



Spatiotemporal Variation on Fertility, Mating System, and Gene Flow in *Vriesea gigantea* (Bromeliaceae), an Atlantic Forest Species

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OPEN ACCESS

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Specialty section:

This article was submitted to
Tropical Forests,
a section of the journal
Frontiers in Forests and Global
Change

Received: 10 March 2022

Accepted: 16 May 2022

Published: 16 June 2022

Citation:

Paggi GM, Palma-Silva C,
Zanella CM, Goetze M, Büttow MV,
Lexer C and Bered F (2022)
Spatiotemporal Variation on Fertility,
Mating System, and Gene Flow
in *Vriesea gigantea* (Bromeliaceae), an
Atlantic Forest Species.
Front. For. Glob. Change 5:893548.
doi: 10.3389/ffgc.2022.893548

Knowledge of the patterns of variation in plant mating systems and fitness, in the context of conservation biology, is essential for understanding microscale differences and their probable causes. The aim of this study was to assess the patterns and determinants of spatiotemporal variation in fertility, mating systems, and gene flow in the epiphytic bromeliad *Vriesea gigantea*, which is endemic to the Atlantic Forest. We studied three wild populations over 2 years in terms of flowering, fruit set, seed production, and seed germination, and used nuclear microsatellite markers to analyse variations in the mating system and contemporary gene flow. We observed high fertility parameter values, with among-population differences only in fruit set and germination rate. Mating system analysis showed moderate outcrossing rates (t_m), which ranged from 0.107 to 1.2, and strong pollen pool genetic structures (Φ_{FT}), of 0.319 to 0.598 across populations and years. We suggest that the observed spatiotemporal variation in fertility, mating systems, and gene flow in this species are attributable to the adaptation to selfing due to edge effects, mainly caused by range expansion during post-glacial migration. Populations on the edge of the *V. gigantea* distribution were the most genetically divergent in the southern limit of the Atlantic Forest biome. Our results may contribute to ongoing debate regarding the characteristic fitness trade-offs that shape variation in plant mating systems and gene flow when species meet their range limits.

Keywords: Atlantic Forest, contemporary gene flow, microsatellite loci, selfing rate, Southern Brazil, Tillandsioideae

INTRODUCTION

Mating systems have important effects on the genetic diversity structure within and among populations in many flowering plants due to gene flow *via* pollen transference and seed dispersion. These factors directly affect the distribution of alleles and contribute to shaping the patterns of genetic diversity within and among populations (Hamrick, 1982; Loveless and Hamrick, 1984; Hamrick and Godt, 1996). Many studies on the reproductive ecology of plants have focused on field

studies of reproductive systems and pollination aspects, along with the estimation of genetic and mating system parameters (Barrett and Eckert, 1990; Cascante-Marín et al., 2006; Eckert et al., 2009; Hmeljevski et al., 2011; Paggi et al., 2015). These studies provide valuable information regarding the spatial variation of mating systems and gene flow patterns in wild species (Moeller et al., 2017).

The outcrossing rate can be estimated by examining naturally-pollinated progeny arrays of plants. This approach excludes self-fertilised progeny from the data and uses a maximum-likelihood model (Ritland and Jain, 1981). In addition, conservation and evolutionary researchers concerned with different microevolutionary processes and the impact of landscape change must measure real-time gene movement. Austerlitz and Smouse (2001) and Smouse et al. (2001), developed a two-generation approach (TwoGener) to study pollen pool genetic structure, by quantifying the heterogeneity among the male gamete pools sampled by maternal plants distributed across populations, and estimating the mean pollination distance and effective neighbourhood size.

Genetic diversity and gene flow analyses are important for understanding the genetic diversity distribution of natural plant populations, which can influence local adaptation and provide valuable information for conservation decision-making (Segelbacher et al., 2021), although genetic diversity has often been neglected by environmental policy agreements (Hoban et al., 2021). Furthermore, genetic diversity is highly relevant to population viability, which can be reflected in survival and fecundity parameters determined by habitat conditions and biotic and abiotic factors (Reed and Frankham, 2003; Caruso et al., 2005; Leimu et al., 2006). Several fertility aspects have been studied to help estimate population viability, including plant size, flower production, fruit and seed set, and pollen and seed viability (Johnson et al., 2004; Paggi et al., 2007, 2013; Palma-Silva et al., 2008; Sampaio et al., 2012; Amat et al., 2013). Plant fruit and seed production can be influenced by the availability of biotic (pollen and pollinators; Barrett and Eckert, 1990; Colas et al., 2001; Johnson et al., 2004) and abiotic resources (Caruso et al., 2005). For example, pollen limitation on seed production can be a result of reduced pollinator visits or a decrease in the quantity or quality of pollen deposited per visit (Colas et al., 2001; Huang and Guo, 2002; Ashman et al., 2004; Buide, 2004; Ohara et al., 2006; Ratto et al., 2018; Bennett et al., 2020). In addition, fertility patterns associated with mating system analysis can provide a better understanding of biotic effects on the reproductive success of natural plant populations, especially when considering species from fragmented habitats (Aizen et al., 2002; Murren, 2002; Aguilar et al., 2006; Ohara et al., 2006), such as the Atlantic Forest (AF) located along the eastern coast of Brazil.

The relationship between plant fitness and patterns of genetic diversity differs between self-incompatible (positively correlated) and self-compatible (independent) species (Barrett, 2014). This relationship also affects population size and is a critical factor in plant ecology, evolution, and conservation. Even though many plant populations are naturally isolated and small, recent anthropogenic fragmentation of habitats has contributed to

population reduction and isolation of several plant species (Leimu et al., 2006). In general, self-compatible species with large populations may experience less pronounced negative effects from habitat fragmentation (Leimu et al., 2006). Several studies have measured inbreeding depression, genetic variation, population size, and fitness of plant species to aid in their conservation (Stockwell et al., 2003; Kramer and Havens, 2009; Angeloni et al., 2011; Jacquemyn et al., 2012; Oakley and Winn, 2012; Frankham et al., 2014; Ottewell et al., 2016; Rodger et al., 2021).

Some species, such as epiphytic bromeliads live in stable, naturally fragmented habitats and are well-adapted to drought and wind, although their colonising ability is likely hindered by landscape structure, with only a limited number of suitable sites available (Benzing, 1990, 2000; Winkler et al., 2007; Victoriano-Romero et al., 2017). Bromeliad species frequently show high genetic differentiation among naturally fragmented populations, which could be a result of local adaptation by the species or genetic drift due to historical colonisation events (Zanella et al., 2012; Cascante-Marín et al., 2014; Hmeljevski et al., 2014; Goetze et al., 2016; Gonçalves-Oliveira et al., 2017; Godoy et al., 2018; Ruas et al., 2020; Vicente-Silva et al., 2021).

The Bromeliaceae family has approximately 3,140 described species (Butcher and Gouda, 2017), being among the most species-rich non-woody plant families in the Neotropics. It presents one of the most remarkable adaptive radiations in the plant world (Givnish et al., 2011, 2014). Around 50 percent of the species are epiphytic; they have evolved floral displays with a great diversity of colors, shapes, and scents that are related to pollinator attraction, with nectar being the usual reward (Zanella et al., 2012). These pollinators are bats, birds, and insects, in self- and outcrossing species; there are also autogamous taxa which do not need pollinator agents (Kessler and Krömer, 2000; Krömer and Kessler, 2006; Krömer et al., 2008; Kamke et al., 2011; Schmid et al., 2011a,b; Marques et al., 2015; Aguilar-Rodríguez et al., 2016). The species are also essential resource for small, hovering vertebrate pollinators in the New World (Fleming and Muchhala, 2008) and food sources for hummingbirds in many Neotropical forest regions (Dziedziuch et al., 2003; Wolowski et al., 2013; Pansarin and de Pedro, 2016; Nunes et al., 2018; Gomes et al., 2019; Lenzi and Paggi, 2020).

In the present study, we investigated patterns of spatiotemporal variation in mating systems and fertility of three populations of *Vriesea gigantea* Gaudichaud from the center of the range of the species to the southern margin over 2 years. *Vriesea gigantea* is a self-compatible species which showed high fertility parameters in one population evaluated (Paggi et al., 2007; Palma-Silva et al., 2008). Paggi et al. (2015) reported a high selfing rate ($s = 0.612$), and Sampaio et al. (2012) observed moderate to low inbreeding depression ($\delta = 0.02\text{--}0.39$) in populations from its southern range margin. In addition to the reported high fertility, pollen limitation was observed due to habitat fragmentation (Paggi et al., 2007), although low pollen viability in some localities could also contribute to this effect (Palma-Silva et al., 2008). Palma-Silva et al. (2009) observed a latitudinal trend

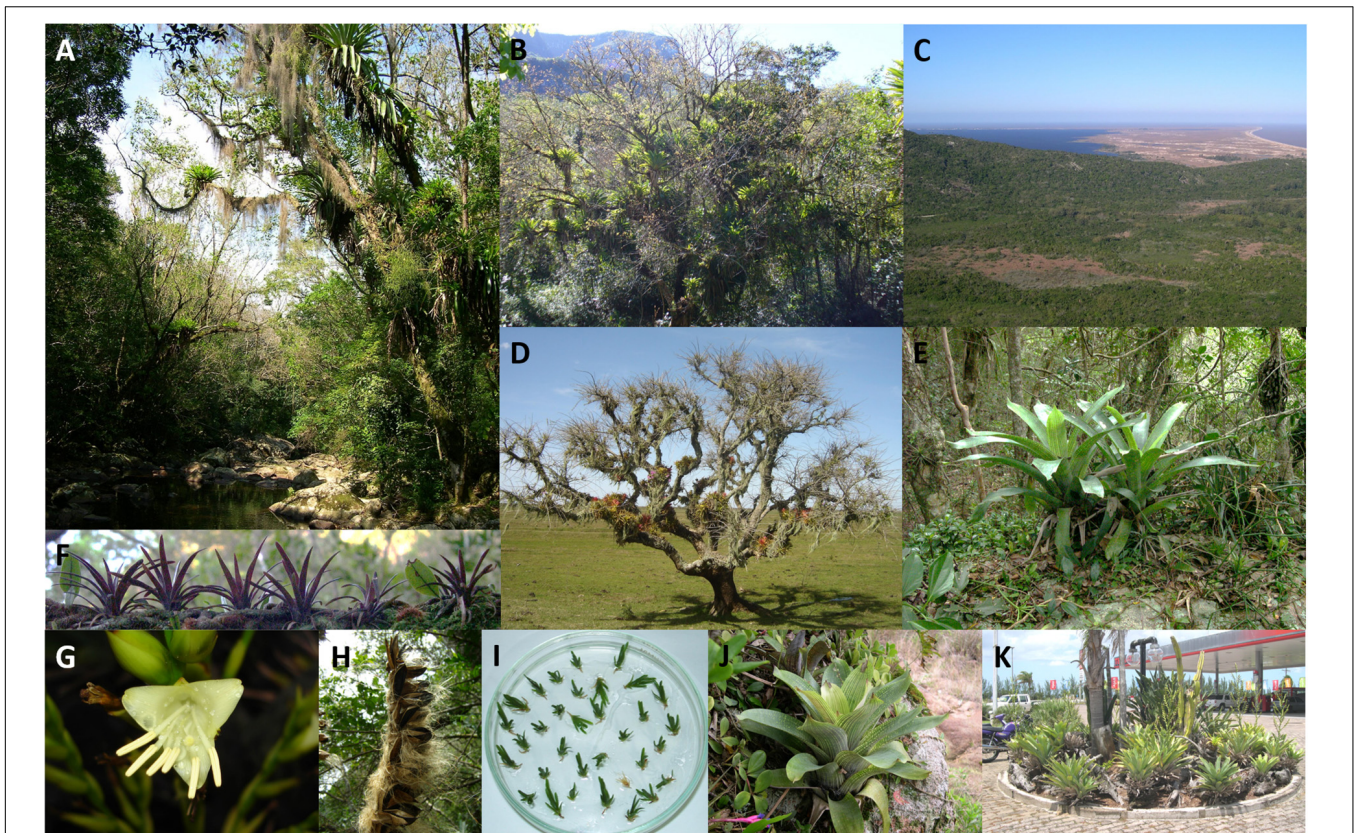


FIGURE 1 | General view of *Vriesea gigantea* natural populations from Rio Grande do Sul state, Brazil, and details of the species. **(A)** Epiphytic individuals of *V. gigantea* in the Maquiné populations, showing individuals of *Tillandsia usneoides* (L.) Nutt. ex Steud., **(B)** Serra Geral Ecological Reserve overview, Maquiné city population, with several epiphytic individuals of *V. gigantea*, **(C)** Itapuá State Park overview, Porto Alegre city, **(D)** Taim Ecological Station, Rio Grande city, in detail an individual of *Erythrina crista-galli* L. Var. *hasskarlii* Backer with several epiphytic individuals of *Aechmea recurvata* (Klotzsch) L.B. Smith, several individuals *Ramalina usnea* (L.) R. Howe, and some individuals of *Usnea* sp. Dill. ex. Adans. **(E)** Lithophytic adult individual of *V. gigantea*, Itapuá population, **(F)** Epiphytic seedlings of *V. gigantea*, Itapuá population, **(G)** Flower detail of *V. gigantea*, Maquiné population, **(H)** Detail of seed dispersal of *V. gigantea*, Itapuá population, **(I)** *V. gigantea* seedlings from seed germinations experiments, **(J)** Lithophytic young individual of *V. gigantea*, Itapuá population, **(K)** Adult individuals of *V. gigantea* used for ornamental purposes at a gas station, BR 101, Maquiné city. Photographs: Clarisse Palma-Silva and Gecele Matos Paggi.

of decreasing genetic diversity from north to south away from the equator, consistent with the prediction of declining within-population and/or increasing among-population genetic diversity toward range margins (Eckert et al., 2008). For *V. gigantea*, Palma-Silva et al. (2009) hypothesised that the observed results are in concordance with the direction of historical forest expansion after the Last Maximum Glacial and the lack of gene flow at the current northern and southern range edges, which is partially explained by restricted seed dispersal (Paggi et al., 2010).

The focus of the present study was to evaluate the potential of plants to evolve local mating system adaptations, such as selfing, in relation to environmental changes (Eckert et al., 2010), for instance, edge effects and habitat loss. Specifically, we used our combined fertility and mating system data across populations and years to address the following questions: (1) Is there spatiotemporal variation in fertility and mating system parameters, and if so, how is this variation distributed in space and time? (2) Is there any correlation between spatiotemporal variation in outcrossing rates and fertility parameters? (3) What

do estimates of the pollen pool genetic structure and the effective number of pollen donors tell us about gene flow among populations in different years?

MATERIALS AND METHODS

Study Species and Sites

Vriesea gigantea is an epiphytic bromeliad species (occasionally lithophytic), which is endemic to the AF and occurs from the Espírito Santo to the Rio Grande do Sul states, Brazil (Figures 1B,E,F,J; Zimmermann et al., 2007; Palma-Silva et al., 2009; Paggi et al., 2015). This species has been described as being bat-pollinated (Sazima et al., 1999), but hummingbirds and bees may also play important roles in its pollination (Paggi et al., 2021), as also observed for *Billbergia horrida* Regel (Marques et al., 2015) and *Tillandsia heterophylla* E. Morren (Aguilar-Rodríguez et al., 2016). Typical inflorescences of *V. gigantea* have a central axis with several branches on each side and one flower on each side of the lateral axis (Reitz, 1983). Flowers

at the base and centre of the inflorescence open first, followed by flowers in the apical position (Reitz, 1983; Benzing, 2000; Paggi et al., 2021). Anthesis may occur during both the day and night (Paggi et al., 2021). The flowers are tube-shaped with three petals, and their yellowish colour corresponds to the chiropterophilous syndrome (**Figure 1G**; Paggi et al., 2021), suggesting that outcrossing is the predominant mating system of this species (Vogel, 1969).

We conducted the present study with three wild populations of *V. gigantea*, Maquiné, Itapuã, and Taim, which were previously evaluated for genetic, mating system and fitness parameters in one reproductive year (Paggi et al., 2007, 2015; Palma-Silva et al., 2008, 2009; Sampaio et al., 2012). We conducted fieldwork on all populations during the flowering and fruiting periods of 2006 and 2007. Maquiné and Itapuã populations are located in the centre of the distribution of the species (Atlantic Forest strict sense), and Taim population at the range margin (Atlantic Forest wide sense), east of Rio Grande do Sul state, southern Brazil [**Table 1** and **Figures 1A–D**; for more details see Paggi et al. (2015)]. These populations comprise a large number of individuals, which are in or close to environmental protection areas [**Table 1** and **Figure 1B**; for more details see Paggi et al. (2015)], although the species is continually threatened by habitat loss and illegal predatory gathering (**Table 1** and **Figure 1K**). The climate in the study region is classified as humid subtropical (Cfa) by the Köppen Climate Classification System.

Fertility Analysis

To evaluate the fertility of each population, we computed the following parameters using different individuals for each year (**Table 2**): (a) total number of flowers per plant; (b) total fruit set (i.e., the fraction of flowers developing into mature fruit) per plant; and (c) number of seeds per fruit (from one fruit randomly collected from 25 plants in each population, $n = 75$, **Figure 1H**). To analyse the seed germination rate, we used two fruits and 30 seeds per fruit per sampled individual (**Table 2**, $n = 60$ seeds per individual, **Figure 1I**). We germinated and monitored seeds daily for 30 days following the methods described by Paggi et al. (2007). We analysed fertility parameters by analysis of variance (ANOVA) followed by Tukey's test to verify differences among populations and years. We tested for possible correlations between the outcrossing rate (t_m) and fruit set considering all populations and years. We performed all statistical analyses using SAS software (Version 8, SAS Institute, Cary, NC, United States).

Mating System Analysis

For genetic analysis, we collected open-pollinated seeds from different mother plants (MPs) each year (**Table 2**) because this species exhibits a semelparous life cycle, which means that an adult plant only blooms once in a lifetime. From each MP, we collected fresh leaves both in the field ($\sim 5 \text{ cm}^2$) and from seedlings germinated *in vitro*. The leaves were stored in liquid nitrogen until DNA extraction. Total genomic DNA was extracted according to the protocol described by Doyle and Dickson (1987). Using a global positioning system (GPS) receiver,

TABLE 1 | Natural populations of *Vriesea gigantea* in the Atlantic Forest, Rio Grande do Sul State, Brazil.

Population (species distribution)	Nearest ECU/city	Geographical coordinates	Maximum altitude (m)	Mean temperature (°C)	Annual rainfall	Species habit	Conservation status	Threat
Maquiné (centre)	Serra Geral ecological reserve/Maquiné	29° 48' S, 50° 16' W	612	21.5	1,650 mm/year	Epiphytic	Partially protected	Habitat loss; predatory gathering
Itapuã (centre)	Itapuã state park/Viamão	30° 21' S, 51° 01' W	252	17.5	1,200 mm/year	Epiphytic; lithophytic	Completely protected	-
Taim (Southern margin)	Taim ecological station/Rio Grande	31° 56' S, 52° 25' W	5	18	1,100 mm/year	Epiphytic	Slightly protected	Habitat loss; agriculture, livestock

Name of the population, nearest Environmental Conservation Unit (ECU), geographical coordinates, maximum altitude, mean temperature, annual rainfall, species habit, and conservation status of populations.

we recorded the location of each MP. The total sample size, considering MPs and seedlings from all years and populations, was 1,465 individuals.

We genotyped four microsatellite loci for data analysis from 2006 and scored three nuclear microsatellite loci previously characterised for *V. gigantea*, VgB06, VgF01, and VgG02 (Palma-Silva et al., 2007); and one locus characterised for *T. fasciculata*, CT5 (Boneh et al., 2003). For the data analysis from 2007, we genotyped two of the most polymorphic loci identified in previous analyses (loci: VgF01 and VgG02), due to the occurrence of monomorphic loci in some sampled populations in the previous year. We labelled the forward primer of each locus at the 5' end with an M13 fluorescent tag (6-FAM). We performed all polymerase chain reaction (PCR) amplifications in a PE Applied Biosystems 9700 thermocycler as described by Palma-Silva et al. (2007). Microsatellite alleles were resolved on a 3100 DNA Analyser (Applied Biosystems, Foster, United States) and precisely sized against an ROX molecular size standard using Genotyper 3.7 software (Applied Biosystems, Foster, United States).

We performed progeny pair analysis using the mixed mating model described by Ritland (2002) with Mltr 3.4 software (Ritland, 1990). We estimated the outcrossing rate (t_m), inbreeding coefficient of maternal parents (F), correlation of selfing (r_s) (Ritland, 2002), correlation of outcrossed paternity (r_p ; which reflects any tendency for siblings to share the same male parent) (Ritland, 2002), and number of effective pollen donors ($N_{ep} = 1/r_p$). We calculated standard errors based on 1,000 bootstraps between individuals within a progeny array. Using one-tailed Student's t -tests, we determined the significance of t_m and F based on the null hypothesis that $t_m = 1$ and $F = 0$ using SAS software.

To analyse the genetic structure of pollen pools (Φ_{FT}) of individual plants relative to the global pollen pool (Smouse et al., 2001) we used the TwoGener procedure, based on the AMOVA approach described by Excoffier et al. (1992). We computed the 99% confidence interval of Φ_{FT} by bootstrapping among the loci with 1,000 replicates. We performed computations using the POLDISP 1.0 software (Robledo-Arnuncio et al., 2007) to estimate the pollen pool structure (Φ_{FT}), average distance of pollination (δ), effective neighbourhood pollination area (A_{ep}), and effective number of pollen donors (N_{ep}).

TABLE 2 | Sample sizes per population and year for fertility (adult plants) and mating system analysis (adult plants/progenies) in the three studied populations of *Vriesea gigantea*.

Population	Fertility			Mating system		
	2006	2007	Total	2006	2007	Total
Maquiné	13	12	25	10/191	14/276	24/467
Itapuã	16	15	57	11/162	14/273	37/617
Taim	12	15	27	5/91	12/212	17/303
Total	41	42	109	26/444	40/761	78/1387

RESULTS

Fertility

The mean number of flowers per plant for all populations and years was 169.2 (standard error [SE] \pm 5.6), with a range of 154.1 (SE \pm 11.3, Itapuã–2006) to 201.2 (SE \pm 20.7, Maquiné–2006), and no statistical differences were observed among populations (Table 3). The fruit set per plant ranged from 23.3% (SE \pm 6.0, Itapuã–2006) to 75.9% (SE \pm 5.6, Taim–2006) with an overall mean of 47.6% (SE \pm 2.5), across all populations and years. We detected differences in fruit set among the populations in each year. The Taim population yielded more fruits in 2006 (75.9 \pm 5.6), while the Itapuã population showed a lower fruit set in 2006 (23.3% \pm 6.0; Table 3). The mean number of seeds per fruit was 598.4 (SE \pm 35.2), and there were no differences among the populations (Table 3). We evaluated seed viability based on the germination rate of *V. gigantea*, which was high in all the populations. After 15 days of culture, an average of 86.8% (SE \pm 2.5) of the seeds germinated (Figure 11). The Taim population had the lowest germination rate of 73.0% (SE \pm 6.3; Table 3).

Mating System

The inbreeding coefficient (F) based on the genotypic frequencies of maternal plants was positive and significantly higher than zero in all populations and years (Table 4, except in Taim–2007), suggesting an excess of homozygotes ($P < 0.0001$). The outcrossing rate (t_m) was variable among populations as well as between years within populations, ranging from 0.107 (Itapuã–2007) to 0.445 (Maquiné–2006; Table 4). A significant self-fertilisation rate ($s = 1 - t_m$) was detected since the t_m was significantly lower than one for all populations and years ($P < 0.0001$). The correlation of selfing (r_s) was high in the Maquiné population, implying that some families were highly selfed and others were highly outcrossed (Table 4). In the other populations, the r_s values were lower, indicating less variation in selfing among the families. The correlation of the outcrossed paternity (r_p) ranged from 0.286 (Itapuã–2007) to 0.714 (Maquiné–2006), suggesting that a high proportion of siblings shared the same male parent. Patterns of fruit set observed in *V. gigantea* populations were not significantly correlated with outcrossing rates; however, a slight increase in fruit set was observed when there was an increased outcrossing rate ($R = 0.2742$, $P = 0.5518$; Figure 2).

The differentiation in allelic frequencies among pollen pools (Φ_{FT}) of *V. gigantea* ranged from 0.319 to 0.598, indicating differences in the distribution of pollen pools from plant to plant across populations and years (Table 5). The average pollination distance (δ) ranged from 77 to 299 m, and the effective neighbourhood pollination area (A_{ep}) varied from 47 to 718 m². The lowest average pollination distance and effective neighbourhood pollination area were observed in Itapuã–2007 when reproductive adult density was highest (17.4 plants/km²; data not shown). The effective number of pollen donors (N_{ep}) for all populations and years was low, ranging from 0.77 to 1.56 (Table 5), with higher values in the more central population

TABLE 3 | Fertility parameters, including mean number of seeds per fruit, germination rate ($n = 30$ seeds per fruit), mean number of flowers per plant, and fruit set of *Vriesea gigantea*, in the three studied populations and the 2 years evaluated.

Parameters	Flower production			Fruit set (%)			Number of seeds	Germination (%)
	2006 ¹	2007 ²	All ³	2006 ⁴	2007 ⁵	All ⁶	2007 ⁷	2007 ⁸
Maquiné	201.2 ± 20.7	174.1 ± 21.0	188.2 ± 14.7	48.2 ± 5.0 b	46.7 ± 5.5 ab	47.5 ± 3.6 b	700.3 ± 117.9	89.8 ± 3.2 a
Itapuã	154.1 ± 11.3	181.7 ± 13.6	166.8 ± 7.4	23.3 ± 6.0 c	42.2 ± 4.4 b	37.1 ± 3.3 b	506.7 ± 65.3	93.6 ± 3.0 a
Taim	178.0 ± 14.5	161.9 ± 10.1	169.1 ± 8.5	75.9 ± 5.6 a	63.2 ± 5.7 a	68.6 ± 4.2 a	601.4 ± 35.9	73.0 ± 6.3 b
All [†]	176.1 ± 9.3	172.5 ± 8.4	169.2 ± 5.6	46.6 ± 4.7	51.0 ± 3.2	47.6 ± 2.5	598.4 ± 35.2	86.8 ± 2.5

Values shown are means ± SE (standard error). ¹ $F = 2.42, P = 0.1021$; ² $F = 0.49, P = 0.6148$; ³ $F = 2.00, P = 0.1403$; ⁴ $F = 21.82, P < 0.0001$; ⁵ $F = 4.93, P = 0.0124$; ⁶ $F = 18.29, P < 0.0001$; ⁷ $F = 1.76, P = 0.1954$; ⁸ $F = 6.63, P = 0.0024$; [†]Differences among populations not tested; *Differences among years: flower production, $F = 1.51, P = 0.2263$; and fruit set, $F = 0.70, P = 0.4988$. Means with the same letter are not significantly different.

TABLE 4 | Mating system parameters (SD—standard deviation in parentheses) of *Vriesea gigantea* populations and years.

Populations	Maquiné		Itapuã		Taim	
	2006	2007	2006	2007	2006	2007
F	0.240 (0.091)	0.267 (0.233)	0.291 (0.107)	0.293 (0.189)	0.611 (0.455)	-0.200 (0.270)
t_m	0.445 (0.087)	0.300 (0.082)	0.296 (0.079)	0.107 (0.079)	0.152 (0.215)	1.200 (0.076)
r_s	0.452 (0.208)	0.710 (0.185)	-0.175 (0.066)	-0.200 (0.427)	-0.200 (0.481)	0.406 (0.235)
r_p	0.714 (0.070)	0.710 (0.192)	0.476 (0.093)	0.286 (0.442)	0.386 (0.309)	0.406 (0.235)
$N_{ep} (1/r_p)$	1.40	1.41	2.10	3.49	2.59	2.46

*Mixed mating system parameters: inbreeding coefficient of maternal parents (F); multilocus outcrossing rate (t_m); correlation of selfing (r_s); correlation of paternity (r_p); effective number of pollen donors ($N_{ep} = 1/r_p$).

(Maquiné). We could not compute the estimates for Taim–2007, since the loci were monomorphic. The mating system parameters exhibited a notable trend from the range centre of the species (Maquiné) toward the southern range margin (Taim), with an increase in the inbreeding coefficient (F) and pollen pool

genetic structure (Φ_{FT}), and a decrease in the outcrossing rate (t_m) (Figure 3).

DISCUSSION

In this study, we observed spatiotemporal variation in fertility, mating systems, and gene flow of wild populations of *V. gigantea* from the centre to the southern range edge of the species. Unfortunately, we found no populations in geographically intermediate localities among the studied populations. No significant differences were observed when comparing temporal variations. Nevertheless, spatial variation was observed from the range centre of the species (north) toward the southern range margin (south), with an evident trend of increasing pollen pool genetic structure, decreasing outcrossing rate and no apparent

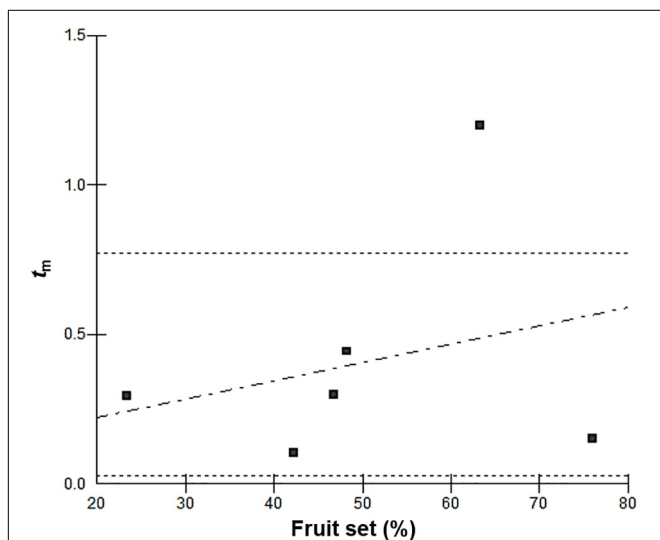
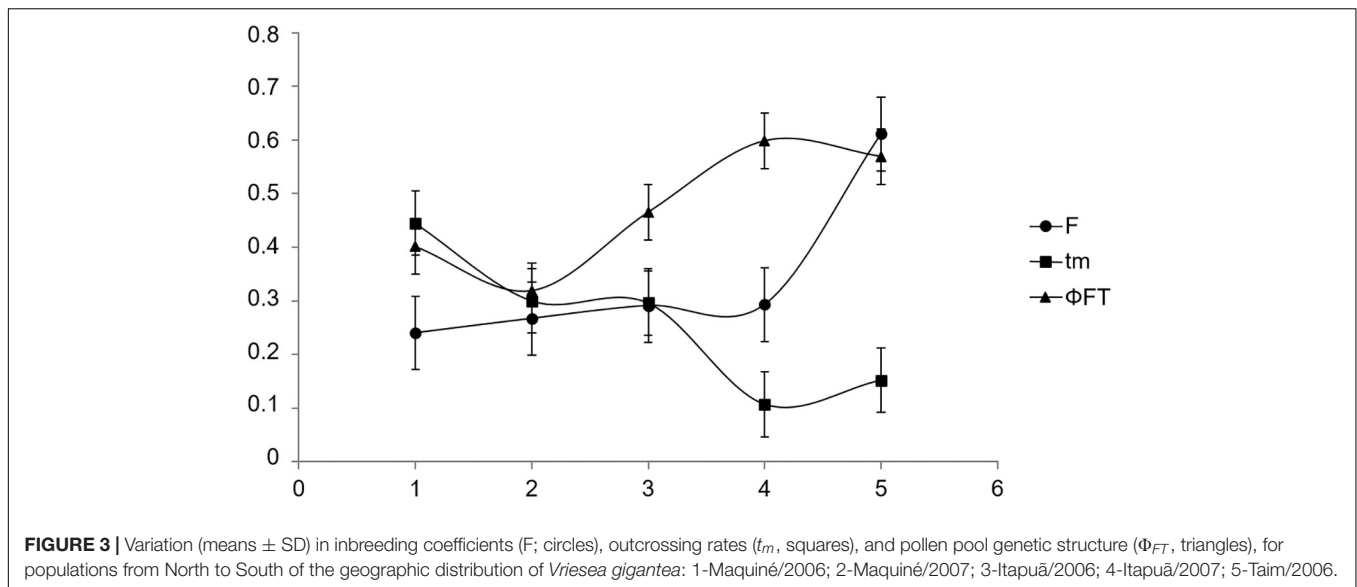


FIGURE 2 | Correlation between fruit set and outcrossing rate in *Vriesea gigantea* ($r = 0.2742, P < 0.5518$) for populations of Maquiné/2006, Maquiné/2007, Itapuã/2006, Itapuã/2007, Taim/2006, and Taim/2007, which were plotted in this order in the graphic. The horizontal dotted line delimits the boundary of one standard deviation.

TABLE 5 | Contemporary gene flow parameters of *Vriesea gigantea* across populations and years.

Populations	Maquiné		Itapuã		Taim	
	2006	2007	2006	2007	2006	2007
Φ_{FT}	0.401	0.319	0.465	0.598	0.569	-
δ (m)	152	235	150	77	299	-
A_{ep} (m ²)	184	441	181	47	718	-
N_{ep}	1.25	1.56	0.77	0.82	0.81	-

*TwoGener model parameters: pollen structure parameter (Φ_{FT}); average distance of pollination (δ); effective neighborhood pollination area (A_{ep}); and effective number of pollen donors (N_{ep}).



changes in fertility parameters with latitude. The sampled population in the extreme southern edge of the distribution (Taim) exhibited the highest selfing rate and strongest pollen pool genetic structure. The results presented here are important for ongoing debate regarding the cost of and evolutionary significance of the adaptation to selfing in plant mating systems.

Across populations and years, mating systems and fertility analyses revealed that the studied populations had both high selfing rates and high fertility values. We observed a large production of flowers, fruits, and seeds, and high seed germination rates in all populations. Nevertheless, a more detailed examination of the results revealed that each population behaved differently in terms of the mating system and fertility. Although the Itapuã and Maquiné populations had the highest t_m values, they showed the lowest fruit production, which could indicate pollen limitation due to ecological factors (Knight et al., 2005). In this case, qualitative (pollen viability) and/or quantitative (pollinator limitation) components, which had been previously identified in the Itapuã population as factors that decrease reproductive fitness, could be responsible for the observed results (Paggi et al., 2007; Palma-Silva et al., 2008).

Both populations showed high germination rates consistent with the low levels of inbreeding depression previously observed [$\delta = 0.05$ in Maquiné, and $\delta = 0.03$ in Itapuã; Sampaio et al. (2012)]. This result may indicate a significant contribution of outcrossing, although some unavoidable geitonogamy is expected to occur in self-compatible species that have stable low-to-intermediate selfing rates (Schoen and Lloyd, 1992; Lloyd, 1992; Porcher and Lande, 2005; Paggi et al., 2007, 2015). In contrast, the Taim population showed the highest fruit set, probably due to autonomous selfing or geitonogamy (Paggi et al., 2015). The results also indicated low cross-pollen competition, since we observed a high proportion of selfing ($s = 0.848$). Together, these features should effectively provide reproductive assurance for the species (Lloyd, 1992; Lloyd and Schoen, 1992); however, the Taim population had the lowest germination rate, likely

due to inbreeding depression ($\delta = 0.02$; Sampaio et al., 2012). Although we used a limited number of SSR loci to estimate these parameters, Palma-Silva et al. (2009) reported very similar results of low genetic diversity levels for Taim population ($H_O = 0.039$ and $F_{IS} = 0.408$) using ten SSR loci.

Variations in outcrossing rates across time and space can be attributed to the historical and biological contexts of each plant species (Loveless and Hamrick, 1984; Schemeske and Lande, 1985; Barrett and Eckert, 1990; Knight et al., 2005; Moeller et al., 2017). Most of this variation occurs in self-compatible species in which environmental and demographic factors can play essential roles in the degree of outcrossing due to differences in pollen quality and pollen vectors (Barrett and Eckert, 1990; Barrett, 2014). Those reproductive outcomes can vary among populations of animal-pollinated plants owing to differences in the availability of effective pollinators and environmental effects on plant phenotypes and their functional consequences for pollinator behavior that governs pollen dispersal (Tian et al., 2022). Here, we observed spatial inter-population variation in the mating system of *V. gigantea*, with an increase in selfing rates from north to south, and different effects on fertility parameters that can occur due to ecological or genetic factors (Barrett, 2014). Previous studies have shown that this species is self-compatible (Paggi et al., 2007) with some level of autonomous selfing and geitonogamy, although it also presents a cryptic self-incompatibility system with delayed germination of self-pollen at the stigma (Paggi et al., 2015). Moreover, Paggi et al. (2021) showed that hummingbirds are effective pollinators in the Southern populations of *V. gigantea*, even though the species is classified as chiropterophilous; the authors also reported only one hummingbird species for Itapuã and Taim populations, which may be related to the high levels of selfing observed here for both populations (Paggi et al., 2021). Additionally, low-to-moderate levels of inbreeding depression have been observed in the same population (Sampaio et al., 2012). Together, these features corroborate our observations of

spatio-interpopulation variation in mating systems and fertility for the studied populations.

Results from the progeny analysis confirmed that selfing occurs at a moderate to high frequency ($0.555 < s < 0.893$; excluding data from Taim–2007), apparently as a consequence of different modes of self-fertilisation that contribute to reproductive assurance in *V. gigantea* populations when outcrossing is not possible. Many functional, ecological, morphological, and physiological factors affect the occurrence of self-fertilisation (Lloyd and Schoen, 1992). In the case of *V. gigantea*, this is more likely due to pollen limitation (lack of pollinator activity or low pollen quality; Paggi et al., 2007, 2021; Palma-Silva et al., 2008); however, demographic factors, such as genetic drift and consequently low genetic diversity could affect populations at the southern range margin, which have the highest selfing rates, and where natural populations have become scarce (personal observation). In many models for the selection of self-fertilisation, pollen limitation has been reported to be responsible for stable mixed mating systems with high selfing rates (Lloyd, 1992; Porcher and Lande, 2005), as was observed with *V. gigantea*. Models of mating system evolution that combine functional and genetic components have demonstrated that intermediate selfing is optimal for achieving the greatest possible fertility, implying that partial selfing may be adaptive and evolutionarily stable (Johnston et al., 2009). However, our results showed a slight positive correlation between outcrossing rates and fruit set in *V. gigantea* (Figure 2), indicating that outcrossing rates still have a significant influence on the fertility of this species, in which we observed lower fitness (germination rate) when high selfing rates occurred (Taim population, Tables 3, 4).

Fragmented habitats have a profound effect on the gene flow, mating patterns, and fertility of natural plant populations (Keller and Waller, 2002; Lienert, 2004; Aguilar et al., 2008). Previous molecular studies have indicated that *V. gigantea* populations at the margin of the species distribution range exhibit increased genetic drift due to reduced gene flow ($N_{em} = 0.688$) among populations, and present smaller effective population sizes (N_e) when compared to central populations located in the continuous forest (Palma-Silva et al., 2009). Additionally, we observed spatial inter-population variation in the outcrossing rates of *V. gigantea*, from low to moderate (south $0.107 < t_m < 0.445$ north; Table 4 and Figure 3), and high levels of pollen pool structure (north $0.319 < \Phi_{FT} < 0.598$ south; Table 5 and Figure 3). These results corroborate the low levels of gene flow among populations from the southern range margin reported by Palma-Silva et al. (2009).

The fertility parameters revealed that, even with low gene flow, low effective population size (Palma-Silva et al., 2009), moderate inbreeding depression (Sampaio et al., 2012), strong pollen pool structure, and low effective number of pollen donors, all populations had high fitness levels (with high levels of fruit set and viable seed production). These results suggest that the observed among-population patterns of fertility, mating systems, and gene flow in *V. gigantea* may be attributable to adaptation for selfing, likely due to edge effects (Baker, 1955; Sun and Ritland, 1998; Pannell et al., 2015) during range

expansion (during post-glacial migration; Palma-Silva et al., 2009; Moeller et al., 2017). However, other adaptive or non-adaptive casual factors cannot be excluded, such as pollinator behaviour or local densities of flowering plants.

CONCLUSION

By examining the spatiotemporal patterns of fertility, mating system, and gene flow parameters for all populations and years, we showed that *V. gigantea* presented a combination of high fertility, high selfing rate, and low gene flow among populations with a geographic distance higher than the average distance of pollination, which also resulted in a strong pollen pool structure. The Atlantic Forest is experiencing rapid ongoing fragmentation, and bottlenecks and low gene flow are expected to contribute to increasing genetic differentiation among populations in addition to high selfing rates. Considering the observed spatiotemporal variation, *V. gigantea* populations from the range edge, such as, the Taim population, should be granted conservation priority, since most of *V. gigantea* individuals occurs outside the conservation unit, and are threatened by habitat loss due to agriculture and livestock actions. Conservation strategies that protect populations at the range edge of the species may contribute to the continued survival and maintenance of the evolutionary potential and local adaptation of this species.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

GP and FB designed the study. GP, CP-S, CZ, MG, and MB collected the material, performed the experiments, and analyzed the data. GP, CL, and FB wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Council of Technological and Scientific Development—CNPq; Pró-Reitoria de Pesquisa da Universidade Federal do Rio Grande do Sul—PROPESQ/UFGRS; Programa de Pós-Graduação em Genética e Biologia Molecular—PPGBM/UFGRS; Kew Latin-American Research Fellowship Program—KLARE, from Andrew W. Mellon Foundation; and the International Foundation of Science—IFS. FB, CP, and GP were granted by a CNPq fellowship (numbers 309517/2015-0, 305398/2019-9, and 301596/2015-8, respectively). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

ACKNOWLEDGMENTS

We thank Thelma Barbará, Eliane Kaltchuk-Santos, Tânia Wendt, Maurício Sedrez dos Reis, and Tatiana Souza-Chies for helpful discussions. We also thank Adriele Guerra, Guilherme Chazan, Jamilla Alves Trindade Sampaio, Lucas Caetano Tieppo da Silveira, and Manuela Bruxel for help with sample collection, fieldwork, and DNA extractions. We

also thank the Itapuã State Park and Taim Ecological Station for logistical support, especially to Mr. Jairo Soares for the fieldwork support. We also thank IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) and SEMA (Secretaria do Meio Ambiente e Infraestrutura, Rio Grande do Sul state) for the processing of collection permits. We also thank Editage (www.editage.com) for English language editing.

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