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ORIGINAL ARTICLE

Comparison of mating system parameters and genetic structure in three natural scenarios of *Acacia visco* (Leguminosae, Mimosoideae)

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Abstract *Acacia visco* is a native South American tree species that has been extensively used for ornamental purposes and in carpentry, bodywork and parquet due to the hardness and durability of its wood. Little is known about genetic diversity and mating system of *A. visco*. The main aims of this study were to (1) estimate outcrossing rates in natural Argentinean populations using AFLP markers, (2) test for any difference in mating patterns among a large a patchy and relict population, and (3) compare the mating system of *A. visco* with other *Acacia* species. The three primer pairs used in the AFLP analysis revealed a total of 569 variable loci. Most genetic variation was observed among individuals within families (61.2 %). The estimate of multilocus outcrossing rate (t_m) was high ($\geq$0.971) in all populations. Average pairwise coancestry between progenies within families for each population ranged from 0.082 to 0.105 or from 0.125 to 0.136, depending on the method θ was estimated. In the three populations studied, the progenies of open pollination were constituted mainly for half-sibs (94.3 %). This work shows a similar mating system in all populations of *A. visco* in spite of their size differences, hypothesizing that the entire species has a similar mating system of outcrossing preferential. Considering the results obtained here where a high percentage of individuals were half-sibs, sampling large numbers of pods from individual trees for *ex situ* conservation will result in a genetically diverse sample as a consequence of high outcrossing rates.

Keywords *Acacia visco* · AFLP · Mating system parameters · Genetic structure · Different natural scenarios

Introduction

The understanding of the mating system of a species is of fundamental importance for genetic improvement and conservation programmes because it allows the outlining of strategies that optimize the sampling of genetic variability and the adoption of genetic-statistical models appropriate for the estimation of genetic parameters. Information about the mating system, diversity and genetic structure, as well as the spatial distribution of genotypes within populations, is important for the establishment of strategies aimed at the effective conservation of any species (Freitas et al. 2004).

The mating system and the genetic structure of a species are directly related to its ecology and genetics (Loveless et al. 1998). In small, recently fragmented populations a departure from mating patterns found in larger undisturbed populations can bring about genetic decline and population extinction (Barrett and Kohn 1991; DeMauro 1993; Frankham 1995; Newman and Pilson 1997). Consequently, an accurate characterization of mating is important for the conservation of the evolutionary potential of natural populations because altered patterns of mating, such as increased inbreeding, may result in future genetic decline

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and population extinction (Frankham 1995; Saccheri et al. 1998). 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). 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. By contrast, 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).

Rangeland deterioration in Argentina started after a period known as the "conquest of the desert". Although human population always remained very low, the new settlers began a livestock production industry across the landscape based on grazing of natural vegetation, with little knowledge or no consideration about environmental impact or ecosystem management techniques. The wildland ecosystems proved to be extremely fragile and easily injured by abusive use; all rangelands in Argentina are currently experiencing some form of deterioration or desertification. Contributing factors to this situation have been deforestation, uncontrolled wood harvesting for fuels, livestock overstocking, and in some areas ploughing of non-arable lands (Fernández and Busso 1997).

Acacia visco is a native South American tree species that has been extensively used for ornamental purposes and in carpentry, bodywork and parquet due to the hardness and durability of its wood, but its poor diameter limits its use in this sense. However, it is mainly used for post and as fuel (Tortorelli 1956). Methanol extract of leafs and bark of *A. visco* has been shown to have short-term and long-term anti-inflammatory effects in mice (Pedernera et al. 2007). Among the class of compounds characterized from *A. visco* leaves, the triterpenoid lupeol, α-amyrin and β-amyrin may be mainly responsible for the pharmacological activities (Pedernera et al. 2010).

Little is known about genetic diversity and mating system of *A. visco*. Mating system analyses in other species of *Acacia* have shown high levels of outcrossing, sometimes together with self-incompatibility systems, and some species are able to form hybrid complexes (Ali and Qaiser 1980; Bernhardt et al. 1984; Kenrick and Knox 1985; Kenrick et al. 1986; Moran et al. 1989; Sedgley et al. 1992; Casiva et al. 2004; Pometti et al. 2011).

Proper estimates of the outcrossing rates are often needed for planning breeding programmes (Ritland and Jain 1981), conservation and management of tropical trees (Loveless 1992). The mating system may be sensitive to plant density and population size, type of pollination vector and abundance, flower colour, size of floral display and anther-stigma separation. As such it is reasonable to expect that outcrossing rates could vary extensively both spatially within and between populations, and temporally within a single population (Muluvi et al. 2004). Traditional methods used for the measurement of mating systems have been based on the analysis of floral morphology, greenhouse crossing experiments, and (where appropriate) the observation of pollinator behaviour (Clegg 1980). The practical use of phenotypic markers in trees is limited by a number of factors such as long time required for progeny to reach maturity for the markers to be scored and lack of consistency between phenotypic markers and outcrossing (Gjuric and Smith 1996). For this reason biochemical and molecular markers have been used extensively to estimate outcrossing rates. Codominant loci are usually preferred because they provide more information per locus than dominant ones. This is particularly relevant for applications that require genotype discrimination, as in the case of outcrossing-rate estimation (Gaiotto et al. 1997). However, Ritland and Jain (1981) demonstrated through simulation studies that the limitation associated with dominant loci could be readily overcome by multilocus estimation using a large number of dominant markers with intermediate gene frequencies. Dominant DNA-based markers such as RAPDs (Gjuric and Smith 1996) and AFLPs (Gaiotto et al. 1997; Krauss 2000; Freitas et al. 2004; Muluvi et al. 2004) have been used to estimate outcrossing rates as they are able to produce very large number of informative loci without previous genetic information about the genetic system in natural populations of tree species.

In this work, Argentinean populations of *A. visco* were studied and the main aims of this study were (1) to estimate outcrossing rates in natural populations of this species using AFLP markers, (2) to test for any difference in mating patterns among a large population, a patchy population and a relict population, and (3) to compare the mating system of *A. visco* with other *Acacia* species.

Materials and methods

Study species

Acacia visco is a leguminous tree, without spines or thorny stipules; it can grow up to 15 m and 50 cm of diameter at breast height (dbh). On its specific form, it has a globose top and semi-perennial foliage. Its flowers are white, not scented and its fruit is a typical legume, of 10–17 cm long and 1.5–2.5 cm wide. Flowers bloom from October to December and the fruits ripen between February and April (Cialdella 1984). Chromosome numbers for this species has been recorded as $2n = 26$ (Zanín et al. 1998) and $2n = 26, 52$ (Covas and Schnack 1946). Pollen is shed in polyads consisting of 16 grains (Cialdella 1984). Seeds are

found in numbers of 8–12 per fruit (Cialdella 1984). *A. visco* is found as native species in Chile, Bolivia and Argentina. In Argentina grows in the provinces of Salta, Jujuy, Tucumán, Catamarca, La Rioja, San Juan and San Luis.

Study sites and sample collection

Three populations of *A. visco* were collected in Northwest Argentina (Table 1). In the case of Cachi and Tapia sampling methodology was that of Vilardi et al. (1988) and Saidman and Vilardi (1993). Approximately 50 seed pods were collected from 11 to 12 mother trees per population that were separated from each other by more than 50 m. The Cachi population, considered the largest population, was situated in the Calchaqui Valley on a steep slope parallel to the west bank of Calchaqui River at about 2,350 m o.s.l. Its size was about 100 trees from which only those with fruits and accessible to be collected were sampled. Tapia, considered the patchy population, was situated on the Northeast slope of San Javier Hill at about 700 m o.s.l. This area is highly degraded by agricultural and livestock activities. This population exhibits a patchy distribution and the sampled site embraces about 20 trees, from which only those with fruits were sampled. Posta de los Hornillos was a relict population situated in the Quebrada de Humahuaca, at about 2,400 m o.s.l., on the west bank of Grande River. It was composed of only a few trees. In this case all trees with fruits were sampled, collecting about 50 pods per tree. The number of seeds analysed per mother plant in all populations was between eight and twenty sampled at random within each family array.

Representative vouchers of each population are deposited at the herbarium SI, Instituto de Botánica Darwinion, San Isidro, Buenos Aires, Argentina.

AFLP methods and data analysis

DNA extraction

Cotiledons were ground to a fine powder in liquid nitrogen and then placed in a microtube. The DNeasy Plant kit (QIAGEN Inc., Valencia, CA, USA) was used for DNA extraction following the manufacturer's instructions. DNA was stored at −20 °C.

The AFLP assay was performed as described by Vos et al. (1995), but with a slight modification. Three selective primers were combined as follows: E + ACA/M + CTT (C1), E + AGG/M + CAG (C3) and E + AAC/M + CAA (C4). In all cases, primers M + 3 were labelled with a fluorescent dye 6-FAM. A complete technical replicate was run for a subset of 24 randomly selected individuals (about 10 % of the total) from the restriction-ligation stage. Negative controls were carried through from the restriction-ligation stage to test for systematic contamination and dye blobs. PCR products were electrophoresed in an ABI313XL (HITACHI) automated DNA sequencer and automatically sized with the size standard GS500 LIZ using GENEMAPPER ver 3.7 (Applied Biosystems). The size of AFLP bands (bins) scored ranged from 50 to 400 bp. All bins were set to a width of two bases pair, and those fragments with peak heights below 50 relative fluorescence units (RFU) were assumed to represent instrument noise and were not scored. To ensure that bin positions were assigned accurately, all bins were then checked manually. Any bins possessing fragments that overlapped with adjacent bins were removed. As well, we adjusted bins assigned off-centre of any peak distributions. Finally, all AFLP profiles were checked manually to ensure successful amplification and were either re-run or removed from analysis if the fingerprint failed to amplify or appeared to possess many unique fragments.

Statistical analyses

Genetic structure

The distribution of genetic variation was analysed by 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: A Language and Environment for Statistical Computing 2011). The levels considered in the hierarchical analysis were populations, families within populations and individuals as nested levels. Signification for variance components and *F* statistics were obtained by 2,000

Table 1 Populations of *Acacia visco* sampled in this study

Population	Population code	Latitude (°S)	Longitude (°W)	Province	Number of adult trees sampled	Number of seeds per tree analyzed
Cachi	CA	25°10′0.8″	66°10′59.3″	Salta	11	10–20
Posta de los Hornillos	PH	23°39′15″	65°25′52″	Jujuy	5	8–10
Tapia	TA	26°36′31.6″	65°20′35.4″	Tucuman	12	8

bootstraps. Also, genetic structure within each population was evaluated by means of analysis of variance components.

Mating system

Estimation of multilocus (t_m) and single locus (t_s) outcrossing rates, correlation of outcrossed paternity (r_p), correlation of t_m among progeny arrays (r_t), and fixation indices of maternal parents (F_M) were calculated. The expectation–maximization (EM) procedure that bound outcrossing rates between 0 and 1 was used for the iterations, and default settings were used with initial values of outcrossing rate $t = 0.9$, parental inbreeding $F_M = 0.1$, and paternity correlation $r_p = 0.1$. The standard errors for these parameters were calculated from 1,000 bootstraps with resampling of individuals within families. Standard error was used to determine weather mating parameters were significantly lower than one or greater than zero. All these estimates were obtained with the software MLTR 3.4 (Ritland 2009). This programme 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. Biparental inbreeding was estimated following Ritland (1990) as $t_m - t_s$. The effective number of pollen donors over all mother trees (N_{ep}) was estimated under sibling pair model (Ritland 1989) by the expression $N_{ep} = 1/r_p$. The proportion of selfing progeny (P_{ss}), half-sibs (P_{hs}), full-sibs (P_{fs}) and self-half-sibs (P_{shs}) was calculated for each population as $P_{ss} = s^2, P_{hs} = t_m^2(1 - r_{p(m)})$, $P_{fs} = t_m^2 r_{p(m)}$ and $P_{shs} = 2st_m$ (Sebben 2006).

The expected inbreeding coefficient in Wright's (1951) equilibrium of seedlings within each population was calculated from the estimated outcrossing rate as

$$F_e = \frac{1 - t_m}{1 + t_m}$$

where t_m is the multi-locus outcrossing rate (Ritland 1983; Lee et al. 2000).

Average coancestry coefficient (θ)

We obtained Ritland (1996) pairwise relatedness estimator (r_{xy}) as $r_{xy} = 0.25(1 + F_p)[4s + (t_m^2 + t_m r_t s)(1 + r_{p(m)})]$, where F_p is the inbreeding coefficient in the parental generation, t_m is the multi-locus outcrossing rate, s is the selfing rate, r_t is the correlation of t_m among progeny arrays and $r_{p(m)}$ is the multilocus paternity correlation. From this estimation, we calculated the average coancestry coefficient (θ_{xy}) within progenies 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). Within family coancestry coefficient (θ_{wf}) was calculated for each family as the average of θ_{xy} for individuals belonging to that family. The θ_{wf} were averaged over families within each population to estimate (θ_{wfp}) and for the whole sample (θ_{wft}). Between families within population (θ_{bfp}) and between populations (θ_{bp}) coancestry coefficients were also calculated.

The variance effective size (N_{ev}) for each population was calculated by (Cockerham 1969)

$$N_{ev} = \frac{0.5}{\theta_{wfp}\left(\frac{n-1}{n}\right) + \frac{1+F_e}{2n}}$$

where n is the average family array size.

The number of seed trees ($\hat{m}$) 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 programme ($N_{e(reference)}$) and the average N_{ev} (Sebben 2006):

$$\hat{m} = \frac{N_{e(reference)}}{\bar{N}_{ev}}.$$

Pollen and ovule allele frequencies were estimated for each locus using the expectation–maximization method. Differences between these frequencies were tested by heterogeneity chi- square test estimated as $\chi^2 = 2NF_{ST}(a-1), df = (a-1)(s-1)$, where N is the number of seedlings examined, a is the number of alleles at the locus considered (in all cases is 2, dominant and recessive allele), s is the number of groups (in our case 2, pollen and ovules), and F_{ST} is the Wright's (1951) measure of the genetic differentiation between pollen and ovule pools (Workman and Niswander 1970; Murawski and Hamrick 1992; Hall et al. 1994). Significance was corrected for multiple comparisons by the method of Benjamini and Hochberg (1995).

Results

The three primer pairs used in the AFLP analysis revealed a total of 569 variable loci. For population structure analysis all these loci were included. As according to Ritland and Jain (1981), alleles with an intermediate or close to intermediate frequency (0.5) are more appropriate for the estimation of multilocus outcrossing rates because they allow a better distinction between plants generated by outcrossing events and those obtained by self-fertilization, only those loci with allele frequencies between 0.4 and 0.6 were selected for the mating system study. Following this criterion, the number of loci included in this analysis was

86 in Cachi, 37 in Posta de los Hornillos and 43 in Tapia populations.

Genetic structure

The hierarchical analysis (Table 2) indicated that the genetic differentiation among populations and among families within populations were highly significant. Expressed as percentages, most of genetic variation was observed among individuals within families (61.2 %), whereas the differentiation among families within populations (18.8 %) and among populations (20.1 %) was similar (Table 2).

Table 2 Hierarchical analysis of components of variance

Source of variation	Var (%)	*P*
Among populations	20.1	5.10^{-4}
Among families within populations	18.8	5.10^{-4}
Among individuals within families (error)	61.2	

The analysis of variance components (Table 3) indicated that genetic diversity among families represented from 18.3 to 26.4 % of total variation within population, in Posta de los Hornillos and Cachi, respectively. This component was highly significant in all cases ($P \leq 0.01$) (Table 3).

Mating system

The estimate of multilocus outcrossing rate (t_m) was high ($\geq$0.971) in all populations. These results indicated that the three populations of *A. visco* studied are mostly outcrossers. The estimated biparental inbreeding rate ($t_m - t_s$) was low in all populations, but significantly different from zero, considering the standard error of the mean, suggesting some tendency to mating between relatives (Table 3). The correlation of t_m within progeny arrays (r_t) was low in all populations, suggesting no differences in outcrossing rates among mother plants. The correlation of outcrossed paternity (r_p) was not significantly higher than zero for any

Table 3 Estimates of mating system parameters in three populations of *A. visco*

Parameters	CA	PH	TA	Species level
Number of progeny	120	48	96	264
t_m	0.992 (0.007)	0.971 (0.024)	1.000 (0.000)	0.988 (0.010)
t_s	0.868 (0.010)	0.847 (0.031)	0.895 (0.008)	0.870 (0.016)
$t_m - t_s$	0.124 (0.012)	0.124 (0.030)	0.105 (0.008)	0.118 (0.017)
r_t	0.040 (0.008)	0.034 (0.011)	0.110 (0.000)	0.061 (0.006)
r_p	0.087 (0.028)	0.000 (0.013)	0.014 (0.018)	0.034 (0.020)
F_e	0.004	0.015	0.000	0.006
F_M	0.001 (0.000)	0.000 (0.000)	0.002 (0.000)	0.001 (0.000)
N_{ep}	11.494	–	71.429	41.462
$\theta_{wfp(N)}$	0.105 (0.044)	0.103 (0.026)	0.082 (0.023)	0.095 (0.032)
$\theta_{bfp(N)}$	0.027 (0.047)	0.066 (0.025)	0.041 (0.023)	0.045 (0.032)
$\theta_{wfp(R)}$	0.136	0.130	0.125	0.130
$N_{ev(N)}$	3.555	3.448	3.745	3.583
$N_{ev(R)}$	3.676	3.846	4.000	3.846
$\hat{m}_{(N)}$	0.008	0.029	0.000	0.012
$\hat{m}_{(R)}$	27.203	26.000	25.000	26.000
P_{hs}	0.898	0.943	0.986	0.943
P_{ss}	0.000	0.001	0.000	0.000
P_{fs}	0.086	0.000	0.014	0.033
P_{shs}	0.016	0.056	0.000	0.024
Var among families (%)	26.4 [24.0–28.7]**	18.3 [15.6–20.9]**	20.2 [18.1–22.5]**	38.8 [36.7–40.8]**

The indices r_t and r_p denote correlation of t_m within progeny array and correlation of outcrossing paternity, respectively

Standard errors between brackets. 95 % CI between square brackets

N number of progeny studied, θ_{wfp} average coancestry within families within populations, θ_{bfp} average coancestry between families within populations, N_{ev} variance effective size of open pollinated families, $\hat{m}$ number of seed—trees necessary for seed collection aiming to retain the effective population size of 100; P_{ss}: $1 - t_m$, P_{hs}: $t_m(1 - r_p)$; P_{fs}: $t_m\, r_p$. *R* and *N* sub-indices indicate Ritland and Nason, respectively

Multilocus (t_m) and single locus (t_s) outcrossing rates, progeny fixation index (F_e) and maternal fixation index (F_M) for *A. visco* populations estimated from progeny arrays

** $P \leq 0.01$

population. These values suggest a high probability that a random chosen pair of progeny from the same array would be half sibs. This estimate then provided a high value for the effective number of pollen donors (N_{ep}) that ranges between 11.5 and 71.43 donors per maternal tree (Table 3). Since in Posta de los Hornillos $r_p = 0$, N_{ep} cannot be estimated from $1/r_p$. Then the result may be interpreted assuming that all trees present in the population would act as pollen donors.

In all cases, F_e in the seed generation (estimated from t_m) was zero or slightly positive, varying from 0.000 to 0.015, indicating no inbreeding in Cachi and Tapia and a low rate of inbreeding in Posta de los Hornillos (Table 3). For maternal population the average single-locus inbreeding coefficient (F_M) was in all cases similar to that estimated for their progeny (Table 3). In all cases, F_M was near zero, suggesting no Hardy–Weinberg deviations in the populations.

In all populations significant differences were observed for several loci between pollen and ovule allelic frequencies. In Cachi, 40 out of 86 loci showed significant differences, and 36 were significant at matrix level; in Posta de los Hornillos, 14 out of 37 loci were significant at individual level and 11 at matrix level; and in Tapia, 22 out of 43 loci were significant at individual level and 20 at matrix level (see Online supplementary material).

Coancestry, variance effective size and number of seed-trees necessary for seed collection

Average pairwise coancestry between progenies within families obtained by Nason for each population ranged from 0.082 to 0.105 (Table 3) and was lower than the value expected for half-sibs progenies ($\theta = 0.125$). The value estimated from Ritland's (1996) relatedness estimator ($\theta_{wfp(R)}$ from 0.125 to 0.136) was higher and closer to the expected value.

The average coancestry coefficient between families within populations by Nason ranged from 0.027 to 0.066 (Table 3). In average between family coefficient was half of within family estimated one. Finally, average coancestry among all populations by Nason was -0.025 ± 0.022 (not different from zero) indicating that individuals from different populations are not related.

The average variance effective size estimated from the average coancestry coefficients within families were close to that expected for progenies of an ideal population with high number of pollen donors per mother plant (where $N_{ev} = 4$), ranging from 3.45 to 3.75 by Nason, and from 3.68 to 4 from Ritland's (1996). The number of seed-trees necessary for seed collection aiming to retain an effective population size of 100 was estimated at the minimum of 25–29 depending on the method θ was estimated.

In the case of the relict population of Posta de los Hornillos where we could sample only the five fructifying trees present, the effective sampling size corresponding to these five seed-trees was $N_{e(reference)} = \hat{m}\, N_{ev} \approx 17$.

The progenies of open pollination of all populations were constituted mainly for half-sibs. In the case of Cachi, they represented 89.8 % (P_{hs}) of the family array, 1.6 % were self-half-sibs (P_{shs}), 8.6 % were full sibs (P_{fs}), and self-sibs were absent. In Posta de los Hornillos, 94.3 % of the progenies were half-sibs, 5.6 % were self-half-sibs (P_{shs}), 0.1 % self-sibs, and full-sibs were absent. In Tapia, 98.6 % were half-sibs whereas the remaining 1.4 % of the progenies were full-sibs with absence of self-sibs and self-half-sibs (Table 3).

Discussion

The use of three primer combinations allowed detecting a high number (569) of variable AFLP loci in *Acacia visco*. This number is much higher than those obtained in similar studies conducted in other shrub and tree species, such as *Eucalyptus urophylla* (Gaiotto et al. 1997), *Persoonia mollis* (Krauss 2000), *Myracrodruon urundeuva* (Freitas et al. 2004) and *Moringa oleifera* (Muluvi et al. 2004). The availability of many polymorphic loci provides the opportunity of selecting the most suitable ones according to the kind of analysis to be conducted. For example, Ritland and Jain (1981), describing the mixed-mating model showed through simulation studies that more than five dominant marker loci with $P = 0.5$ are necessary to reach as low a variance on the estimate of outcrossing rate as that obtained with two co-dominant triallelic marker loci with $P = 0.33$ and that dominant loci with allele frequencies close to 0.5 should be preferred to get more accurate estimates of mating system parameters.

The information on population structure and mating system is paramount for developing strategies for rational use and conservation programmes of native species as they contribute to define the breeding units in the wild. The hierarchical analysis of population structure in this work showed that the main component of genetic variance corresponds to the variation among individuals within families. This result is compatible with the contribution of many pollen donors to each progeny array and low rate of selfing. Total variation within populations (that is the sum of variation within families and among families) accounts for 80 % of total population variation. This result is consistent with the observation of a large proportion of genetic variation within populations in other *Acacia* species, including the African *A. senegal* (86–91 %) (Chiveu Chemulanga et al. 2008; Omondi et al. 2010) and the South American *A. caven* (68.5 %) (Pometti et al. 2012).

The differentiation among populations and among families within populations were highly significant and similar to each other in magnitude. A sample with purposes of germplasm conservation should then balance the number of populations and families within each population.

Although the number of populations studied in this species is low, there is a trend suggesting that the among families variance is associated with population size, as it is highest in Cachi and the lowest in the relict population of Posta de los Hornillos.

The distribution of genetic variability within and among populations and the inbreeding rates in plants are highly dependent on the mating system. The estimates of mating system parameters in *A. visco* obtained in the present paper may be compared with those obtained in other natural populations of acacias with different molecular markers (Table 4) as well as for other tropical tree species (Murawski and Hamrick 1991; Freitas et al. 2004; Sobierajski et al. 2006). The general trend observed is that *Acacia* species are predominantly outcrossers, with high t_m estimates. The estimates of t_m for *A. visco* obtained here were high, indicating a predominantly outcrossing system. Self-incompatibility is considered an important outbreeding mechanism in *Acacia* (Kenrick and Knox 1989), with many species being highly self-incompatible (Fleming et al. 2007).The presence of only low amounts of inbreeding in a number of *Acacia* species has been attributed to the self-incompatibility system (Bernhardt et al. 1984).The exception seems to be the tetraploid *A. nilotica* subsp. *leiocarpa* where t_m is highly significantly <1; similar results were obtained in other tetraploid species *A. tortilis* (Olng'otie 1991, cited by Mandal et al. 1994) where t_m estimate was also low (0.35), but not in *A. nilotica* subsp. *kraussiana*.

Inferences about inbreeding other than selfing (e.g., biparental mating) can be made from the comparison between multi-locus and average single-locus outcrossing rates. Single-locus outcrossing rates are expected to be biased downward by any inbreeding in addition to selfing; thus the mean of such single-locus outcrossing rate is expected to be lower than the multi-locus outcrossing rate (which is less affected by violations of the mixed-mating model assumptions) when mating among relatives occurs (Lee et al. 2000). Comparison of these two values in *A. visco* ($t_m - t_s$) indicated that this expectation was generally

Table 4 Comparison among mating system parameters of *Acacia* species

	Species	Marker	t_m	t_s	$t_m - t_s$	r_t	r_p	F_M	Reference
American species	*A. caven* var *caven*	IE	0.987–1.000	0.981–1.000	0.006–0.000	0.047–0.109	0.047–0.101	0.000–0.016	Pometti et al. (2011)
	A. caven var *dehiscens*	IE	0.954–0.957	0.926–0.954	0.013–0.031	0.121–0.294	0.055–0.068	0.000	Pometti et al. (2011)
	A. aroma	IE	0.914–1.000	0.719–0.963	0.037–0.195	0.110–0.147	0.363–0.986	(−0.329)–0.000	Casiva et al. (2004)
	A. macracantha	IE	0.977–1.000	0.931–0.999	0.001–0.046	0.110–0.267	0.819–0.931	0.000–0.003	Casiva et al. (2004)
African species	*A. nilotica* subsp. *leiocarpa*	IE	0.384	0.358	NA	NA	NA	NA	Mandal et al. (1994)
	A. nilotica subsp. *kraussiana*	IE	0.983	0.971	NA	NA	NA	NA	Mandal and Ennos (1995)
Australian species	*A. auriculiformis*	IE	0.93	0.88	NA	NA	NA	0.098	Moran et al. (1989)
	A. crassicarpa	IE	0.99	1.01	NA	NA	NA	0.037	Moran et al. (1989)
	A. melanoxylon	IE	0.86–0.88	NA	NA	NA	NA	NA	Muona et al. (1991)
	A. saligna subsp. *saligna*	SSR	0.98	NA	NA	NA	0.234	NA	Millar et al. (2008)
	A. anfractuosa	IE	0.85–0.89	0.81–0.89	0.01–0.05	0.08–0.12	0.06–0.15	(−0.49)–(−0.33)	Coates et al. (2006)
	A. sciophanes	IE	0.61	0.54	0.07	0.15	0.25	–0.44	Coates et al. (2006)

Marker: *IE* = isozymes, *SSR* = microsatellites

factual with a biparental mating rate of 0.105–0.124. This positive difference between t_m and t_s, may be explained by mating among relatives or any other kind of genetic structuring of the populations leading to positive assortative matings (Ritland and Jain 1981; Ennos and Clegg 1982). The biparental inbreeding estimate in *A. visco* was similar to the highest estimates obtained in *A. aroma* (Table 4). The correlation of t_m within progeny arrays (r_t) was highly variable among species and also among populations within species (Table 4). In *A. visco* the highest r_t estimate was obtained in Tapia. This may be associated with the patchy distribution of trees in this population. It may be expected that in more dense patches the outcrossing rate might be higher than in patches where trees are more distant from each other. In the first case the distance to which a pollinator should move from one individual tree to a neighbour is lower, favouring crossing between different trees. Correlated paternity (r_p) was highly variable among species, from 0 (in the population PH of *A. visco*) to near 1 in *A. aroma* and *A. macracantha*. This estimate is generally indicative of spatial genetic structuring within natural populations, but the values found in these studies are more likely to be due to the nature of *Acacia* floral biology. In most of acacias, flowers are grouped into complex inflorescences, and pollen from the same paternal tree may regularly fertilize more than one flower within a cluster. In addition, pollen grains are compound in nature occurring as polyads of 4, 8, 16 and 32 grains, and there is correlation between the number of ovules within a flower and the number of pollen grains per polyad across *Acacia* species (Kenrick and Knox 1982). Hence, one polyad may be sufficient to sire all progeny within a pod. A correlation between polyad grain number (pgn) and maximum pod seed number (mpsn) was suggested by Kenrick and Knox (1982), who observed that pgn $\geq$ mpsn and proposed that polyads in *Acacia* could be a mechanism helping to ensure seed set following a single pollination event (a single polyad fertilizing all available ovules at a single ovary). This observation agrees with the fact that *A. visco* has 16 pollen grains per polyad and presents 8–12 seeds per fruit (Cialdella 1984). These aspects of floral biology have been shown to produce high levels of correlated paternity in another *Acacia* species (Muona et al. 1991). In the case of *A. visco,* the correlated paternity increases its value with the population size. The low r_p estimate in PH could be attributed to the little size of this relict population that only allows more sporadic contacts among mother trees. Therefore, population density may affect the rate of correlation of paternity in *A. visco*. In other words, high-density populations have higher paternity correlation than less dense populations. Similar results were found in *Araucaria angustifolia* by several authors (Sousa et al. 2005; Bittencourt and Sebbenn 2008). The r_p estimates in *A. visco* observed in the present work yielded then high numbers of effective pollen donors (N_{ep}), which ranged between 11.5 and 71.43 donors per maternal tree.

Estimation of population outcrossing rates suggested that there was a low level of or no selfing at all. This was further supported by fixation indices for maternal trees ($F_M = 0.000$) and the progeny ($F_e = 0.000$–0.015). Both coefficients indicated the presence of panmixia in the progeny arrays and no Hardy–Weinberg deviations in mother trees for all populations. The values of F_M were similar to those found in other American *Acacia* species as *A. caven*, *A. aroma,* and *A. macracantha* (Table 4).

Significant differences in pollen and ovule allele frequencies were detected for some loci in all populations of *A. visco*. These observed discrepancies in allele frequencies between the ovule and outcrossing pollen pool could have been caused by relative differences in male and female function among trees, migration of pollen from outside of the population, selection between the time of pollination and progeny sampling, or most probably, non-random mating of genotypes during outcrossing events (Murawski and Hamrick 1992; Lee et al. 2000; Ribeiro and Lovato 2004). The relative low number of mother genotypes may also cause a bias in ovule allele frequencies that may explain differences between ovule and pollen allele frequencies. The consequence of this violation is not readily measurable, but as shown by Ritland and Jain (1981), it has a minor effect on the multilocus estimates of the population outcrossing rates.

Families from open-pollinated crosses are mostly considered as random mating, resulting in half-sibs. This result was reinforced by the proportion of half-sibs present in the three populations of *A. visco* studied here, 94.3 % in average, showing a little percentage of full, self-half and self-sibs. However, the proportion of full sibs might be slightly underestimated because the magnitude of the estimated mean coancestry within families ($\theta_{wfp(N)} = 0.095$ in average) was lower than that expected for half-sibs progenies (0.125), suggesting some downward bias in coancestry estimation with the Nason method. Nevertheless, when the average coancestry coefficient within progenies was estimated from Ritland's (1996) relatedness coefficient, it was near to that expected for half-sibs progenies ($\theta_{wfp(R)} = 0.130$ in average), in concordance with the result found here that a higher proportion of the progeny were half-sibs.

The average variance effective size measures the genetic representation of a population by means of its progenies, and it is a valuable measurement for the monitoring of the evolutionary potential of populations in improvement programmes and conservation genetics. The variance effective size of an ideal population (infinite size, random matches, absence of selection, migration and mutation) corresponds to 4 ($N_{ev} = 4$) non-related individuals per

progeny array. However, deviations of assumptions that characterize the ideal population cause reduction in the variance effective size in real populations (Sobierajski et al. 2006). In *A. visco* we obtained N_{ev} estimates close to that expected for progenies of an ideal population with high number of pollen donors per mother plant (3.58 and 3.85 in average, with Nason and Ritland method, respectively). This implies that sample sizes closer or slightly bigger to those studied here would be needed for the maintenance of genetic variability in programmes of genetic improvement and conservation.

A. visco is currently considered as lower risk/least concern species (IUCN 2009); however, all rangelands in Argentina, including those where this species grow, are currently experiencing some form of deterioration or desertification. In consequence, the results here presented have important implications for future seed collection strategies for ex situ conservation, tree breeding and reforestation of this species. Based on the variance effective size, we estimated that to retain the effective size of 100 for ex situ conservation, seeds need to be collected from at least 25–29 seed-trees in populations of Cachi and Tapia. However, since in the relict population of Posta de los Hornillos we could sample only the five fructifying trees present, the effective sampling size corresponding to these five seed-trees was equivalent to 17 ($N_{e(reference)} = \hat{m}\, N_{ev}$).

The present work shows a similar mating system in the three populations of *A. visco* in spite of their size differences, allowing us to hypothesize that the entire species could have a similar mating system of outcrossing preferential. These results also were in agreement with those found in other American, African and Australian species of *Acacia*.

Although *A. visco* seems to be able to persist in relatively small populations (PH and TA) any significant decline in numbers will also require management intervention such as population augmentation or translocation based on ex situ seed collections.

Considering the results of the present work where a high percentage of individuals were half-sibs, sampling large numbers of pods from individual trees will result in a genetically diverse sample as a consequence of high outcrossing rates. The presence of internal genetic structure suggested that seeds need to be collected from seed-trees spatially separated to avoid duplications in the sample.

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