Genetics and Molecular Biology, 42, 1, 87-94 (2019)

DOI: http://dx.doi.org/10.1590/1678-4685-GMB-2017-0293

Research Article

Cross-genera SSR transferability in cacti revealed by a case study using *Cereus* (Cereeae, Cactaceae)

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Abstract

The study of transferability of simple sequence repeats (SSR) among closely related species is a well-known strategy in population genetics, however transferability among distinct genera is less common. We tested cross-genera SSR amplification in the family Cactaceae using a total of 20 heterologous primers previously developed for the genera *Ariocarpus, Echinocactus, Polaskia* and *Pilosocereus,* in four taxa of the genus *Cereus*: *C. fernambucensis* subsp. *fernambucensis*, *C. fernambucensis* subsp. *sericifer*, *C. jamacaru* and *C. insularis*. Nine microsatellite loci were amplified in *Cereus* resulting in 35.2% of success in transferability, which is higher than the average rate of 10% reported in the literature for cross-genera transferability in eudicots. The genetic variation in the transferred markers was sufficient to perform standard clustering analysis, indicating each population as a cohesive genetic cluster. Overall, the amount of genetic variation found indicates that the transferred SSR markers might be useful in large-scale population studies within the genus *Cereus*.

Keywords: Cactaceae, *Cereus,* cross-genera, SSR markers, Transferability.

Received: September 13, 2017; Accepted: June 13, 2018.

Simple sequence repeats (SSR) or microsatellites are, in general, non-coding regions commonly found in Eukaryote genomes composed of tandemly arranged repeat motifs from 1 to 6 base pairs (Oliveira *et al.*, 2006). SSRs are useful molecular markers for several applications in population genetics and breeding studies, as they frequently exhibit high levels of polymorphism, in addition to their abundance and random distribution across and throughout genomes. In plants, SSR loci have been used for several purposes, for example, estimates of genetic diversity (Zhu *et al.*, 2016), intra- and interspecific gene flow (Palma-Silva *et al.*, 2011; Pinheiro *et al.*, 2014), biogeographical distributions (Beatty and Provan 2011), phylogenetic relationships (Mehmood *et al.*, 2016), genetic mapping (Tan *et al.*, 2016), and conservation (Gómez-Fernández *et al.*, 2016).

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An alternative to overcome time consuming and costly development of a new set of SSR primers for a target species is to carry out the transferability of SSR primers among related species (Barbará *et al.*, 2007; Lavor *et al.*, 2013; Nogueira *et al.*, 2015). The rate of success in this approach (i.e., heterologous amplification) depends on the nucleotide similarity among the flanking regions of different species. Therefore, it is expected that there will be a higher rate in heterologous amplification among taxa with recent divergence times. In plants, this technique has been widely adopted for a great variety of eudicots (e.g., Haerinasab *et al.*, 2016; Mengistu *et al.*, 2016), where the average rate of success at infrageneric level is around 60% (Barbará *et al.*, 2007). The rate of cross-genera transferability is around 10% in eudicots (Barbará *et al.*, 2007), but the levels of success may reach values above 50% in some plants (Satya *et al.*, 2016).

Taking into account the recent divergence within Cactaceae, as well as its emergence as an informative model to study diversification in xeric habitats (Arakaki *et al.*, 2011), the aim of this study was to perform cross-genera

SSR amplification in four closely related taxa of the genus *Cereus* (Cactaceae; Cereeae) occurring in eastern Brazil: *C. fernambucensis* subsp. *fernambucensis*, *C. fernambucensis* subsp. *sericifer*, *C. insularis* and *C. jamacaru.* A previous phylogenetic analysis based on plastid DNA placed *C. jamacaru* as a member of a polytomic clade, sister of the monophyletic clade composed by *C. fernambucensis* and *C. insularis* (Franco *et al.*, 2017a). In this study, we selected a set of 11 SSR loci originally described for *Ariocarpus bravoanus* (Hughes *et al.*, 2008), *Echinocactus grusonii* (Hardesty *et al.*, 2008) and *Polaskia chichipe* (Otero-Arnaiz *et al.*, 2004) that were recently transferred to *Cereus* species cultivated in different urban areas, including *C. hildmannianus* (Martin 2011; Fernandes *et al.*, 2016). An additional nine SSR loci described for *Pilosocereus machrisii* (Perez *et al.*, 2011) were included in this investigation.

We sampled 122 individuals from representative populations of *C. jamacaru*, *C. insularis* and *C. fernambucensis* (Table 1), besides one individual of *C. hildmannianus* (Salto, SP; 23.99, 47.33; SORO 2746) as a positive control in the initial tests. Genomic DNA was extracted from the radicular tissue using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). As the samples from localities S82 and S83 are geographically (~34 km) and genetically close, sharing the same unique alleles and comprising a cohesive genetic group, we decided to join the individuals from the two populations in a single sample, hereinafter referred to as S82/S83.

Initial amplification tests were performed using a subsample of 12 individuals, with slight modifications on PCR conditions as described by Albert and Schmitz (2002), Don *et al.* (1991), and Perez *et al.* (2011). The reactions were performed in 10 μL of total PCR volume including 0.5 U of *Taq* DNA Polymerase (Promega), 1X *Taq* Buffer (5X Colorless GoTaq® Flexi Buffer), 0.2 μM dNTPs, and primer and $MgCl_2$ concentrations varying when necessary. We considered a locus successfully transferred when the PCR products were clearly visualized in 3% agarose gels and showed a product size compatible with the range described for that locus. The loci successfully amplified were then genotyped in the total sample (Table 1) using PAGE (denaturing polyacrylamide gel) with concentrations varying between 6% to 9%, according to expected allele size. To visualize the alleles, the gels were stained with silver nitrate. The percentage of transferability success was estimated according to the number of individuals amplified in each locus.

The occurrence of null alleles, allele drop-out, and stutter bands was evaluated with Micro-Checker 2.2.3 software (Van Oosterhout *et al.*, 2004). The number of alleles per locus (N_a), effective number of alleles (n_e), expected (H_e) and observed (H_o) heterozygosities, private alleles, and percentage of polymorphic loci were estimated using GenAlEx 6.5 software (Peakall and Smouse, 2012). The inbreeding coefficient (F_{IS}) per population was calculated using FSTAT 2.9.3.2 (Goudet, 1995), assuming $\alpha = 0.01$ and $\alpha = 0.001$ (Lavor *et al.*, 2013; Ribeiro *et al.*, 2014). Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were investigated using the Arlequin 3.5.1.3 program (Excoffier and Lischer, 2010). We used the sequential Bonferroni correction for multiple testing with $\alpha = 0.05$ (Rice, 1989) to minimize statistical errors. Genetic differentiation among populations was quantified by F_{ST} (Weir and Cockerham, 1984) estimated in FSTAT 2. 9. 3. 2 (Goudet, 1995) and corrected for null alleles in FreeNA (Chapuis and Estoup, 2007). The pairwise chord distances (Dc, Cavalli-Sforza and Edwards, 1967) between populations was estimated in FreeNA software (Chapuis and Estoup, 2007), and the resulting matrix was

Table 1 - Geographical localities of the populations from three species of *Cereus* used in this work.

Species	Voucher	Geographic Coordinates (S, W)	N
C. fernambucensis subsp. *fernambucensis* Lem.			
Arraial do Cabo, RJ (S80) - Southern group*	SORO 2663	-22.97, -42.03	19
Maracajaú, RN (S104) - Northern group*	SORO 4529	-5.39, -35.31	20
Una, BA (S114) - Northern group*	SORO 2675	-15.11, -39.00	20
C. fernambucensis subsp. *sericifer* Ritt.			
Santa Maria Madalena, RJ (S82) - Southern group*	SORO 2665	-21.95, -42.03	12
Itaoacara, RJ (S83) - Southern group*	SORO 2666	-21.65, -42.09	4
Águia Branca, ES (S88) - Northern group*	SORO 2749	-19.06, -40.69	10
C. jamacaru DC.			
Conceição de Feira, BA (S113)	HUEFS 33711	-12.59, -38.99	18
C. insularis Hmsl.			
Fernando de Noronha, PE (S115D)	SORO 2677	-3.85, -32.40	19

***** The classification in Southern and Northern group is based on phylogeographic data available for *C. fernambucensis* (Franco *et al.*, 2017b) / N = number of individuals per populations.

then used as input to generate a Neighbor-Joining dendrogram (NJ) (Saitou and Nei, 1987) in Populations 1.2.32 software (Langella, 1999). To explore genetic structure in our data we performed: a Principal Coordinate Analysis (PCoA) in GenAlEx 6.5 (Peakall and Smouse, 2012); a global and a hierarchical Analysis of Molecular Variance (AMOVA) in Arlequin 3.5.1.3 (Excoffier and Lischer, 2010); and a Bayesian clustering analysis in STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). The latter was implemented using 10 simultaneous and independent runs with 10^6 generations of MCMC (25% as burn-in). The K-values tested ranged from 1 to 8. To find the best K we used ΔK statistics (Evanno *et al.*, 2005) in Structure Harvester. The results of the independent runs for the best K were combined in Clumpp (Jakobsson and Rosenberg, 2007), and were graphically displayed with Distruct (Rosenberg, 2004).

From the 20 tested loci (Table S1), nine (*mAbR 28* from *A. bravoanus*; *mEgR 02*, *mEgR 76* and *mEgR 78* from *E. grusonii* and *Pmac82*, *Pmac84*, *Pmac108*, *Pmac146* and *Pmac149* from *P. machrisii*) showed positive results in transferability for at least one species (Table S2), resulting in 35.16% of success in transferability. Except for *mEgR 02*, the allele size for all loci was congruent with the expected size (Table S2). We were not able to amplify five SSR loci previously transferred to *Cereus* (*Pchi 21*, *Pchi 47*, *Pchi 54*, *mAbR 42* and *mAbR 77*) (Martin, 2011; Fernandes *et al.*, 2016), even after several attempts to modify PCR conditions (Table S3). This result is likely related to nucleotide differences among the flanking regions of the samples used in this work, preventing primer annealing.

The percentage of polymorphic loci ranged from 44.4% (populations S113 and S82/S83) to 77.8% (location S80) (Table 2). In contrast with the expectation of reduced levels of genetic diversity at transferred SSR loci (e.g. Goldstein and Pollock, 1997; Jan *et al.*, 2012; Moodley *et al.*, 2015), we found higher levels of polymorphism for some loci (*Pmac82* in all populations, *mEgR 78* in S88 and *mEgR 02* in S80) than those reported in the original description (Table 2). No locus showed significant heterozygosity deficiency in relation to expectations of HWE after Bonferroni correction. Inbreeding coefficient estimates (F_{IS}) provided no significant result (Table 2). The locus *Pmac108* showed high levels of observed heterozygosity in all populations, excepting S82/S83 (Table 2). Private al-

Table 2 - Genetic diversity indices: Number of samples (N), Number of alleles (*Na*), Effective allele number (*Ne*), Observed heterozygosity (*Ho*), Expected heterozygosity (*He*), absence (-) and presence (+) of null alleles, and F_{IS} values per loci and population are shown.

Locus	N	*Na*	*Ne*	*Ho*	*He*	Null Allele	F_{IS}	*p*-value
Population S113							0.06	0.26
Pmac82	18	3	1.57	0.33	0.36	-		
Pmac108	13	5	2.38	0.62	0.58	-		
Pmac149	18	5	1.81	0.50	0.45	-		
mEgR 02	16	1	1.00	0.00	0.00	-		
mAbR 28	16	1	1.00	0.00	0.00	-		
mEgR 76	18	2	1.12	0.00	0.11	-		
Média	16.5	2.83	1.48	0.24	0.25			
Population S80							0.08	0.13
Pmac82	19	2	1.17	0.16	0.15	-		
Pmac84	18	3	2.66	0.78	0.62	-		
Pmac108	18	5	2.61	0.67	0.62	-		
Pmac146	17	3	2.34	0.41	0.57	-		
mEgR 02	16	3	1.68	0.25	0.41	-		
mAbR 28	14	4	1.57	0.43	0.36	-		
mEgR 76	17	1	1.00	0.00	0.00	-		
mEgR 78	17	2	1.12	0.00	0.11	-		
Média	17.0	2.56	1.77	0.34	0.36			
Population S82/S83							0.05	0.35
Pmac82	16	2	1.44	0.38	0.30	-		
Pmac84	15	1	1.00	0.00	0.00	-		
Pmac108	13	1	1.00	0.00	0.00	-		
Pmac146	12	5	1.71	0.17	0.42	++		
mEgR 02	14	1	1.00	0.00	0.00	-		
mAbR 28	11	1	1.00	0.00	0.00	-		
mEgR 76	14	2	1.51	0.00	0.34	++		
mEgR 78	16	2	2.00	1.00	0.50	-		
Média	13.88	1.88	1.33	0.19	0.19			

Locus	N	*Na*	*Ne*	*Ho*	*He*	Null Allele	F_{IS}	*p*-value
Population S88							0.24	0.01
Pmac82	10	5	3.13	0.40	0.68	++		
Pmac84	10	2	1.60	0.10	0.38	-		
Pmac108	8	6	4.74	0.50	0.79	++		
Pmac146	9	4	2.00	0.56	0.50	-		
mEgR 02	7	1	1.00	0.00	0.00	-		
mAbR 28	5	1	1.00	0.00	0.00	-		
mEgR 76	10	1	1.00	0.00	0.00	-		
mEgR 78	9	4	3.00	0.89	0.67	-		
Média	8.50	3.00	2.18	0.31	0.38	-		
Population S115D							0.59	1.00
Pmac82	19	2	1.05	0.05	0.05	-		
Pmac84	19	2	1.70	0.58	0.41	-		
Pmac108	19	2	1.98	0.89	0.49	-		
Pmac146	18	6	3.27	1.00	0.69	-		
mEgR 02	18	2	1.74	0.61	0.42	-		
mAbR 28	15	1	1.00	0.00	0.00	-		
mEgR 76	17	1	1.00	0.00	0.00	-		
mEgR 78	18	2	2.00	1.00	0.50	-		
Média	17.88	2.25	1.72	0.52	0.32	-		
Population S104							0.18	0.99
Pmac82	20	4	1.60	0.45	0.37	-		
Pmac84	20	2	1.72	0.60	0.42	-		
Pmac108	20	6	3.15	1.00	0.68	-		
Pmac146	18	5	2.19	0.61	0.54	-		
mEgR 02	20	1	1.00	0.00	0.00	-		
mAbR 28	20	2	1.66	0.25	0.40	-		
mEgR 76	20	1	1.00	0.00	0.00	-		
mEgR 78	19	1	1.00	0.00	0.00	-		
Média	19.63	2.75	1.67	0.36	0.30	-		
Population S114							0.01	0.52
Pmac82	20	2	1.05	0.05	0.05	-		
Pmac84	20	4	2.02	0.55	0.50	-		
Pmac108	19	4	2.06	0.68	0.52	-		
Pmac146	13	2	1.83	0.54	0.45	-		
mEgR 02	20	1	1.00	0.00	0.00	-		
mAbR 28	20	1	1.00	0.00	0.00	-		
mEgR 76	20	1	1.00	0.00	0.00	-		
mEgR 78	15	2	1.92	0.27	0.48	-		
Média	18.38	2.13	1.48	0.26	0.25			

$p < 0.01$ / 0.001 = significant, and $p > 0.01$ / 0.001 = not significant.

leles were found in populations S113, S88, S115D, S114, S104 and S82/83 (Table S4). The LD analysis results between polymorphic loci were not statistically significant after Bonferroni correction.

The FreeNA-corrected estimate of global F_{ST} was 0.44, ranging from 0.12 (*Pmac82*) to 0.79 (*mEgR 02*) (Table S5). Clustering analyses (NJ, PCoA, STRUCTURE) have shown somewhat distinct results (Figure 1 and Figure S1). However, the results from AMOVA suggest that the three clusters recovered by STRUCTURE better explain the genetic variation structuring in our data (Table 3), as follows: 1) S113, S014 and S115D populations; 2) S80, S88 and S114 populations; 3) S82/S83 (Figure 1a). To investigate sub-structuration within our data, we performed a STRUCTURE analysis for each cluster, which resulted in each location being a cohesive genetic group (Figure 1b). Although we have not done an extensive geographic sampling for each studied taxon, and the number of markers is relatively low, some clustering results recovered here agree with previous phylogeographic hypotheses established for *C. fernambucensis* and *C. insularis* based on cpDNA and the *PHYC* gene (Franco *et al.*, 2017b). The close relationship of *C. jamacaru* and *C. fernambucensis* subsp. *fernambucensis* (S104) deserves additional investigation,

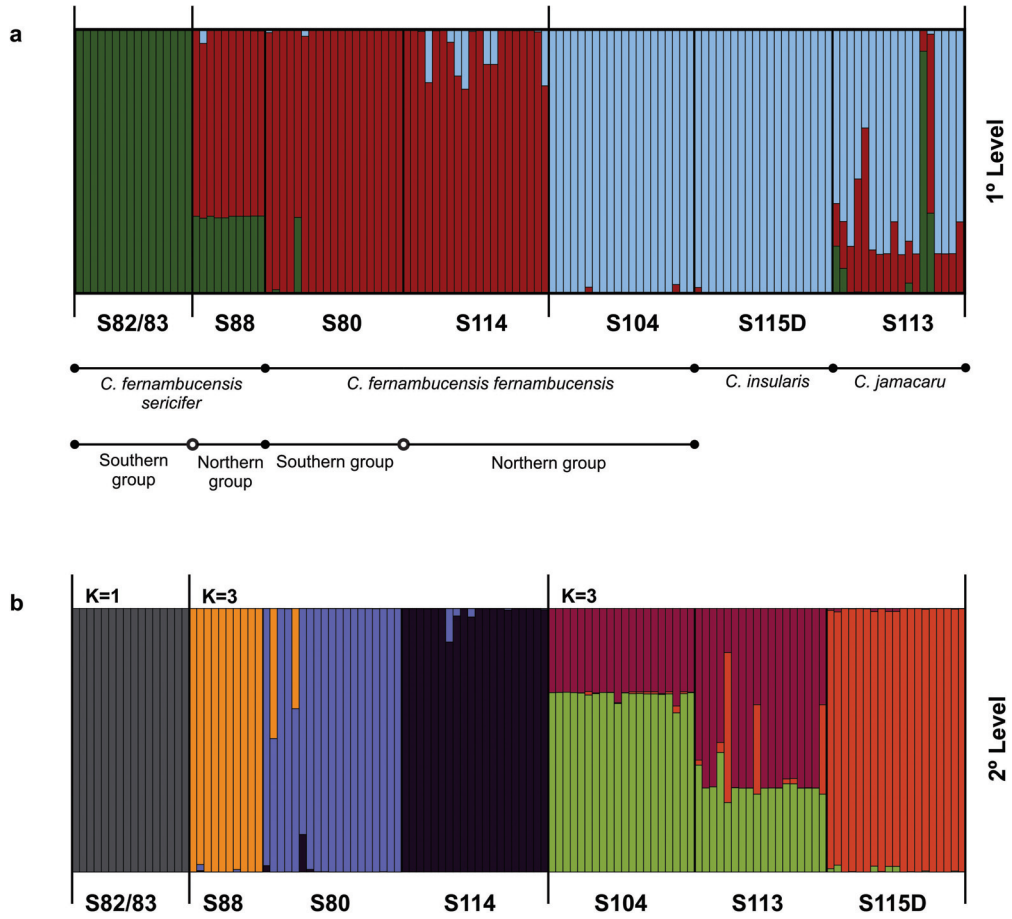

Figure 1 - Population differentiation in STRUCTURE, (a) results for K = 3 on the first level structure, and (b) separating each population as a distinct genetic group. The southern and northern population groups of C. *fernambucensis* subsp. *sericifer* and *C. fernambucensis* subsp. *fernambucensis* are based on phylogeographic circumscription (Franco *et al.*, 2017b).

Table 3 - Global and hierarchical Analysis of Molecular Variance (AMOVA). For hierarchical AMOVA the a priori groups are based on taxonomic circumscription, NJ phenogram, PCoA and STRUCTURE.

Groups	Fixation Indexes	Source of Variation	Percentege of Variation
Global AMOVA			
All populations only one group	Φ_{ST} = 0.34460	Among populations within group	34.46
Taxonomic circumscription			
S113, S115D, S82/S83 with S88, S80 with S104 and S114	Φ_{CT} = 0.01385	Among groups	1.38
	Φ_{SC} = 0.33731	Among populations within groups	33.26
NJ Phenogram			
S113, S80 with S82/83 and S88, S104 with S115D, S114	Φ_{CT} = -0.25331	Among groups	-25.33
	Φ_{SC} = 0.45546	Among populations within groups	57.08
PCoA			
	Φ_{CT} = -0.26648	Among groups	-26.65
S113, all others populations (S80, S82/83, S88, S104, S114 and S115D)	Φ_{SC} = 0.38531	Among populations within groups	48.80
STRUCTURE			
S82/S83, S80 with S88 and S114, S104 with S113 and S115D	Φ_{CT} = 0.34737	Among groups	34.74
	Φ_{SC} = 0.09764	Among populations within groups	6.37

but seems to be a spurious grouping as a result of the reduced number of sampled populations.

The estimated success in transferability observed in this study (35.16%) was higher than the average of 10% found in cross-genera transferability studies published between 1997 and mid-2006 (see Barbará *et al.*, 2007). However, this is not an uncommon result, as similar findings or even higher levels of cross-genera transferability were observed in some groups of plants (Satya *et al.*, 2016). In the family Iridaceae, for example, a success of 77% was observed in cross-amplification between genera (Miz *et al.*, 2016). In the family Malvaceae cross-genera SSR transferability varied from 71% to 92% (Satya *et al.*, 2016), while in Euphorbiaceae these percentages ranged from 9.5% to 59.1% (Whankaew *et al.*, 2011). Evidently, genera are taxonomic categories mainly based on morphological instead of genetic information, and different levels of phylogenetic divergence must be embedded within each genus. Therefore, the success in cross-genera transferability may vary highly depending on the target organism. On the other hand, it is expected that the success in cross-amplification should be a function of phylogenetic distance, at least regarding genetic differentiation (Barbará *et al.*, 2007).

This expectation was not clearly observed here considering cactus phylogeny (Hernández-Hernández *et al.*, 2014). We observed similar success in heterologous amplification using primers described for relatively distantly (*A. bravoanus* and *E. grusonii* – four transferred of nine tested) or closely related species (*P. machrisii* – five transferred of 11 tested). It is worth highlighting that for those loci from *A. bravoanus*, *E. grusonii,* and *P. chichipe* we had a previous expectation of positive cross-amplification, as they were formerly transferred for some *Cereus* species (Martin, 2011; Fernandes *et al.*, 2016). Nevertheless, higher levels of cross-genera amplification in the cactus family might be a widespread tendency, as the main lineage divergences and species radiation events within this family are thought to have occurred in the last 10 Myr (Arakaki *et al.*, 2011; Hernández-Hernández *et al.*, 2014; Silva *et al.*, 2018). Even with remarkable morphological distinctness among cactus species, resulting in more than 120 recognized genera (Hunt *et al.*, 2006), these recent divergence times increase the possibility of heterologous amplification in the family Cactaceae due to the expected similarities in the flanking SSR regions among different species. Evidently, this is not a rule, as even here we found discordance between some results obtained by Martin (2011) and Fernandes *et al.* (2016), which were likely due to nucleotide differences in flanking regions of the distinct samples. At any rate, this information should be taken into consideration to encourage cross-genera transferability studies in Cactaceae, which, despite their potential, are still relatively scarce in this family (Table S6).

The genus *Cereus* constitutes an interesting biological model to perform evolutionary studies, and efforts were employed to screen informative molecular markers in this genus (Silva *et al.*, 2016; Romeiro-Brito *et al.*, 2016) to solve species level phylogeny (Franco *et al.*, 2017a) and to investigate population differentiation and phylogeography (Franco *et al.*, 2017b; Silva *et al.*, 2018). Our results are in line with these endeavors, supplying additional molecular markers that can be useful for estimating genetic diversity and gene flow in target *Cereus* species. Furthermore, considering the rate of success in transferability, our results should encourage cactus researchers interested in using the increasing number of SSR loci that have been described for representatives of this highly diverse and relatively overlooked plant family (e.g., Bonatelli *et al.*, 2015; Fava *et al.*, 2016).

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 (fellowship to J.R.B.). This work was also supported by grants from São Paulo Research Foundation (FAPESP) to F.F.F. (2010/25227-0, 2014/25227-0). To sampling *Cereus insularis*, we had support from Fernando de Noronha Marine National Park (ICMBIO/PARNAMAR) and governmental administration of Fernando de Noronha (DEFN). We thank Maria de Fatima P.S. Machado (State University of Maringá, Brazil) for supplying valuable information about SSR transferability in *Cereus*. We thank Manolo F. Perez for critical comments in a preliminary version of this work.

Conflict of interest

The authors declare that they have no conflict of interest associated with this study.

Author contributions

F.F.F. and J.R.B. conceived and designed the study; J.R.B. conducted the experiments; J.R.B., I.A.S.B and G.A.R.S. analyzed the data; F.F.F. and J.R.B. wrote the manuscript; E.M.M, D.C.Z. and N.P.T contributed in data interpretation, writing and grammar review, all authors read and approved the final version.

References

Albert S and Schmitz J (2002) Characterization of major royal jelly protein-like DNA sequences in *Apis dorsata*. J Apic Res 41:75-82.

Arakaki M, Christin PA, Nyffeler R, Lendel A, Eggli U, Ogburn RM, Spriggs E, Moore MJ and Edwards EJ (2011) Contemporaneous and recent radiations of the world's major succulent plant lineages. Proc Natl Acad Sci U S A 108:8379–8384.

Barbará T, Palma-Silva C, Paggi GM, Bered F, Fay MF and Lexer C (2007) Cross-species transfer of nuclear microsatellite markers: potential and limitations. Mol Ecol 16:3759-3767.

Beatty GE and Provan J (2011) Comparative phylogeography of two related plant species with overlapping ranges in Europe, and the potential effects of climate change on their intraspecific genetic diversity. BMC Evol Biol 11: 29

Bonatelli IAS, Carstens BC and Moraes EM (2015) Using Next Generation RAD sequencing to isolate multispecies microsatellites for *Pilosocereus* (Cactaceae). PLoSOne 10: e0142602.

Cavalli-Sforza LL and Edwards AWF (1967) Phylogenetic analysis: Models and estimation procedures. Am J Hum Genet 19:233-257.

Chapuis MP and Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631.

Don RH, Cox PT, Wainwright BJ, Baker K and Mattick JS (1991) Touchdown PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008.

Evanno G, Regnault S and Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611-2620.

Excoffier L and Lischer HE (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 3:564-7.

Fava WS, Paggi GM, Zanella CM and Lorenz-Lemke AP (2016) Development and characterization of microsatellite markers for *Echinopsis rhodotricha* and cross-amplification in other species of Cactaceae. Biochem Syst Ecol 66:19-23.

Franco FF, Rodrigues GAS, Marsola EM, Taylor NP, Zappi DC, Jojima CL and Machado MC (2017a) Plio-Pleistocene diversification of *Cereus* (Cactaceae, Cereeae) and closely allied genera. Bot J Linn Soc 183:199-210.

Franco FF, Jojima CL, Perez MF, Zappi DC, Taylor NP and Moraes EM (2017b) The xeric side of the Brazilian Atlantic Forest: The forces shaping phylogeographic structure of cacti. Ecol Evol 7:9281–9293.

Fernandes VNA, Neves AF, Martin PG, Mangolin CA and Machado MFPS (2016) Genetic structure and molecular divergence among samples of mandacaru (*Cereus* spp.; Cactaceae) as revealed by microsatellite markers. Biochem Syst Ecol 64:38-45.

Goldstein DB and Pollock DD (1997) Lauching microsatellites: A review of mutation processes and methods of phylogenetic interference. J Hered 88:335-342.

Gómez-Fernández A, Alcocer I and Matesanz S (2016) Does higher connectivity lead to higher genetic diversity? Effects of habitat fragmentation on genetic variation and population structure in a gypsophile. Conserv Genet 17:631–641.

Goudet J (1995) FSTAT (version 1.2): A computer program to calculate F-statistics. J Hered 86:485-486.

Haerinasab M, Rahiminejad MR and Ellison NW (2016) Transferability of Simple Sequence Repeat (SSR) markers developed in red clover (*Trifolium pretense* L.) to some *Trifolium* species. Iran J Sci Technol Trans Sci 40:59-62.

Hardesty BD, Hughes L, Rodriguez VM and Hawkins JA (2008) Characterization of microsatellite loci for the endangered cactus *Echinocactus grusonii*, and their cross-species utilization. Mol Ecol Res 8:164-167.

Hernández-Hernández T, Brown JW, Schlumpberger BO, Eguiarte LE and Magallón S (2014*)* Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World succulent biome. New Phytol 202:1382-1397.

Hughes SL, Rodriguez VM, Hardesty BD, Luna RTB, Hernández HM, Robson RM and Hawkins JA (2008) Characterization of microsatellite loci for the critically endangered cactus *Ariocarpus bravoanus*. Mol Ecol Res 8:1068-1070.

Hunt D, Taylor N and Charles G (2006) The new cactus lexicon. DH Books, Milborne Port, 526 pp.

Jakobsson M and Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.

Jan C, Dawson DA, Altringham JD, Burke T and Butlin RK (2012) Development of conserved microsatellite markers of high cross-species utility in bat species (Vespertilionidae, Chiroptera, Mammalia). Mol Ecol 12:532–548.

Lavor P, Van Den Berg C and Versieux LM (2013) Transferability of 10 nuclear microsatellite primers to *Vriesea minarum* (Bromeliaceae), a narrowly endemic and threatened species from Brazil. Braz J Bot 36:165-168.

Martin PG (2011) Transferibilidade de microssatélites de cactáceas para a análise de regenerantes clonais (R0) de *Cereus peruvianus* Mill. (Cactaceae). M. Sc. Thesis, Universidade Estadual de Maringá, Maringá.

Mehmood A, Luo S, Ahmad NM, Dong C, Mahmood T, Sajjad Y, Jaskani MJ and Sharp P (2016) Molecular variability and phylogenetic relationships of guava (*Psidium guajava* L.) cultivars using inter-primer binding site (iPBS) and microsatellite (SSR) markers. Genet Resour Crop Evol 63:1345–1361.

Mengistu FG, Motoike SY, Caixeta ET, Cruz CD and Kuki KN (2016) Cross-species amplification and characterization of new microsatellite markers for the macaw palm, *Acrocomia aculeata* (Arecaceae). Plant Genet Resour 14:163–172.

Miz RB, Tacuatiá LO, Cidade FW, de Souza AP, Bered F, Eggers L and de Souza-Chies TT (2016) Isolation and characterization of microsatellite loci in *Sisyrinchium* (Iridaceae) and cross amplification in other genera. Genet Mol Res 15:38474.

Moodley Y, Masello JF, Cole TL, Calderon L, Munimanda GK, Thali MR, Alderman R, Cuthbert RJ, Marin M, Massaro M *et al.* (2015) Evolutionary factors affecting the cross-species utility of newly developed microsatellite markers in seabirds. Mol Ecol 15:1046–1058.

Nogueira AM, Ferreira A and Ferreira MFS (2015) Transferability of microsatellites from *Psidium guajava* to *Eugenia*, *Myrciaria, Campomanesia, and Syzygium Species* (Myrtaceae). Plant Mol Biol 34:249–256.

Oliveira EJ, Pádua JG, Zucchi MI, Vencovsky R and Vieira MLC (2006) Origin, evolution and genome distribution of microsatellites. Genet Mol Biol 29:294-307.

Otero-Arnaiz A, Cruse-Sanders J, Casas A and Hamrick JL (2004) Isolation and characterization of microsatellites in the columnar cactus: *Polaskia chichipe* and cross-species amplification within the Tribe Pachycereeae (Cactaceae). Mol Ecol Notes 4:265-267.

Palma-Silva C, Wendt T, Pinheiro F, Barbará T, Fay MF, Cozzolino S and Lexer C (2011) Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. Mol Ecol 20:3185–3201.

Peakall R and Smouse PE (2012) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295.

Perez MF, Téo MF, Zappi DC, Taylor NP and Moraes EM (2011) Isolation, characterization, and cross-species amplification of polymorphic microsatellite markers for *Pilosocereus machrisii* (Cactaceae). Am J Bot 98:204-206.

Pinheiro F, Cozzolino S, Draper D, Barros F, Félix LP, Fay MF and Palma-Silva C (2014) Rock outcrop orchids reveal the genetic connectivity and diversity of inselbergs of northeastern Brazil. BMC Evol Biol 14:1471-2148.

Pritchard JK, Stephens M and Donnelly P (2000) Inference of population structure using multilocus genotype data. Genet Soc Am 155:945-959.

Ribeiro PCC, Muller LAC, Lemos-Filho JP and Lovato MB (2014) Transferability and characterization of nuclear microsatellite markers in populations of *Annona coriacea* (Annonaceae), a tree from the Brazilian Cerrado. Bot Soc Sao Paulo 37:353-356.

Rice WR (1989) Analyzing tables of statistical tests. Evol 43:223-225.

Romeiro-Brito M, Moraes EM, Taylor NP, Zappi DC and Franco FF (2016) Lineage-specific evolutionary rate in plants: Contributions of a screening for *Cereus* (Cactaceae). Applic Plant Sci 4:1500074.

Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. Mol Ecol Notes 4:137-138.

Saitou N and Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.

Satya P, Paswan PK, Ghosh S, Majumdar S and Ali N (2016) Confamiliar transferability of simple sequence repeat (SSR) markers from cotton (*Gossypium hirsutum* L.) and jute (*Corchorus olitorius* L.) to twenty two Malvaceous species. Biotech 6:65-70.

Silva GAR, Jojima CL, Moraes EM, Antonelli A, Manfrin MH and Franco FF (2016) Intra and interspecific sequence variation in closely related species of *Cereus* (CACTACEAE). Biochem Syst Ecol 65:137-142.

Silva GAR, Antonelli A, Lendel A, Moraes EM and Manfrin MH (2018) The impact of early Quaternary climate change on the diversification and population dynamics of a South American cactus species. J Biogeogr 45:76-88.

Tan LQ, Wang LY, Xu LY, Wu LY, Peng M, Zhang CC, Wei K, Bai PX, Li HL, Cheng H and Qi GN (2016) SSR-based genetic mapping and QTL analysis for timing of spring bud flush, young shoot color, and mature leaf size in tea plant (*Camellia sinensis*). Tree Genet Genomes 12:52-64.

Van Oosterhout C, Hutchinson WF, Wills DPM and Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Whankaew S, Kanjanawattanawong S, Phumichai C, Smith DR, Narangajavana J and Triwitayakor K (2011) Cross-genera transferability of (simple sequence repeat) SSR markers among cassava (*Manihot esculenta* Crantz), rubber tree (*Hevea brasiliensis* Muell. Arg.) and physic nut (*Jatropha curcas* L.). Afr J Biotechnol 10:1768-1776.

Weir BS and Cockerham CC (1984) Estimating F-statistics for the analysis of population-structure. Evolution 38:1358-1370.

Zhu XH, Cheng SP, Liao T and Kang XY (2016) Genetic diversity in fragmented populations of *Populus talassica* inferred from microsatellites: Implications for conservation. Genet Mol Res 15:27899.

Internet Resources

Structure Harvester, http://taylor0.biology.ucla.edu/structureHarvester/ (accessed 10 August 2016)

Langella O (1999) Populations 1.2.32, http://bioinformatics.org/~tryphon/populations/ (accessed 05 August 2016)

Supplementary material

The following online material is available for this article:

Figure S1 - Neighbor-joining phenogram.

Table S1 - Characteristics of 20 microsatellite loci tested for transferability.

Table S2 - Transferability results of the SSR markers for populations.

Table S3 - PCR conditions for the SSR markers transferred.

Table S4 - Private alleles (> 10% frequency) found in each locus per population.

Table S5 - F-statistics by locus for all populations and FST corrected by ENA method.

Table S6 - Results of a literature survey.

Associate Editor: Dario Grattapaglia