp., *Casuarina* sp., *Populus* sp., *Prosopis* sp., *Cynodon dactilon*, *Sorghum halepensis*)

biomath.trinity.edu/; search term, *Prosopis*) were labeled with a fluorescent dye (Invitrogen); three loci were labeled with 6-FAM and three with HEX. The PCR amplifications were carried out in a 50 μl reaction volume containing 10–30 ng DNA, 0.6 μM each primer, 0.2 mM dNTPs, 0.3 U Taq DNA polymerase (Invitrogen), and 1.5 mM MgCl$_2$. A MyCycler Thermal Cycler (BioRAD Laboratories) was used for amplifications where the cycling profile was: initial denaturation at 94°C for 5 min followed by 30 cycles at 94° for 45 s denaturation, primer-specific annealing temperature (56°–59°) for 45 s and at 72°C for 45 s extension, and a final extension step at 72° for 10 min. PCR products were electrophoresed in an ABI313XL (HITA-CHI) automated DNA sequencer and automatically sized using GENEMARKER ver 1.91 (SoftGenetics LLC™ www.softGenetics.com).

Statistical analyses

Analyses of genetic variation and population structure

Possible genotypic associations among the studied loci were tested using the program Multilocus 1. 3 (Agapow and Burt 2001) by the index of association (I_A) and a slightly modified statistic which is independent of the number of loci (r_d). In brief, the "distance" (number of loci at which they differ) between all pairs of individuals is calculated, and the variance of these distances compared to that expected if there is no linkage disequilibrium.

Genetic variation was quantified by means of the number of detected alleles per locus (A), effective number of alleles (N_e), observed (H_o), and expected heterozygosity under Hardy–Weinberg equilibrium (H_e). The distribution of genetic variation was analyzed by nonhierarchical and hierarchical F statistics and analysis of variance components using the software FSTAT (Goudet et al. 1995) and the package HIERFSTAT (Goudet 2005) for R software (R Development Core Team 2011).

The levels considered in the hierarchical analysis were: zone, family, pod, and individual. The fixation index (F_{IS}) was estimated in adult and offspring samples. The significance of the nonhierarchical F_{ST} was estimated using the likelihood ratio G statistic (with 2,000 replicates). Confidence intervals for variance components and F statistics were obtained by bootstrap with 2,000 pseudoreplicates. The significance of each level of the hierarchical F statistics was evaluated by between, between-within, and within F test.

Fine analysis of structure at family level

The sample collected is composed of individuals involving different relationship classes. As mother plants were separated more than 50 m from each other, they, as well as their progeny, are assumed as nonrelated following the Saidman and Vilardi (1993) criterion, which is based on field observations (Palacios, personal communication) that trees isolated by 50 m or larger distance do not produce fruits. As seeds proceed from open pollination, the progeny obtained from each mother tree should involve half sibs, full sibs, self half sibs, and self full sibs (Squillace 1974).

Three approaches were applied in order to asses mating system parameters, pollen dispersal and the structure of each family group.

1. *Mating system*

Estimates of multilocus (t_m) and mean single locus (t_s) outcrossing rates, correlation of *tm* within progeny arrays (r_t), the correlation of outcrossed paternity (r_p), and fixation index of maternal parents were calculated using the MLTRwin software (Ritland 2002) This program is based on the multilocus mixed-mating model and the estimation procedure of Ritland and Jain (1981) which assumes that progeny are derived from either random mating (outcrossing) or self-fertilization. The estimation of mating system parameters was made by the expectation–maximization method to assure convergence and 10,000 bootstraps were used to get standard errors for each parameter. Biparental inbreeding was estimated following Ritland (1990) as $t_m - t_s$. The effective number of pollen donors over all mothers trees were estimated under sibling pair model (Ritland 1989) by the relation effective number of pollen donors $N_{ep} = 1/r_p$.

In a first analysis, all seeds within each family were considered a single group and all parameters were estimated. As seeds within mother trees are grouped in pods, a second analysis was conducted considering the pod as an additional level within family.

2. *Estimation of pollen dispersal parameters based on assumed pollen distribution curves*

Pollen dispersal parameters were fit using two indirect methods based on the spatial genetic structure of the pollen pool: TwoGener (Smouse et al. 2001; Austerlitz and Smouse 2002) and KinDist (Robledo-Arnuncio et al. 2006) using the POLDISP 1.0 software (Robledo-Arnuncio et al. 2007). The TwoGener method is based on the relationships between physical distances among a sample of mother trees and pairwise intraclass genetic correlations (Φ_{FT}) for the pollen pools sampled by these mothers. To fit pollen dispersal using the TwoGener approach, an independent estimate (or a joint estimation) of the effective density of pollen donors (de) is needed (Austerlitz and Smouse 2002), which can complicate the accurate estimation of two parameters. In addition, this method assumes uniformity in male fecundity and synchronous flowering. The KinDist method is based on a normalized measure of correlated paternity (ψ) between female pairs

(Robledo-Arnuncio et al. 2006). The estimates of pollen dispersal parameters obtained by KinDist are generally more accurate than those from TwoGener because KinDist does not rely on effective density, a parameter, which is very difficult to measure in the field. Joint estimation of *de* with TwoGener is associated with the usual statistical problems of increasing the dimensionality of the parameter space. The KinDist method is also robust for the levels of male fecundity variation and asynchronous flowering commonly observed in forest tree populations (Robledo-Arnuncio et al. 2006)

The estimate of the pollen structure parameter due only to limited pollen dispersal (Φ_{FT}) was corrected using the formula described in Austerlitz and Smouse (2001):

$$\Phi'_{FT} = \Phi_{FT}(1 + F)$$

where, F is the adult (mother tree) inbreeding coefficient. The corrected value was used to estimate N_{ep} under the pollen structure model by the relation:

$$N_{ep} = \frac{1}{2\,\Phi'_{FT}}$$

The estimate of Φ_{FT} was also used to further calculate the derivative measure of the average distance of pollination (δ) using normal and exponential curves with the Subprogram TwoGener.

If we assume a bivariate normal distribution of pollen dispersal, the estimate of pollen structure Φ_{FT} is inversely related the variance of pollen dispersal (σ^2) and reproductive adult density (d),

$$\Phi_{FT} = \frac{1}{8\pi\sigma^2 d}$$

Given that Φ_{FT} is known from genetic data and d from ecological observation, it follows that the variance in pollen dispersal can be computed from the expression above.

Next, σ^2 is used to estimate the effective number of pollen donors as:

$$N_{EP} = 4\pi\sigma^2 d$$

The effective neighborhood pollination area (A_{EP}) is:
$A_{EP} = 4\pi\sigma^2$ (Austerlitz and Smouse 2001)

3. *Average coancestry coefficient (θ) and variance effective population size*

We obtained Ritland (1996) and Queller and Goodnight (1989) relatedness estimator (r) for all the individual pairs using GENALEX (Peakall and Smouse 2006). From this estimation, we calculated the coancestry (θ_{xy}) matrix following the equation $r_{xy} = 2\theta_{xy}$ (Lynch and Walsh 1998). We also estimated pairwise coancestry (θ_{xy}) coefficients using the Nason (Loiselle et al. 1995) method with the

software SPAGeDI (Hardy and Vekemans 2002). We calculated within family θ as the average of θ_{xy} for individuals belonging to the same family, the average of θ within family, and for the whole sample.

The variance effective population size (N_{ev}) for each family was calculated by:

$$N_{ev} = \frac{0.5}{\theta\left(\frac{n-1}{n}\right) + \frac{1+Fo}{2n}}$$

(Cockerham 1969)

where, n is the size family and F_o is the inbreeding coefficient in offspring. The total variance effective population size of progeny arrays ($N_{e(t)}$) was estimated using the same equation with θ_t instead of θ.

The number of seed trees (m) necessary for seed collection was calculated by the method of Sebben (2006) based on the relationship between the proposed effective population size of the conservation program ($N_{e(reference)}$) and the average N_{ev}:

$$\widehat{m} = \frac{N_{e(reference)}}{\widehat{N}_{ev}}$$

(Sebben 2006)

Analysis of spatial genetic structure

Correlation between the Ritland (1996), Queller and Goodnight (1989), and Nason (Loiselle et al. 1995) relatedness matrices and the spatial distance matrix was tested using a Mantel (1967) test (with 2,000 permutations) using the package ade4 of R software (R Development Core Team 2011; www.r-project.org).

The association between relatedness and geographic distance was also evaluated using an additive generalized model (gam) using the package mgcv (Wood 2001) of R. Upper and lower confidence limits for the 95% about the null hypothesis of no spatial structure were plotted based on 2,000 permutations of the data set.

Results

Genetic diversity

The genotypes for six loci were determined for 12 individuals of each of the 16 families (making a total of 192). All loci were polymorphic with three to nine (average, 6.5) alleles per locus with a total of 39 alleles. Multilocus linkage disequilibrium coefficients $I_A=0.041$ and $r_d=0.0084$ were nonsignificant ($P=0.13$), indicating that the studied loci are in linkage equilibrium. The allelic richness varied from 2.48 to 5.77 (3.9), the observed heterozygosity ranged from 0.169 to

0.688 (mean, 0.460; Table 1). Expected heterozygosity within families (H_S) varied between 0.155 and 0.584 (mean=0.394). Although individual tests of Hardy–Weinberg departures were nonsignificant, multilocus F_{IS} estimate (−0.169) was significant (CI_{95}=−0.194/−0.133) indicating a significant heterozygote excess in the whole population.

Genetic structure

Heterozygote observed frequencies in the adult (mother) population did not differ from expected (F_{IS}=−0.068, CI_{95}= −0.205/0.200). Genetic structure of progeny generation was studied through a nonhierarchical and a hierarchical (Table 2) analysis of variance. The first analysis showed a significant heterozygote excess within families (F_{IS}=−0.14, CI_{95}= −0.162/−0.116) and a highly significant differentiation among families (F_{ST}=0.172, CI_{99}=0.093/0.243).

The hierarchical analysis (Table 2) indicated that the genetic differentiation among zones was nonsignificant, whereas the differentiation among families (within zones) and pods (within families) were highly significant. Expressed as proportion, most of genetic variation was observed among families within zones (65.5%), whereas the lowest proportion corresponded to the differentiation among zones (2.8%; Table 2). A highly significant heterozygote excess was observed within pods.

Mating system and pollen disperal

The analysis of the mating system showed an almost complete outcrossing rate (Table 3). The difference $t_m - t_s$ was low but significant indicating a low proportion of mating among relatives. The correlation of t_m within progeny arrays (r_t) was high, suggesting that outcrossing rates differ among mother plants. The correlation of outcrossed paternity was evaluated at three levels: global, among fruits, and within fruits. In all cases, r_p was significantly higher than 0. The r_p estimate was maximum for sib sampled from the same fruit (r_p=0.638) but for different fruits on the same plants the value dropped in about 85% (0.098/0.638). These results indicate that the proportion of full sibs is about 64% for seeds proceeding from the same pod, whereas this proportion is only about 10% for seeds from different pods. Effective number of pollen donors, estimated as ($1/r_p$) indicated that about six pollen donors are siring each progeny array (Table 3). Pollen pool structure analysis with POLDISP gave a Φ_{FT} estimate of about 10%, which translated into an effective N_{ep} represents about five pollen donors per mother plant (Table 3).

Applying KinDist, the average pollen dispersal distance was estimated in 5.36 m when assuming a normal distribution and 11.96 when assuming exponential one of the pollen dispersal distance curve. The model based on exponential distribution curve fits only slightly better than the normal one as described by least square residuals of 26.51 and 26.43, respectively. Therefore, both the normal and exponential curves were used for further analysis using TwoGener.

The estimates made by TwoGener yields estimates of pollen distances a little higher than those obtained with KinDist. Considering a normal distribution, pollen dispersal distances varied depending on the estimation method between 13.74 and 23.74 m. When the same estimates were done using exponential curves, distance estimates varied between 15.5 and 30.92 m.

Effective densities of pollen donor was de=0.0010 and 0.0007 m^2 for both kinds of curves. These estimates are 31% and 24%, respectively, lower than d counted in the field. Finally, the A_{ep} was estimated in 0.150 ha, that is 22 m radiuses circle around each maternal tree.

Number of pollen donors, paternity correlation, coancestry, variance of effective population, and number of seed trees necessary for seed collection

The effective number of N_{ep}, r_p, coancestry coefficient θ, and the variance effective size of open pollinated families (N_e)

Table 1 Characterization of six microstellites loci from *Prosopis* in the population sampled

Locus name	N	H_O	H_s	H_T	K	A	F_{IS}
MO13	192	0.688	0.584	0.693	6	4.598	−0.158
MO05	192	0.169	0.155	0.158	5	2.172	−0.048
MO09	192	0.219	0.189	0.214	3	2.479	−0.294
MO08	192	0.536	0.446	0.556	8	4.111	0.175
MO16	192	0.688	0.573	0.653	9	5.772	−0.158
MO07	192	0.464	0.416	0.584	8	4.186	−0.114
Mean		0.460	0.394	0.476	6.5	3.9	−0.169*
SD		0.225	0.185	0.231	2.26	1.35	0.157

Given is the arithmetic mean over loci along with the standard deviation

H_O observed heterozygosity, H_S expected heterozygosity within families, H_T total heterozygosity, K number of detected alleles per locus, A effective richness, N sampled size

Table 2 Hierarchical analysis of components of variance

	F	var (%)	P
Zone/total	0.0068 (−0.0094/0.0268)	0.0198 (2.8)	0.16
Family/zone	0.1613** (0.0993/0.2183)	0.4651 (65.5)	5.10^{-4}
Pod/family	0.0930** (0.0554/0.1244)	0.2248 (31.7)	5.10^{-4}
Individual/pod	−0.2495* (−0.2800/−0.1986)	−1.047	–
Error		3.7396	

*$P<0.05$, **$P<0.01$

were estimated for each of the 16 families (Table 4). The average paternity correlation was 0.1896 (CI_{95}=−0.061/0.44), which represents a mean effective number of pollen donors of about seven. The average coancestry coefficient within families estimated from Ritland's (1996) relatedness estimator (θ=0.053, CI_{95}=−0.007/0.113) was lower than the expected for panmictic population (θ=0.125). The estimate obtained by Queller and Goodnight (1989) and Nason methods were higher (respectively, θ_{QG}=0.168 CI_{95}=0.029/0.307 and θ_N=0.165 CI_{95}=−0.007/0.336) and closer to the expected value. The average variance effective population size estimated from the coancestry coefficients were respectively 8.1, 2.83, and 3.26 for Ritland, Queller and Goodnight, and Nason. The number of seed trees necessary for seed collection aiming to retain an effective population size of 100 was estimated at the minimum of 16–39 individuals depending on the method θ was estimated (Table 4).

Spatial genetic structure

The pairwise Ritland (1996), Queller and Goodnight (1989), and Nason (Loiselle et al. 1995) relatedness matrices were compared with the corresponding geographic distance matrix by mantel test. In all cases, the correlation was significant (R^2=−0.11, P=0; R^2=−0.066, P=0.013; and R^2=−0.105, P=0, respectively, for RI, QG, and N). The plot of relatedness as function of geographic distance shows a deep decrease at short distances, following a gam, suggesting that the relatedness is null for distances over 100 m (Fig. 2).

Discussion

Detrimental effects of habitat fragmentation on species survival are largely dependent on gene flow between spatially isolated populations (Lander et al. 2010). Genetic

Table 3 Indirect estimations. Results from mixed mating system, sibling pair model, KinDist and TwoGener pollen structure model analysis

Model	Parameter	Estimate
Mixed mating (MLTR)	Multilocus outcrossing rate (t_m)	1.000 (0.000)
	Single locus outcrossing rate (t_s)	0.951 (0.018) (0.916/0.986)
	Biparental inbreeding ($t_m - t_s$)	0.049 (0.018)* (0.014/0.0843)
	Correlated outcrosing rate (r_t)	0.107 (0.000)*
	Correlated paternity (r_p)	
	Global	0.166 (0.028) (0.111/0.221)
	Among fruits	0.098 (0.029) (0.041/0.155)
	Within fruits	0.638 (0.056) (0.528/0.748)
	Effective number of pollen donor under sibling pair model (N_{ep})	5.9
Pollen pool structure (KinDist/TwoGener)	Φ_{FT} (pollen structure parameter)	0.10473
	Φ'_{FT}	0.09771
	Efective number of pollen donor under pollen structure model (N_{ep})	5.11
	Average distance of pollination (δ)	
	KinDist	
	Normal	5.36 m
	Exponential	11.96 m
	TwoGener	
	Normal	13.74[a]/13.81[b]/23.74 m[c]
	Exponential	15.5[a]/15.58[b]/30.92 m[c]
	Effective neighborhood pollination area (A_{EP})	0.150 ha

Number in parenthesis for the MLTR output corresponds to standard error of estimates under 10,000 bootstraps

[a] Distance estimated obtained from the global Φ'_{FT} value

[b] Distance estimated obtained from the pairwise Φ_{FT} values assuming the density equals the census density (de)

[c] Distance estimated obtained from the joint estimate of the effective de and pollen dispersal variance (σ^2)

a, b and c estimates were obtained using TwoGener

Table 4 Genetic structure of open pollinated families from *P. alba* trees

Family	N	r_p	N_{ep}	θ_R	$N_{ev\,(RI)}$	θ_{QG}	$N_{ev\,(QG)}$	θ_N	$N_{ev\,(N)}$	m_R	m_{QG}	m_N
1	12	0.498	2.010	0.068	5.102	0.196	2.323	0.236	1.923	19.60	43.05	51.99
2	12	0.198	5.042	0.043	6.699	0.288	1.667	0.194	2.251	14.93	59.99	44.43
3	12	0.273	3.660	0.060	5.527	0.216	2.136	0.295	1.594	18.09	46.82	62.74
4	12	0.109	9.153	0.011	10.777	0.153	2.844	0.029	6.983	9.28	35.17	14.32
5	12	0.190	5.253	0.112	3.611	0.170	2.604	0.235	1.927	27.70	38.40	51.88
6	12	0.023	42.753	0.030	7.886	0.240	1.957	0.122	3.204	12.68	51.09	31.21
7	12	0.122	8.193	0.037	7.166	−0.006	16.761	0.122	3.201	13.95	5.97	31.24
8	12	0.024	42.017	0.025	8.517	0.238	1.967	0.141	2.879	11.74	50.84	34.73
9	12	0.132	7.581	0.018	9.476	0.088	4.280	0.062	4.905	10.55	23.37	20.39
10	12	−0.029	−34.223	0.074	4.825	0.147	2.935	0.131	3.037	20.73	34.07	32.93
11	12	0.225	4.438	0.013	10.378	0.094	4.101	0.010	9.085	9.64	24.38	11.01
12	12	0.206	4.861	0.080	4.589	0.160	2.745	0.119	3.259	21.79	36.44	30.68
13	12	0.236	4.238	0.062	5.415	0.208	2.207	0.182	2.370	18.46	45.31	42.20
14	12	0.258	3.881	0.105	3.780	0.153	2.836	0.290	1.617	26.45	35.26	61.86
15	12	0.256	3.910	0.056	5.720	0.209	2.199	0.202	2.181	17.48	45.47	45.84
16	12	0.313	3.194	0.054	5.833	0.137	3.098	0.262	1.763	17.14	32.27	56.72
Mean		0.190	7.248	0.053	8.102	0.168	2.837	0.165	3.261	16.89	37.99	39.01
Upper CI$_{95}$		−0.061	−25.96	−0.007	3.693	0.029	−4.216	−0.007	−0.817	5.96	12.57	7.79
Lower CI$_{95}$		0.440	40.45	0.113	12.511	0.307	9.890	0.336	7.340	27.81	63.42	70.23

*N* sample size, N_{ep} effective number of pollen donors under sibling pair model, r_p paternity correlation, θ coancestry coefficient within families, N_{ev} variance effective size of open pollinated families, *m* number of seed trees necessary for seed collection aiming to retain the effective population size of 100±95%, CI_{95} calculated by mean±1.96 SE, being SE the standard error. *R*, *QG*, and *N* sub-indices indicates Ritland, Queller and Goodnight and Nason, respectively

isolation would result in inbreeding depression, genetic drift, and loss of adaptive potential (Young et al. 1996; Lowe et al. 2005). However, due to their long life cycle, demography, ecology, and reproductive biology, the response of forest tree species to fragmentation may exhibit certain peculiarities in comparison with the expectations according to

classical theoretical models (Kramer et al. 2008). Information on the mating systems and parameters of pollen dispersal is paramount to predict the consequence of fragmentation on native forest species.

A fragmented population of the "algarrobo blanco", *P. alba* was analyzed in order to study population structure, pollen

Fig. 2 Plot showing the relationship between Ritland (*R*), Queller and Goodnight (*QG*), and Nason (*N*) relatedness estimates and geographic distance fitted by a generalized additive model (gam)
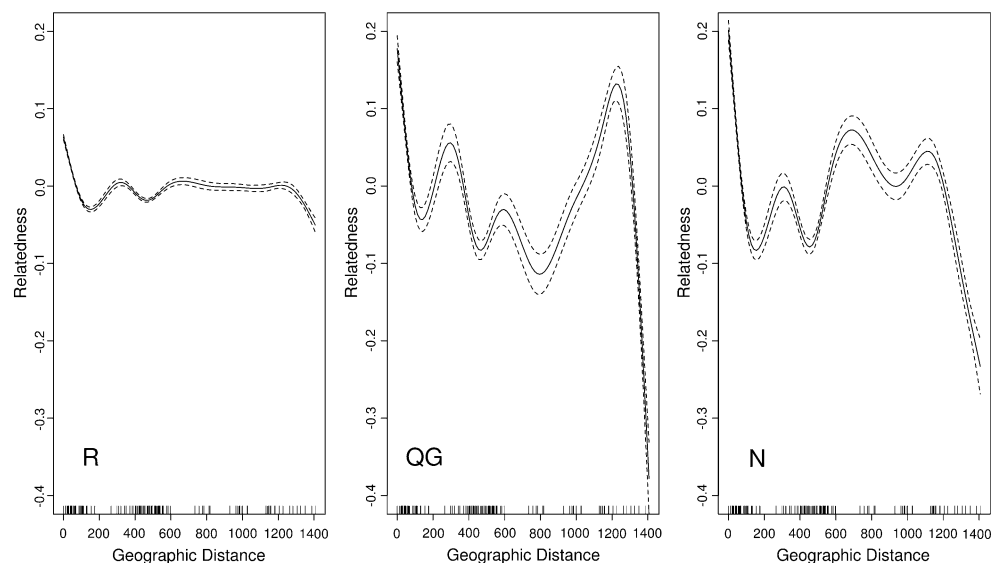
dispersal distance, and mating system parameters. A significant heterozygote excess ($F_{IS}<0$) was found within families suggesting that the pollen cloud differs genetically from the corresponding mother tree. This heterozygote excess suggests high outcrossing rates (see below) and would contribute to reduce the effect of fragmentation on the reduction of genetic variability and increase of inbreeding.

The effect of fragmentation on the distribution of genetic diversity was evaluated by a hierarchical population structure analysis. The differentiation among the three zones recognized in the population ($F_{zone/total}$) was nonsignificant, whereas the differentiation among families (within zones; $F_{family/zone}$) and pods (within families; $F_{pod/family}$) were highly significant. Within pods, a highly significant heterozygote excess was observed ($F_{individual/pod}$). This result indicates that although pollen dispersal insures that allele frequencies differ between mother plants and pollen donors producing negative F_{IS} and $F_{pod/family}$ estimates, pollen dispersal is not enough as to prevent the differentiation among families within zones. The lack of differentiation among zones is evidence that the genetic variation preserved within each zone is not different up to now from that of the original unfragmented population, most probably due to the fact that fragmentation is a process relatively recent in terms of number of generations, considering that average life span of *P. alba* is over 100 years.

Further than the evidence from heterozygotes excess within progenies, the relative importance of family structure and outcrossing can be weighed by estimating mating system parameters. The population analyzed is characterized by a complete outcrossing ($t_m=1$). The low (roughly 5%), but significant, degree of biparental inbreeding (t_m-t_s) observed may be caused by the seed dispersion system. *Prosopis* species have an endozoic seed dispersal system mediated by native small herbivorous and livestock (Mares et al. 1977; Hafez 1962). Although livestock are able to disperse seeds over larger distances (up to 4–6 km) in comparison with small rodents (approximately 50 m; Reynolds 1954, Keys 1993; Keys and Smith 1994), in all cases herbivorous tend to eat as many pods as they can from each single tree, and scarified seeds after passing through the animal gut are deposited in dungs forming groups of related individuals (Keys 1993; Bessega et al. 2000). As an example in *Prosopis glandulosa*, 75% of the cattle dung pats contained an average of 4.2 seedlings per pat (Brown and Archer 1987). Therefore, a relatively high relatedness is expected for neighbor trees, which are most probably involved in reciprocal matings.

The number of pollen donors siring each mother plant was near six in average (the number varies depending on the estimation method), but there is a great variation occurring among mother plants. Difference in the number of pollen donors per mother plant may be associated to demographic causes. In low-density areas, a lower number of fathers per family is expected respect to more dense regions. This trend was not observed in the present study if the N_{ep} is compared among zones 1–3. Another cause for differences in N_{ep} estimates might be the family structure. If the trees in the neighborhood are highly related, underestimation of the actual number of pollen donors might occur because of the increased difficulty of differentiating pollen donors with similar genotypes.

The joint analysis of outcrossing rates, correlation of outcrossed paternity within r_p at different levels (global, among, and within fruits), the correlation of outcrossing rate within progeny arrays (r_t) and average distance of pollination yielded several conclusions.

First, the proportion of full sibs is about 64% for seeds proceeding from the same pod whereas this proportion is only 10% for seeds from different pods. This may be consequence of the pollinator insect behavior and inflorescence anatomy. The pollinator species associated to *Prosopis* (*A. mellifera*, Meliponini sp., *Xylocopa ordinaria*, *Xylocopa splendidula*, *Caupolicana ruficollis*, *Caupolicana mendocina*, *Colletini* sp.; Genisse et al. 1990) usually focus their foraging efforts on densely flowering plants and limit their interplant movement to nearest neighbors (Levin and Kerster 1974). Consequently, each pollination event involves pollen from only one or a few pollen donors fertilizing each inflorescence, favoring the occurrence of full sibs in each pod. By contrast, different inflorescences would be fertilized by different insects determining that seeds from different pods tend to be half sibs.

Second, the average distance of pollination estimated was from 5 to 30 m depending of the method used. This range agrees with the field observation by Palacios (personal communication) that trees isolated by 50 m from other trees do not produce fruits. Pairwise relatedness was also observed to decrease steeply with distance. According to Dick et al. (2008), a fine-scale spatial genetic structure characterized by a decrease of relatedness between individuals with distance is often interpreted as limited seed dispersal. Under scenarios of long distance seed dispersal with limited pollen flow, there should be no structure because closely related individuals (FS or HS) will be broadly spaced. On the other hand, restricted seed dispersal is always expected to produce a fine-scale genetic structure even if pollen movement is random and long distance because sibs will be aggregated. In *P. alba*, limited pollen and seed dispersal would account for fine-scale genetic structure.

Third, limited pollen dispersal can produce an increase in the coancestry coefficients within progenies reducing the variance effective population size and a small neighborhood pollination area. The estimated effective neighborhood area of pollination was small, representing a 22 m radius circle around each maternal tree. Coancestry coefficient within

families estimated by Ritland (1996; $\theta_{xy}=0.05$) was much lower than the minimum expected for sibs families ($\theta_{xy}=$ 0.125), and the corresponding variance effective population size of open pollinated families was $N_{ev}=8.10$, twice as large as the maximum theoretically expected N_{ev} (4). By contrast, Queller and Goodnight (1989) and Nason (Loiselle et al. 1995) methods produced estimates much closer to the expected. The corresponding values of θ_{xy} (0.168 and 0.165) are very similar to each other and agree with the observation of a significant proportion of full sibs within progenies, giving a more conservative N_{ev} (2.83 and 3.26). With our sample, no significant differences were observed between Nason and Queller and Goodnight methods in spite of the fact that the latter assumes HW equilibrium and the former does not.

Fourth, the results here presented have important implications for seed collection strategies for ex situ conservation, tree breeding, and reforestation of *P. alba*. The spatial genetic structure at short distances indicates that relatedness at distances further than 100 m is negligible. This means that this distance would be enough to avoid duplications in the sampling due to relatedness among harvested seed trees. Seeds for ex situ conservation or tree breeding programs should be collected from trees at least 100 m apart would reduce relatedness among harvested seed trees. An additional caution to be considered is that seeds collected from each mother plant should be taken from different pods to reduce the proportion of full sibs within progeny arrays. Finally, considering that each progeny array represents approximately three non-inbred individuals, the sample aiming to retain an effective population size of 100 should include at least 38 seed trees.

Finally, according to the present results, both pollen and seed dispersal in *P. alba* would be limited, however, the differentiation among zones was relatively low. Previous works on *P. alba* geographically distant populations (Ferreyra et al. 2004, 2007, 2010) have also shown that most genetic variation occurs within populations and that the differentiation among populations and regions is rather low. The ability to maintain high genetic coherence in spite of limited dispersal ability may be associated with the long life span of *Prosopis* species together with large populations' sizes and continuous distribution that characterized forests in the Chaqueña Region before the habitat fragmentation produced by human activities. Current patchy distribution may still not show the expected effects of drift and inbreeding because of the long life span of *P. alba* trees. In relict populations, 300-year age trees can be found, which means that, in terms of number of generations, habitat fragmentation is too recent as to have produced statistically significant effects. Also, if patches were separated by shorter distances than the maximum that can be reached either by pollen or seed dispersal vectors, these semi-isolated 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 model is consistent with the hypothesis that gene flow, following a stepping stone model with scattered trees or small patches, would delay the genetic effects of fragmentation in *P. alba*. Consequently, a strategy to preserve in situ diversity of *P. alba* compatible with a rational use of resources would imply the conservation of patches connected by pollinators and seed disperser in agroforestry systems combined with livestock breeding.

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