

Transferability of microsatellite loci to *Vellozia plicata* (Velloziaceae), a widespread species on Brazilian inselbergs

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Abstract Inselbergs are isolated, mainly granitic and gneissic rock outcrops, forming old landscape elements on crystalline continental shields on all continents. Many Velloziaceae which occur specifically as mat-formers on these rock outcrops are well adapted to harsh environmental conditions and do not occur in the surrounding matrix. *Vellozia plicata* Mart. is a widespread desiccation-tolerant mat-forming species on inselbergs in southeastern Brazil, with naturally fragmented populations. Here, we tested the transferability of 53 microsatellite loci previously developed for two *Vellozia* species (*V. gigantea* N. L. Menezes & Mello-Silva and *V. squamata* Pohl) in two populations of *V. plicata*. We succeeded in the amplification of 11 loci, and six of them were polymorphic. The number of alleles per locus ranged from 2 to 17, the expected and observed heterozygosities ranged from 0.000 to 0.875 and from 0.000 to 0.895, respectively. Our results show that cross-amplification works within the genus *Vellozia*, but at a lower level when compared to other monocot families on inselbergs, such as Bromeliaceae and Orchidaceae. This work might help in further studies on population genetics of *V. plicata*, in order to guide conservation actions in the future and also to promote further

investigations about speciation processes of inselberg specialists.

Keywords Conservation genetics · Cross-amplification · Monocots · Population genetics

Introduction

In many tropical regions, granitic and gneissic inselbergs form ancient landscape elements (Porembski 2007). Monocotyledonous mats (monocot mats) are one of the most typical vegetation types, in which predominantly long-lived monocots occur on open rocky slopes, frequently forming isolated circular patches (Porembski et al. 1998). Four families of monocots are typical mat-formers: Bromeliaceae, Cyperaceae, Poaceae and Velloziaceae (Porembski et al. 1998; de Paula et al. 2016).

The Velloziaceae form a Gondwanan link between African, Malagasy and South American inselbergs, and in particular *Vellozia* (mainly Brazil) and *Xerophyta* (Africa/Madagascar) are very speciose (Mello-Silva et al. 2011). Many Velloziaceae, which occur as mat-formers specifically on inselbergs, are desiccation-tolerant and well adapted to harsh environmental conditions (such as lack of soil and water, high insolation) and do not occur in the surrounding matrix (Porembski and Barthlott 2000), showing a high morphological plasticity. For these mat-formers, inselbergs form terrestrial habitat islands that are characterized by various degrees of geographic isolation (Porembski et al. 2000).

In Brazil, few studies investigated reproductive systems of *Vellozia*, which showed that species occurring either on high altitude quartzitic grasslands (Jacobi and del Sarto 2007) and/or on “Cerrados” (Oliveira et al. 1991) were

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mostly self-incompatible. Besides that, information on potential pollinators is also scarce, but the available studies pointed mainly pollination by birds and bees (Sazima and Sazima 1990; Oliveira et al. 1991; Jacobi and del Sarto 2007). Concerning the genetic structure of *Vellozia* species, up to now there are no clear patterns within the genus. For example, it was demonstrated high values of genetic diversity for bee-pollinated species growing on pebbly or sandy soil and low values for species occurring on quartzitic outcrops, pollinated by bees and hummingbirds (Franceschinelli et al. 2006). Furthermore, it was shown that populations of high vulnerable species with a narrow distribution on quartzitic outcrops showed high genetic diversity (Lousada et al. 2011). Interesting results were also observed for a species occurring on ferruginous and on quartzitic outcrops, populations from the former outcrops presented about half the genetic diversity of those from the later (Lousada et al. 2013). Nevertheless, even though *Vellozia* species are common members of Brazilian granitic and gneissic rocks, up to now we lack information concerning taxonomic, ecological and molecular aspects of the species occurring on these outcrops.

Here, we tested the transferability of microsatellite loci previously developed for two *Vellozia* species, which occur on “campos rupestres”, in *Vellozia plicata* Mart., a widespread species on Brazilian inselbergs. Cross-amplification is a good alternative to avoid expenses in generating new markers, besides being less time-consuming. We believe that this study will help in further investigations concerned with the relationship between geographic isolation and population genetic differentiation of this species and also guide studies with other species belonging to this genus.

Materials and methods

Plant material and DNA extraction – We sampled young leaves of 20 individuals of *V. plicata* (Fig. 1) from two inselberg populations (totaling 40 individuals), named BOCA (BO; 17°54'58.87"S, 41°11'15.05"W; voucher L. de Paula 1145) and SIVA (SI; 17°46'49.61"S, 41°11'54.93"W; voucher L. de Paula 1146), and subsequently they were stored in silica gel. The inselbergs sampled were about 15 km away from each other and were located in the Mucuri Valley, northeastern Minas Gerais, Brazil. Total DNA was extracted following the protocol described by Štorchová et al. (2000). All vouchers are deposited in RB herbarium in Rio de Janeiro Botanical Garden.

PCR amplification and genotyping – We initially tested the potential cross-amplification of 53 microsatellite (simple sequence repeats, SSR) loci previously developed for *V. gigantea* N. L. Menezes & Mello-Silva (Martins

et al. 2012) and *V. squamata* Pohl (Duarte-Barbosa et al. 2015) (see Online Resource 1) in a set of eight individuals (from both sampled populations) of *V. plicata*. All polymerase chain reaction (PCR) amplifications were performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems) in a reaction volume of 10 µL containing ~10 ng of DNA, 2.5× GoTaq Master Mix (Promega), 0.5 µM forward primer, 1 µM reverse primer and 1 µM universal M13 (5'CACGACGTTGTAAAACGAC-3') primer tagged with FAM, VIC, PET or NED fluorochromes. A touchdown cycle program was used as described by Palma-Silva et al. (2007). The amplification products were analyzed by electrophoresis on 1% agarose gel stained with GelRed (Biotium, Hayward, California, USA). The loci were considered successfully amplified when a band of the expected size was clearly visualized. The PCR products of the well-amplified loci were subjected to fragment analysis on an ABI PRISM 3500 sequencer (Applied Biosystems) and sized in accordance with GeneScan 500 LIZ (Applied Biosystems) using GENEMARKER v1.95 software (SoftGenetics). Raw alleles sizes of the polymorphic loci were then automated binned into discrete classes using FlexiBin (<http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm>) (Amos et al. 2007) and manually inspected.

Data analyses – The Micro-Checker 2.2.3 program (van Oosterhout et al. 2004) was used to assess genotyping errors due to the presence of null alleles, stuttering and allele dropout. For each population and locus, we estimated the number of alleles (*A*) and observed (H_O) and expected (H_E) heterozygosities according to the Hardy–Weinberg equilibrium (HWE), using GenAlEx 6.5 (Peakall and Smouse 2012); within-population inbreeding coefficient (F_{IS}) and allelic richness (*AR*) were accessed using FSTAT (Goudet 1995). Deviations from HWE were evaluated using exact tests, as implemented in GENEPOP on the Web (Raymond and Rousset 1995). Linkage disequilibrium between all pairs of loci in each population was tested in FSTAT. The statistical significance was adjusted for multiple testing using a sequential Bonferroni correction (Rice 1989).

Results and discussion

From the 53 primers tested in this study, 19 showed amplification products, but eight of them were nonspecific or had weak amplification. From the 11 successfully amplified loci, six of them were polymorphic for *V. plicata* (all of them from *V. squamata*; for details see Online Resource 1), displaying an average allele number of seven (ranging from 2 to 17) (Table 1). Among the six analyzed loci, three loci (Vsqs25, Vsqs30 and Vsqs34) in BO

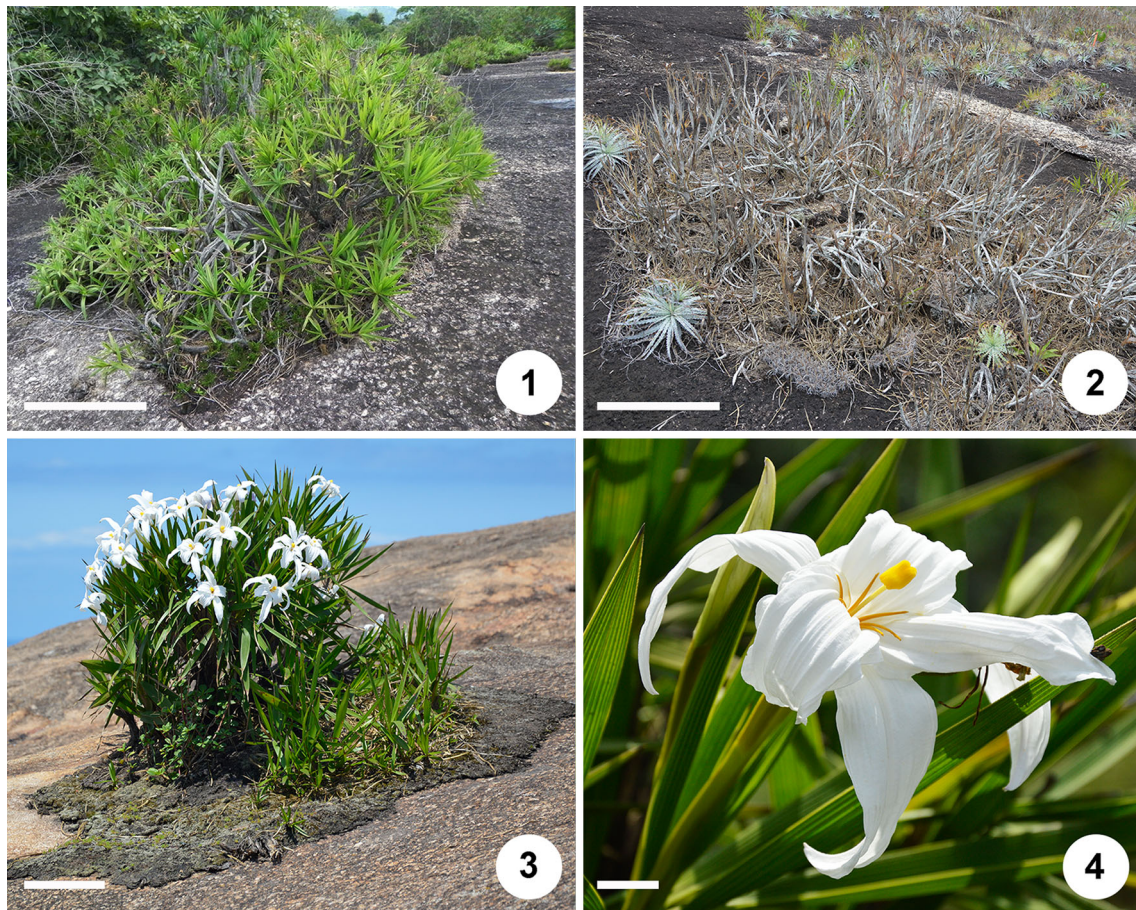


Fig. 1 1–4 Overview of *Vellozia plicata* mats on inselbergs in southeastern Brazil. **1** Mats with fully green leaves during the wet season. **2** Mats during the dry season reflecting the desiccation tolerance strategy of this species. **3** Habit. **4** Flower. Bars 50 cm (1, 2), 20 cm (3), 2 cm (4)

Table 1 Microsatellite loci transferred to *Vellozia plicata* (Velloziaceae), followed by the species in which the marker was developed, reference, size range of the PCR products, repeat motif and number of alleles (A)

Locus	Species	References	Size range (bp)	Repeat motif	A
Vsq25	<i>Vellozia squamata</i>	Duarte-Barbosa et al. (2015)	188–226	(AC)8	7
Vsq30	<i>Vellozia squamata</i>	Duarte-Barbosa et al. (2015)	267–275	(AC)10...(TG)6	4
Vsq34	<i>Vellozia squamata</i>	Duarte-Barbosa et al. (2015)	159–243	(CT)15	17
Vsq39	<i>Vellozia squamata</i>	Duarte-Barbosa et al. (2015)	265–267	(AC)7	2
Vsq47	<i>Vellozia squamata</i>	Duarte-Barbosa et al. (2015)	217–219	(AC)11	2
Vsq48	<i>Vellozia squamata</i>	Duarte-Barbosa et al. (2015)	207–229	(CA)14	10

population and one locus (Vsq34) in SI population showed significant deviation from HWE due to heterozygous deficiency ($P < 0.05$; Table 2). In BO population, expected heterozygosity ranged from 0.053 to 0.895 (an average of 0.399) and observed heterozygosity ranged from 0.051 to 0.875 (an average of 0.534). In SI population, expected heterozygosity ranged from 0.000 to 0.889 (an average of 0.355) and observed heterozygosity ranged from 0.000 to 0.789 (an average of 0.415). Both populations showed significant deviation from HWE due to heterozygous deficiency ($P < 0.0001$), inbreeding coefficients were also high and significant for both populations ($F_{IS} = 0.286$ in

BO and 0.183 in SI, $P < 0.0001$). The excess of homozygous found in this study might be due to inbreeding and/or Wahlund effect, although we could not discard the presence of null alleles in loci Vsq30, Vsq 34 and Vsq39. No linkage disequilibrium was detected in any pair of loci.

The percentage of primer transferability for *V. plicata* found in this study ($\sim 11\%$) is lower than those reported for other *Vellozia* species from “campos rupestres”. From the seven markers developed for *V. gigantea*, all loci amplified in *V. auriculata* Mello-Silva & N. L. Menezes, while 86 and 43% of the markers amplified in *V. compacta* Mart. ex Schult. f. and *V. hirsuta* Goeth. & Henrard, respectively (Martins et al.

Table 2 Characterization of the six polymorphic microsatellite markers transferred to two populations, named BOCA (BO) and SIVA (SI), of *Vellozia plicata* (Velloziaceae)

Locus	BO (<i>N</i> = 20)					SI (<i>N</i> = 20)				
	A	AR	H_O	H_E	F_{IS}	A	AR	H_O	H_E	F_{IS}
Vsq25	5	4.2	0.895	0.634	-0.388*	4	3.3	0.778	0.529	-0.447
Vsq30	4	3.7	0.214	0.617	0.674*	2	1.9	0.188	0.170	-0.071
Vsq34	14	11.4	0.357	0.875	0.615*	6	6	0.000	0.760	1.000*
Vsq39	2	1.9	0.167	0.239	0.329	2	1.9	0.278	0.239	-0.133
Vsq47	2	1.5	0.053	0.051	0.000	1	1	0.000	0.000	NA
Vsq48	8	6.5	0.706	0.789	0.135	7	6.2	0.889	0.789	-0.099
Mean	6	5	0.399	0.534	-	4	3	0.355	0.415	-
All	35	-	-	-	0.286*	22	-	-	-	0.183*

Number of alleles per locus (A), allelic richness per locus (AR), observed heterozygosity (H_O), expected heterozygosity (H_E) and within inbreeding coefficient (F_{IS}) are shown

NA not applied

* Significant departure from Hardy–Weinberg equilibrium ($P < 0.05$)

2012). *Vellozia plicata* has a very special position in the phylogeny of the genus: it is the sister clade of all the other Brazilian *Vellozia* species (Mello-Silva et al. 2011), being even considered in another genus in previous studies (*Nanuzia plicata*: Smith and Ayensu 1976; Mello-Silva 2005). In this sense, the studied species is phylogenetically distant from *V. gigantea* and *V. squamata*, the two species in which the tested primers were developed. Therefore, that might help in explaining the low primer transferability found here.

Even though for monocots cross-amplification rates are relatively high within species belonging to same genus (~40%, Barbará et al. 2007b), the rate for marker transferability found in this study was considerably low. For other monocot families occurring on inselbergs, cross-amplification works even for species belonging to different subfamilies in Bromeliaceae (Palma-Silva et al. 2007; Barbará et al. 2007a) and within the same genus in Orchidaceae (e.g., *Epidendrum* spp., Pinheiro et al. 2009b). Bromeliaceae show relatively recent adaptive radiations with low levels of DNA sequence divergence (Barbará et al. 2007b) while *Epidendrum* comprise relatively recent species (Pinheiro et al. 2009a), and that contrasts with the ancient Gondwanan origin of Velloziaceae, suggested by its distribution (Mello-Silva et al. 2011). Thus, that might have also influenced different levels of sequence divergence within Velloziaceae, making it more difficult the cross-amplification within the genus *Vellozia* for phylogenetic distant species.

Studies on neotropical inselberg-adapted species, using microsatellite marker approaches, have showed high population differentiation, high genetic diversity levels and strong phylogeographic structure in this naturally fragmented environment (e.g., Barbará et al. 2007a; Palma-Silva et al. 2011; Pinheiro et al. 2014). However, these studies are still restricted to species belonging to few plant families (mainly Bromeliaceae and Orchidaceae). Thus, as one of the most predominant members on inselbergs

(Porembski 2007), we claim that molecular studies on Velloziaceae are urgently needed, especially because this family can be used as a model to understand patterns of speciation at broader scales, since it is distributed on terrestrial islands throughout the tropics. Furthermore, species with patchy distribution usually experience reduced gene flow, significant genetic drift and high levels of population divergence, supporting the view of inselbergs as centers of species diversity and endemism.

In this context, threats to inselbergs and consequentially its plant populations are rapidly increasing, due to quarrying, mining and invasive species (de Paula et al. 2015; Porembski et al. 2016). Therefore, conservation efforts to preserve these populations include describing the levels of genetic diversity and structure, still largely unknown for Velloziaceae species (but see Barbosa et al. 2012; Lousada et al. 2013). We hope that the markers reported in this study will promote further investigations about conservation status and speciation processes within this notoriously difficult group of inselberg specialists.

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