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Mating System and Genetic Structure Across All Known Populations of *Dyckia brevifolia*: A Clonal, Endemic, and Endangered Rheophyte Bromeliad

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Abstract

Dyckia brevifolia is an endemic rheophyte bromeliad that occurs exclusively in patches on rocky banks of the Itajaí-Açu River in southern Brazil. The genetic diversity of all known populations was carried out using allozyme markers and the total numbers of rosettes, reproductive rosettes, and clumps per population were characterized. The mating system was also investigated. Most rosettes were aggregated in groups, and the populations differed significantly in number of rosettes and reproductive rosettes per population. The outcrossing rate obtained was 8.2%, with predominant selfing. The populations presented an average of 1.4 alleles per locus and 27% of polymorphisms. The mean expected genetic diversity was 0.067. Downstream populations showed the highest genetic diversity which could be attributed to hydrochory (unidirectional river flow). Most genetic diversity is distributed among populations ($\hat{F}_{ST}$ = 0.402). Natural habitats of *D. brevifolia* are not recommended for the construction of hydroelectric plants given that it would seriously complicate in situ conservation of this species. Based on the results of this study, it can be concluded that between 35 and 161 reproductive rosettes must be collected for effective ex situ conservation, depending on the targeted population, or seeds collected from 157 seed-rosettes per population.

Subject area: Conservation genetics and biodiversity; Population structure and phylogeography
Key words: allozyme, conservation, founder event, genetic diversity, metapopulation, selfing

Riparian plant populations occur as discrete patches as a result of the irregular distribution of suitable habitats along river banks (Honnay et al. 2010). Small and isolated habitat patches may reduce the levels of genetic diversity in these populations, in turn elevating the costs of fitness as a result of inbreeding, and, finally, increase the risk of local extinction for this kind of species (Reed and Frankham 2003). The colonization process often involves a marked founder event, which can also significantly impact genetic diversity (Barrett 1996;

Cain et al. 2000). Gene flow strongly influences the spatial scale by which genetic differentiation will be observed (Slatkin 1985) and the mechanism of seed dispersal is a major determinant of the spatial genetic structure of plant populations (Shimamura et al. 2007). Long-distance seed dispersal is a crucial determinant for within-population genetic variability and among-population genetic differentiation in metapopulations (Jacquemyn et al. 2006). The mating system is another factor that can influence the spatial distribution of genetic variation within and among populations (Loveless and Hamrick 1984; Tero et al. 2003). Clonal propagation and/or sexual reproduction can also significantly affect the demography and genetic diversity of natural populations (Murawski and Hamrick 1990; Arnaud-Haond et al. 2007).

In riparian populations, several studies have found higher levels of genetic diversity in downstream populations in comparison with upstream populations (Kudoh and Whigham 1997; Gornall et al. 1998; Lundqvist and Andersson 2001; Liu et al. 2006; Markwith and Scanlon 2007). This phenomenon has been termed the "unidirectional diversity hypothesis" (Ritland 1981). However, other studies with riparian species have shown no evidence of downstream accumulation of genetic diversity (Tero et al. 2003; Honnay et al. 2010; Hmeljevski et al. 2011). Some authors have found indirect genetic evidence for local extinction and founder event along larger rivers, suggesting that metapopulation processes in stream ecosystems are much more common than previously thought (Tero et al. 2003; DeWoody et al. 2004; Prentis et al. 2004).

Dyckia brevifolia (Bromeliaceae) is a rare, endemic and endangered rheophyte from southern Brazil. It occurs in discrete patches on the banks of the Itajaí-Açu River, about 80 km from Lontras City, going toward Blumenau City, in the State of Santa Catarina in Brazil (Rogalski and Reis 2009). According to Rogalski and Reis (2009), this species occupies an area smaller than two hectares. Both the self-compatibility of *D. brevifolia* and the behavior of its floral visitors indicate that selfing is the main form of pollination (Rogalski et al. 2009). Seeds are dispersed by gravity and wind within population and mainly by water flow among population (Rogalski and Reis 2009; Rogalski et al. 2009). Therefore, this species has interesting features to test again the "unidirectional diversity hypothesis."

Moreover, construction of the Salto Pilão hydroelectric power plant and the possible construction of yet another hydroelectric power plant in the Itajaí-Açu River Basin are both concerning imminent risks for in situ conservation of *D. brevifolia*, primarily from the loss of its natural habitat. Thus, studies of *D. brevifolia* populations and genetic diversity will provide a scientific foundation for the development of viable strategies for its in situ and ex situ conservation.

To pursue this goal in the present article, we asked 1) what level of genetic diversity is present among natural populations of *D. brevifolia* and how it is distributed among these populations, 2) how genetic diversity is distributed along the river, 3) what correlations might exist between population size and genetic diversity based on total number of rosettes, number of reproductive rosettes, or number of clumps per population, 4) what correlations might exist between genetic diversity and geographic distances, and 5) what constitutes the mating system of *D. brevifolia*. To address these questions, we characterized *D. brevifolia* populations along the Itajaí-Açu River, focusing on the mating system and genetic diversity using allozyme markers.

Materials and Methods

Study Area

This study was carried out in natural areas of *D. brevifolia* occurrence along the Itajaí-Açu River located in the Itajaí hydrographic basin, Santa Catarina State, Brazil. The area of the Itajaí hydrographic basin is about 15 500 km^2, and its main affluent is the Itajaí-Açu River.

According to the Köeppen classification, the region presents a humid and mesothermic climate, with rain distributed throughout the year (Bacic et al. 1994). The relative humidity is about 85% during the year.

Population Characterization

This study was conducted on the banks of the Itajaí-Açu River, and all patches of *D. brevifolia* (hereinafter termed as populations) were studied (Figure 1).

For each population, population size was determined by counting all rosettes. The rosettes were also classified into reproductive or nonreproductive. The spatial distribution of the plants was determined by isolated rosettes or rosette clumps.

Sampling and Laboratory Procedures

Samples of reproductive rosettes of *D. brevifolia* were collected from all populations along the Itajaí-Açu River. Since genetic similarity was unknown within each group or even within the whole population, the sampling strategy involved the collection of one reproductive rosette per clump in order to avoid sampling the same genet. For each population, 50 reproductive rosettes belonging to different clumps were sampled, totaling 361 individuals. In 2 populations
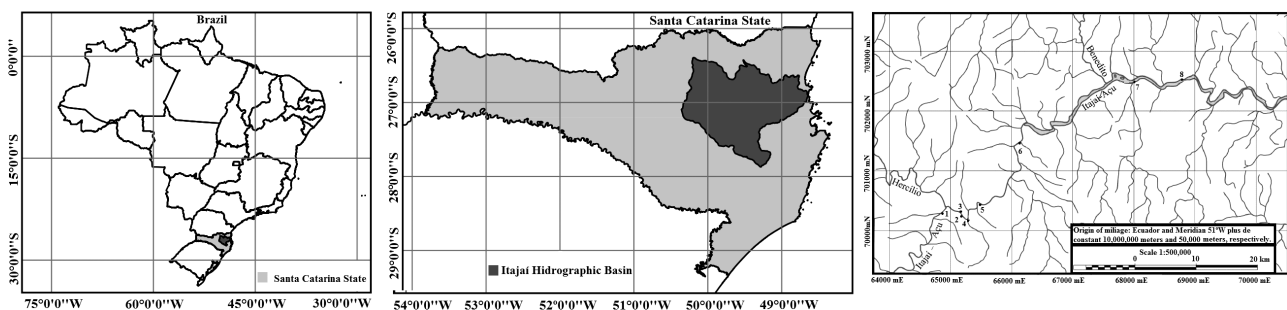

Figure 1. Distribution of *Dyckia brevifolia* natural population along the Itajaí-Açu River, Itajaí Hydrographic Basin, Santa Catarina State, Brazil. Locations of populations and points of material collect. 1. Ressacada; 2. Subida/Apiúna I; 3. Subida/Ibirama I; 4. Subida/Apiúna II; 5. Morro Santa Cruz; 6. Ascurra; 7. Encano; 8. Salto Waissbach.

(P1-35 and P7-26), the number of clumps collected with reproductive rosettes was lower than 50 (Table 2).

To determine the mating system, seeds of 45 seed-rosettes were collected randomly in the populations of Salto Waissbach (15), Ressacada, Subida/Apiúna I, and Encano (10 each). Seeds were germinated under standard greenhouse conditions on vermiculite substrate. After 1 year of germination, leaf samples were collected from 25 seedlings per seed-rosette. To compensate the low number of loci and the lack of variation per locus, a relatively high number of seedlings per seed-rosette were used.

Genetic characterization was carried out using allozyme markers, according to Kephart (1990) and Alfenas et al. (1991). The enzymes were extracted from leaf apex tissues and ground in a mortar containing extraction solution 1, according to Alfenas et al. (1991). The migration of enzymes was analyzed by horizontal eletrophoresis on gel prepared with 13% hydrolyzed starch (penetrose 30) with constant electric current.

For the analyses, 10 enzymatic systems with good resolution were used. Histidine buffer was used to resolve the enzymatic systems as follows: acid phosphatase (EC 3.1.3.2; locus *Acp*-1), diaphorase (EC 1.8.1.4; locus DIA-1), malate dehydrogenase (EC 1.1.1.40; loci *Mdh*-1 and *Mdh*-2), malic enzyme (EC 1.1.1.40; locus *Me*-1), and phosphoglucomutase (EC 5.4.2.2; locus *Pgm*-1). Tris citrate buffer was used to resolve the enzymatic systems as follows: fluorescent esterase (EC 3.1.1.1; loci *Est*-1 and *Est*-2), glutamate oxaloacetate transaminase (EC 2.6.1.1; locus *Got*-1), phosphoglucoisomerase (EC 5.3.1.9; locus *Pgi*-1), peroxidase (EC 1.11.1.7; loci *Po*-1 and *Po*-2), and superoxido dismutase (EC 1.15.1.1; locus *Sod*-1). To determine the mating system, 7 loci of the 10 enzymatic systems were used (*Acp*-1, *Est*-2, *Dia*-1, *Me*-1, *Mdh*-2, *Po*-1, and *Po*-2).

Data Analysis

Genetic Diversity and Structure

Genetic diversity was analyzed using the estimated allele frequencies. The mean number of alleles per locus ($\hat{A}$), percentage of polymorphic loci ($\hat{P}$), observed heterozygosity ($\hat{H}_o$), expected heterozygosity ($\hat{H}_E$) in Hardy–Weinberg equilibrium, and the fixation index ($\hat{f}$) were estimated to reproductive rosettes and progenies using the GDA software (Lewis and Zaykin 2002). The statistical significance (5%) of allelic frequencies and fixation index were obtained by using the Fstat 2.9.3.2 software (Goudet 2002). Confidence interval (95%) for $\hat{H}_o$ and $\hat{H}_E$ were estimated in R language, with 1000 bootstraps, using the "PopGenKit" package (Paquette 2012).

F-statistics (Wright 1951) were estimated according to Weir and Cockerham (1984). The statistical significance (5%) was calculated by the bootstrapping method using the Fstat 2.9.3.2 software (Goudet 2002). The effective population size of reproductive individuals was determined according to Li (1976).

The association between pairwise population genetic distance $\left(\hat{F}_{ST} / 1 - \hat{F}_{ST} \right)$ and the linear geographic distances (km) among populations was investigated, and the Mantel test was applied by using the TFPGA program, version 1.3 (Miller 1997).

Mating System

The mating system was analyzed on the basis of the mixed mating model and correlated mating model, using the Multilocus MLTR software, version 3.4 (Ritland 2009). For each population, the following were estimated: the multilocus outcrossing rate ($\hat{t}_m$) by the expectation–maximization method, single outcrossing rate, outcrossing between related individuals ($\hat{t}_m - \hat{t}_s$), the correlation of selfing ($\hat{r}_s$),

and the correlation of paternity ($\hat{r}_p$). The mean standard for the estimates described above was obtained by 1000 bootstraps, where the resampling units were considered the plants within families.

The correlation coefficient among offspring ($\hat{r}_{xy}$) within progenies was estimated according to Ritland (1989), as $\hat{r}_{xy} = 0.25(1 + \hat{F}_p)[4\hat{s} + (\hat{t}^2 + \hat{r}_s \cdot \hat{s} \cdot \hat{t})(1 + \hat{r}_p)]$, where $\hat{F}_p$ is parental inbreeding, $\hat{s}$ is selfing rate $(1 - \hat{t}_m)$, $\hat{t}$ is outcrossing rate, $\hat{r}_s$ is selfing correlation, and $\hat{r}_p$ is paternity correlation. From the $\hat{r}_p$ parameter, it was possible to estimate the effective number of pollen donors $N_{e(v)} = 0.5 / \hat{r}_{xy}$, as described by Ritland (1989). The coancestry coefficient ($\hat{\theta}_{xy}$) within progenies was estimated from the coefficient correlation among offspring, as $\theta_{xy} = (r_{xy}(1 + \hat{F}_p)) / 2$ (Cockerham 1969). The variance of effective size ($N_{e(v)}$) of the progenies was estimated by $N_{e(v)} = 0.5 / \theta_{xy}$ according to Cockerham (1969). The required number of seed-rosettes for seed collection in order to maintain a reference effective population size of 100 was estimated as $\hat{m} = N_{(reference)} / \hat{N}_{e(v)}$, according to Sebbenn (2002).

Results

Population Characterization

All populations along the Itajaí-Açu River were studied (Figure 1). The number of rosettes per population of *D. brevifolia* ranged from 508 (Encano) to 7187 (Subida/Ibirama I), with a mean of 3398.9 (Table 1). The mean percentage of reproductive rosettes was 14.4% and ranged from 5.4% to 20.1%. In addition, no isolated rosette was classified as reproductive.

Spatially, the rosettes in the different populations showed an aggregated distribution. Typically, the number of clumped rosettes was significantly larger than the number of isolated rosettes (Table 1). The clump number of rosettes per population ranged from 37 (Encano) to 686 (Subida/Ibirama I) (Table 1).

Genetic Diversity

Ten enzyme systems were revealed and scored 13 loci, of which 8 were polymorphic (*Acp*-1, *Dia*-1, *Est*-1, *Est*-2, *Mdh*-2, *Me*-1, *Po*-1, and *Po*-2) (Table 2). The maximum number of alleles per locus was 4 (Table 2).

Five alleles were private: allele 1 in loci *Mdh*-2, *Me*-1 (Salto Waissbach I) and *Po*-2 (Subida/Apiúna I), allele 2 in locus *Est*-1 (Ascurra), and allele 4 in locus *Acp*-1 (Salto Waissbach) (Table 2).

Dyckia brevifolia revealed a number of alleles per locus of 2.0, a percentage of polymorphic loci of 0.615, and expected heterozygosity of 0.106 (Table 1). The mean number of alleles per locus was 1.4, and the percentage of polymorphic loci ranged from 0.154 (Ressacada, Subida Apiúna I, and Morro Santa Cruz) to 0.538 (Salto Waissbach I), with mean of 0.269 (Table 1). The expected heterozygosity ranged from 0.011 (Ressacada) to 0.123 (Salto Waissbach I), and the mean was 0.067 (Table 1).

Downstream populations 6, 7, and 8 (mean $\hat{H}_E = 0.102$) from the Itajaí-Açu River presented the highest genetic diversity if compared with upstream populations 1–5 (mean $\hat{H}_E = 0.048$), considering that each clump is constituted by at least one genet the population effective size ranged from 40 to 909 with a mean of 340 individuals (Table 1).

The fixation index ($\hat{f}$) ranged from –0.638 (Morro Santa Cruz) to 0.610 (Subida/Ibirama I), with a mean of –0.039 (Table 2), considering that 95.8 individuals, on average, are required for a population effective size ($\hat{N}_e$) of 100, ranging from 35 (Subida/Ibirama I) to 161 reproductive rosettes (Morro Santa Cruz) (Table 1).

The correlation between population size and genetic diversity ($\hat{H}_E$, $\hat{H}_o$, and $\hat{f}$) was weak and nonsignificant (–0.262, –0.258, and 0.090,

respectively; $P \leq 0.05$). The correlation between number of clumps per population and genetic diversity ($\hat{H}_E$, $\hat{H}_O$, and $\hat{f}$) was nonsignificant (-0.273, -0.254, and 0.109, respectively; $P \leq 0.05$). As observed for the other mentioned parameters, the correlation between the number of reproductive rosettes and genetic diversity ($\hat{H}_E$, $\hat{H}_O$, and $\hat{f}$) was also nonsignificant (-0.355, -0.502, and 0.309, respectively; $P \leq 0.05$).

When considering only the 8 polymorphic loci of the reproductive rosettes, 4 loci had highly skewed allele frequencies, and a fifth had a frequency higher than 0.9 for the most common allele, thus showing very little genetic diversity at these 5 loci (Table 2).

The polymorphic loci presented an average of 1.7 alleles per locus and 50% of polymorphism for the progenies (Table 3). The mean expected heterozygosity was 0.139, while the mean observed heterozygosity was 0.035 (Table 3). Based on the same loci used for the progenies, the reproductive individuals presented similar diversity indexes ($\hat{A} = 1.9$; $P = 58.3\%$; $\hat{H}_E = 0.145$) if compared with the progenies, except for the observed heterozygosity ($\hat{H}_O = 0.128$) (Table 3). However, if we consider each population, the expected heterozygosity was higher in the progenies, except for the Encano population. The fixation indexes ($\hat{f}$) were higher in the progenies than in reproductive individuals for the 4 populations analyzed (Table 3).

The data showed similar allelic frequencies for the progenies, as well as reproductive rosettes (Table 4). However, alleles 1 and 3 of the *Acp*-1 locus occur in the progenies, but not in the reproductive rosettes in the Ressacada population and the allele 1 of the *Po*-2 locus occur in the progenies but not in Encano population (Table 4).

Genetic Structure

F-statistics showed a considerable degree of divergence in the analyzed populations. The $\hat{F}_{IS}$ value was negative (-0.023), but did not differ significantly from zero, indicating absence of fixation within populations. The $\hat{F}_{IT}$ (0.388) and $\hat{F}_{ST}$ (0.402) values differed significantly from zero, indicating that fixation could be attributed to the structure of the populations (disjunctive populations and selfing). The Ressacada and Subida/Apiúna I populations did not present divergence, indicating the occurrence of high gene flow.

The Mantel test revealed a negative and nonsignificant isolation ($r = -0.006$; $P < 0.05$) between populations of *D. brevifolia* along the Itajaí-Açu River (Figure 2).

Mating System

The number of polymorphic loci for the progenies ranged from 3 (Ressacada) to 7 (Salto Waissbach) (Table 5). The estimation of multilocus outcrossing rate ($\hat{t}_m$) was different from zero for all populations and revealed a variation between 0.057 (Ressacada) and 0.113 (Salto Waissbach I) with an average of 0.082 (Table 5).

The differences between the multilocus and single rates ($\hat{t}_m - \hat{t}_s$) were different from zero for all populations and ranged from 0.019 (Subida/Apiúna I) to 0.040 (Salto Waissbach) (Table 5), with a mean of 0.025. The selfing correlation was different from zero for all populations, ranging from 0.077 (Encano) to 0.295 (Salto Waissbach I), with mean of 0.173 (Table 5). The estimation of paternity correlation did not differ significantly from unity for any population studied (Table 5).

The coancestry coefficient and the variance of effective size showed an average of 0.632 and ranged from 0.513 to 0.957.

Table 1. Genetic diversity of the rheophyte *Dyckia brevifolia* Baker (Bromeliaceae), Itajaí-Açu river, Santa Catarina State

Populations[a]	Number of rosettes/population	Number of reproductive rosettes	Clumps per population	Isolated rosettes	N	$\hat{A}$	$\hat{P}$	$\hat{H}_O$ (CI)	$\hat{H}_E$ (CI)	$\hat{f}$	n	$\hat{N}_e$
1	606	109	39	7	35	1.154	0.154	0.011 (0.009)	0.011 (0.009)	−0.024	98	40
2	4860	973	249	56	50	1.462	0.308	0.029 (0.012)	0.074 (0.018)	0.610[b]	161	155
3	7185	1000	686	195	50	1.231	0.154	0.015 (0.007)	0.020 (0.010)	0.245[b]	125	551
4	5773	311	394	105	50	1.385	0.231	0.111 (0.013)	0.078 (0.006)	−0.434[b]	57	697
5	4702	831	318	112	50	1.308	0.154	0.077 (0.008)	0.047 (0.005)	−0.650[b]	35	909
6	664	65	58	84	50	1.538	0.385	0.083 (0.012)	0.078 (0.013)	−0.066[b]	94	63
7	508	102	37	14	26	1.462	0.231	0.127 (0.020)	0.108 (0.017)	−0.184[b]	82	46
8	2893	291	297	95	50	1.692	0.538	0.106 (0.021)	0.123 (0.019)	0.139[b]	114	261
Mean	3398.9	460.3	259.8	83.5	45.1	1.404	0.269	0.070	0.067	−0.039	95.8	—
Overall	27191	3682	2078	668	361	2.000	0.615	—	0.106	—	—	—

Sample size (N); mean numbers of alleles per locus ($\hat{A}$); percentage of polymorphic loci ($\hat{P}$); observed heterozygosity ($\hat{H}_O$); expected heterozygosity ($\hat{H}_E$); fixation indexes ($\hat{f}$); number of reproductive rosettes ($\hat{f}$); number of reproductive rosettes to retaining a $\hat{N}_e = 100$ (n); effective size based on the number of groups. 1. Ressacada; 2. Subida/Apiúna I; 3. Subida/Ibirama I; 4. Subida/Apiúna II; 5. Morro Santa Cruz; 6. Ascurra; 7. Encano; 8. Salto Waissbach I. CI = confidence interval.

[a]Populations positioned according to Itajaí-Açu River flow.

[b]Value different from zero.

Aiming to maintain an effective size of 100, the data showed the need to collect seeds from 103 to 157 seed-rosettes according to the population analyzed in the present study (Table 5).

Discussion

In general, *D. brevifolia* presents lower genetic diversity if compared to most bromeliad species (Zanella et al. 2012 and references therein; Lavor et al. 2014; Hmeljevski et al. 2015). This species also revealed lower overall expected heterozygosity ($\hat{H}_E$ = 0.106) than the rheophyte congeners *Dyckia ibiramensis* ($\hat{H}_E$ = 0.219 with mixed mating system; Hmeljevski et al. 2011) and *Dyckia distachya* ($\hat{H}_E$ = 0.202; Wiesbauer 2008), but showed a value of genetic divergence similar to that found for selfing polycarps (mean G_{ST} = 0.394; Loveless and Hamrick 1984).

The low within-population genetic diversity can be explained by the reproductive system of this species. As normally observed in selfing species, *D. brevifolia* has fewer alleles per locus and more skewed allele frequencies (Table 2). In general, low frequency allozyme alleles are far more abundant than alleles at intermediate frequency in undisturbed natural populations (Chakraborty et al. 1980).

The outcrossing estimation was 8.2% ($\hat{t}_m$), indicating that this species is predominantly selfing with very few matings among related individuals (Table 5). The estimations of selfing correlation were low (Table 5), indicating that some seed-rosettes tend weakly and flexibly to produce more descendants from selfing (or matings) than others. The selfing correlation was significantly affected in all populations, and the estimation of paternity correlation did not differ significantly for the unity of any population (Table 5), indicating that offspring are composed of full-sibs. High estimations of

Table 2. Allelic frequencies for 13 allozyme loci in 8 populations of *Dyckia brevifolia* in Itajaí-Açu River, State of Santa Catarina

Loci	Alleles	Populations								Mean
		1	2	3	4	5	6	7	8	
Acp-1	1		0.040		0.010		0.010	0.077	0.080	0.027
	2	1.000	0.810	1.000	0.980	1.000	0.250	0.827	0.130	0.750
	3		0.150		0.010		0.740	0.096	0.750	0.218
	4								0.040	0.005
Dia-1	1		0.070		0.344		0.020		0.440	0.109
	2	1.000	0.930	1.000	0.656	1.000	0.980	1.000	0.560	0.891
Est-1	1	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	0.999
	2						0.010			0.001
Est-2	1				0.010			0.100		0.014
	2	0.029	0.660	0.080	0.530	0.520	0.550	0.440	0.020	0.354
	3	0.971	0.340	0.920		0.030	0.400	0.420	0.650	0.466
	4				0.460	0.450	0.050	0.040	0.330	0.166
Got-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-1	1								0.030	0.004
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.970	0.996
Mdh-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	1								0.030	0.003
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.970	0.997
Pgi-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Po-1	1	0.957	0.990	0.940	1.000	0.960	0.990	0.654	0.940	0.929
	2	0.043	0.010	0.040		0.020	0.010	0.346	0.060	0.066
	3			0.020		0.020				0.005
Po-2	1								0.020	0.003
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.980	0.998
Sod-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

1. Ressacada; 2. Subida/Apiúna I; 3. Subida/Ibirama I; 4. Subida/Apiúna II; 5. Morro Santa Cruz; 6. Ascurra; 7. Encano; 8. Salto Waissbach.

Table 3. Mean numbers of alleles per locus ($\hat{A}$), percentage of polymorphic loci ($\hat{P}$), observed heterozygosity ($\hat{H}_o$), expected heterozygosity ($\hat{H}_E$), fixation indexes ($\hat{f}$) for progenies (P), and reproductive individuals (R), using 7 polymorphic loci, in 4 populations of *Dyckia brevifolia*

Populations	$\hat{A}$		$\hat{P}$		$\hat{H}_o$		$\hat{H}_E$		$\hat{f}$	
	P	R	P	R	P	R	P	R	P	R
Ressacada	1.43	1.33	28.6	33.3	0.008	0.024	0.101	0.023	0.921[a]	−0.024
Subida/Apiúna I	1.71	1.83	57.1	66.7	0.026	0.053	0.129	0.139	0.798[a]	0.620[a]
Encano	1.71	2.00	42.9	50.0	0.049	0.275	0.108	0.233	0.546[a]	−0.184
Salto Waissbach	2.00	2.33	71.4	83.3	0.056	0.163	0.217	0.184	0.742[a]	0.112
Mean	1.71	1.87	50.0	58.3	0.035	0.129	0.139	0.145	0.752[a]	0.113

[a]Value different from zero.

Table 4. Allelic frequencies for 7 polymorphic loci in progenies (P) and reproductive rosettes (R) for 4 population of *Dyckia brevifolia*

Loci	Alleles	Ressacada		Subida/Apiúna I		Encano		Salto Waissbach	
		P	R	P	R	P	R	P	R
Acp-1	1	0.016		0.008	0.040	0.006	0.077	0.005	0.080
	2	0.884	1.000	0.688	0.810	0.878	0.827	0.209	0.130
	3	0.100		0.304	0.150	0.116	0.096	0.687	0.750
	4							0.099	0.040
Dia-1	1			0.004	0.007			0.901	0.440
	2	1.000	1.000	0.996	0.930	1.000	1.000	0.099	0.560
Est-2	1					0.067	0.100		0.020
	2	0.464	0.029	0.762	0.660	0.780	0.440	0.512	0.650
	3	0.536	0.971	0.238	0.340	0.153	0.420	0.488	0.330
	4						0.040		0.030
Mdh-2	1							0.195	0.030
	2	1.000	1.000	1.000	1.000	1.000	1.000	0.805	0.970
Me-1	1							0.027	0.030
	2	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.970
Po-1	1	1.000	0.957	1.000	0.990	0.902	0.654	1.000	0.940
	2		0.043		0.010	0.098	0.346		0.060
Po-2	1							0.050	0.020
	2	1.000	1.000	1.000	1.000	1.000	1.000	0.950	0.980

paternity correlations are common in species with clumped distribution (Kageyama et al. 2003).

The phalanx growth form displayed by *D. brevifolia* (Rogalski and Reis 2009) is believed to be advantageous in optimizing resource capture and space occupation (de Kroon et al. 1994); however, this architecture can be expected to increase geitonogameous pollination and decrease mate availability (Charpentier 2002; Ruggiero et al. 2005).

Dyckia brevifolia is self-compatible, predominantly selfing, and most of its rosettes occur as clumped. The clumps are mainly constituted of ramets from clone propagation, and pollinator behavior indicates that the main pollination forms are autogamy and/or geitonogamy (Rogalski et al. 2009).

Analyzing all populations studied here, 5 have negative $\hat{f}$ values, and 4 showed deviation from Hardy–Weinberg equilibrium and an excess of heterozygosity, suggesting positive selection for individuals with more heterozygous loci (Table 1). The fixation index between the progenies and reproductive rosettes indicates that selection against inbred progeny is occurring. According to Kang et al. (2005), 2 factors are usually involved in driving selection in favor of heterozygotes: environmental stress and inbreeding depression.

Theoretically, inbreeding will result in an excess of homozygotes, but the proportion may be reduced at different life stages (Kang et al. 2005) as a consequence of clonal propagation, which could contribute to the maintenance of homozygous/heterozygous frequencies in adults. Inbreeding depression may reduce the recruitment of selfed progenies within populations of self-compatible plants, which, in turn, could strongly reduce the inbreeding coefficient of the adult population (Goodwillie et al. 2005). In small and fragmented populations, the homozygous seeds produced by inbreeding may have lower germination rates and higher mortality in comparison with heterozygous seeds, which are affected by outbreeding. In addition, homozygous seedlings may exhibit lower vigor than heterozygous seedlings (Kang et al. 2005).

An excess of heterozygous adults has been found, particularly in long-lived species, suggesting selection for heterozygotes (Tonsor
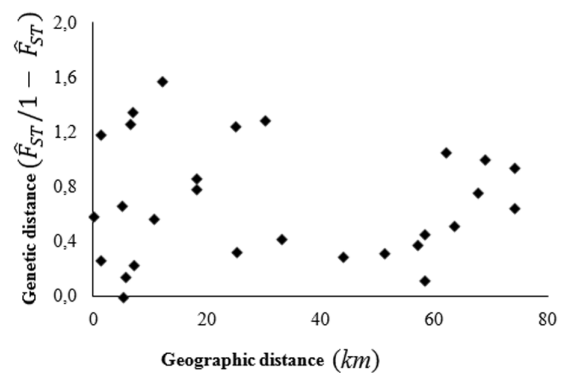

Figure 2. Correlation between genetic distance $\left(\hat{F}_{ST} / 1 - \hat{F}_{ST} \right)$ and geographic distance (*km*) for population pairs of *Dyckia brevifolia*, Itajaí-Açu River, Santa Catarina.

et al. 1993; Luijten et al. 2000; Mariot et al. 2002; González-Astorga et al. 2003; González-Astorga and Castillo-Campos 2004; Kang et al. 2005; Fernández-M and Sork 2007; Mathiasen et al. 2007). The heterozygote advantage may be an important demographic-genetic mechanism to conserve genetic variation during population bottlenecks in long-lived perennial plants (Luijten et al. 2000).

The predominance of autogamy and geitonogamy indicates that gene flow by pollen occurs preferentially within population (Rogalski et al. 2009) or, eventually, nearby populations. Since pollen flow occurs practically within populations, the gene flow among populations may be limited to hydrochory. Hydrochory has the potential to affect both short- and long-distance transport of seeds (Cain et al. 2000; Kudoh et al. 2006). In this case, a newly founded area will possibly show few individuals. If the number of founders is limited to colonization, genetic drift would take place after colonization. Otherwise, even if differential survival and spread of genotypes (clone propagation) occur, the populations would still consist of a few genotypes, resulting in lower genetic diversity within populations and divergence among populations.

Table 5. Estimates of the mating system parameters of *Dyckia brevifolia* in Itajaí-Açu River, State of Santa Catarina

Estimates	Ressacada	Subida/Apiúna I	Encano	Salto Waissbach
Multilocus outcrossing rate ($\hat{t}_m$)	0.057[a] (0.057), CI: ±0.007	0.065[a] (0.079), CI: ±0.010	0.092[a] (0.172), CI: ±0.021	0.113[a] (0.083), CI: ±0.008
Single-locus outcrossing rate ($\hat{t}_s$)	0.038[a] (0.051), CI: ±0.006	0.044[a] (0.074), CI: ±0.009	0.072[a] (0.186), CI: ±0.023	0.073[a] (0.080), CI: ±0.008
Outcrossing between related individuals ($\hat{t}_m - \hat{t}_s$)	0.019[a] (0.012), CI: ±0.001	0.021[a] (0.013), CI: ±0.002	0.020[a] (0.053), CI: ±0.007	0.040[a] (0.019), CI: ±0.002
Selfing correlation ($\hat{r}_s$)	0.167[a] (0.283), CI: ±0.035	0.153[a] (0.294), CI: ±0.036	0.077[a] (0.367), CI: ±0.045	0.295[a] (0.208), CI: ±0.021
Paternity correlation ($\hat{r}_p$)	0.990 (0.541), CI: ±0.067	0.990 (0.512), CI: ±0.063	0.990 (0.505), CI: ±0.063	0.990 (0.229), CI: ±0.023
Number of pollen donors ($\hat{N}_{ep}$)	1.0	1.0	1.0	1.0
Coancestry coefficient within progeny ($\hat{\theta}_{xy}$)	0.672	0.782	0.513	0.561
Variance effective size ($\hat{N}_{e(v)}$)	0.744	0.640	0.975	0.891
Number of seed-rosettes to retain the effective size of 100 ($\hat{m}$)	135	157	103	113

Values in parentheses indicate standard deviation.

[a]Significant to confidence interval of 0.95.

In selfing plants like *D. brevifolia*, most of the genetic variation is found among populations (Loveless and Hamrick 1984; Hamrick and Godt 1989). Even though a greater part of genetic diversity is distributed among populations ($\hat{F}_{ST}$ = 0.402), this value is lower than those found for predominately disjunctive selfing species (mean G_{ST} = 0.510, Hamrick and Godt 1989) and their rheophyte congener *D. ibiramensis* (G_{ST} = 0.674) that presents disjunctive populations and a mixed mating system ($\hat{t}_m$ = 0.743) (Hmeljevski et al. 2011). Other studies with riparian species also found significant genetic differences among populations (Tero et al. 2003; Honnay et al. 2010), most likely resulting from the patchy distribution that limits gene flow and also the founder event. Species with occurrence across discontinuous ranges also tend to have greater genetic differences among their populations compared to their congeners with more continuous ranges (Hamrick and Godt 1996).

Rivers are generally characterized by unidirectional water movement which would facilitate large-scale seed dispersal of riparian species in the downstream direction (Boedeltje et al. 2004). In the Itajaí-Açu River, downstream populations (Ascurra, Encano, and Salto Waissbach) of *D. brevifolia* showed higher levels of diversity, which could likely be explained by the arrival of seeds or rosettes brought by water (hydrochory) from upstream populations (unidirectional flow). In the downstream populations, the higher probability of seed and propagule arrival by water flow may accumulate diversity and, consequently, decrease the effects of the founder event, genetic drift and endogamy, resulting in higher diversity.

The lack of correlation between population size and genetic diversity relative to clump number, or number of reproductive rosettes per population, could suggest that either colonization or founder event plays an important role in the distribution of genetic diversity of *D. brevifolia*.

The populations did not form a genetically uniform unit with free gene flow. The correlation between geographic and genetic distances for population pairs did not differ significantly. Therefore, the groups formed from genetic distance showed low correlation with geographical distance between populations; consequently, the stepping-stone model of gene flow cannot be assumed. In this model, given equilibrium, the genetic distance between subpopulations should increase monotonically with the increase of geographical distance between them (Kimura and Weiss 1964). These 2 analyses corroborate the hypothesis that the founder event plays an important role in the constitution of the populations by hydrochory.

It is possible that *D. brevifolia* represents a metapopulation model with formation of demes based on its environmental selectivity, occurrence in patches, founder effects, mating system, low gene flow and population structure. The model is also influenced by unidirectional river flow, as observed by the founding of new populations and gene flow among populations, which may be limited to hydrochory. Taken together, these data suggest a tendency toward diversity accumulation in downstream populations.

The population structure of *D. brevifolia* may be affected by the levels of extinction and colonization rates, the number of the individuals colonizing new areas, the number of individuals migrating among populations and the number of colonizers coming from different source populations (Pannell and Charlesworth 2000; Bohrer et al. 2005), or by pollen flow, seed and propagule dispersal. Population structure may also be influenced by the capacity of individual recruitment and competition and by the capacity of resource capture and space occupation, both of which are influenced by genotypic constitution and clonal propagation.

Bidirectional gene flow (pollen and seed) is the main form of distribution of genetic variation in the populations, permitting the similarity and the divergence between the populations. The restrictive distribution of this species, together with specific microhabitat occupation, indicates that this species is very vulnerable. The maintenance of populations prevents the risks of genetic erosion given that the river acts as a corridor allowing gene flow and enabling (re)colonization. Considering the data related to population effective size, as determined by the number of clumps per population and the diversity indexes (Table 1), the populations of Subida/Ibirama I, Subida/Apiúna I, Subida/Apiúna II, Morro Santa Cruz, and Salto Waissbach are priorities for in situ conservation.

The construction of the Salto Pilão hydroelectric power plant and the possibility of construction of still another hydroelectric power plant in the Itajaí-Açu River Basin make ex situ conservation of this species a necessity. To maintain an effective size of 100, our results suggest that it is necessary to collect from 35 to 161 reproductive rosettes, depending on the population affected by the dam, or collect seeds from 157 seed-rosettes, considering the population that requires the higher number of seed-rosettes, per affected population.

In both cases, sampling within groups must be prevented because of clonal propagation.

Data Availability

We have deposited the primary data underlying these analyses as follows: Allozyme genotypes: Dryad (doi: 10.5061/dryad.sv72c).

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