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Genetic structure and variability of the endemic and vulnerable *Vellozia gigantea* (Velloziaceae) associated with the landscape in the Espinhaço Range, in southeastern Brazil: implications for conservation

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Abstract The Espinhaço Range, in eastern Brazil, has a peculiar landscape that has influenced the vegetation pattern of the region because of its valleys, canyons, ranges and disjunct rock outcrops found at high elevations. In this region, the vegetation type known as *campos rupestres* (rupestrian fields), which occurs in the disjunct outcrops, has high levels of species richness and endemism. *Vellozia gigantea*, a 6-m tall dracenoid monocot, is a vulnerable species endemic to this vegetation and has a narrow distribution that extends approximately 27 km. This region is located in a disturbed area, where populations are divided into three geographical groups, separated by a canyon and a valley. For this study, we used ISSR markers to measure the genetic diversity of the species and test the hypothesis that the canyon and the valley constitute geographical barriers to gene flow in *V. gigantea*. Nine populations and 173 individuals were analyzed using nine ISSR primers, which produced 89 fragments. In spite of being a vulnerable species with a narrow distribution, the populations of *V. gigantea* have high genetic diversity (mean percentage of polymorphic loci = 56.6%; mean Shannon's index of diversity = 0.278; mean expected heterozygosity = 0.183). Genetic divergence among populations was high ($\Phi_{ST} = 0.28$), and principal coordinate, neighborjoining and Bayesian analyses showed that only the canyon may constitute a partial barrier to gene flow in this species. Groups of populations separated by the canyon should be managed separately because they contain different gene pools.

Keywords Velloziaceae · Espinhaço Range · *Campos rupestres* · Conservation genetics · Threatened species · Genetic structure

Introduction

The Espinhaço Mountain Range is a highly biodiverse region in eastern Brazil (in the states of Minas Gerais and Bahia) extending approximately 1,000 km in a North–South direction. The southern portion of this region has been designated as part of the Biosphere Reserve (UNESCO 2005). Within the Espinhaço Range there are several high-elevation regions with high biodiversity, including Chapada Diamantina in Bahia and Serra do Cipó in Minas Gerais states. The Espinhaço Range has a vegetation type called *campos rupestres* (rupestrian fields or rocky fields) that is known for its species richness and high endemism, and convergence of vegetative habit is common among several families (Joly 1970; Giulietti and Pirani 1988). It has been estimated that approximately 30% of the taxa from the *campos rupestres* are restricted to this geological formation, especially within some plant groups, such as Velloziaceae and Eriocaulaceae. Due to the discontinuity of the mountains of the Espinhaço Range, the plant species are distributed in disjunct populations, especially those

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occurring on isolated rock outcrops. This disjunction has been considered one of the main factors that lead to the high species diversity of the *campos rupestres* (Giulietti and Pirani 1988).

The Velloziaceae comprise approximately 240 species that are predominantly Neotropical. The majority of the diversity and endemism within the family occurs in the eastern mountains of Brazil, especially in the *campos rupestres* of the Espinhaço Range (Menezes et al. 1994). *Vellozia*, with about 105 species, is the largest genus in the family. The species of this genus are locally known as *canela-de-ema* and are common in the *campos rupestres* (Giulietti and Pirani 1988). Several *Vellozia*, especially the shrub and arborescent (dracenoid) species, are important phorophytes for many taxa, such as species of the Orchidaceae, and some of these epiphytes are exclusive to the stems of *Vellozia* (Barros and Lourenço 2004).

Vellozia gigantea N.L. Menezes & Mello-Silva is a relatively recently described species (Mello-Silva and Menezes 1999), considered vulnerable according to Biodiversitas (2005), and has been given a deficient data conservation status by the *Ministério do Meio Ambiente* (MMA 2008). It is a narrowly endemic species with a linear area of distribution that extends approximately 27 km and occurs in a region that is disturbed by recurrent fires associated with cattle farming and urbanization. When it was first described only one population was known to exist, in the Serra do Cipó National Park (Fig. 1) (Mello-Silva and Menezes 1999). *Vellozia gigantea* is the tallest species in the family, reaching more than 6 m. Its stems are often densely covered with many species of epiphytes (mainly orchids, bromeliads and pteridophytes), which are extremely sensitive to over-collecting. *Vellozia gigantea* has great potential to be a "flag-species" because its image is closely linked to the tourism industry in Serra do Cipó, due to its unusual habit and beauty. Mello-Silva and Menezes (1999) highlighted that the flowering cycle of the species is irregular and recruitment of new individuals appears to be limited; however, mass flowering is often induced by fires that are usually caused by humans

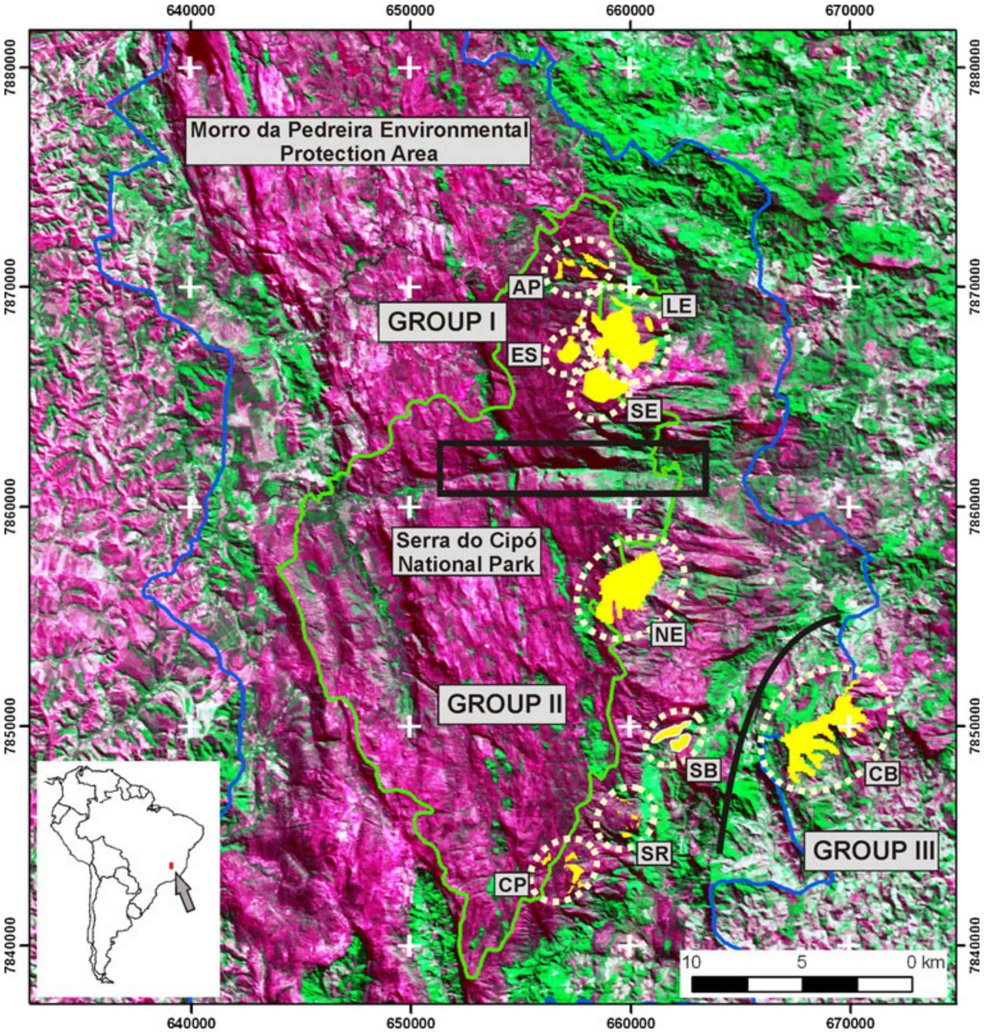

Fig. 1 Geographic distribution of the *Vellozia gigantea* populations in the Serra do Cipó, Espinhaço range, southeastern Brazil. Populations are indicated as clear patches inside *dotted circles*. *Upper rectangle* = Travessão Canyon, separating geographic group I; *lower arc* = Serra do Lobo valley, separating group III; *inner outline* = Serra do Cipó National Park limits; *outer outline* = Morro da Pedreira Environmental Protection Area limits. For population names see Table 1

(ICMBio 2009). Recently, eight other populations were discovered in the Serra do Cipó region at elevations between 1,200 and 1,500 m. Three major groups of *V. gigantea* populations can be recognized based on their distribution in relation to two geological formations, a canyon (Canyon Travessão) and a valley between a range (Serra do Lobo) and the continuous massive rocky area of the Serra do Cipó region (Fig. 1). These geological formations may constitute significant barriers to the gene flow between groups of populations, promoting genetic differentiation among them.

The biology and life history of *V. gigantea* are almost unknown, with only seed germination studies carried out for this species (Garcia and Diniz 2003). However, studies on other *Vellozia* species showed that they are perennial, desiccation-tolerant, well adapted to fire and self-incompatible (Sazima 1978; Oliveira et al. 1991; Alves 1994; Ibisch et al. 1995; Porembski and Barthlott 1995; Franceschinelli et al. 2006). Bees are the major pollinators (Jacobi and del Sarto 2007), but other groups, such as hummingbirds and Lepidoptera, pollinate some species (Franceschinelli et al. 2006). The seeds of *Vellozia* are small and are dispersed by gravity (Franceschinelli et al. 2006) or wind, but they are not carried over long distances (Ibisch et al. 2001).

Life history characteristics of plants, such as taxonomic status, life form, geographical range, breeding system, seed dispersal and successional status have been related to genetic diversity and its spatial distribution (Hamrick and Godt 1996; Nybom and Bartish 2000; Nybom 2004). On average, annual, self- or mixed-breeding, early- or mid-successional species show lower levels of intra-population diversity than long-lived perennial, outcrossing, late-successional taxa. Moreover, annual, self-breeding, gravity- and attachment-dispersed plants show higher levels of inter-population diversity than long-lived perennial, outcrossing, wind-, water- and animal-dispersed plants. Another important factor influencing the genetic diversity and genetic structure of plant species is the demographic history of the populations. Threatened species with fragmented and small populations generally show low intra-population diversity and high differentiation among populations (Young et al. 1996; Frankham et al. 2002).

All *V. gigantea* populations are vulnerable because of the occurrence of illegally set fires, and even within the National Park, because of the presence of cattle, their trails, and the collection of the stems for the orchids that grow on them. To create a plan for its conservation and management, in order to ensure the preservation of the species, it is essential to know the structure and history of the existing populations, the existence of demographic partitions throughout its distribution and the hierarchical distribution of its genetic diversity (Excoffier et al. 1992; Moritz 1995). By using highly variable DNA markers (ISSR), we tested two hypotheses in this study: (1) Both Canyon Travessão and Serra do Lobo valley constitute geographical barriers to gene flow in *Vellozia gigantea*, and the genetic variability of the species is structured according to the three geographical groups; (2) this species and its populations show low genetic diversity due to narrow distribution and human disturbance.

Materials and methods

Plant material

Young leaves of 173 individuals from all nine known populations of *V. gigantea* were collected in the *campos rupestres* of the Serra do Cipó, at Espinhaço Range in Minas Gerais state, southeastern Brazil (Table 1). Almost all populations are inserted either in the National Park of Serra do Cipó or in the Environmental Protection Area (Área de Proteção Ambiental—APA) Morro da Pedreira (Fig. 1). One of these populations (CB) covers a small disjunct mountain range (Serra do Lobo) and is situated outside of the APA, close to a mining area. The nine populations were clustered in three groups (I, II and III) according to their spatial distribution in relation to a canyon (Canyon Travessão) and a valley between a range (Serra do Lobo) and the continuous massive rocky of Serra do Cipó region (Fig. 1). Canyon Travessão separates group I in the north (AP, LE, ES and SE populations) from group II in the south (NE, SR, CP and SB populations). Group III (CB population) is located in a small isolated mountain range, Serra do Lobo and separated from Group II by the valley (Fig. 1). Leaf samples from 15 to 20 individuals per population were collected and stored on silica gel until DNA extraction.

Table 1 Populations of *Vellozia gigantea* occurring in the Serra do Cipó, Espinhaço range, southeastern Brazil, used in this study

Municipality	Code	Group	Coordinates	*N*
Morro do Pilar	AP	I	19°14′50″S/43°30′37″W	20
Morro do Pilar	LE	I	19°16′26″S/43°29′34″W	15
Morro do Pilar	ES	I	19°16′38″S/43°30′09″W	20
Morro do Pilar	SE	I	19°18′07″S/43°28′48″W	20
Itambé do Mato Dentro	NE	II	19°22′49″S/43°29′22″W	20
Itabira	SR	II	19°28′35″S/43°28′23″W	18
Itabira	CP	II	19°29′41″S/43°30′14″W	20
Itambé do Mato Dentro	SB	II	19°26′00″S/43°27′00″W	20
Itabira	CB	III	19°26′28″S/43°23′48″W	20

N = number of individuals sampled. For group and population locations see Fig. 1

Table 2 Primers used for ISSR amplification of *Vellozia gigantea*

Primer name	Primer sequence[a]	T °C	*N* (*P* %)
Bechy	$(CA)_7YC$	50.1	6 (83.3)
Chris	$(CA)_7YG$	47.6	7 (85.7)
John	$(AG)_7YC$	47.6	11 (100)
Manny	$(CAC)_4RC$	50.1	14 (85.7)
Mao	$(CTC)_4RC$	50.1	14 (100)
UBC 827	$(AC)_8G$	46.1	14 (100)
UBC 840	$(GA)_8YT$	50.1	8 (75)
UBC 848	$(CA)_8RG$	50.1	9 (55.5)
UBC 899	$(CA)_6RG$	50.1	6 (100)
Total			89 (88.8)

[a] Y = C or T; R = A or G

T °C = melting temperature; *N* = number of fragments; *P*% = percentage of polymorphic fragments

DNA isolation and ISSR-PCR

Approximately 150 mg of dry leaves were used for DNA extraction according to Doyle and Doyle (1987) protocol, slightly modified as suggested by Ferreira and Grattapaglia (1995). The cetyltrimethylammonium bromide (CTAB) protocol uses the following buffer: 100 mM Tris pH 8.0, 20 mM ethylenediamine tetraacetic acid (EDTA), 1.4 M NaCl, 2% of CTAB, 1% of polyvinylpyrolidone (PVP) and 2% of ß-mercaptoethanol. The concentration of DNA was visually quantified using 0.8% agarose gel by comparison with standard DNA concentrations. DNA was diluted in TE buffer to a final concentration of approximately 5 ng/µl prior to PCR amplifications.

PCR amplifications were carried out in a total volume of 19 µl, containing 20 ng of DNA template, 2.0 µl 10x PCR buffer, 0.21 mM dNTPs, 0.32 µM primer, 1 unit of *Taq* polymerase (Phoneutria) and double-distilled water. The reactions were performed in a Mastercycler thermocycler (Eppendorf). The program consisted of an initial denaturation of 94°C for 4 min, followed by 37 cycles of 1 min at 94°C, 2 min at 46.1–50.1°C (depending on the primer), 2 min at 72°C and a final extension of 7 min at 72°C. A negative control, in which DNA template was omitted, was included in each PCR. Amplification products were electrophoretically separated at a constant voltage of 60 V for 4 h in 1.5% agarose gels with 0.5x TAE buffer, stained with ethidium bromide and photographed under UV light. A 100 bp DNA ladder was used to estimate the molecular size of the fragments. Thirty primers were tested to identify those that produce sharp and reproducible markers, and nine of them were selected for use (Table 2). Positive controls were used, including DNA from individuals of other populations and other species (*V. compacta*), of which amplification profiles were already known from previous procedures. This procedure allowed us to compare gel photographs and monitor the reproducibility of the technique.

Data analysis

The fragments amplified by ISSR were visually scored assuming that amplified products of similar molecular size, amplified with the same primer, were homologous. ISSR bands were scored as 1 (presence) or 0 (absence) and a matrix of ISSR phenotypes was constructed. Only data from unambiguous intensely stained clear bands were used for data analyses. The software POPGENE v. 1.32 (Yeh et al. 1999) was used to obtain genetic diversity parameters: percentage of polymorphic loci (*P*), Shannon's index of phenotypic diversity (*I*) and expected heterozygosity (H_e). Shannon's index was estimated as $I = -\Sigma p_i \, \mathrm{Ln} p_i / n$, (where p_i is the frequency of the band and n is the number of markers evaluated) and expected heterozygosity as $H_e = 1 - \Sigma p_i^2$. Nei's unbiased genetic distance (1978) was calculated (POPGENE v.1.32) for all population pairs and used to construct a phenetic tree with neighbor-joining as grouping algorithm using MEGA 4.1 (Kumar et al. 2004) and to carry out a Principal Coordinate Analysis (PCO) with Genealex 6 (Peakall and Smouse 2006). The Bayesian algorithm in AFLP-SURV (Vekemans et al. 2002) was used to generate 1,000 dissimilarity matrices, which were used to construct 1,000 neighbor-joining trees using the NEIGHBOR module in PHYLIP 3.69 (Felsenstein 2006). The CONSENSE module was then used to compute a majority-rule consensus tree, from which bootstrap values were obtained.

Arlequin 3.0 (Excoffier et al. 2005) was used to perform Mantel test and Analysis of Molecular Variance (AMOVA). Mantel test was used to test correlations between the matrices of pairwise $F_{ST}/(1\text{-}F_{ST})$ and Ln of geographical distances (Slatkin 1995). Five AMOVAs were performed. One considered only two hierarchical levels and analyzed the partition of total genetic diversity among and within populations. Two AMOVAs tested for geographical barriers to gene flow, one for Canyon Travessão, which clustered populations into two groups (I and II + III) and another for both barriers, Canyon Travessão and Serra do Lobo valley, which clustered populations in three geographical groups (I, II and III). The remaining two AMOVAs grouped populations according to results of the Bayesian analysis. Statistical significance of the covariance components and fixation indices were determined through permutation tests against a null distribution generated by the data.

A Bayesian analysis was performed using the software STRUCTURE 2.2 (Falush et al. 2007) to infer the number of genetic clusters (*K*). The number of genetic clusters

Table 3 Parameters of genetic diversity of nine populations of *Vellozia gigantea*, occurring in the Serra do Cipó, Espinhaço range, southeastern Brazil, based on 89 ISSR loci

Population	P (%)	I (±SD)	H_e (±SD)
AP	68.5	0.334 (±0.279)	0.222 (±0.198)
LE	58.4	0.285 (±0.279)	0.188 (±0.195)
ES	66.3	0.339 (±0.280)	0.225 (±0.197)
SE	61.8	0.307 (±0.280)	0.203 (±0.196)
NE	57.3	0.236 (±0.256)	0.150 (±0.176)
SR	52.8	0.267 (±0.288)	0.178 (±0.201)
CP	47.2	0.240 (±0.280)	0.159 (±0.194)
CB	48.3	0.216 (±0.260)	0.140 (±0.180)
SB	49.4	0.266 (±0.297)	0.180 (±0.208)
Mean	56.6	0.278 (±0.046)	0.183 (±0.030)
Species	88.8	0.395 (±0.235)	0.256 (±0.176)

P = percentage of polymorphic loci; I = Shannon's index of diversity; H_e = expected heterozygosity. Standard deviation in parentheses. For population names see Table 1

(K) was set of $K = 1$ to $K = 9$ and 15 independent runs were made for each K. Each run was pursued for 1,000,000 Markov chain Monte Carlo (MCMC) iterations, with an initial burn-in of 100,000 iterations, with the admixture model with alleles correlated among populations. To infer the number of genetic clusters (populations), we calculated the average of each K likelihood value, 'log of probability' (LnP(D)), through all runs as suggested by Pritchard et al. (2000) and the statistic ΔK according to Evanno et al. (2005). As done in several other studies (e.g., Pérez-Collazos et al. 2008; Segarra-Moragues and Catalán 2010), the F_{ST} value obtained in ARLEQUIN was used to estimate the number of populations needed to represent a given proportion (P) of the genetic diversity according to the modified equation of Ceska et al. (1997): $P = 1 - (F_{ST})^n$, where n is the number of the populations that are necessary to represent a proportion P of the among-population genetic diversity. A proportion of 99.9% of the total genetic diversity was set for *V. gigantea.*

Results

The nine primers used resulted in 89 fragments with good resolution. Each primer amplified 6–14 fragments ranging from 200 to 2,200 bp (Table 2). The percentage of polymorphic fragments at the species level was 88.8%, and at the population level the polymorphism ranged from 47.2 to 68.5% (Table 3). For the species, the expected heterozygosity (H_e) and Shannon's index (I) were 0.256 and 0.395, respectively. Populations showed relatively high genetic variability, with mean H_e and I equal to 0.183 and 0.277, respectively. Population CB had the lowest genetic diversity ($H_e = 0.140$, $I = 0.216$) and population ES and AP had the highest diversity ($H_e = 0.225$ and 0.222, $I = 0.339$ and 0.334, respectively) (Table 3).

The AMOVA that considered only two hierarchical levels (Table 4) showed that most of the variation was found within populations (72.0%, $\Phi_{ST} = 0.28$). In the AMOVA clustering the populations in two groups (one with populations of geographic group I and another with

Table 4 Analysis of Molecular Variance (AMOVA) for different hierarchical levels of nine populations of *Vellozia gigantea*, occurring in the Serra do Cipó, Espinhaço range, southeastern Brazil

Source of variation	*d.f.*	Sum of squares	Variance components	% Total variance	P-value
Vellozia gigantea s.l.					
Among populations	8	582.180	3.348	28.0	<0.000
Within populations	164	1,406.168	8.574	72.0	<0.000
Three goups: north Canyon Travessão (I), south Canyon Travessão (II) and Serra do Lobo (III)					
Among groups	2	223.029	0.976	8.0	<0.030
Among pops. within groups	6	359.151	2.688	22.0	<0.000
Within populations	164	1,406.167	8.574	70.0	<0.000
Two main clusters according to Bayesian analysis					
Among groups	1	167.764	1.368	10.9	<0.007
Among pops. within groups	7	414.416	2.645	21.0	<0.000
Within populations	164	1,406.167	8.574	68.1	<0.000
Three clusters according to Bayesian analysis					
Among groups	2	280.470	1.608	13.0	<0.000
Among pops. within groups	6	301.711	2.177	17.6	<0.000
Within populations	164	1,406.167	8.574	69.4	<0.000

For groups see Table 1 and Fig. 1

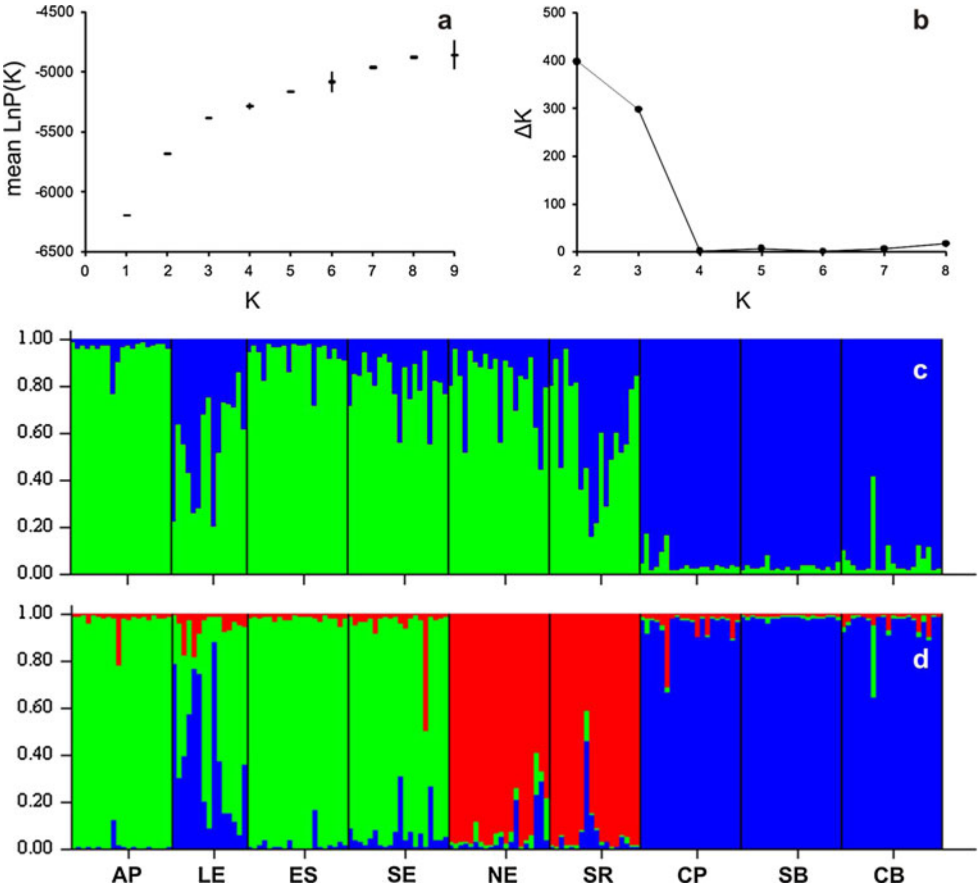

Fig. 2 Bayesian analysis of 173 individuals from nine populations of *Vellozia gigantea*, occurring in the Serra do Cipó, southeastern Brazil, based on 89 ISSR loci. **a** LnP(D) graphic for 15 runs. Values presented as mean ± standard deviation. **b** ΔK graphic. **c, d** Graphic representation of the different genetic pools for $K = 2$ (**c**) and $K = 3$ (**d**). Populations are separated by *vertical bars*. For population names see Table 1. The real number of genetic clusters corresponds to the higher value of ΔK and the stabilization of LnP(D)

the remaining populations (groups II + III)), 7.8% of the total variation was found between the two groups and 22.8% among populations within the groups (data no shown). In the analysis that considered the three geographic groups, north and south of Canyon Travessão and Serra do Lobo, 8.0% of the total variation was found among the groups and 22.0% among populations within groups (Table 4).

The graphic with the average values of LnP (K) from the Bayesian analysis, carried out in STRUCTURE, showed a large increase in the values at $K = 2$, and the beginning of a stabilization at $K = 3$, when the curve had regular increments of values (Fig. 2a). Likewise, the results of ΔK statistics showed a higher peak at $K = 2$ but also a very high value for $K = 3$, with values close to zero from $K = 4$ (Fig. 2b). These results indicate a main structure in two major genetic clusters, with a secondary structure in one of these clusters. Considering $K = 2$ (main structure), we observed a predominant genetic cluster containing populations AP, ES, SE and NE and the other with populations CP, SB and CB, with populations LE and SR in both genetic clusters (Fig. 2c). For $K = 3$, NE and SR populations constituted a third genetic cluster (Fig. 2d), and one of the three genetic clusters contained only populations of the geographical group I (AP, LE, ES and SE). Considering $K = 3$, only population LE exhibited strong structuring. AMOVA reinforced the results obtained by STRUCTURE, and showed that 10.9% of the total variation is found between the two main clusters, and 13.0% of total variation is found among the three clusters (Table 4).

The neighbor-joining dendrogram clustered the populations into two main clusters, one corresponding to

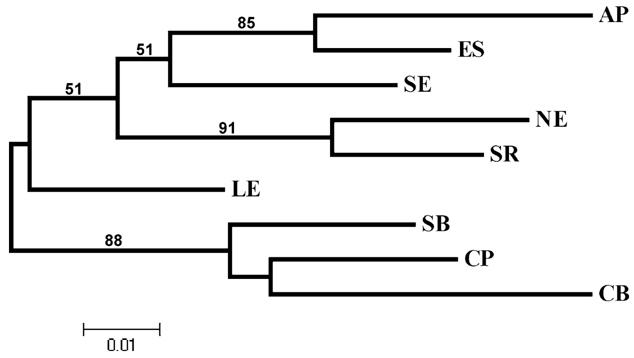

Fig. 3 Neighbor-joining dendrogram showing the phenetic relationships among nine populations of *Vellozia gigantea*, occurring in the Serra do Cipó, southeastern Brazil, constructed using the matrix of genetic distances (Nei 1978; unbiased estimate) based on 89 ISSR loci. Bootstrap percentages (50% or more) are above branches. For population names see Table 1

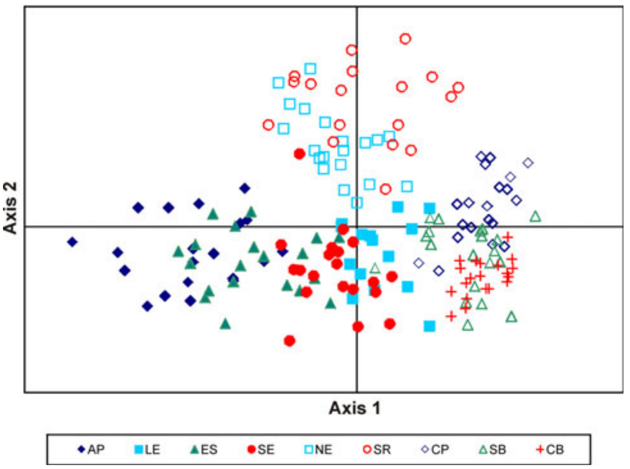

Fig. 4 Representation of the scores on the first two axes of the principal coordinate analysis (PCO) from the matrix of genetic distances of 173 individuals from nine populations of *Vellozia gigantea*, occurring in the Serra do Cipó, southeastern Brazil, based on 89 ISSR loci. Percentage of variance accumulated on the first two axes = 55% (axis 1 = 34.7%, axis 2 = 20.3%). *Solid symbols* correspond to geographic group I, *empty symbols* to group II and *crosses* to group III (see Fig 1). For population names see Table 1

populations of geographical group I with populations SR and NE (group II) and another containing populations SB, CP (group II) and CB (group III) (Fig. 3). The first cluster is subdivided into small clusters, one containing populations SR e NE, as observed in the Bayesian analysis. Population LE is located in an intermediate position between the two main groups in the dendrogram, probably due to its mixed gene pool as shown in the Bayesian analysis. AP and CB are the most divergent populations.

In the PCO analysis, 55.0% of the total variability is explained by the first two axes (axis 1 = 34.7% and axis 2 = 20.3%) (Fig. 4). This analysis was concordant with the Bayesian analysis and cluster analysis, which ordered the populations into the same three groups and showed the genetic separation of geographical group I from the other populations. The second cluster was comprised of populations SR and NE (geographical group II) and the third by populations SB, CP (group II) and CB (group III). Similar to the previous analysis, PCO showed the intermediate position of population LE, due to individuals sharing the genetic pool with the three geographical groups. The Mantel test showed a positive and significant correlation ($r = 0.474$ and $P = 0.002$) between pairwise $F_{ST}/(1 - F_{ST})$ and geographical distances. In spite of the small geographical distance between populations (1.1–27.3 km), the pairwise F_{ST} ranged from 0.149 to 0.411, with population AP and CB showing the highest values (Table 5). According to the F_{ST} value (0.28), the conservation of six (5.43) populations is necessary in order to represent 99.9% of the total diversity of the species.

Discussion

The *Vellozia gigantea* populations showed genetic diversity levels similar to other mixed/outcrossing species analyzed with RAPD markers (Nybom and Bartish 2000; Nybom 2004). As ISSR, RAPD are dominant markers and estimates derived by them are similar and comparable (Nybom 2004). The breeding system of *V. gigantea* is not known, but self-incompatibility has been found for some species in the genus (Sazima 1978; Oliveira et al. 1991; Jacobi and del Sarto 2007). Unexpectedly, the intra-population diversity was higher than that found in the widely distributed congener *V. compacta*, which was evaluated using the same set of ISSR primers (Lousada 2010). The comparison of narrowly endemic species with congenerics that have regional distributions, within the Espinhaço Range, showed lower levels of intra-population genetic diversity for the narrowly distributed species, both in *Vellozia* (Franceschinelli et al. 2006) and in *Chamaecrista* (Silva et al. 2007). In this same geological formation, Jesus et al. (2001, 2009) and Lambert et al. (2006a, b) also found low genetic variability for endemic species of Asteraceae

Table 5 Matrix of population pairwise F_{ST} (bellow diagonal) based on 89 ISSR loci and geographic distance in km (above diagonal) among nine populations of *Vellozia gigantea* occurring in the Serra do Cipó, Espinhaço range, southeastern Brazil

Population	AP	LE	ES	SE	NE	SR	CP	CB	SB
AP	–	3.48	3.42	6.90	14.89	25.62	27.33	24.64	21.53
LE	0.259	–	1.09	3.39	11.79	21.11	24.44	21.07	18.19
ES	0.172	0.171	–	3.61	11.49	22.24	24.05	21.26	18.12
SE	0.225	0.209	0.156	–	8.73	19.30	21.46	17.71	14.87
NE	0.282	0.234	0.217	0.232	–	10.76	12.73	11.84	7.18
SR	0.309	0.295	0.302	0.277	0.149	–	3.82	8.89	5.34
CP	0.394	0.222	0.337	0.284	0.304	0.277	–	12.69	8.83
CB	0.411	0.271	0.356	0.273	0.369	0.387	0.269	–	5.65
SB	0.364	0.207	0.315	0.259	0.333	0.324	0.205	0.212	–

For population names see Table 1

and Cactaceae. On the other hand, Borba et al. (2001, 2007), Azevedo et al. (2007) and Ribeiro et al. (2008) found high genetic diversity in rupicolous orchids from *campos rupestres* in the Espinhaço Range. It was expected that *V. gigantea*, which has a very restricted distribution, would display low levels of genetic diversity. One explanation for the moderate to high levels of genetic diversity found in this species could be related to its long life cycle and to its relatively large populations. Apparently, human disturbances (such as fires related to cattle farming, tourists, and the illegal collection of *V. gigantea* stems for the orchids that grow on them or, in the past, as a fuel source) have not seriously affected the diversity of *V. gigantea* yet, or the partition of this diversity.

The fixation index of *V. gigantea* ($\Phi_{ST} = 0.28$) was similar to outcrossing species (0.27), long-lived perennial species (0.25) and species with wind-dispersed seeds (0.25), analyzed with RAPD markers (Nybom 2004). However, the genetic divergence among *V. gigantea* populations was high for a narrowly endemic species and partially confirmed our first hypothesis that geological formations constitute partial barriers to gene flow. The PCO, cluster and Bayesian analyses showed that the Canyon Travessão might constitute a partial barrier to gene flow in *V. gigantea*. However, the Serra do Lobo valley does not appear have restricted the gene flow in this species. Several genetic studies with plant species from the *campos rupestres* of the Espinhaço Range have shown moderate to high genetic divergence among populations, such as Orchidaceae (Borba et al. 2001; Ribeiro et al. 2008), Asteraceae (Jesus et al. 2001, 2009), Cactaceae (Lambert et al. 2006a, 2006b), Velloziaceae (Lousada 2010) and Leguminosae (Silva et al. 2007). These studies reinforce the hypothesis that the discontinuity of the mountains of the Espinhaço Range is one of the main factors that lead to the divergence among populations. High divergence among populations of *Antirrhinum valentinu*m (Scrophulariaceae), a narrow endemic plant in southeastern Spain, was also associated with habitat discontinuity (Mateu-Andrés and Segarra-Moragues 2000).

Another factor that appears to influence the genetic structure of *V. gigantea* is isolation by distance, which is evident when looking at the correlation between geographical distance and pairwise F_{ST} in the Mantel test. Apparently, despite being light and flat, the seeds of *V. gigantea* are not dispersed by wind over long distances, like those of two other species of *Vellozia* (Franceschinelli et al. 2006). Although measurements were not made, most of the seeds produced by *V. gigantea* appear to fall near the parent plant and large clusters of seedlings can be found in some of these places. The pollinators of *V. gigantea* are not known; however, the results of the genetic structure obtained in this study suggest that gene flow through pollen is limited.

Implications for conservation

According to our results, special attention needs to be given to conserve each of the main genetic groups, one constituting the geographical group I and the other geographical groups II and III. Loss of one of the two main genetic groups could affect the genetic diversity of *V. gigantea*. Estimates from the F_{ST} value indicated that six populations would adequately represent 99.9% of the genetic diversity attributable to most common alleles. Based on genetic divergence of the others, genetic variation and degree of threat, priority populations to monitor could be AP, LE (cluster 1), NE, SR (cluster 2), CB and CP (cluster 3). Population LE is the closest large population to the Travessão Canyon and, because it has a mixed ancestry to geographic groups I and II it represents a source of variation for other populations from group I. It may also represent a connection between these groups, and its disappearance could lead to complete isolation between these two groups. Population AP is one of the most differentiated populations and it deserves immediate concern. Even though it is in the National Park, it is vulnerable due to its proximity to the main road inside the National Park. Currently this population is threatened by the presence of tourists, illegal collection of epiphytes, illegally set fires, and because there are cattle ranches on the other side of the road (Ribeiro et al. unpublished data).

Populations NE and SR are also priorities, because they represent a different genetic cluster, and NE is a large population that by its intermediate spatial position can represent a stepping-stone for gene flow between populations. Therefore, population CB (geographical group III), which has the lowest level of genetic diversity and is located outside the two protected areas (the Serra do Cipó National Park and the APA Morro da Pedreira) near an iron ore mine, must be urgently protected. The first management plan of the National Park was recently approved (ICMBio 2009). It proposes an increase in the limits of the National Park, which includes all populations except CB. However, if constant monitoring does not occur, the populations will continue to suffer from the current threats. Population CP, which showed one of the lowest values of genetic diversity, is also of special concern due to anthropogenic activities. This population is composed of scattered subgroups, where some of the patches of plants are found within the Park and many others are found outside of it, and all of the patches are heavily affected by fires.

The conservation of *Vellozia gigantea* will probably ensure the survival of other species, such as several epiphytes. Some of these species occur exclusively on its stems, such as the very rare orchid *Grobya cipoensis* (Barros and Lourenço 2004). The monitoring of these populations will also contribute to the preservation of the *campos rupestres*, which has a highly diverse and endemic flora.

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