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BIOLOGICAL SCIENCES

Genetic and demographic aspects of *Varronia curassavica* Jacq. in a heterogeneous coastal ecosystem

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Abstract: The *restinga* is a threatened Brazilian ecosystem and a highly heterogeneous environment. This work aimed to evaluate demographic and genetic aspects of *Varronia curassavica* and whether environmental heterogeneity can influence the studied population parameters. Three annual evaluations were carried out in an area of *restinga* in Florianópolis-SC, Brazil. Demographic data were analyzed using descriptive statistics, and the spatial distribution pattern was calculated by Ripley's K-function and correlated with environmental characteristics. To characterize diversity and genetic structure, eight microsatellite markers were used. This work demonstrated that variations in the distribution of individuals and genotypes can be related to specific environments. Dry lowlands were environments favorable to population development, and flooded lowland and mobile dunes were unfavorable. The fixation indices were distinct between environments, evidencing a tendency toward preferential crosses in favor of heterozygotes. We found absence of spatial genetic structure, indicating that genotypes are randomly distributed and that gene flow may be related to such genetic factors as the presence of autoincompatibility mechanisms. This diversity of environments contributed to the aggregate distribution and is relevant for the maintenance of demographic and genetic processes of the species in *restingas*, and this aspect should be considered for *in situ* conservation.

Key words: *in situ* conservation, population dynamics, *restinga*, spatial distribution, spatial genetic structure.

INTRODUCTION

Varronia curassavica Jacq. (Boraginaceae) is an important medicinal species widely used in folk medicine and also by the pharmaceutical industry owing to its anti-inflammatory, analgesic, and healing properties (Lorenzi & Matos 2008, Melo et al. 2008, Bolzani et al. 2012). It is a perennial subshrubby tetraploid species, insect-pollinated and dispersed mainly by birds (Opler et al. 1975, Hoeltgebaum et al. 2018), that exhibits self-incompatibility mechanisms, such as heterostyly and protogyny (Hoeltgebaum & Reis 2017, Hoeltgebaum et al. 2018). It is found along most of the Brazilian coastline, as characteristic vegetation of coastal tropical and subtropical moist broadleaf forest, locally known as *restinga* (Smith 1970, Judd et al. 2009).

As suggested, *restingas* comprise the entire vegetation complex established in sandy soils of marine origin (Castro et al. 2007, Brasil 2016), and they are extremely sensitive and susceptible to environmental disturbances (Falkenberg 1999). Based on their coastal location, *restingas* have experienced a continuous degradation of natural characteristics (Barcelos et al. 2012), with a strong reduction in original area (SOS Mata Atlântica 1998, 2012-2013, Rocha et al. 2003, Marques et al. 2015). The *restinga* is considered one of the most anthropically threatened ecosystems in the Atlantic Forest (Strohaecker 2008, Barcelos et al. 2012).

Extractivist collection practices are still considered the main way of obtaining medicinal plants from nature (Reis & Siminski 2011). In general, when coupled with habitat fragility, the results can lead to an extensive reduction of their natural populations, and/or loss of genetic variability, compromising the maintenance capacity of these species. Consequently, understanding the ability of *V. curassavica* to maintain population dynamics in this context and understanding how individuals of this species are genetically structured would be fundamental to subsidizing and improving conservation and use initiatives (Epperson 1992), as well as adding to knowledge about flora of the *restingas*.

Demographic studies are an important tool for evaluating population processes and mechanisms (Griffith et al. 2016), especially when focused on the conservation of plant species and their populations, since they allow quantification of the regenerative potential through processes of birth, mortality and reproduction (Pavlik & Barbour 1988, Méndez et al. 2004).

The literature has consistently demonstrated the importance of integrating genetic and demographic studies in our efforts to understand different mechanisms that define the population structure of plants, along with their interactions (e.g., Connell et al. 1984, Martins 1987, Diaz & Oyama 2007, Gonçalves et al. 2010, Lara-Romero et al. 2016). This knowledge is important for improving both management and conservation strategies of plant genetic resources (Frankham et al. 2014), evaluating the impact of habitat exploration and fragmentation (Young & Merriam 1994, Mona et al. 2014), and supporting activities leading to the restoration of plant populations (Kettenring et al. 2014).

To date, no studies have integrated population genetic and demographic parameters for *V. curassavica*. Indeed, few studies have reported on *restinga* shrub species. In general, the population dynamics of shrub species is not as well known when compared to herbaceous and arboreal groups. Moreover, different methods are required to perform these studies (Crawley & May 1987, Kyncl et al. 2006).

Restingas are very heterogeneous areas with several soil gradients that contribute to a mosaic-like phytophysiognomy with spotty vegetation in the landscape and, hence, uneven vegetation coverage (Waechter 1985, Castro et al. 2007), resulting in the formation of different local habitats.

Environmental heterogeneity is a relevant factor to be considered in demographic and genetic studies of plants (e.g. Janzen 1970, Loveless & Hamrick 1984, Ferreira et al. 2010), since it may influence the distribution of genetic variation in populations (Gram & Sork 2001) and the spatial distribution of plants (Bernasol & Lima-Ribeiro 2010).

In this context, this work aimed to evaluate aspects of population dynamics, spatial distribution of individuals and genotypes, genetic diversity and structure of *V. curassavica*. Due to the singularity of the *restingas*, we also sought to evaluate if the environmental heterogeneity, typical of this ecosystem, may have influenced the studied population parameters. That is, is it possible to detect environments that are limiting or favor population occurrence and development? Which characteristics of these distinct environments can be related to the presence or absence of individuals of the species? Are there differences in the distribution of allelic richness or genetic structure among individuals from different environments? We expect that our results would positively inform efforts to provide subsidies for the *in situ* conservation of this species.

MATERIALS AND METHODS

Study area

This study was carried out in the Parque Municipal Dunas da Lagoa da Conceição (PMDLC) (27°37'46.19"S; 48°27'1.59"W), a *restinga* area that covers almost 563 hectares. It is located at Joaquina Beach on the east side of Florianópolis City, Santa Catarina, Brazil. This region is classified as "Cfa mesothermal humid" (Koeppen 1948). The predominant vegetation is herbaceous shrubs associated with dunes and some sections formed by small trees or a few trees in the driest areas. Even though this area is presumably protected by law, it still is under anthropogenic pressure from tourism and real estate development.

Demographic and environmental characterization

To evaluate the aspects of population dynamics, annual surveys were carried out during three consecutive years, using a permanent plot of two hectares (100 m x 200 m) subdivided into 200 subplots of 10 m x 10 m. All individuals present within the plot were measured and located through a Cartesian coordinate system (x and y). This species exhibits a densely branched pattern and partial burial of the branches. Thus, for this study, we considered an individual to be a single axis of branches visible above the soil line. The height of all individuals was measured and also diameter at soil level of 30 individuals, representative from each height class were evaluated. The presence of reproductive structure was recorded in order to identify developmental stage.

To test if the distribution of the species was correlated with environmental characteristics, we evaluated the degree of vegetation coverage, the altimetric profile and the type of environment for each subplot (10 x 10 m). The classification of the environment followed Guimarães (2006) who proposed these classes: itinerant dune, semi-settled and settled dune; dry, humid and flooded lowland, and transition areas between them. The degree of vegetation coverage was visually estimated by means of an interval scale of scores (0 - 4), with amplitude of 25% between each interval (Fournier 1974). Each subplot was divided into four quadrants with the help of a wooden cross positioned at the central point of the subplots. One score was assigned to each quadrant, and the subplot value was the average of quadrant. Altimetric profile was evaluated with the aid of laser tape with readings every 5 meters in the plot. The height of each point was estimated from the horizontal distance between the points, and the slope was calculated with the neighborhood method.

Characterization of diversity and genetic structure

For genetic characterization of the population in the different environments of the *restinga*, leaf samples were collected from the individuals sampled in the demographic survey. For extraction of genomic DNA from the leaves, the Nucleospin Plant II kit was used (Macherey-Nagel GmbH & Co. KG), according to the manufacturer's instructions. Eight polymorphic microsatellite loci developed for the species by Figueira et al. (2010) were amplified by polymerase chain reaction (PCR) to genotype individuals. PCR reaction, DNA was diluted in water at 1:9 µL, and the KAPA PCR kit (KAPA

Biosystems) was used with a volume of 10 µL per reaction. Each primer for each locus was labeled with the fluorochromes FAM, PET, NED and VIC for the eight microsatellite loci in two multiplex systems (MCvCIRCPQ14, MCvCIRCPQ3, MCvCIRCPQ8, MCvCIRCPQ6 - multiplex 1 - MCvCIRCPQ11, MCvCIRCPQ7, MCvCIRCPQ16, MCvCIRCPQ15 - multiplex 2), according to Hoeltgebaum & Reis (2017). The set of cycles and temperatures used were 95 °C for 3 min of denaturation and 30 cycles of three phases: 95 °C for 30 s, 61 °C for 30 s, and 72 °C for 30 s, with a final elongation step at 72 °C for 30 min. Capillary electrophoresis was performed using 1 µL of diluted PCR product in water (2:15µL), 0.25 µL of GS600 LIZ® and 8.75 µL of formamide HIDI™. Reading of the alleles was carried out in an ABI 3500XL Sequencer (Applied Biosystems). Gene Mapper v.3.2 (Applied Biosystems) was used to interpret the electropherograms.

Demographic data analysis

The total number of individuals (reproductive and nonreproductive) per hectare, height averages and diameter at soil level (DAS) were estimated, and incremental differences between years were estimated using the Student's *t*-test (t; $p < 0.05$). The population growth rate was calculated by subtracting the individuals that were dead and not found from the recruits. For individuals with multiple ramifications, the diameter at soil level was unified by calculating the root of the sum of the squares.

The spatial distribution pattern was estimated using Ripley's K Function (1977) and carried out in R software, version 3.3.0 (R Development Core Team 2016), with the Spatstat package (Baddeley & Turner 2005). The values obtained for K (h) were transformed by the function L (d) for better interpretation of the data. The deviations from the Complete Spatial Randomness were tested using confidence envelopes ($p < 0.001$) constructed from 1000 Monte Carlo simulations.

The relationship between the number of individuals and vegetation coverage, as well as altimetric profile, was evaluated by means of Spearman correlation. For vegetation coverage, the number of sampled *V. curassavica* individuals was correlated with the average percentage of cover obtained in each subplot. For the altimetric profile, the correlation between altimetric profile and presence of individuals was made from the slope angle obtained from 5 x 5 m subplots and the number of individuals present in them. The association between environment type and prensence of individuals was verified by comparing the frequency of occurrence of the individuals in the different environments, using the contingency table and chi-square test (χ^2). Differences between height increase averages and DAS and environments were tested by means of confidence intervals ($p < 0.05$). In transition zone subplots, the predominant environment was considered.

We used confidence intervals ($p < 0.05$) to test for potential differences in the growth of individuals (height and diameter) for the different environments, using the differences in average increment for each environment.

Analysis of genetic data

To verify possible genetic differences in the different environments, the following indices of genetic diversity were estimated for each environment: number of alleles per locus ($\hat{A}$) and per individuals ($\hat{A}_i$); genotypic richness, which measures the number of genotype with four alleles per locus ($\hat{G}$); observed ($\hat{H}_O$) and expected ($\hat{H}_E$) heterozygosities and fixation index ($\hat{f}$) for all loci, calculated as 1 - ($\hat{H}_O / \hat{H}_E$). Each locus deviating from the Hardy-Weinberg Equilibrium (HWE) model was tested using the chi-square test (χ^2). Genetic diversity parameters were calculated using the Autotet program (Thrall & Young 2000), which is specific to polyploid organisms and calculates the observed heterozygosity by weighting five different classes of possible genotypes per locus inversely against the probability of any two alleles will be identical by descent; $\hat{H}_E$ and $(\hat{f})$ were calculated on the basis of random chromosome segregation (Li 1955). The confidence intervals at 95% probability were obtained by Jackknife resampling between loci for each environment.

The partition of genetic diversity within and among environments was calculated using the genetic diversity statistics of Nei (1973), based on the following parameters: $\hat{H}_T$ – total genetic diversity; $\hat{H}_S$ – genetic diversity average within environments; $\check{D}_{ST}$ – genetic diversity among environments and $\hat{G}_{ST}$ – proportion of the total genetic diversity among environments, using DISPAN software (Ota 1993).

Spatial distribution of genotypes was estimated using the coancestry coefficient ($\hat{\theta}_{xy}$, Loiselle et al. 1995) between pairs of individuals and was obtained from SPAGEDI software, v. 1.4 (Hardy & Vekemans 2003). Confidence intervals (95%) for $\hat{\theta}_{xy}$ values in each distance interval were obtained by 1000 permutations of individual locations among all individuals.

RESULTS

Population dynamics

In the first year of evaluation (2014), 425 individuals (213 ind.ha^{-1}) were found, of which about 10% (42 individuals) were in the reproductive phase, and from 2014 to 2016, 125 individuals were recruited to the population (63 ind.ha^{-1}). We found 12 deads ind.ha^{-1}, and 37 ind. ha^{-1} were not found, totaling 248 ind.ha^{-1} survivors in 2016, representing an annual growth rate of 8%. Of these survivors, 26% were recorded in the reproductive phase. Of individuals who entered the population after 2014, four were not found and three died. Of those that died, 75% were in flooded areas. At the end of the first year, no reproductive structures were observed for the recruits of 2015.

Individuals of *V. curassavica* presented mean height of 0.57 m (± 0.30 SD), with no significant change between the years (t = - 0.89; p = 0.37) (Table I), ranging from 0.05 to 2.15 m. The recruits had height between 0.08 m and 1.32 m, with an average of 0.49 m (± 0.29), and the highest growth was 0.74 m / year. Reproductive individuals had, on average, a height ranging from 0.75 m (± 0.31) to 0.83 m (± 0.33) between evaluation years. Reproductive structures, such as floral buds, were recorded for individuals from 0.12 m in height.

Most *V. curassavica* individuals (67%) were concentrated in the first two height classes, but reduced in number as heights increased from 35 cm (Figure 1a). With respect to DAS, the mean ranged from 1.77 cm (SD = ± 0.92) to 2.75 cm (SD = ± 1.97) between the evaluated years. The maximum and minimum DAS values were 9.11 cm and 0.60 cm, respectively. The species had a frequency of individuals greater than 50% in the first diameter class, but as diameter increased, the number of individuals decreased during the first year of evaluation (Figure 1b). This pattern differed in subsequent years, with a shift in the number of individuals in the classes, owing to the increase in diameters. For both height and DAS classes, the population presented a tendency toward a J-inverted curve (Figure 1a, b).

Spatial distribution and relationship with the environment

This species had an aggregate spatial structure (Figure 2). The function L (d) determined that the aggregation distances of individuals were constant and peaked at a distance of ~ 40 m.

Table I. **Mean, minimum and maximum heights by classes of individuals of *Varronia curassavica* Jacq. (Boraginaceae), which were evaluated between 2014 and 2016 in the *restinga* population of Joaquina - Florianópolis/SC.**

Classes	N. Individuals			Min. height (m)			Max. height (m)			Mean height ± SD (m)		
Years	**2014**	**2015**	**2016**	**2014**	**2015**	**2016**	**2014**	**2015**	**2016**	**2014**	**2015**	**2016**
Recruits	-	54	71	-	0.08	0.1		0.98	1.32	-	0.57 ± 0.32	0.41 ± 0.26
Non Reprod.	383	322	367	0.05	0.06	0.08	1.85	1.31	1.8	0.54 ± 0.27	0.49 ± 0.23	0.51 ± 0.28
Reproductive	42	119	128	0.39	0.12	0.22	1.7	2.15	1.78	0.83 ± 0.33	0.75 ± 0.31	0.78 ±. 34
Total /Mean	425	441	495	-	-	-	-	-	-	0.57 ± 0.29	0.56 ± 0.30	0.58 ± 0.32

N = Number; Min = minimum; Max = maximum; SD = standard deviation; Reprod. = Reproductive.

The correlation values between number of individuals and environmental characteristics were low, but significant. The highest value was for vegetation coverage ($r = 0.26$; $p = 0.0003$), and for slope, the correlation was negative ($r = - 0.146$; $p = 2.21 \cdot 10^{-5}$).

Inside the plot, vegetation coverage was unevenly distributed. About 50% of the analyzed subplots (97 subplots) had, on average, 76-100% vegetation coverage; 58 subplots between 51-75%, and 36 subplots between 26-50% vegetation coverage values. Only 4% (9 subplots) of the evaluated area showed 0-25% vegetation coverage values. For qualitative classification of the environment (Table II), dry lowland occurred in 26% of the plots and predominated in 10% of transition zone subplots. Itinerant dunes occurred in 33% of the studied area, while fully covered with vegetation fixed occurred only in 11 subplots. Dune areas occurred in 51% of the plots, and the remaining 49% are lowlands.

The species exhibited preferential environments for occurrence, recruits and deaths (Figure 3). Of the sampled individuals, 99% were found on dry lowland (DL), itinerant dunes (ID), and transition zones between them (LTZ) ($\chi^2 = 669.88_{(2014)}$; $618.24_{(2015)}$; $805.40_{(2016)}$; DF = 3; $p < 0.05$). Among these environments, 77% of individuals occurred in dry lowlands. For the remaining classes, the numbers of individuals were < 1%.

Similarly, 94% of individuals entered the population in the following environments: DL, ID, LTZ, and DTZ ($\chi^2 = 37.65_{(2015)}$; $99.64_{(2016)}$; DF = 3; $p < 0.05$), of which 66% occurred in dry lowlands. The number of dead individuals in dry lowlands was also higher for the last year evaluated ($\chi^2 = 3.67_{(2015)}$; $21.26_{(2016)}$; $p < 0.05$; DF = 3).

Mean DAS increment for the evaluated period (three years) was significantly different between the environments of higher occurrence of the species. In the transition zones from dune to lowlands, the average increment was 0.314 cm (CI = ±0.28). In the itinerant dunes, the values were negative (-1.46 cm; CI = ±0.28), as well as on dry lowlands and lowland transition zones, where the increment was similar (0.80 cm and 0.87 cm; CI = ±0.28). Mean height increment was higher in the dry lowlands and itinerant dunes (0.05 cm; CI = ±0.01).

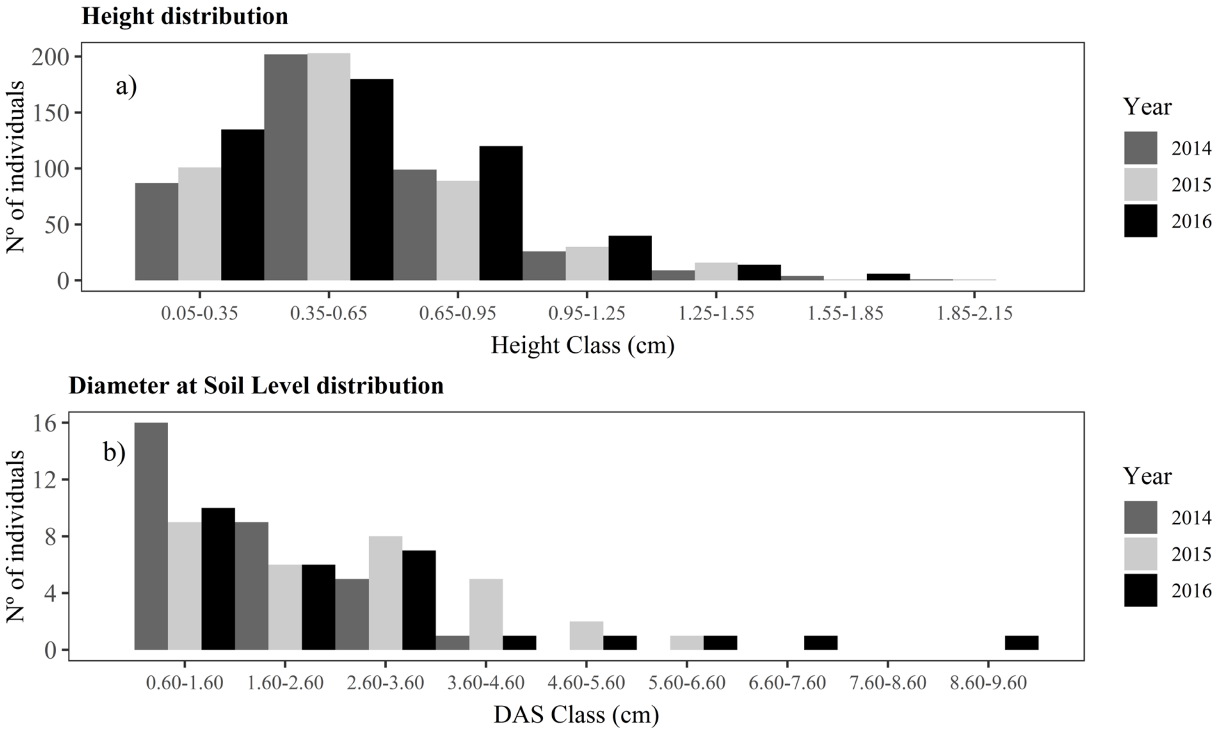

Figure 1. a) Distribution of individuals of *Varronia curassavica* Jacq. (Boraginaceae) by height intervals (top); b) Distribution of individuals in diameter to soil level classes (30 individuals) (bottom). Results represent evaluations from 2014 to 2016 in the *restinga* area of Joaquina - Florianópolis/SC.

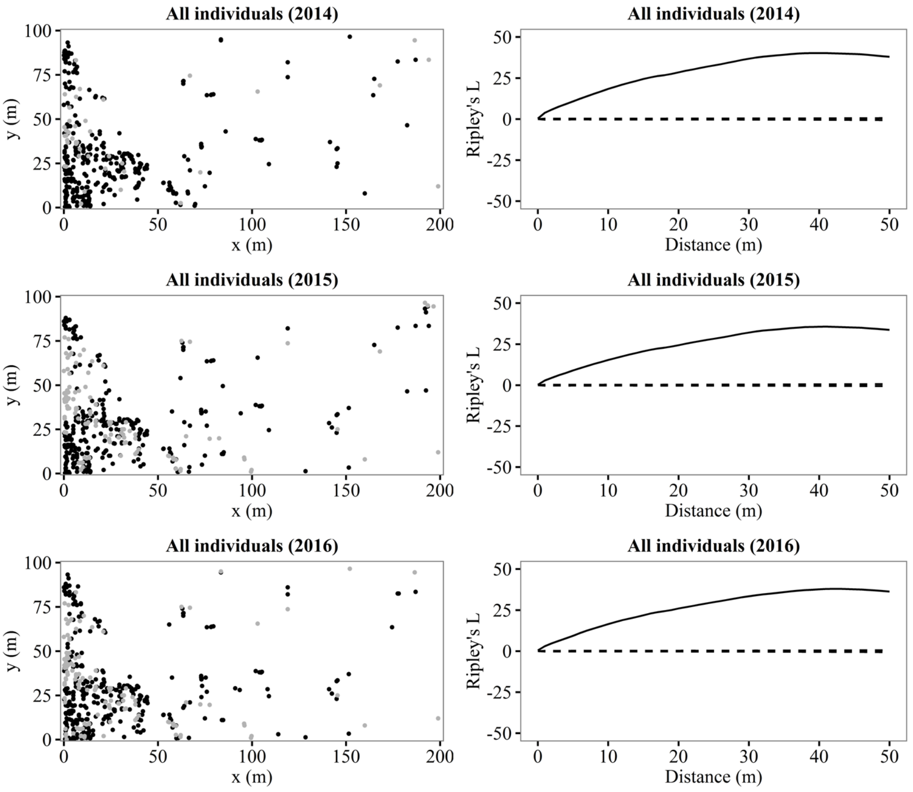

Figure 2. Spatial distribution of individuals of *Varronia curassavica* Jacq. (Boraginaceae), between 2014 and 2016 (nonreproductive individuals represented in black and reproductive represented in gray), sampled in the *restinga* of Joaquina, Florianópolis / SC (left) and analysis of the spatial pattern by Ripley's K function (right). Confidence interval represented by dotted lines.

Table II. Environment type in the subplots sampled in the evaluation of demographic parameters of *Varronia curassavica* Jacq. (Boraginaceae) in the *restinga* of Joaquina, Florianópolis/SC.

Environment type					
Lowland	**N° SP**	**N° SP TZ**	**Dune**	**N° SP**	**N° SP TZ**
Flooded	3	1	Itinerant	14	11
Humid	10	14	Semi-settled	42	24
Dry	51	19	Settled	3	8

N° SP = Number of subplots; N° SP TZ = Number of subplots in transition zone.

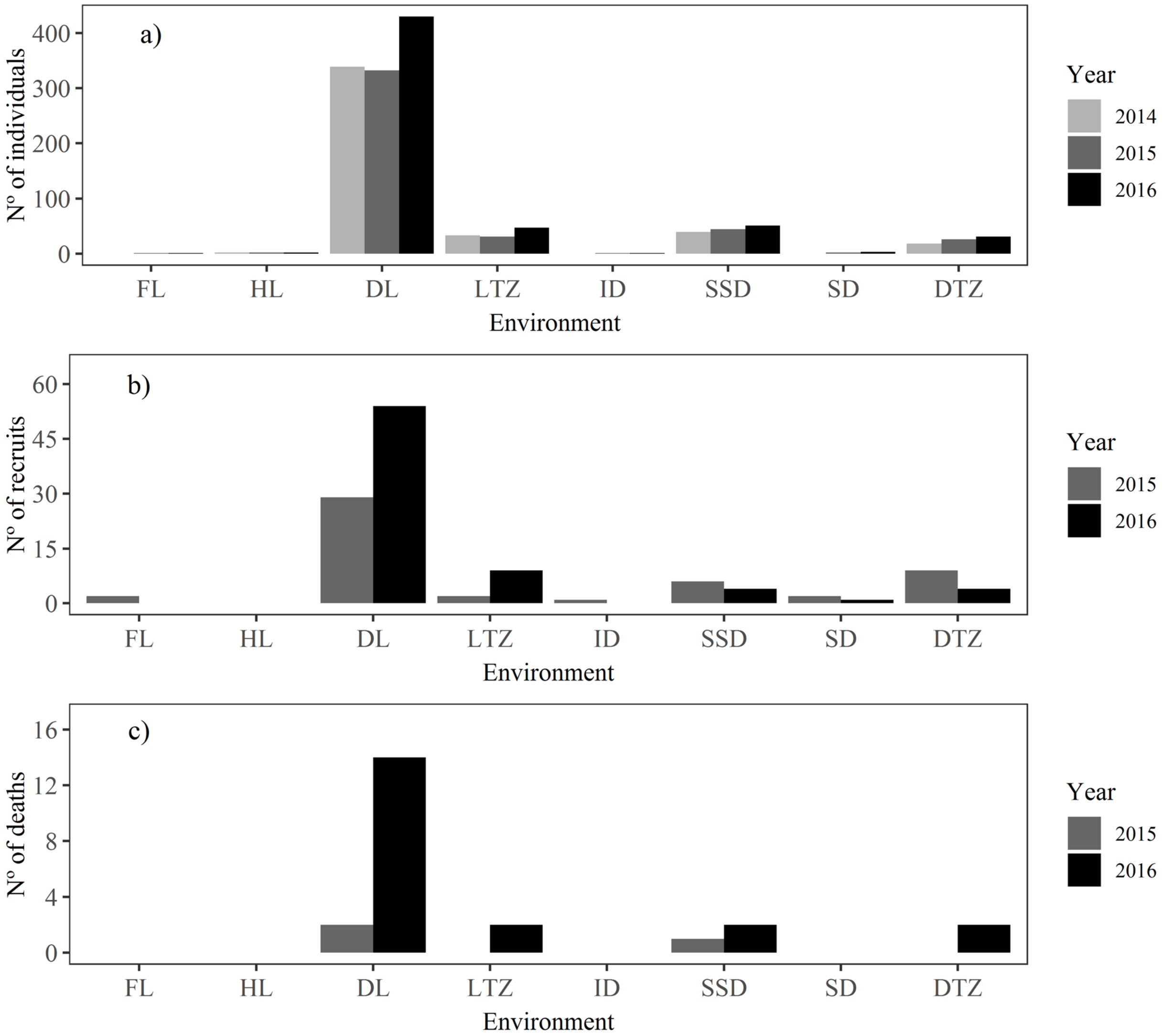

Figure 3. Frequency of (a) individual, (b) recruits and (c) deaths of *Varronia curassavica* Jacq. (Boraginaceae) from 2014 through 2016 in the different environments of the *restinga* of Joaquina / SC. FL = Flooded lowland; HL = Humid lowland; DL = Dry lowland; LTZ = Lowland transition zone; ID = Itinerant dune; SSD = Semi-settled dune; SD = Settled dune; DTZ = Dune transition zone.

Diversity and genetic structure among environments

Seventy alleles were detected in eight polymorphic loci in the *V. curassavica* population. The mean number of alleles per locus was 9.9, ranging from 3 to 19 (Table III).

The allelic frequencies were heterogeneous among environments (Table SIV -Supplementary Material). Rare alleles (frequency less than 5%) were detected in all environments with the highest occurrence of the species. For the population, 41% of alleles occurred with a frequency of less than 5%. This value was maintained for individuals in the dry lowlands, but in other environments, the values were lower than 21%. Private alleles were found only for the dry lowland environment (11 alleles at five loci).

The mean observed heterozygosity in the population ($\hat{H}_O$) was 0.711, and the expected heterozygosity average ($\hat{H}_E$) was 0.716, ranging from 0.305 to 0.914 in the eight loci evaluated (Table III). Fixation index ($\hat{f}$) was significantly different from zero in seven of the eight loci evaluated ($p < 0.001$), suggesting HWE deviations. The mean index of fixation of the population, however, was not different from zero ($p < 0.01$). Allelic richness ($\hat{A}$) ranged from three to nineteen alleles per locus, while the mean number of alleles per individual / loci ($\hat{A}_i$) ranged from 1.5 to 3.76 (tetraploid; Hoeltgebaum & Reis 2017).

In the different environments (Table III), the mean observed heterozygosity ($\hat{H}_O$) ranged from 0.703 to 0.737, with no significant differences among the environments, and the ranged from 0.675 to 0.730, where it was higher in semi-settled dune when compared to the dune transition zone, but showed no difference compared to the other environments. The highest mean allelic richness (9.88) was found in dry lowland, where the highest number of individuals were also found, while the mean number of alleles per individual / loci ($\hat{A}_i$) was 2.90 to 2.98, with no significant difference among environments. The fixation indices ($\hat{f}$) did present differences

Table III. Total genetic variation by environment of *Varronia curassavica* Jacq. (Boraginaceae) in the *restinga* population of Joaquina- Florianópolis/SC- Brazil. Mean values (first row) and confidence interval (second row) by environment.

Environment	**N**	$\hat{A}$	$\hat{A}_i$	$\hat{G}$	$\hat{H}_0$	$\hat{H}_E$	$\hat{f}$
Dry lowland	306	9.88	2.93	63.75	0.709	0.718	0.012
		[9.05-10.7]	[2.83-3.02]	[56.42-71.08]	[0.678-0.740]	[0.692-0.744]	
Dry lowland transition	32.4	7.25	2.91	14.13	0.703	0.699	-0.006*
		[6.7-7.8]	[2.8-3.01]	[12.96-15.29]	[0.669-0.736]	[0.673-0.724]	
Semi-settled dune	34.5	7.88	2.98	14.88	0.737	0.730	-0.009*
		[7.25-8.5]	[2.89-3.07]	[13.67-16.08]	[0.71-0.764]	[0.709-0.751]	
Dune transition	19	6.25	3.02	9.25	0.712	0.675	-0.054*
		[5.79-6.71]	[2.89-3.15]	[8.62-9.88]	[0.678-0.745]	[0.643-0.708]	
Total	396	9.9	2.9	74.9	0.711	0.716	0.01

N = number of individuals; $\hat{A}$ = allelic richness; $\hat{A}_i$ = allelic richness within the individual; $\hat{G}$ = mean number of individuals with four distinct alleles; = $\hat{H}_0$ heterozygosity observed; $\hat{H}_E$ = expected heterozygosity; $\hat{f}$ = fixation index. $p < 0.001$*.

among environments, being negative and significant for dry lowland transition, semi-settled dune and dune transition, but no different from zero in dry lowland.

The analysis of genetic structure indicated low divergence between the environments ($\check{D}_{ST}$ = 0.015; Table V). The estimated interpopulation divergence ($\hat{G}_{ST}$) indicated that around 2% of the allelic variation found was not shared among the different *restinga* environments. When analyzing the structure among environments (peer-to-peer), the values were found to closely range from 0.009 to 0.015 (Table V). For semi-settled dune and dune transition, however, the divergence was higher than the other environments ($\hat{G}_{ST}$ = 0.021), evidencing a trend towards greater divergence between dunes.

Spatial genetic structure

The population did not present significant spatial genetic structure for the distance classes evaluated, suggesting that the genotypes are distributed randomly in the area (Figure 4). When analyzing the different environments, this pattern was maintained with no variation among them.

DISCUSSION

The population of *V. curassavica* presented positive growth rate over the years, with a number of regenerants higher than the number of dead individuals andnot found, indicating that the population is expanding. Although the seedling stage, which is considered the most critical phase of the life cycle of many species, with greater structural fragility (Kozlowski 1971), the number of deaths and individuals not found in this class was low. This aspect is extremely important for the establishment of the population, especially because the species presents a considerable number of aborted flowers and fruits (Hoeltgebaum et al. 2018), aside from being frequently subjected to trampling (men and animals) and herbivory.

Most seedling-stage deaths can be associated with “mobile flooding”. On the other hand, the number of individuals not found, is, in general, reflective of methodological difficulties observed in the demographic evaluation of species, such as the pattern of disordered growth of the branches and their easy detachment, which, apart from burial, result from movement of dunes.

Table V. **Nei’s (1973) genetic diversity statistics of eight microsatellite loci for the *Varronia curassavica* Jacq. (Boraginaceae) population and among different environments of the *restinga*, Joaquina / SC- Brazil.**

Environment	$\hat{H}_T$	$\hat{H}_S$	$\hat{G}_{ST}$
Population	0.720	0.705	0.020
DL x SSD	0.731	0.723	0.009
DL x LTZ	0.716	0.708	0.011
DL x DTZ	0.705	0.696	0.012
LTZ x SSD	0.725	0.714	0.015
LTZ X DTZ	0.696	0.687	0.012
DTZ X SSD	0.718	0.703	0.021

$\hat{H}_T$ = total genetic diversity; $\hat{H}_S$ = mean genetic diversity within populations; $\hat{G}_{ST}$ = proportions of total genetic diversity between populations; DL = Dry lowland; LTZ = Lowland transition zone; SSD = Semi-settled dune; DTZ = Dune transition zone.

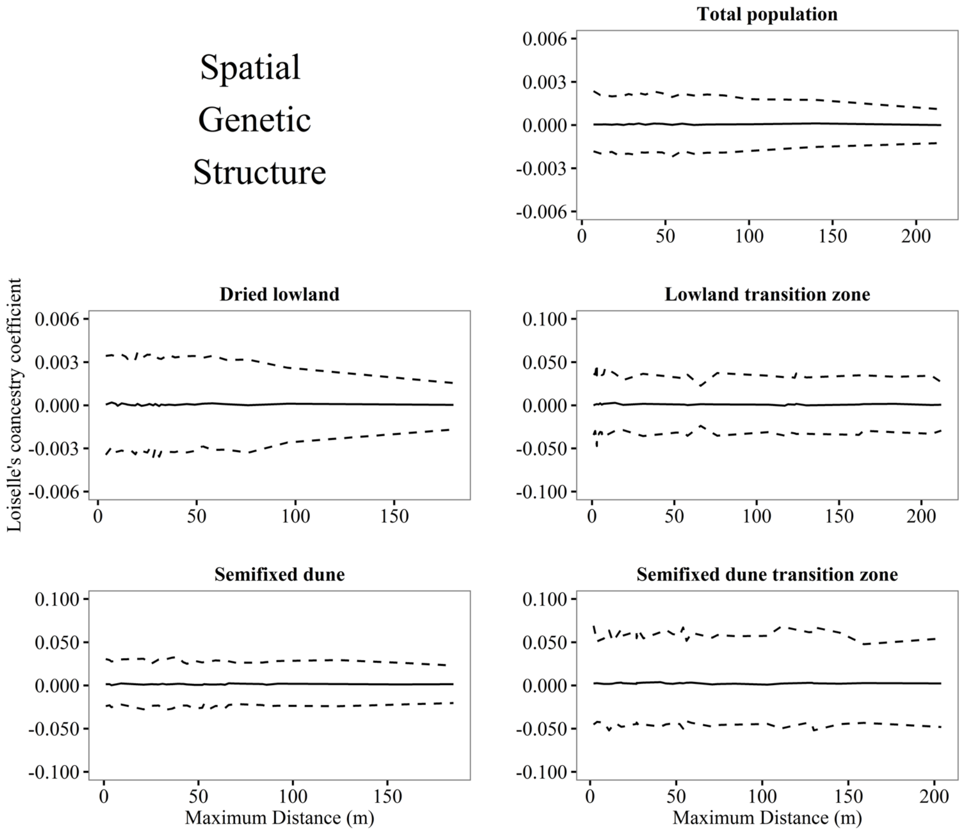

Figure 4. **Spatial genetic structure (SGE) of *Varronia curassavica* Jacq. (Boraginaceae) individuals in the different *restinga* environments of Joaquina/SC. The continuous line represents the estimate of the mean coancestry coefficient, and the dotted lines represent the estimate of the confidence interval at 95% probability of the hypothesis of absence of genetic structure (H0: θxy = 0).**

In terms of diameter and height structure, the pattern that trends toward "J-inverted" indicates a positive balance between recruitment and mortality, which is characteristic of populations in regenerative equilibrium. However, the establishment of the classes proposed herein (recruits, nonreproductive, and reproductive) does not necessarily reflect cohorts in the population, since it was not possible to identify morphological characteristics by which to classify individuals sampled among seedlings, juveniles and adults. Sometimes, branches with regenerating aspects could be observed in mature plants. Depending on the degree of burial of the plant, older branches may not have been found. Likewise, individuals observed in the first class were observed in reproduction.

In the studied area, the species showed an aggregate distribution pattern. As evidenced in this study, the occurrence of environmental heterogeneity, in terms of habitats favorable to the propagation of plant species, as well as the discontinuity of the landscape in terms of physiognomy and vegetation coverage, has been commonly cited in order to explain the distribution of vegetation in *restinga* areas (Castro et al. 2007, Pereira 2009, Ferreira et al. 2010). In arid and semi-arid environments, the pattern of mosaic aggregation in plants has also been well documented (Montaña 1992, Tirado & Pugnaire 2005, Kyncl et al. 2006).

Relationships between the occurrence of individuals of this species and different environmental variables of the *restinga* showed that higher slopesand lower degree of vegetation coverage are associated with lower frequency of individuals. Dry lowlands showed the highest occurrence. Although this *restinga* area has about the same proportion available between dunes and lowland environments

(51% and 49% respectively), about 80% of the individuals of this *V. curassavica* population occur in dry lowlands. The transition zones are responsible for 19% of the population. On the other hand, the environments less favorable to the occurrence of the species are the flooded areas, as well as itinerant dune. Mobile dunes have little to no vegetation, whereas in settled dune, it is possible to find different densities of vegetation with shrub and tree species that can form canopies. The exposed has high temperature ranges, and water availability is low, generally limiting the establishment of several species (see: Pinheiro & Borghetti 2003, Mantovani & Iglesias 2010).

However, environments with dense vegetation may not favor germination since the species is heliophyta (Smith 1970), and shading in these areas may be one of the main factors influencing the establishment of a new individual. In addition to limited establishment, greater mortaility of seedlings occurs in environments subject to flooding, indicating that such conditions are unfavorable for the establishment of individuals. Recruits were also more frequent in dry lowlands (66%), as well as the number of deaths (68%), possibly by the higher number of individuals.

Apart from being areas of preferential occurrence of the species, lowland environments, along with the semi-settled dune, were places where the species presented the highest average increase in height within the observed period, while the increase of the average DAS was significantly higher in the dune transition zones. These results reinforce the importance of dry lowland environments and semifixed dune (and transition zones) for the establishment and development of the species.

Therefore, it can be concluded that the aggregate spatial distribution found for *V. curassavica* in this study results from the preference of this species for certain habitats (DL and SSD) and, consequently, their distribution in the *restinga*.

The analysis of genetic diversity also showed heterogeneity in allelic frequencies associated with differences among environments. Dry lowlands, environments with the highest number of individuals, also presented higher allelic richness. Rare alleles were sampled in all environments, but in the dry lowlands these represented 40%. Private alleles were found only in the dry lowlands. The presence of rare and private alleles is indicative of the importance and function of these environments in terms of species conservation and also the prioritization of areas to be conserved, since low frequency alleles are more susceptible to loss, mainly due to effects of genetic drift (Frankel et al. 1995). However, for a fair comparison, allelic richness data would need to be rarefied, since richness data tends to increase by sampling, and this result should be interpreted with care. Nevertheless, such an analysis could not be performed since the population sampled is tetraploid (Hoeltgebaum & Reis 2017), and most of the softwares do not deal with this data.

In the population as a whole and in the dry lowland environment specifically, the fixation index was not significantly different from zero, evidencing the absence, or reduced effects, of inbreeding and genetic drift. This aspect can be explained by the reproductive system of the species, which is typically allogamic, with mechanisms of self-incompatibility, such as heterostyly and protogyny (Hoeltgebaum et al. 2018). However, for the other environments, these values were negative and significant, indicating a tendency to preferential crosses or selection in favor of heterozygotes for this population. The selection in favor of heterozygotes has advantages for the species, increasing its genetic plasticity, allowing their survival and distribution in diverse environmental conditions.

In plants, spatial distribution and kinship degree may be associated. As a consequence of the aggregate pattern found for *V. curassavica*, we expected to find family structure at close range. Contrary to expectation, we found an absence of structure, indicating that genotypes are randomly distributed in the area. This result suggests that the gene flow within this population may be more related to genetic factors, such as the presence of self-incompatibility mechanisms (Hoeltgebaum et al. 2018), and is not associated to environmental heterogeneity. Levin (1981) demonstrated that the movement of genes within populations is conditioned by the degree of genetic compatibility between individuals. According to the author, the presence of autoincompatibility may prevent certain types of crosses and increase the probability of crossing between more distant plants, considering that the compatibility of pollen-pistil may be smaller between neighbors than between moderately spaced plants. The influence of heterostyly on spatial distribution of genes is also discussed by Martins (1987) for *Cordia goeldiana* Huber, where heterostyly, associated with population density and flowering, has implications for intra and interpopulation gene flow patterns. For *V. curassavica*, the possible preference of crosses between more distant individuals, owing to heterostyly, would act as a promoter of the gene flow within the population, thus reducing the formation of internal genetic structure. In addition, gene flow for this species is also favored by an ample guild of insects that pollinate it (Hoeltgebaum et al. 2018).

Thus, the presence of mechanisms of self-incompatibility and the gene flow favored by generalist pollination may favor the random distribution of genotypes in the population, which would not otherwise be expected for the species owing to the aggregated spatial pattern. Dependence on genetically compatible neighbors implies larger areas for conservation.

This study demonstrates the quality of monitoring and evaluating the demographic and genetic dynamics of a representative number of individuals of the species. The diversity of environments, ranging from mobile dunes to fixed dunes, from flooded areas to dry dunes, exemplifies the heterogeneity that can be found in the Brazilian *restingas*. Therefore, it can demonstrate that variations in the distribution of individuals and genotypes can occur due to different environments.

Dry lowlands are of great relevance for the maintenance of the demographic and genetic processes of the species in *restingas*. On the other hand, areas such as flooded lowland and and mobile dunes were examples of environments unfavorable to the development of the species. This diversity of environments contributed to the aggregate distribution of the species, but did not influence the internal genetic structure. Although the population has evidenced the absence or reduced effects of inbreeding and genetic drift, in some environments the fixation indices indicate a tendency to preferential crosses or selection in favor of heterozygotes for this population. For *V. curassavica*, this selection may favor alleles or genotypic combinations more favorable to the diversity of environments found in the *restinga*, allowing their survival and distribution in diverse environmental conditions.

Thereby, the environmental heterogeneity, typical of *restingas*, is especially relevant for the establishment of the species and should be considered in deciding the minimum areas for conservation and effective population size when defining strategies for *in situ* conservation, and management approaches of the species.

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SUPPLEMENTARY MATERIAL

Table SIV.

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M.S. REIS conceived of the presented idea and supervised the project.

M.B. LAUTERJUNG maked substantial contributions to acquisition of data, and analysis and interpretation of data.

T. MONTAGNA, R.C. RIBEIRO, W. VIEIRA, A. BERNARDI, C. CRISTOFILINI help in fieldwork, and provided critical feedback and helped shape the research, analysis and manuscript.