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ORIGINAL ARTICLE

Mating system parameters and genetic structure in Argentinean populations of *Acacia caven* (Leguminosae, Mimosoideae)

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Abstract *Acacia caven* (Mol.) Mol. is native to South America. The species is a leguminous, woody small tree that is considered to have certain potential as a managed silvopastoral crop. Six varieties have been described for the species based on both morphological traits and molecular markers. Little information is available on its mating system. The main objectives of this work were to test the hypothesis that *A. caven* is an outcrosser and to estimate parameters of its mating system and population structure on the basis of isozyme markers. In the four populations studied, a high homozygote excess was found in the progeny population but not in the mother plant genotypes. The estimate for the multi-locus outcrossing rate (t_m) was high ($\geq$0.957) in all populations, indicating that *Acacia caven* is a predominantly outcrosser species. The results of genetic structure analysis within each population indicated that differences in allelic frequencies among families in all of the populations studied are highly significant. The difference in F estimates between progeny and mother plants suggests some selection favouring heterozygotes between the seedling and adult stages. Therefore, a strategy for ex situ conservation might emphasise sampling more populations with a relative large number of trees per site.

Keywords *Acacia caven* · Outcrosser · Mating system parameters · Genetic structure

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Introduction

Acacia caven (Mol.) Mol. (Leguminosae, Mimosoideae) belongs to the genus *Acacia*, subgenus *Acacia*. It is a native of South America, being found in six countries (Argentina, Chile, Paraguay, Uruguay, Brazil and Bolivia) (Aronson 1992). In Argentina, it is distributed from central to northern regions.

A. caven is a leguminous, woody small tree that is considered to have certain potential as a managed silvopastoral crop. The pods, seeds and leaves are consumed by domestic animals (Ovalle et al. 1990). A nitrogen fixer, this species exhibits wide altitudinal and latitudinal tolerance (Peralta et al. 1992). Six varieties have been described for this species (*A. caven* var. *caven*, *A. caven* var. *dehiscens*, *A. caven* var. *sphaerocarpa*, *A. caven* var. *stenocarpa*, *A. caven* var. *microcarpa* and *A. caven* var. *macrocarpa*) based on both morphological traits (Aronson 1992; Pometti et al. 2007) and molecular markers (Pometti et al. 2010). Argentina is the only country where all varieties are fouund (Aronson 1992).

While a good deal is known about the dispersal (Gutiérrez and Armesto 1981), seed germination capacity (Fuentes et al. 1990) and genetic diversity (Pometti et al. 2010) of *A. caven*, little information is available on its mating system. 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; Kenricket al. 1986; Moranet al. 1989; Sedgley et al. 1992; Casiva et al. 2004). As in some other acacias (Arroyo 1981; Tybirk 1989), female-sterile flowers are found in the inflorescence of *A. caven* (Burkart 1967). This species showed a small amount of self-compatibility, although this

level was within the range generally accepted for highly self-incompatible species (Peralta et al. 1992).

The understanding of the mating system of a species is of fundamental importance for genetic improvement and conservation programmes because it permits the outlining of strategies that optimise 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).

An efficient method to evaluate mating system parameters in natural populations is based on the multi-locus mixed mating model and the estimation procedure of Ritland and Jain (1981). The method requires grouping the population samples in family arrays and the use of neutral (preferably) codominant loci that segregate independently.

The objective of the present paper was to test the hypothesis that *A. caven* is an outcrosser, to estimate parameters of mating system and population structure and to evaluate the existence of differences in the mating system between two varieties of this species, *A. caven* var. *caven* and *A. caven* var. *dehiscens,* on the basis of isozyme markers.

Materials and methods

Species and populations sampled

Four Argentinean populations of *A. caven* were collected (Table 1) using the methodology of Vilardi et al. (1988) and Saidman and Vilardi (1993). For each population, approximately 50–80 seed pods were collected from six to ten mother trees that were separated from each other by more than 50 m. All seeds (300–500) from each plant were placed in a single bag and then chosen at random for isozyme analysis of progeny arrays. The number of seeds analysed per mother plant (family array size) was about ten. For all four populations, the number of mother trees sampled was the maximum possible to fulfill sampling conditions because the populations have been seriously degraded by anthropic causes.

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

Description of the species studied

Acacia caven can grow as a tree or shrub up to 10 m high but is usually much smaller. The branches have small, thin, conical thorny stipules, and the flowers are arranged in globose inflorescences. Flowers bloom in August and September, and fruits ripen between January and April. This species has an extremely wide range and probably originated in the warm temperate to subtropical biogeographical region known as the Gran Chaco of Southern South America, where the species shows high morphological diversity (Aronson and Ovalle 1989). In Argentina, it is distributed from central to northern regions and is the most common species of *Acacia* in these areas. In addition, this species shows remarkable climatic tolerance and ecologic adaptability and is able to colonise areas degraded by anthropic causes (intensive agriculture, shepherding or fire). Because of its high plasticity, it is used in reforestation of degraded ecosystems (Karlin et al. 1997).

Here two populations of *A. caven* var. *dehiscens* (Pan de Azúcar and Vaquerías) and two of *A. caven* var. *caven* (Campo Quijano and La Caldera) were studied (Table 1).

1. *Acacia caven* var. *caven*: Stipular spines 0.4–2.5 cm long, white or gray, borne in pairs at every node. Leaves are bipinnate, deciduous, usually with one petiolar gland. Pods are brown, indehiscent, oblong-elongate or subglobose, usually not striate, generally with a short peduncle, acuminate in the tip (Cialdella 1984; Aronson 1992).
2. *Acacia caven* var. *dehiscens*: Its characteristics correspond with those of var. *caven*, but it is differentiated by its mature dehiscent pods. Burkart (unpublished) considered that dehiscence of the fruit in this variety can not be attributed to the dry climate of the region where it grows because in more arid zones (Mendoza, for instance) *A. caven* var. *caven* pods are indehiscent (Cialdella 1984).

Table 1 Locations of *Acacia caven* var. *caven* and *A. caven* var. *dehiscens* sampled in Argentina

Variety	Population	Population abbreviation	Latitude (°S)	Longitude (°W)	Province
A. caven var. *caven*	Campo Quijano	CQ	24°55′12.00″	65°39′0.00″	Salta
	Ruta 9	R9	24°39′48.00″	65°22′49.00″	Salta
A. caven var. *dehiscens*	Pan de Azúcar	PA	31°15′58.90″	64°20′28.60″	Córdoba
	Vaquerías	VA	31°23′38.93″	63°51′30.87″	Córdoba

Isozyme methods

Seven isozyme systems were assayed: alcohol dehydrogenase (ADH), (Enzyme Commission) E.C. 1.1.1.1; glutamate oxalacetate transaminase (GOT), E.C. 2.6.1.1; isocitric dehydrogenase (IDH), E.C. 1.1.1.42; peroxidases (PRX), E.C. 1.11.1.7; shikimate dehydrogenase (SKD), E.C. 1.1.1.25; 6-phosphogluconic dehydrogenase (6-PGD), E.C. 1.1.1.43; and superoxide dismutase (SOD), E.C. 1.15.1.1 (IUPAC-IUB, 1979). Horizontal electrophoresis on 5% (GOT) or 7% (remaining systems) polyacrylamide gels was used. Buffers and electrophoretic conditions are described elsewhere (Saidman 1990). Homogenates were made from 7-day-old cotyledons for all systems except ADH. This system could not be studied in the same individuals as the former ones because analysis requires 10- to 12-h-old seedlings (the age of cotyledons is measured from the moment the seeds are placed into a wet chamber to stimulate germination). Isozyme loci were named according to the decreasing mobility, relative to bromophenol, of their corresponding bands. Superscript numbers were used to name allelic variants according to their relative mobility.

Because seeds from controlled crosses are not available, open-pollinated progenies were used to infer the genetic control of each isozyme system. Bands that did not conform to Mendelian segregation patterns were omitted from the analysis.

For mating system analysis, the six polymorphic loci (*Sod-1*, *Sod-3*, *Sod-4*, *6Pgd-2*, *Idh-1* and *Skd-1*) that could be studied simultaneously in the same individuals (seedlings) were chosen.

Data analysis

Allelic frequencies and unbiased expected heterozygosity (H_e; Nei 1978) of the whole seedling population sample were estimated including all available loci. The bias from Hardy-Weinberg expectations was evaluated by means of the F fixation index (Wright 1951) estimated by the method of Nei (1977) using the software Biosys-1 (version 1.7) (Swofford and Selander 1981).

Genetic structure within each population was evaluated by means of analysis of molecular variance (AMOVA) using the software GeneticStudio (Dyer 2008).

The following mating system parameters were calculated: multi-locus (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). The expectation-maximisation 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. All these estimates were obtained with the software MLTR 3.4 (Ritland 2009).

Because the mixed mating model assumes independent segregation of alleles at different marker loci, we tested possible genotypic association among loci using GDA 1.1 (Lewis and Zaykin 2001). We used the estimator of linkage disequilibrium of Burrows (Δ_{ii}) (Weir 1996). This estimator is more appropriate in species where the possibility exists that gametes have been joined in a non-random way (Weir and Cockerham 1984). The null hypothesis H_0: $\Delta_{ii} = 0$ was tested with the chi-squared test proposed by Weir (1996).

The inbreeding coefficient of seedlings was also 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).

Pollen and ovule allele frequencies were estimated for each locus using the expectation-maximisation method. Differences between these frequencies were tested by heterogeneity chi-squared 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, s is the number of groups (in our case 2, pollen and ovules) and F_{ST} is Wright's (1951) measure of the genetic diversity between pollen and ovule pools (Workman and Niswander 1970; Murawski and Hamrick 1992; Hall et al. 1994).

Results

Genetic diversity and population structure

Allelic frequencies, average heterozygosities, and fixation indices for the whole seed (progeny) sample of each population are listed in Table 2. Of 14 isozyme loci assayed, 8 proved to be variable. However *Idh-1* was found to be polymorphic only in populations belonging to *A. caven* var. *dehiscens*, and *Sod-1*, *3* and *4* were found to be polymorphic only in *A. caven* var. *caven* populations.

Heterozygosity estimates (H_e) ranged from 0.171 in Vaquerías to 0.255 in Campo Quijano (Table 2).

In all cases, F was positive and highly significant, ranging from 0.039 to 0.397, indicating a general trend towards homozygote excess within populations.

Among-family variances within each population estimated by AMOVA are listed in Table 2. Genetic diversity among families represented from 13.5% of total variation in Campo Quijano to 82.7% in Ruta 9. This component was highly significant in all cases ($p \leq 0.01$) (Table 2).

Table 2 Allele frequencies and measures of genetic variability at 14 isozyme loci in four Argentinean populations of *Acacia caven*

Allele	Population			
	CQ	R9	VA	PA
Adh-1^{51}	0.273	0.426	0.063	0.218
Adh-1^{48}	0.727	0.574	0.788	0.436
Adh-1^{46}	0	0	0.15	0.345
Adh-2^{29}	0.485	0.5	0.28	0.692
Adh-2^{26}	0.515	0.5	0.72	0.308
Skd-1^{30}	0.333	0.167	0.185	0.317
Skd-1^{27}	0.548	0.5	0.548	0.35
Skd-1^{25}	0.119	0.333	0.266	0.333
6pgd-2^{24}	0.211	0.5	0.306	0.365
6pgd-2^{22}	0.211	0	0.081	0.095
6pgd-2^{16}	0.474	0.478	0.613	0.516
6pgd-2^{14}	0.105	0.022	0	0.024
Idh-1^{30}	0	0	0.119	0.046
Idh-1^{28}	0	0	0.216	0.246
Idh-1^{26}	1	1	0.664	0.708
Sod-1^{1}	0.214	0	0	0
Sod-1^{91}	0.786	1	1	1
Sod-3^{79}	0.526	1	1	1
Sod-3^{69}	0.474	0	0	0
Sod-4^{57}	0.447	0.25	0	0
Sod-4^{53}	0.553	0.75	1	1
Got-1^{64}	1	1	1	1
Got-2^{57}	1	1	1	1
Got-3^{45}	1	1	1	1
Got-4^{37}	1	1	1	1
Prx-3^{46}	1	1	1	1
Prx-4^{41}	1	1	1	1
N	39	46	67	65
He	0.255 (0.074)	0.181 (0.068)	0.171 (0.065)	0.199 (0.076)
F	0.039**	0.111**	0.397**	0.275**
Var. among families (%)	13.5**	82.7**	29.9**	40.1**

N Sample size, H_e estimated heterozygosity, *Var. among families (%)* percentage of variance explained by AMOVA for each population among families

Standard errors are shown *in parentheses*. For abbreviations, see Table 1

** $p \leq 0.01$

Mating system parameters

The test of linkage disequilibrium detected little evidence of linkage between the isoenzymatic loci evaluated in *A. caven* (Table 3). Of a total of 60 tests comparing locus pairs, Bonferroni sequential test (5%) showed 6 with significant association (10%) and 3 of these with matrix-wide significance. When genotypic associations were tested within each population individually, neither of the two populations belonging to *A. caven* var. *dehiscens* showed significant association between loci, and Ruta 9 presented only one association.

The estimate for the multi-locus outcrossing rate (t_m) was high ($\geq$0.957) in all populations. These results indicate that the two varieties of *A. caven* studied are mostly outcrossers. The estimated biparental inbreeding rate ($t_m - t_s$) was low in all populations and not significantly different from zero, considering the standard error of the mean, suggesting low tendency to mate between relatives (Table 4). 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 population. These values suggest a high probability that a randomly chosen pair of progeny from the same array would be half-sibs.

The average single locus inbreeding coefficient of maternal parents (F_M) was in all cases lower than that estimated for their progeny (Tables 2 and 4). In all cases, F_M was near zero, suggesting no Hardy-Weinberg deviations in the populations.

Table 3 *p* values for each loci pair in the four populations of *A. caven* studied, applying the Burrows test of independence of genotypes between loci

Loci	Population			
	Pan de Azúcar	Vaquerías	Campo Quijano	Ruta 9
6Pgd2/Idh1	0.346	0.392	1.000	1.000
6Pgd2/Skd1	0.521	0.234	0.021*	0.140
6Pgd2/Sod1	1.000	1.000	1.000	1.000
6Pgd2/Sod3	1.000	1.000	0.000**[a]	1.000
6Pgd2/Sod4	1.000	1.000	0.054	1.000
Idh1/Skd1	0.845	0.124	1.000	1.000
Idh1/Sod1	1.000	1.000	1.000	1.000
Idh1/Sod3	1.000	1.000	1.000	1.000
Idh1/Sod4	1.000	1.000	1.000	1.000
Skd1/Sod1	1.000	1.000	1.000	1.000
Skd1/Sod3	1.000	1.000	0.001**	1.000
Skd1/Sod4	1.000	1.000	0.000**[a]	0.000**[a]
Sod1/Sod3	1.000	1.000	0.574	1.000
Sod1/Sod4	1.000	1.000	1.000	1.000
Sod3/Sod4	1.000	1.000	0.007**	1.000

* $p < 0.05$, ** $p < 0.01$

[a] *p* values that showed significance at the matrix level

In some populations, significant differences were observed for several loci between pollen and ovule allelic frequencies (Table 5).

Discussion

Inbreeding in plants is one of the major determinants of the extent of genetic variability within and differentiation among populations. The available estimates of outcrossing rates in natural populations of acacias (Moran et al. 1989; Muona et al. 1991; Mandal and Ennos 1995; Casiva et al. 2004) are high, indicating a predominantly outcrossing system. The presence of only low amounts of inbreeding in a number of *Acacia* species has been attributed to a strong self-incompatibility system (Bernhardt et al. 1984).

The present study showed that estimation of multi-locus outcrossing rate (t_m) was high ($\geq$0.957) and not significantly different from 1.0, indicating that *A. caven* is a predominantly outcrosser species. These values are comparable to those reported for other *Acacia* species (Moran et al. 1989; Muona et al. 1991; Mandal and Ennos 1995; Casiva et al. 2004) as well as for other tropical tree species (Murawski and Hamrick 1991; Freitas et al. 2004; Sobierajski et al. 2006). Outcrosser species with wide geographic range have higher levels of genetic diversity than selfer and endemic species. Likewise, species whose seeds are dispersed by animal ingestion or by wind tend to maintain high levels of within-population genetic variability (Hamrick and Godt 1990).

The results obtained here indicated relatively high heterozygosity for isozyme loci ($H_e = 0.171$–0.255), consistent with those estimates obtained from RAPD loci by Pometti et al. (2010) for the six varieties of *A. caven* ($H_e = 0.16-0.33$). Likewise, the present analysis indicated that most variability occurs within populations ($1 - F_{ST} = 0.88$; data not shown). In fact, in most loci, the allele with the highest frequency is common to all populations.

This is consistent with the previous results obtained by AMOVA from RAPD data (Pometti et al. 2010) where about 61% of total variation occurred within populations. These results also agree with the trends described by Hamrick and Godt (1990) and Gutiérrez and Armesto (1981) who observed that animals play an important role in the dispersal of *A. caven* seeds.

Some differences were observed with respect to allelic frequencies among populations. For some loci certain populations are monomorphic as the least frequent allele is absent. Interestingly, *Idh-1* is monomorphic in both populations of *A. caven* var. *caven* and polymorphic in both populations of *A. caven* var. *dehiscens* and the opposite occurs for *Sod-4*. This result might be explained by genetic drift and limited gene flow among populations, both yielding to the fixation of one allele in these populations.

The results of genetic structure within each population indicated that differences in allelic frequencies among families in all the populations studied are highly significant. Population substructuring may explain why the

Table 4 Multi-locus (t_m) and single locus (t_s) outcrossing rates, progeny fixation index (F_e) and maternal fixation index (F_M) for *A. caven* var. *caven* and *A. caven* var. *dehiscens* populations estimated from progeny arrays

Population	t_m	t_s	$t_m - t_s$	r_1	r_p	F_e	F_M
CQ	0.987 (0.092)	0.981 (0.033)	0.006 (0.074)	0.047 (0.084)	0.047 (0.084)	0.006	0.000 (0.000)
R9	1.000 (0.000)	1.000 (0.000)	0.000 (0.000)	0.109 (0.000)	0.101 (0.133)	0.000	0.016 (0.010)
PA	0.957 (0.060)	0.926 (0.051)	0.031 (0.045)	0.294 (0.163)	0.055 (0.054)	0.002	0.000 (0.000)
VA	0.954 (0.056)	0.954 (0.042)	0.013 (0.033)	0.121 (0.064)	0.068 (0.064)	0.017	0.000 (0.000)

The indexes r_t and r_p denote correlation of t_m within progeny array and correlation of outcrossing paternity respectively. Standard errors are shown *in parentheses.* See Table 1 for abbreviations

Table 5 Pollen and ovule allele frequencies estimated from *Acacia caven* populations and χ^2 test results for the differences between these frequencies

Population	Allele	Pollen	Ovule	F_{ST}	χ^2
Campo Quijano	*Sod-1*^{1}	0.000 (0.000)	0.428 (0.133)		
	Sod-1^{91}	1.000 (0.000)	0.572 (0.133)	0.272	3.812
	Sod-3^{79}	0.621 (0.165)	0.608 (0.136)		
	Sod-3^{69}	0.379 (0.165)	0.392 (0.136)	0.000	0.007
	Sod-4^{57}	0.503 (0.262)	0.246 (0.195)		
	Sod-4^{53}	0.497 (0.262)	0.754 (0.195)	0.070	2.679
	Skd-1^{30}	0.175 (0.151)	0.717 (0.133)		
	Skd-1^{27}	0.568 (0.161)	0.283 (0.133)		
	Skd-1^{25}	0.257 (0.107)	0.000 (0.000)	0.046	2.937
	6pgd-2^{24}	0.393 (0.108)	0.000 (0.000)		
	6pgd-2^{22}	0.271 (0.085)	0.180 (0.050)		
	6pgd-2^{16}	0.336 (0.087)	0.624 (0.065)		
	6pgd-2^{14}	0.000 (0.000)	0.195 (0.055)	0.012	1.331
Ruta 9	*Sod-1*^{1}	0.016 (0.024)	0.216 (0.046)		
	Sod-1^{91}	0.984 (0.024)	0.784 (0.046)	0.098	7.997*
	Sod-3^{79}	1.000 (0.000)	0.905 (0.029)		
	Sod-3^{69}	0.000 (0.000)	0.095 (0.029)	0.050	4.588*
	Sod-4^{57}	0.201 (0.114)	0.450 (0.079)		
	Sod-4^{53}	0.799 (0.114)	0.550 (0.079)	0.071	6.213*
	Skd-1^{30}	0.245 (0.103)	0.087 (0.059)		
	Skd-1^{27}	0.413 (0.111)	0.612 (0.071)		
	Skd-1^{25}	0.342 (0.065)	0.302 (0.042)	0.008	1.376
	6pgd-2^{24}	0.933 (0.033)	0.160 (0.022)		
	6pgd-2^{22}	0.022 (0.020)	0.000 (0.000)		
	6pgd-2^{16}	0.000 (0.000)	0.839 (0.022)		
	6pgd-2^{14}	0.045 (0.027)	0.000 (0.000)	0.001	0.169
Pan de Azúcar	*6pgd-2*^{24}	0.775 (0.059)	0.000 (0.000)		
	6pgd-2^{22}	0.065 (0.036)	0.255 (0.019)		
	6pgd-2^{16}	0.161 (0.051)	0.662 (0.019)		
	6pgd-2^{14}	0.000 (0.000)	0.083 (0.000)	0.342	135.591**
	Idh-1^{30}	0.095 (0.036)	0.000 (0.000)		
	Idh-1^{28}	0.143 (0.051)	0.500 (0.000)		
	Idh-1^{26}	0.762 (0.055)	0.500 (0.000)	0.103	26.868**
	Skd-1^{30}	0.365 (0.084)	0.269 (0.045)		
	Skd-1^{27}	0.315 (0.087)	0.275 (0.042)		
	Skd-1^{25}	0.320 (0.084)	0.456 (0.050)	0.011	1.328
Vaquerías	*6pgd-2*^{24}	0.000 (0.000)	0.500 (0.000)		
	6pgd-2^{22}	0.181 (0.049)	0.000 (0.000)		
	6pgd-2^{16}	0.819 (0.049)	0.500 (0.000)		
	6pgd-2^{14}	0.000 (0.000)	0.500 (0.000)	0.194	49.002**
	Idh-1^{30}	0.138 (0.045)	0.168 (0.011)		
	Idh-1^{28}	0.249 (0.054)	0.171 (0.018)		
	Idh-1^{26}	0.613 (0.065)	0.661 (0.021)	0.004	1.181
	Skd-1^{30}	0.161 (0.065)	0.283 (0.053)		
	Skd-1^{27}	0.509 (0.083)	0.549 (0.053)		
	Skd-1^{25}	0.330 (0.058)	0.168 (0.009)	0.018	4.351*

F_{ST} is the genetic difference between populations. Standard errors are shown *in parentheses*

* $p < 0.05$, ** $p < 0.01$

homozygote excess observed in the seedlings (F) is higher than that expected from the outcrosssing rate (F_e).

The fixation indexes in adult trees (F_M) were virtually zero in all populations. Moreover, F_M approximates to the equilibrium inbreeding coefficient (F_e). In the progeny, F was higher than in mother plants, mainly due to population structuring. The reduction in inbreeding in mother plants compared to progeny suggests the existence of selection against homozygotes between the time of seed dispersal and growth to reproductive age. Similar results were found by Lee et al. (2000) in the tropical species *Shorea leprosula* Miq. (Dipterocarpaceae), by Sobierajski et al. (2006) in the species *Mimosa scabrella* (Leguminosae) and by Bessega et al. (2000) in *Prosopis* species. Possible explanations for these results and those found in this work could be that selection favouring and increasing the proportion of heterozygotes can occur in predominantly outcrossed populations as a result of heterosis and overdominance, or by discriminating against the products of selfing or biparental mating, which tend to have higher levels of homozygosity (Lee et al. 2000).

Significant differences in pollen and ovule allele frequencies were detected for *Sod-1*, *Sod-3* and *Sod-4* in one population belonging to *A. caven* var. *caven* (Ruta 9), and for *6pgd-2, Idh-1* and *Skd-1* in populations belonging to *A. caven* var. *dehiscens* (Pan de Azúcar and Vaquerías). 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. It also may have been affected to some degree by the low sample sizes of the maternal plants and unequal progeny number per family (Murawski and Hamrick 1992; Lee et al. 2000; Ribeiro and Lovato 2004). The consequence of this violation is not readily measurable, but as shown by Ritland and Jain (1981), it has a minor effect on the multi-locus estimates of the population outcrossing rates.

The present work shows a similar mating system in the four populations of *A. caven*, independently of the variety they belong to, allowing us to hypothesize that the entire species could have a similar mating system of outcrossing preferentially.

Considering the results obtained here together with those of the previous work on genetic variability of the six varieties of *A. caven* (Pometti et al. 2010), sampling large numbers of pods from individual trees will result in a genetically diverse sample as a consequence of high outcrossing rates. Furthermore, trees within populations

should be spatially separated to avoid duplications in the sample as a consequence of internal structuring.

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