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The Genetic Diversity of Two Brazilian *Vellozia* (Velloziaceae) with Different Patterns of Spatial Distribution and Pollination Biology

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• *Background and Aims* The genetic structure and variability of two species of *Vellozia* (Velloziaceae) with restricted distribution in high-altitude quartzitic fields in south-eastern Brazil were studied. *Vellozia epidendroides* is short, grows on pebbly or sandy soil, and is pollinated by bees. *Vellozia leptopetala* is arborescent, grows on rock outcrops, and is pollinated by bees and hummingbirds. Both are self-incompatible and have a short, massive flowering strategy. The study aimed to associate differences in their genetic diversity and structure with their microhabitat distribution and pollination ecology.
• *Methods* Leaves from 106 and 139 plants of *V. epidendroides* and *V. leptopetala*, respectively, were collected from five patches of each species and prepared for electrophoretic analyses.
• *Key Results* Five enzyme systems could be reliably scored for both species. *Vellozia epidendroides* showed 100 % of the loci polymorphic for almost all patches. The average number of alleles per locus ranged between 2·2 and 2·4 among patches. The Wright's fixation index (F) for this species was 0·226. A significant θ_p value indicates that there is a reasonable genetic divergence among patches. *Vellozia leptopetala* presented 47·5 % of polymorphic loci. All levels of P, A, A_p and of heterozygosities were lower than those of *V. epidendroides*. *Vellozia leptopetala* showed high inbreeding within patches.
• *Conclusions* The relatively high values of genetic diversity indices found for *V. epidendroides* may be associated with its large and widespread populations. On the other hand, the low values of genetic diversity found for *V. leptopetala* may be related to physical isolation on outcrops and intensive foraging by territorial hummingbirds, which may hinder gene flow among patches, aggravated by the very restricted seed dispersal characteristic of the genus, that facilitates sibling mating. It is important to stress the need to preserve the specific habitats of these species of *Vellozia*, in particular those of *V. leptopetala* that has lower genetic diversity and is restricted to rock outcrop environments.

Key words: Brazil, endemic species, genetic diversity, isozyme, rupestrian fields, Serra do Cipó, tropical plant, *Vellozia epidendroides*, *Vellozia leptopetala*.

INTRODUCTION

Velloziaceae is a small family of approximately 250 tropical species distributed in four genera (Mello-Silva, 1995). Its members are characteristic landscape elements in arid biomes, in particular high-altitude fields and outcrops of South America, Africa and Madagascar (Alves and Kolbek, 1994; Ibisch *et al*., 1995; Porembski and Barthlott, 2000). They are perennial, desiccation-tolerant and well adapted to fire (Alves, 1994; Ibisch *et al*., 1995; Porembski and Barthlott, 1995) to the point of sometimes being referred to as resurrection plants (Gaff, 1987; Ibisch *et al*., 1995, 2001).

The majority of species are found in the 'campo rupestre' vegetation (high-altitude rupestrian fields) of south-eastern Brazil (Joly, 1998), associated with a quartzitic formation known as Espinhaço Range. *Vellozia* in particular is well represented in these environments (about 140 species, Mello-Silva, 1996), with many endemic species restricted to portions of the Espinhaço Range.

A peculiar phenological feature in many *Vellozia* species is that the flowering season is characteristically short (cornucopia blooming *sensu* Gentry, 1974), usually a few weeks for a whole population. This strategy is useful to attract pollinators, or to avoid herbivore damage through predator satiation. On the other hand, and given the high level of self-incompatibility in the genus (Sazima, 1978; Oliveira *et al*., 1991), a large floral display is usually detrimental for seed set, because it increases the amount of self-pollination (Rademaker and de Jong, 1998; Ohashi and Yahara, 1999; Vrieling *et al*., 1999). Another mechanism that contributes to reduced pollen export and therefore gene flow in *Vellozia* is cross-pollination among siblings, a consequence of gravity-assisted dispersal of the tiny seeds (but see Ibisch *et al*., 2001).

Restricted gene flow may affect the genetic structure of a population, by reducing neighbourhood size and area (Levin and Kerster, 1974; Crawford, 1984). A low number of out-crossers may favour genetic drift, increasing the chances of reduced genetic variation within populations. This reduction is due to allele loss after some generations, besides reducing heterozigosity (Wright, 1943). The lack of genetic variability in a population may, for example, reduce its resistance to parasites and predators, and its potential for adaptation to changing environmental conditions (Lewontin, 1974).

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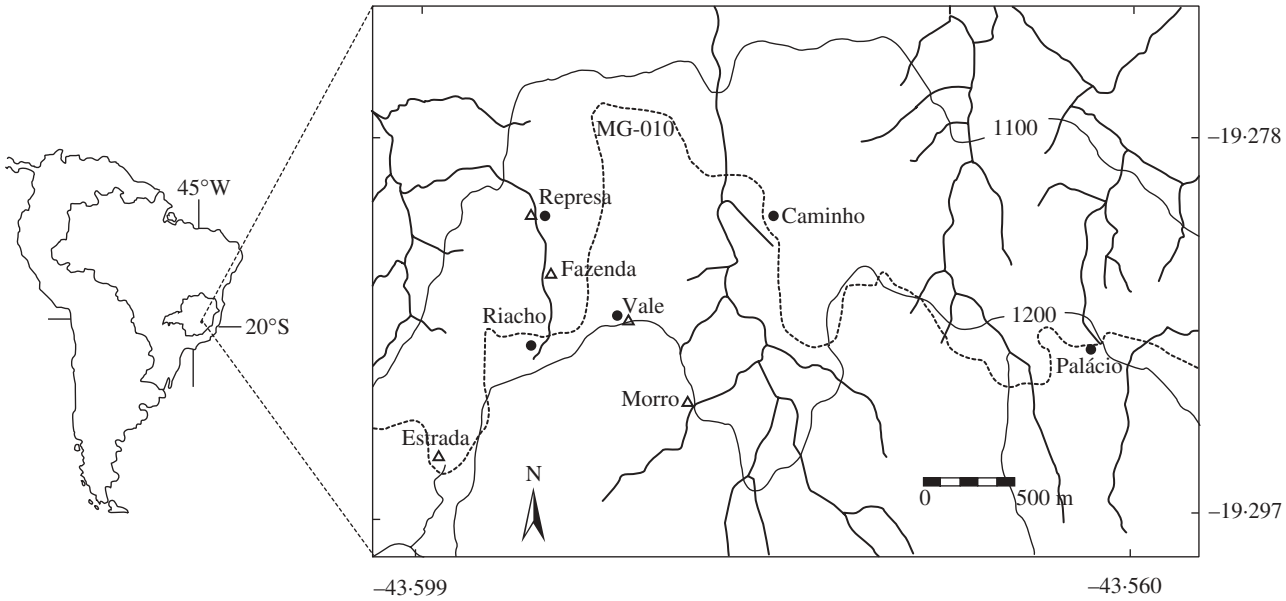

FIG. 1. Location of the studied patches for *Vellozia epidendroides* (circles) and *V. leptopetala* (triangles). The dotted line is interstate road MG-010. Georeference: André Hirsch, Zoology Dept., ICB/UFMG (source: ESRI, 2001; IBGE, 1998).

Species with a restricted geographical distribution tend to have low genetic diversity (e.g. Loveless and Hamrick, 1984; Godt *et al*., 1996). However, some rare species may show higher genetic variability than widespread ones (Smith and Pham, 1966; Gitzendanner and Soltis, 2000; Kang *et al*., 2000). In addition, phylogenetic history is another very important component determining levels of genetic variability (Felsenstein, 1985; Karron, 1987, 1997). Gitzendanner and Soltis (2000) found high degrees of correlation within genera for all measures of genetic diversity. These authors suggest comparing levels of genetic variation among congeneric species with different ranges of geographical distribution to evaluate levels of genetic variability in rare plants.

Levels of genetic diversity may also depend on population size, pollination dynamics and plant density in the area (Barrett and Kohn, 1991; Ellstram and Elam, 1993; Franceschinelli and Bawa, 2000). For example, rare species with large populations may show high levels of genetic diversity (Ellstram and Elam, 1993). However, endemic species with a particular microhabitat requirement would be likely to have a disjoint distribution and thus be more prone to suffer the effects of founder members and selective pressures.

We aimed to compare the genetic structure and diversity of populations of *Vellozia epidendroides* and *V. leptopetala* that occur in high-altitude fields of the Espinhaço Range, using allozyme markers. These species were chosen because, although they share some important reproductive biology characteristics that lead to restricted gene flow, their level of genetic isolation may differ on account of their different microhabitat requirements and pollination dynamics. Botanical studies of rupestrian Espinhaço Range Velloziaceae have traditionally focused on their anatomy and taxonomy, with a few exceptions (Sazima, 1978; Sazima and Sazima, 1990; Landau *et al*., 1998). This is the first attempt to examine the genetic diversity and structure of two species of *Vellozia*, and to associate these results with their reproductive strategy and habitat distribution.

MATERIALS AND METHODS

Study area and plant species

The study took place in rupestrian fields of Serra do Cipó, at the southern end of the Espinhaço Range, south-easthern Brazil (19°12′–19°20′S, 43°30′–43°40′W). The region is characterized by quartzitic mountains with altitudes varying between 1000–1400 m, reaching 1800 m in certain areas (Menezes and Giulietti, 1986). The climate in the region is marked by wet, mild summers and a dry winter season, which can last from 3 to 5 months. The mean annual rainfall is 1500 mm (Nimer, 1989). Rupestrian fields replace the cerrado (savanna) vegetation from around 900–1000 m (Giulietti and Pirani, 1988).

Vellozia leptopetala Goeth. et Henr. has an arborescent pseudostem 0·5–1·5 m high. Individuals grow exclusively on rock outcrops, which are distinct, isolated landscape features. The flowering period is short and associated with the beginning of the rainy season, usually between October and December. Large plants may bear up to 200 newly opened flowers when in full bloom. *Vellozia epidendroides* Mart. ex Schult. & Schult. has a short pseudostem (30–40 cm high) and occurs in dense and sometimes very large mats in open fields. Its flowering season is less synchronized than that of *V. leptopetala*, generally in April and May.

Populations of both species are distributed throughout Serra do Cipó (see Fig. 1); however, *V. epidendroides* extends approximately 200 km north beyond that region. The floral display of both species is quite conspicuous in the rupestrian landscape. Flowers are large, white, bisexual and short-lived (1–3 d). Pollen, exposed and abundant, is

TABLE 1. *Geographic distances (m) among the five patches of (a)* Vellozia epidendroides *and (b)* V. leptopetala

	Palacio	Caminho	Riacho	Vale	Represa
(a) *V. epidendroides*					
Palacio	–				
Caminho	1,858	–			
Riacho	3,036	1,492	–		
Vale	1,571	995	506	–	
Represa	3,099	1,302	711	706	–
	Estrada	Fazenda	Morro	Vale	Represa
(b) *V. leptopetala*					
Estrada	–				
Fazenda	1,153	–			
Morro	1,373	1,006	–		
Vale	1,253	474	543	–	
Represa	1,410	347	1,315	776	–

easily gathered by several bee species, both solitary and social. *Vellozia leptopetala* also produces a small amount of nectar that attracts hummingbirds (Sazima and Sazima, 1990). Hand self- and cross-pollination experiments showed that both species are self-incompatible; their main pollinators are leaf-cutting bees and, in *V. leptopetala*, also the territorial hummingbird *Augastes scutatus* (C.M. Jacobi, unpubl. res.). Seeds are very small (1·1–1·5 mm in diameter), spherical, and show no particular dispersal mechanism.

The study site was thoroughly surveyed for patches of both species. Although outcrops were abundant, many of them were not populated by *V. leptopetala*. However, the spatial scale of sampling did not differ much for the studied species (Table 1).

Allozyme analyses

Material for electrophoretic analyses, consisting of young leaves from five different patches of each species, was collected in the Serra do Cipó region at altitudes between 1115 and 1210 m (Fig. 1), totalling 106 individuals of *V. epidendroides* and 139 individuals of *V. leptopetala*. Because both species have clonal growth, care was taken to sample only individuals that were physically distinct. This reduced the chances of resampling the same individual, but at the risk of omitting genuinely distinct genets, particularly in the case of *V. epidendroides*. However, the material collected most likely represents 70–90 % of the individuals in each patch of this species, and 90 % of *V. leptopetala*. The location of each patch was determined with GPS. Individuals were marked with numbers in the field and leaf samples were wrapped in aluminium foil and refrigerated in ice for transportation. Samples were stored at −80 °C until enzyme extraction.

A small piece of each leaf was ground in liquid nitrogen and the powdered tissue was mixed with extraction buffer #1 of Alfenas *et al*. (1998) and absorbed onto filter paper wicks. Allozyme electrophoresis was carried out on horizontal 13 % starch-gels. Seventeen enzyme systems were screened with three electrophoretic buffers: morpholine citrate, lithium borate (Alfenas *et al*., 1998) and histidine (O'Malley *et al*., 1980).

Five enzyme systems showed simple banding patterns and could be reliably scored for both species. For *V. epidendroides*, the systems used were UGPP (two loci, UGPP1 and UGPP2), PGM, 6PG and AAT; for *V. leptopetala*, UGPP (two loci, UGPP1 and UGPP2), 6PG, GDH and AAT. Uridine diphosphoglucose pyrophosphorylase, IDH, PGM and 6PG were resolved on a morpholine citrate buffer system at pH 6·5 (Alfenas *et al*., 1998). Aspartate aminotransferase and GDH were assayed on a lithium borate buffer at pH 8·3 (Soltis *et al*., 1983). Locus banding patterns were consistent with typical subunit structures. Different loci and alleles for a given system were designated sequentially, with the lowest number corresponding to the most anodally migrating locus or allele.

Data analyses

Standard measures of allozyme diversity were calculated: the proportion of polymorphic loci ($P_{0.99}$), mean number of alleles per locus (A) and per polymorphic locus (A_p), observed heterozygosity (H_o) and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$). An estimate of inbreeding levels for each population was obtained using Wright's fixation index ($F = 1 - H_o/H_e$; Wright, 1922). Chi-square tests were used to check if frequencies of homozygotes and heterozygotes per population deviated from those expected under Hardy–Weinberg equilibrium. Nei's genetic distances (Nei, 1978) among populations were calculated. All these parameters were calculated using BIOSYS-2 (original version by Swofford and Selander, 1989; modified by Black, 1997).

Wright's (1943) F-statistics (f, θ_p and F) were used to measure hierarchical population structure and were calculated by the methods of Weir and Cockerham (1984). Jackknifing and bootstrapping were used for combining information over alleles and loci, and for estimating sample variances and confidence intervals, using the FSTAT program version 2.9.3.2 (Goudet, 2002).

Dendrograms were constructed to show relationships among patches, and Mantel tests were used to compare pairwise genetic distances against the geographical distances between patches. Both of these analyses were based on Nei's genetic distance (Nei, 1978) and performed with the FSTAT program version 2.9.3.2 (Goudet, 2002). An indirect estimate of gene flow was made based on the equation: $Nm = (1 - \theta_p)/4{\cdot}\theta_p$, where Nm is the number of migrants per generation (Wright, 1931).

RESULTS

Vellozia epidendroides

Significant deviations from Hardy–Weinberg expectations were detected for PGM in almost every patch (Represa, Riacho, Vale and Caminho), for 6PG in Represa, and UGPP1 in Riacho (Table 2). This indicates that crosses are not occurring randomly in the studied populations.

The percentage of polymorphic loci was 100 % for almost all patches, except for one that showed 80 % of polymorphic

TABLE 2. *Test for significant deviations from Hardy–Weinberg expectations verified through chi-square test within (a)* Vellozia epidendroides *and (b)* V. leptopetala *patches*

	Represa			Palacio			Riacho			Vale			Caminho		
Loci	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
(a) *Vellozia epidendroides*															
UGPP-1	–	–	–	3·487	1	0·062	6·858	1	0·009	0·831	1	0·362	3·227	1	0·072
UGPP-2	0·003	1	0·959	2·143	3	0·543	0·262	1	0·609	1·588	1	0·208	2·272	3	0·518
6PG-1	5·158	1	0·023	0·130	1	0·719	0·654	1	0·419	0·503	1	0·478	0·379	1	0·538
PGM-1	9·143	1	0·002	0·152	1	0·696	4·800	1	0·028	13·091	1	0·000	8·807	1	0·003
AAT-1	0·043	3	0·998	1·448	3	0·694	1·514	3	0·679	0·043	3	0·998	0·479	3	0·924
	Fazenda			Vale			Represa			Morro			Estrada		
Loci	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
(b) *V. leptopetala*															
UGPP-1	3·359	1	0·067	10·867	1	0·001	6·364	1	0·012	20·413	1	0·000	6·296	1	0·012
UGPP-2	–	–	–	17·171	1	0·000	15·180	1	0·000	–	–	–	–	–	–
6PG-1	0·694	1	0·405	0·013	1	0·909	0·135	1	0·713	2·779	1	0·096	2·042	1	0·153

TABLE 3. *Genetic diversity parameters for the five patches of* Vellozia epidendroides

Diversity indices	Represa	Palacio	Riacho	Vale	Caminho	Mean	Total
Average sample size (*N*)	12·8 (0·2)	21·8 (0·8)	31·6 (1·3)	11 (1·1)	17·8 (0·8)	19	95
Expected heterozygosity (H_e)	0·27 (0·088)	0·413 (0·058)	0·476 (0·017)	0·384 (0·075)	0·476 (0·021)	0·404	0·464
Observed heterozygosity (H_o)	0·177 (0·088)	0·42 (0·076)	0·378 (0·034)	0·251 (0·081)	0·343 (0·053)	0·314	0·341
Average number of alleles per locus (*A*)	2·00 (0·32)	2·40 (0·24)	2·20 (0·2)	2·20 (0·3)	2·40 (0·24)	2·24	2·4
Average number of alleles / polymorphic loci (A_p)	2·25	2·4	2·2	2·2	2·4	2·29	2·4
Percentage of polymorphic loci (*P*) (0·95 and 0·99)	80	100	100	100	100	96	100
Wright's *F*	0·355	−0·020	0·209	0·353	0·285	0·226	0·266

Sample size (*N*) refers to the number of individual plants. Standard deviations are in parenthesis.

TABLE 4. *Genetic diversity parameter estimates of each locus in* Vellozia epidendroides and V. leptopetalas

	V. epidendroides								*V. leptopetalas*						
Loci	N	*P*	*A*	A_p	H_e	H_o	*F*	Loci	N	*P*	*A*	A_p	H_e	H_o	*F*
UGPP1	105	1·0	2·0	2·0	0·453	0·229	0·496	UGPP1	132	1·0	2·0	2·0	0·479	0·197	0·590
UGPP2	86	1·0	3·0	3·0	0·510	0·454	0·112	UGPP2	139	1·0	2·0	2·0	0·158	0·043	0·728
6PG	94	1·0	2·0	2·0	0·503	0·415	0·175	6PG	129	1·0	2·0	2·0	0·486	0·403	0·171
PGM	94	1·0	2·0	2·0	0·502	0·255	0·493	GDH	139	0	1·0	*	0	0	0
AAT	96	1·0	3·0	3·0	0·353	0·354	−0·003	AAT	139	0	1·0	*	0	0	0
All	95	1·0	2·4	2·4	0·464	0·341	0·266	All	135·6	0·6	1·6	2·0	0·225	0·129	0·439

See Table 3 for parameter definitions.

loci (Table 3). The average number of alleles per loci ranged between 2·2 and 2·4 for the studied patches. The Wright's fixation index was high for four patches and the average value among patches was 0·226.

Considering the statistical analysis for each locus (Table 4), UGPP and PGM showed large differences between expected and observed heterozygosity and consequently high fixation indices. On the other hand, AAT showed a very low difference between expected and observed values, and had a correspondingly low value for the fixation index (−0·003). Considering all loci, a high fixation estimate (0·266), and a high difference between expected and observed heterozigosities were observed.

The significant θ_p value (0·101) indicates that there is a reasonable genetic divergence among patches. According to Hartl (1988), θ_p values of 0·05–0·15 indicate moderate genetic differentiation. Gene flow calculated among these populations was 2·23 migrants per generation. The fixation index within each patch (*f*) is high, as it is for each of their individuals (*F*; Table 5).

The analysis of the genetic distances among patches of *V. epidendroides* (Fig. 2) shows a high genetic proximity (identity index = 0·990) between Riacho and Caminho. Riacho and Represa are the most distant genetically (0·836). Geographic distances among patches did not correspond to genetic distances. Accordingly, the Mantel

test showed a low, non-significant inverse correlation ($r = -0{\cdot}29$, $P = 0{\cdot}42$).

Vellozia leptopetala

None of the five patches presented polymorphism in all loci, and the mean value was 0·48 among patches and 0·6 for the species (Table 6). This low polymorphism is the result of two or more monomorphic loci in all the analyses (Table 4). All levels of P, A, A_p and of heterozygosities were lower than those of *V. epidendroides*. The levels of expected and observed heterozygosities were low in all patches, particularly Morro, resulting in a high Wright's fixation index.

Significant deviations from Hardy–Weinberg expectations were detected in UGPP1 for all patches and UGPP2 for patches Morro and Estrada (Table 2). Considering that two out of five analysed loci were monomorphic, the significant deviation from the Hardy–Weinberg expectations detected in UGPP loci may indicate that crossings are not occurring randomly in the studied patches.

Results of Wright's F-statistic for *V. leptopetala* are presented in Table 5. There is high inbreeding within the patches, particularly because of the excess of homozygotes showed by the two bands of UGPP. There is no significant genetic divergence among the patches, with $\theta_p = 0{\cdot}028$. The number of migrants Nm was 8·68 migrants per generation.

Figure 3 illustrates the proximity relations among the different patches. There is no clear relationship between the genetic distances among patches and their geographic distances, as was also seen in *V. epidendroides*. Here, the Mantel test also showed a low, non-significant inverse correlation ($r = -0{\cdot}35$, $P = 0{\cdot}32$).

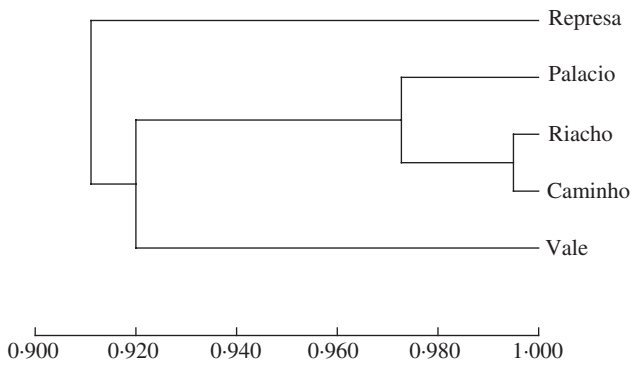

FIG. 2. Dendrogram constructed according to values of Nei's similarity indices found for patches of *Vellozia epidendroides* from Serra do Cipó, Minas Gerais State, Brazil.

TABLE 5. *Estimates of Wright's* F-*statistics* (f, F *and* θ_p) *described for each polymorphic locus and for all patches of* Vellozia epidendroides *and* V. leptopetala

	V. epidendroides			*V. leptopetala*		
Loci	*F*	*f*	θ_p	*F*	*f*	θ_p
UGPP1	0·516	0·402	0·195	0·582	0·600	0·037
UGPP2	0·116	0·062	0·058	0·627	0·236	0·703
6PG	0·174	0·139	0·037	0·176	0·168	-0·010
PGM	0·480	0·413	0·099	–	–	–
AAT	−0·011	−0·032	0·020	–	–	–
Total	0·285	0·202	0·101	0·390	0·412	0·028
s.e.	0·101	0·086	0·034	0·207	0·211	0·036
P (*t*-test)	0·01	0·05	0·05	NS	NS	NS

f = mean fixation index of individuals relative to their population; θ_p = populations co-ancestrality coefficient; F = mean overall inbreeding coefficient of an individual.

DISCUSSION

The genetic diversity of the studied species was shown to be different. The allelic diversity of *V. epidendroides* was not low ($A = 2{\cdot}4$) compared with values of other tropical plants. Hamrick and Godt (1989), in a review of 653 genetic diversity studies using allozymes, found the following values of allelic diversity (number of alleles per locus): 2·19 for woody long-lived species, 2·29 for widely distributed species, 1·81 for tropical species, and 1·99 for allogamous animal-pollinated species. The diversity of *V. leptopetala* ($A = 1{\cdot}6$) was comparatively lower, and similar to values found for endemic species ($A = 1{\cdot}8$), according to Hamrick and Godt (1989).

Hamrick and Murawski (1991) compared levels of allozyme diversity of 16 uncommon neotropical tree species with 16 common ones, and observed significantly higher values in this last group. The common species showed 61 % of polymorphic loci and a mean of 3·17 alleles per polymorphic locus. The rare species presented 42 % of polymorphic loci and a mean of 2·14 alleles per polymorphic locus. *Vellozia epidendroides* showed 100 % of polymorphic loci with a mean of 2·4 alleles per polymorphic locus, while *V. leptopetala* had 60 % of polymorphic

TABLE 6. *Genetic diversity parameters for the five patches of* Vellozia leptopetala

Diversity indices	Fazenda	Vale	Represa	Morro	Estrada	Mean	Total
Average sample size (N)	9·8 (0·2)	22·2 (0·6)	34·2 (0·8)	36·8 (0·2)	32·6 (1·2)	27·12	135·6
Expected heterozygosity (H_e)	0·184 (0·114)	0·227 (0·106)	0·259 (0·107)	0·201 (0·123)	0·193 (0·118)	0·213	0·225
Observed heterozygosity (H_o)	0·104 (0·065)	0·136 (0·094)	0·165 (0·085)	0·099 (0·071)	0·126 (0·078)	0·126	0·129
Average number of alleles per locus (A)	1·40 (0·24)	1·60 (0·24)	1·60 (0·24)	1·40 (0·24)	1·40 (0·24)	1·48	1·6
Average number of alleles per polymorphic loci (A_p)	2·0	2·0	2·0	2·0	2·0	2·0	2·0
Percentage of polymorphic loci (P) (0·95 and 0·99)	0·40	0·60	0·60	0·40	0·40	0·48	0·6
Wright's F	0·444	0·409	0·365	0·509	0·350	0·412	0·439

Sample size (N) refers to the number of individual plants. Standard deviations are in parenthesis.

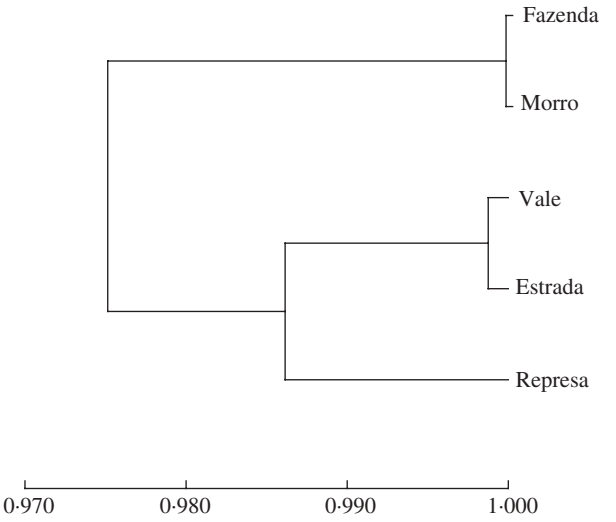

FIG. 3. Dendrogram constructed according to values of Nei's similarity indices found for patches of *Vellozia leptopetala* from Serra do Cipó, Minas Gerais State, Brazil.

loci and 2·0 alleles. These last values are close to the typical ones found for rare and/or endemic species (Godt *et al*., 1996, 2004; Mateus-Andrés and Segarra-Moragues, 2003).

The estimated rates of expected and observed heterozygosity were much higher in *V. epidendroides* (0·464 and 0·341, respectively) than *V. leptopetala* (0·225 and 0·129, respectively) and the Wright's fixation index was higher for the last species (0·439 versus 0·266). The expected heterozygosity rate found for *V. epidendroides* is similar to other long-lived, non-endemic tropical species (Hamrick and Godt, 1989; Moraes, 1992; Santos, 1994; Darrigo *et al*., 2002; Ribeiro, 2002; Vasconcelos, 2002; Carmo, 2005). On the other hand, values of expected and observed heterozygosity found for *V. leptopetala* were low and usually associated with endemic species (Godt *et al*., 1996, 2004; Mateus-Andrés and Segarra-Moragues, 2003).

High genetic diversity has been found in endemic species (Smith and Pham, 1966; Kang *et al*., 2000), including two from Serra do Cipó whose genetic variability is maintained by mechanisms of their reproductive strategies (Borba *et al*., 2001; Gomes *et al*., 2004). Overall, however, most endemic species have shown a significant lower percentage of polymorphic loci, mean number of alleles per locus and observed heterozygosity than their widespread congeners (Gitzendanner and Soltis, 2000). Similar results were found in our work. *Vellozia epidendroides*, with a wider geographical range, larger populations and less restricted habitat requirements had higher levels of genetic diversity than *V. leptopetala*, which has a reduced geographical range and grows exclusively on physically isolated rock outcrops. All those factors may contribute to gene flow within a small neighbourhood in *V. leptopetala*.

Wright's fixation index for *V. leptopetala* is one of the highest found among perennial plant species, suggesting that it is undergoing strong inbreeding. This inbreeding is probably biparental, since the species is self-incompatible. Possibly, the fact that *Vellozia* seeds have a very restricted dispersal promotes the establishment of kin seeds close to their parents, which facilitates sibling mating. Furthermore, the high fixation index and the non-random mating within patches could be associated with intensive foraging by the territorial hummingbird *Augastes scutatus*, one of its main pollinators.

However, indirect estimates of the number of migrants among patches—calculated by means of the θ_p index—were higher for *V. leptopetala* (*Nm* = 8·68) than for *V. epidendroides* (*Nm* = 2·23). Indirect estimates of gene flow reflect historical rates of flow that have occurred over many generations (Loveless, 1992). In our work, individuals could have come from different reproductive cycles and establishment; that is, from supra-annual variations in seed set, with different selective pressures on progeny and seedlings. Besides, it is possible that the high number of migrants *Nm* and low values of θ_p in *V. leptopetala* are also a consequence of the low genetic diversity found for this species in general, making it difficult to distinguish its populations.

Given the longevity of many Velloziaceae (Alves, 1994), the lack of correlation between genetic and geographic distance in our study could be attributed to the previous existence of a wider range than nowadays (Giulietti and Pirani, 1988). In any case, the genetic distances between the patches of both species are very small (especially for *V. epidendroides*) and would not be easy to detect geographically. Considering their restricted distribution and their microhabitat preference, it is important to stress the need to preserve the specific habitats of these species of *Vellozia*, in particular those of *V. leptopetala*. The protection of campo rupestre vegetation may prevent further increases in its inbreeding rates and its extinction due to lack of genetic diversity.

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