



Hybridization and geographic distribution shapes the spatial genetic structure of two co-occurring orchid species

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Abstract

Multiple ecological and life-history traits shape the fine-scale spatial genetic structure (FSGS) of a given population. The occurrence in core versus peripheral populations, levels of outcrossing, pollen and seed dispersal, and hybridization are important biological properties that influence the kinship of individuals within populations. We examined spatial genetic structure within 15 populations of *Epidendrum fulgens* and *E. puniceoluteum* distributed along a linear gradient of Brazilian coastal vegetation, including both allopatric and sympatric populations where the two orchid species hybridize. We analyzed 581 mapped specimens using nine simple sequence repeat loci, aiming to investigate how geographic distribution and hybridization shape within-population FSGS. A significant increase in FSGS was found towards peripheral populations, compared to core populations. Analysis of short-distance and long-distance components of FSGS identified biparental inbreeding and higher levels of FSGS at peripheral populations, when compared to core populations. In contrast, the relatively high density of reproductive adults in core populations potentially leads to highly overlapping seed and pollen movement, decreasing FSGS. Hybridization was an important factor shaping within-population spatial genetic structure at sympatric sites, decreasing the FSGS observed in parental species. Our results indicate that different ecological forces act in concert to create a gradient of FSGS along species distribution ranges, shaped by extensive levels of intraspecific and interspecific gene exchange.

Introduction

In natural plant populations, fine-scale spatial genetic structure (FSGS) is shaped by multiple ecological and life-history traits that influence the patterns of seed and pollen dispersal (reviewed in Vekemans and Hardy 2004). Higher levels of FSGS have been reported for selfing and clonal species, and in low-density populations (Vekemans and

Hardy 2004; Volis et al. 2010; Binks et al. 2015). However, typical life-history traits related to dispersal mechanisms, such as pollen and seeds dispersed by wind or animals (Petit et al. 2005), can show low power to predict the magnitude of FSGS (Vekemans and Hardy 2004), suggesting that additional biotic and abiotic interactions within populations may act in concert restricting gene exchange over small spatial scales. For instance, different life-history stages, such as seedlings versus adult plants, may increase (Jacquemyn et al. 2006) or decrease (Berens et al. 2014) FSGS due to the interaction of local selection and the historical context of populations. Variation in local kinship patterns was also found among successive stages of population succession, comprising expansion, maturation, and senescence (Chung et al. 2007). When multiple populations are sampled, different ecological and evolutionary processes are revealed, clarifying why patterns of FSGS change along species distribution ranges. Variation in mating systems (Barbará et al. 2008; Meeus et al. 2013) and different edaphic and climatic conditions (Kay et al. 2018; Mosca et al. 2018) may translate into substantial differences in FSGS.

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One way to investigate the origin of contrasting FSGS consists of comparing patterns among populations occurring in different ecological contexts, such as comparisons between core and peripheral populations. Several empirical studies have shown that genetic diversity decreases towards species range limits (Eckert et al. 2008; Guo 2012), due to a decrease in gene exchange and increased levels of drift. Lower population densities and increased levels of inbreeding are also expected in marginal populations, when compared to core populations. Consequently, the population structure of geographic range margins is a key issue for understanding how range limits are established due to the occurrence of potential local adaptation and demographic changes (Sagarin and Gaines 2002; Eckert et al. 2008). Species showing linear distributions over larger latitudinal gradients provide excellent opportunities to compare core versus marginal populations, because range limits are imposed by different ecological factors in opposite margins of the geographical distribution. Indeed, species distributed along coastal vegetation communities have revealed contrasting demographic and genetic patterns at their southern and northern range limits (Herlihy and Eckert 2005; Darling et al. 2008; Samis and Eckert 2009; Pinheiro et al. 2011; Meireles and Manos 2018). Moreover, FSGS studies have detected demographic changes at core, northern, and southern ranges of species distributed along linear latitudinal gradients (Gapare et al. 2005; Pandey and Rajora 2012; Torroba-Balmori et al. 2017).

Patterns of genetic diversity and demography may change due to the presence of hybrid zones along species distribution ranges (Cannon and Lerchau 2015). Species ranges can be limited by hybrid zones because interspecific gene exchange may produce negative gene interactions, decreasing fitness and densities of parental species and hybrids at contact sites (Bridle and Wines 2007). Conversely, hybridization may facilitate species range expansion, including the colonization of new habitats (Lewontin and Birch 1966; Pfennig et al. 2016). The introgression of adaptive alleles may increase the adaptive potential of parental species, which can expand their geographic range, increasing genetic diversity and effective population sizes, and decreasing extinction risks (Cannon and Lerchau 2015; Pfennig et al. 2016). In this situation, hybrids act as bridges for gene exchange between parental species, and overall levels of FSGS within sympatric populations, including parental species and hybrids, are expected. When parental taxa occur in sympatry but in segregated habitats, introgression will expand the spatial limits of heterospecific alleles in the recipient species, decreasing the FSGS observed in hybrid zones (Valbuena-Carabaña et al. 2007). Unfortunately, few studies have attempted to study hybrid zones using an FSGS approach (Cornman et al. 2004; Van Loo et al. 2008; Field et al. 2011).

To test the hypothesis that geographical location influences the genetic relatedness of individuals within the population, we compared the extent of FSGS in core and peripheral populations of the abundant and widespread terrestrial orchid *Epidendrum fulgens*. Previous research has shown a significant decrease in genetic diversity and the occurrence of bottlenecks at marginal populations of *E. fulgens* (Pinheiro et al. 2011). In the central portion of its distribution, *E. fulgens* co-occurs with *E. puniceoluteum*, where hybridization and introgression has been reported (Pinheiro et al. 2010; Moraes et al. 2013). At sympatric sites, each parental species occurs in different but adjacent vegetation types. Hybrids do not exhibit such ecological preferences and are found growing together with both parental species (Pinheiro et al. 2010). These findings suggest that hybrids may change the overall levels of FSGS at sympatric populations. Therefore, we also tested the hypothesis that hybrids bridge gene exchange between *E. fulgens* and *E. puniceoluteum*, spatially connecting both species despite their occurrence in different habitats. Our hypothesis will allow us to address the following questions: (1) Do populations of these species exhibit significant FSGS, and if yes, what are the relative roles of pollen versus seed dispersal, and biparental inbreeding or selfing rates? (2) To what extent do peripheral and core populations differ in their FSGS? (3) What can the genetic patterns tell us about the role of hybrids in connecting parental species on a spatial scale? We use our data to obtain insights into the microevolutionary patterns and processes in two closely related Neotropical orchid species. We expect higher levels of inbreeding and FSGS in the marginal populations of *E. fulgens*, and a decrease in the FSGS in hybrid zones due to the hybridization and introgression between *E. fulgens* and *E. puniceoluteum*. We highlight the need to study historical gene dispersal patterns in different species and populations with varying geographic distributions and levels of reproductive isolation, contributing to the debate on the value of peripheral populations and hybridization in species evolution.

Materials and methods

Study species

E. fulgens and *E. puniceoluteum* are two species inhabiting coastal sand vegetation distributed along the Brazilian Atlantic Forest and Pampas vegetation domains (Supplementary Fig. 1; Pinheiro et al. 2010; Moraes et al. 2013). The two species are clearly distinguishable based on phylogenetic, morphometric, and cytogenetic data (Pessoa et al. 2012). According to the most recent phylogenetic analysis of *Epidendrum* (Cardoso-Gustavson et al. 2018), *E. fulgens*

and *E. puniceoluteum* are closely related but not sister species, belonging to the Atlantic Clade within subgenus *Amphylottium*. Both species are self-compatible and pollinated by several butterfly species. Due to the absence of nectar reward in flowers from both species (Cardoso-Gustavson et al. 2018), pollinators potentially avoid plants in the same patch, decreasing the levels of inbreeding and genetic differentiation among populations (Pinheiro et al. 2010, 2011). Hybrids between these two species (Supplementary Fig. 1) were detected in several sympatric populations, with patterns of asymmetric introgression towards *E. puniceoluteum* (Pinheiro et al. 2010). High levels of seed sterility are observed in crosses between hybrids, but seed fertility increases when hybrids backcross with parental species (Pinheiro et al. 2010). Despite their co-occurrence, *E. fulgens* and *E. puniceoluteum* show different ecological preferences. Populations of *E. fulgens* occur in shrubby sand dune vegetation not subject to flooding. By contrast, *E. puniceoluteum* occurs in the interdune vegetation zone, composed of sedge swamp communities that are flooded during the rainy season (November–April), and located in depressions between successive beach ridges (Supplementary Fig. 1 and Fig. 1b). Within sympatric populations, hybrids show no preference for any particular vegetation, occurring in flooded and non-flooded sites (Pinheiro et al. 2010). While *E. puniceoluteum* occurs in the same vegetation throughout its geographic distribution, *E. fulgens* is more abundant and widespread, also occurring in rock outcrops and at comparatively higher elevations in its southern distribution (Fig. 1). For this reason, only *E. fulgens* was used to test for the occurrence of different FSGS patterns in peripheral versus core populations. To explore how hybridization influences the FSGS of parental species, we used allopatric and sympatric populations of *E. fulgens* and *E. puniceoluteum*.

Study sites and sampling

During the summers of 2008 and 2009, we sampled 15 sympatric and allopatric populations of *E. fulgens* and *E. puniceoluteum* along an 1100-km latitudinal transect from the southern range limit in Rio Grande do Sul, Brazil (−30.90°S), to the northern range limit in São Paulo, Brazil (−44.95°S; Fig. 1; see Table 1). Range limits were determined from intensive geographic surveys of site occupancy and analysis of location data from herbarium specimens collected over the last 100 years (Pinheiro et al. 2011). Sympatric and allopatric populations of *E. fulgens* and *E. puniceoluteum* were collected along the distribution ranges of both species (Table 1, Fig. 1). At sympatric populations, samples of both species and hybrids were collected, except for population Florianópolis, where only *E. fulgens* and hybrids were found. Individuals were collected randomly

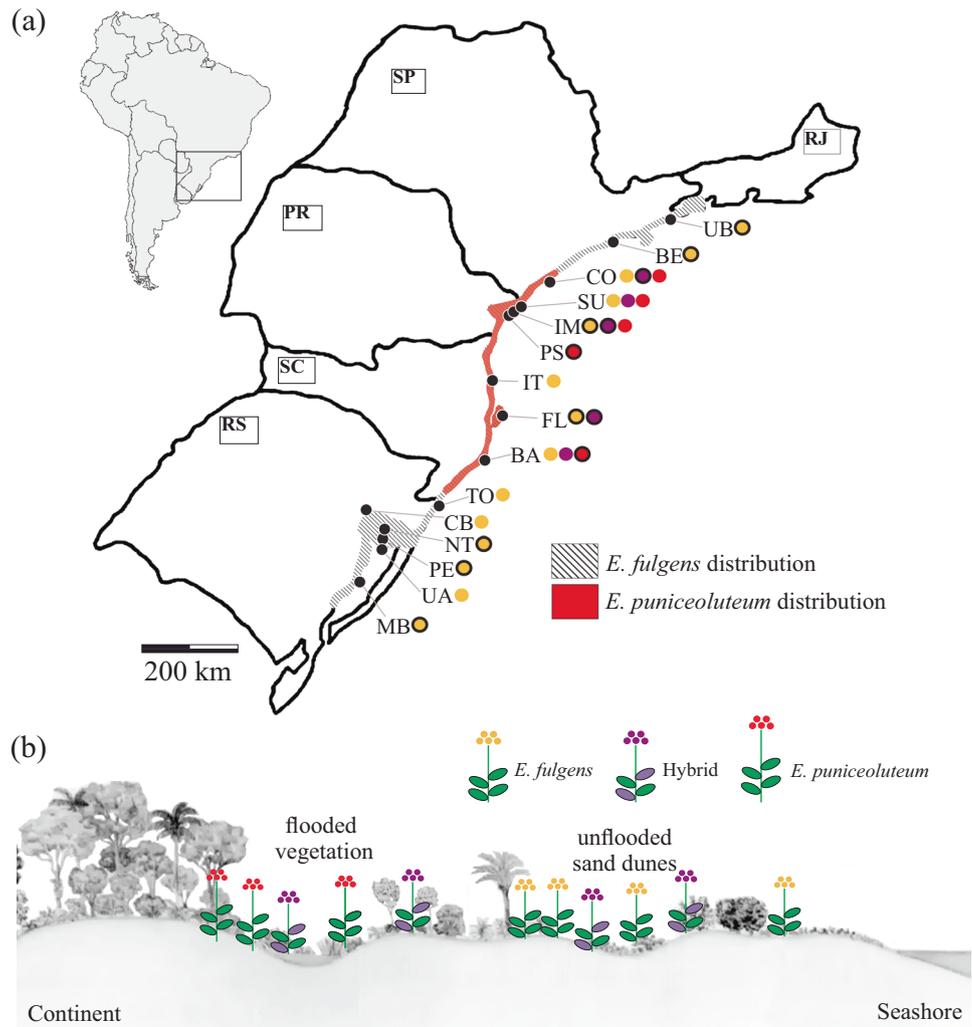
with a minimum sampling distance of 10 m. Clonal plants (individuals growing with many stems close to each other) were avoided during sampling, thus allowing us to focus the present study on the effects of hybridization in the FSGS of parental species and hybrids. Samples were previously genotyped by Pinheiro et al. (2010, 2011) and here were used to test for the first time the effects of latitudinal gradient and hybridization on the FSGS of *E. fulgens* and *E. puniceoluteum*. Genetic data is based on nine nuclear simple sequence repeat loci (eff26, eff29, eff43, eff45, eff61, eff70, epp10, epp18, epp86; Pinheiro et al. 2008a, 2008b) genotyped by Pinheiro et al. (2010, 2011). We included all populations for which detailed GPS-based geographic coordinates were available for each plant (1–2 accuracy), thus facilitating FSGS analysis. Sample sizes, names, and geographical coordinates of each population sampled are given in Table 1. For each individual plant collected, fresh leaves were dried in silica gel and stored at room temperature until laboratory analysis. In total, 581 individuals from 15 populations were analyzed.

Data analysis

We analyzed the FSGS of the following groups of individuals from each sample site: (i) pure *E. fulgens* from all populations; (ii) pure *E. puniceoluteum* from all populations; (iii) sympatric *E. fulgens* and *E. puniceoluteum*; (iv) sympatric *E. fulgens*, *E. puniceoluteum*, and hybrid samples. Parental species and hybrids were classified based on genetic assignment tests using the program STRUCTURE version 2.2 (Pritchard et al. 2000), previously reported by Pinheiro et al. (2010). Assignment values for each individual used in our FSGS analysis were included in Supplementary Table 1.

For the FSGS analysis, we estimated the spatial autocorrelation using the kinship coefficient between pairs of individuals (F_{ij}), using Nason's estimator, as it weighs the allele contribution and is not biased by low-frequency alleles (Loiselle et al. 1995). Average pairwise F_{ij} estimates were plotted against pairwise spatial distances. Distance classes were defined with variable intervals, maximizing the number of pairs of individuals analyzed in each class. For each distance interval, the standard deviation (SD) of the average pairwise F_{ij} estimates was obtained using the jackknife method with 1000 replications of loci, which was also used to calculate the 95% confidence interval of the pairwise spatial autocorrelation for the null hypothesis of no genetic structure ($F_{ij} = 0$). The overall extent of spatial genetic structure in each population was quantified by calculating $S_p = -b \cdot \log/(1 - F_1)$, in which $b \cdot \log$ is the slope of the linear regression between the pairwise kinship and the logarithm of spatial distance between pairs of individuals, and F_1 is the average pairwise kinship between all

Fig. 1 Distribution map of *Epidendrum fulgens* and *E. puniceoluteum* based on field collection and herbarium material, and location of the populations studied. **a** Yellow, red, and purple circles indicate the presence of *E. fulgens*, *E. puniceoluteum*, and hybrids, respectively. Outlined circles indicate significant FSGS. **b** Schematic representation of a transect from the beach to inland, indicating the habitats where *E. fulgens*, *E. puniceoluteum*, and hybrids occur. PR Paraná, RS Rio Grande do Sul, SC Santa Catarina, SP São Paulo states



individuals in the first distance class, which includes all the neighboring pairs (Vekemans and Hardy 2004). The null hypothesis of absence of structure ($b\text{-log} = 0$) was tested using the Mantel test and significance was obtained from 1000 bootstrap replications. All computations were carried out using the SPAGeDi 1.2 program (Hardy and Vekemans 2002).

We investigated short-distance and long-distance limitations of intrapopulation gene flow by analyzing the curvature of the FSGS plots (Heuertz et al. 2003). We obtained the residuals of a linear regression of F_{ij} on the logarithm of distance and fitted it to a third-degree polynomial curve, $y = a + b \ln(x) + c(\ln(x))^2 + d(\ln(x))^3$. The shape of the polynomial regression indicates whether there is a short-distance or long-distance limitation of gene dispersal. This shape is given by $k = 2c + 6d \ln(x)$. If k is larger than zero (concave shape), there is an indication of restricted short-distance gene dispersal, while negative k indicates no restriction at this scale.

We calculated Wright's fixation index (F_{is}) as an inbreeding estimator (Weir and Cockerham 1984) using the *hierfstat* package (Goudet, 2005) in R (R Core Team 2015). We compared f (inbreeding coefficient) with F_{ij} to assess the contribution of selfing and biparental inbreeding to total inbreeding, using a one-sided non-parametric Kolmogorov–Smirnov test in R (R Core Team 2015). Significant differences indicated that inbreeding was mainly due to selfing (Barbará et al. 2008).

The hypothesis that *E. fulgens* populations at range margins show contrasting patterns of FSGS when compared to core populations was tested by assessing the regression between latitudes against f and the S_p statistic. The relationships between latitude and genetic parameters were tested by linear, quadratic, and cubic regression models, and the most likely model was selected based on the highest percentage variance explained ($PVE = R^2$). Analyses were performed using the SPSS 11.0 software (SPSS, Inc., Chicago, IL, USA).

Table 1 Allopatric (A) and sympatric (S) populations of *Epidendrum fulgens*, *E. puniceoluteum*, and respective hybrids sampled for this study, including sample sizes (*N*), population codes, geographical coordinates, and habitat description

Population	Species (sample size)	<i>N</i>	ID	Latitude S	Longitude W	Habitat
Ubatuba (A)	<i>E. fulgens</i>	18	UB	23°22'	44°57'	Sand dune vegetation
Bertioga (A)	<i>E. fulgens</i>	20	BE	23°46'	45°57'	Sand dune vegetation
Ilha Comprida (S)	<i>E. fulgens</i>	18	CO	24°51'	47°42'	Sand dune vegetation
	<i>E. puniceoluteum</i>	26				
	Hybrids	25				
Ilha de Superagui (S)	<i>E. fulgens</i>	18	SU	25°27'	48°13'	Sand dune vegetation
	<i>E. puniceoluteum</i>	10				
	Hybrids	22				
Ilha do Mel (S)	<i>E. fulgens</i>	23	IM	25°31'	48°17'	Sand dune vegetation
	<i>E. puniceoluteum</i>	19				
	Hybrids	25				
Pontal do Sul (A)	<i>E. puniceoluteum</i>	33	PS	25°39'	48°26'	Sand dune vegetation
Itajaí (A)	<i>E. fulgens</i>	20	IT	26°56'	48°37'	Sand dune vegetation
Florianópolis (S)	<i>E. fulgens</i>	53	FL	27°37'	48°27'	Sand dune vegetation
	Hybrids	15				
Imbituba (S)	<i>E. fulgens</i>	45	BA	28°10'	48°41'	Sand dune vegetation
	<i>E. puniceoluteum</i>	9				
	Hybrids	34				
Torres (A)	<i>E. fulgens</i>	25	TO	29°22'	49°45'	Sand dune vegetation
Morro do Cabrito (A)	<i>E. fulgens</i>	22	CB	29°37'	51°39'	Arenitic rock outcrop
Morro Santana (A)	<i>E. fulgens</i>	20	NT	30°03'	51°07'	Granitic rock outcrop
Morro São Pedro (A)	<i>E. fulgens</i>	21	PE	30°11'	51°06'	Granitic rock outcrop
Itapua (A)	<i>E. fulgens</i>	37	UA	30°21'	51°02'	Granitic rock outcrop
Arambaré (A)	<i>E. fulgens</i>	22	MB	30°54'	51°29'	Sand dune vegetation

Populations are indicated as shown on the map in Fig. 1a

Results

Half of the populations analyzed showed significant FSGS (Table 2, Supplementary Fig. 2). For only four populations of pure *E. fulgens*, the kinship coefficient was significantly larger than zero in the first distance class (Supplementary Fig. 2). However, the regression of F_{ij} on the logarithm of distances was significant for these four and other populations of both species and hybrids. Similar Sp values were obtained for *E. fulgens* (−0.0002 to 0.0593, mean: 0.014) and *E. puniceoluteum* (−0.0025 to 0.0610, mean: 0.020). Lower Sp values were observed for hybrids (0.000–0.0149, mean: 0.005). For combined populations of *E. fulgens* and *E. puniceoluteum*, we observed higher F_{ij} than values obtained for pure species populations. F_{ij} for combined populations varied from 0.0138 to 0.2450, with values significantly different from zero for three out of four groups analyzed (Table 2, Fig. 2). We observed Sp values for these groups from 0.007 to 0.119. For groups that included both species and hybrids, we observed lower F_{ij} (0.0020–0.1286) and Sp values (0–0.053), as observed in Table 2 and Fig. 2.

Only *E. fulgens* populations showed f estimates significantly larger than zero (Table 2). The estimated f varied from −0.009 to 0.180 for *E. fulgens* populations, and from

−0.034 to 0.219 for *E. puniceoluteum*. Hybrid populations showed the smaller values for f , from −0.079 to 0.045 and the only f value significantly smaller than zero. Most populations did not show significant differences between f and F_{ij} (Table 2). Only one population of *E. fulgens* showed $f < F_{ij}$, indicating the occurrence of selfing. It was possible to investigate the limitation of intrapopulation gene dispersal in four *E. fulgens* populations, in which $k < 0$ was observed, indicating a limitation of long-distance gene flow (Table 3). For the remaining populations, FSGS was not significant or was not strong enough for robust analysis. For most populations with significant FSGS, it was not possible to fit the residuals of the linear regression of F_{ij} on the logarithm of distance to a polynomial curve. Without the constants of the polynomial equation, it was not possible to calculate k .

The distribution of inbreeding coefficients across the range of the sampled populations of *E. fulgens* was not associated with latitude when considering linear ($P = 0.700$), quadratic ($P = 0.473$), or cubic equations ($P = 0.487$). By contrast, the relationship between Sp and latitude was best described by the cubic model ($R^2 = 0.512$, $P < 0.05$). The model indicated higher values of FSGS at

Table 2 Characterization of the genetic structure in pure and combined populations of *Epidendrum fulgens*, *E. puniceoluteum*, and hybrids, including the mean distance of the first distance class (DC), kinship coefficient (F_{ij}), logarithmic regression slope (b_{log}), S_p statistic for each group, inbreeding (f), and one-sided Kolmogorov-Smirnov test (KS test) to compare f and F_{ij}

Sample site	Species	Number of pairs	Mean distance (m) of 1st DC	F_{ij}	b_{log}^a	S_p	f^b	KS test ^c
Ubatuba	<i>E. fulgens</i>	38	65.5	0.0285	-0.03**	0.030	0.059 (-0.120 to 0.245)	0.333 ^{ns}
	<i>E. fulgens</i>	38	90.6	0.0157	-0.015*	0.016	0.180 (0.095 to 0.378)**	0.444 ^{ns}
	<i>E. fulgens</i>	30	257.0	0.0070	-0.006 ^{ns}	0.006	0.015 (-0.050 to 0.133)	0.333 ^{ns}
Ilha Comprida	Hybrids	30	105.8	0.0083	-0.015**	0.015	0.045 (-0.091 to 0.113)	0.333 ^{ns}
	<i>E. puniceoluteum</i>	27	75.5	-0.0101	-0.007 ^{ns}	0.007	0.128 (-0.068 to 0.123)	0.429 ^{ns}
Ilha Superagui	<i>E. fulgens</i> and <i>E. puniceoluteum</i>	86	71.8	0.1151	-0.042***	0.045		
	All	221	66.7	0.0418	-0.018**	0.019		
	<i>E. fulgens</i>	30	205.0	0.0006	-0.001 ^{ns}	0.001	0.080 (0.006 to 0.173)*	0.444 ^{ns}
	Hybrids	46	226.9	0.0107	-0.005 ^{ns}	0.005	0.000 (-0.212 to 0.074)	0.222 ^{ns}
	<i>E. puniceoluteum</i>	9	63.7	-0.0119	-0.002 ^{ns}	0.002	-0.034 (-0.102 to 0.037)	0.125 ^{ns}
	<i>E. fulgens</i> and <i>E. puniceoluteum</i>	35	45.0	0.1217	-0.022**	0.025		
Ilha do Mel	All	117	49.6	0.0537	-0.009*	0.010		
	<i>E. fulgens</i>	39	85.3	0.0218	-0.006*	0.006	0.064 (-0.018 to 0.179)	0.333 ^{ns}
	Hybrids	42	180.5	0.0227	-0.005*	0.005	-0.079 (-0.161 to -0.033)	0.000 ^{ns}
	<i>E. puniceoluteum</i>	34	206.6	-0.0183	0.003 ^{ns}	-0.003	0.075 (-0.096 to 0.373)	0.286 ^{ns}
Pontal do Sul	<i>E. fulgens</i> and <i>E. puniceoluteum</i>	85	83.4	0.2450	-0.090***	0.119		
	All	252	104.1	0.1286	-0.046***	0.053		
	<i>E. puniceoluteum</i>	75	92.1	0.0091	-0.030***	0.030	-0.014 (-0.154 to 0.138)	0.333 ^{ns}
Itajaí	<i>E. fulgens</i>	38	59.6	-0.0103	-0.014 ^{ns}	0.001	-0.007 (-0.101 to 0.102)	0.444 ^{ns}
	<i>E. fulgens</i>	114	81.6	0.0171	-0.004*	0.004	0.040 (-0.016 to 0.102)	0.333 ^{ns}
Florianópolis	Hybrids	35	112.8	0.0178	0.00008*	0.000	0.027 (-0.183 to 0.150)	0.444 ^{ns}
	<i>E. fulgens</i>	99	120.5	-0.0061	-0.001 ^{ns}	0.001	0.112 (0.039 to 0.166)**	0.889**
	Hybrids	66	68.7	-0.0030	0.002 ^{ns}	-0.002	0.020 (-0.072 to 0.064)	0.333 ^{ns}
Tomres	<i>E. puniceoluteum</i>	9	85.6	0.1125	-0.054*	0.061		
	<i>E. fulgens</i> and <i>E. puniceoluteum</i>	52	36.1	0.0138	-0.007 ^{ns}	0.007	0.219 (-0.256 to 0.657)	0.333 ^{ns}
	All	201	32.8	0.0020	0.000 ^{ns}	0.000		
Morro do Cabrito	<i>E. fulgens</i>	42	59.9	-0.0080	0.000 ^{ns}	0.000	0.058 (-0.045 to 0.121)	0.556 ^{ns}
	<i>E. fulgens</i>	33	15.4	0.0219	-0.001 ^{ns}	0.001	0.102 (-0.019 to 0.218)	0.556 ^{ns}
	<i>E. fulgens</i>	38	19.2	0.0580	-0.008***	0.009	0.102 (0.037 to 0.158)**	0.333 ^{ns}
Morro São Pedro	<i>E. fulgens</i>	42	30.0	0.0391	-0.018***	0.019	0.168 (0.037 to 0.312)**	0.333 ^{ns}
	<i>E. fulgens</i>	66	80.6	0.0009	0.001 ^{ns}	0.059	0.129 (-0.003 to 0.225)	0.556 ^{ns}
Arambaré	<i>E. fulgens</i>	33	26.4	0.0685	-0.025***	0.027	-0.009 (-0.113 to 0.129)	0.111 ^{ns}

^{ns}Not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^aAsterisks indicate significant FSGS

^bSignificant values indicate departures of within-population inbreeding coefficients (f) from the Hardy-Weinberg equilibrium (HWE)

^cConsidering the KS test, significant values that indicate selfing is the main factor responsible for inbreeding; non-significant values indicate the prevalence of biparental inbreeding

Fig. 2 Interspecific autocorrelograms of *Epidendrum fulgens* and *E. puniceoluteum* in four sympatric populations, considering only parental species (triangles and dotted lines) and both species jointly with hybrids (circles and solid lines)

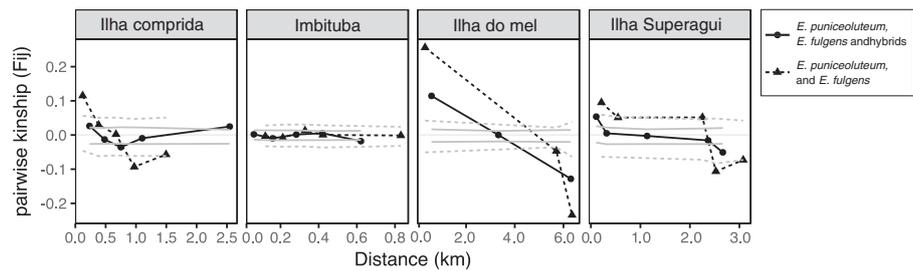


Table 3 Estimates for the limitation of intrapopulation gene flow analysis, where k represents the shape of the polynomial regression, which indicates limitation of gene dispersal; c and d are constants of the polynomial curve used to calculate k

Sample site	Mean distance (m) of 1st DC	k	c	d
Florianópolis	81.6	-0.647	-1.608	0.354
Morro Santana	19.2	-1.495	-0.544	0.197
Morro São Pedro	30.0	-2.357	-3.630	0.811
Arambaré	26.4	-4.300	-7.607	1.874

Negative values of k indicate restrictions of gene exchange over long distances, suggesting low pollen dispersal

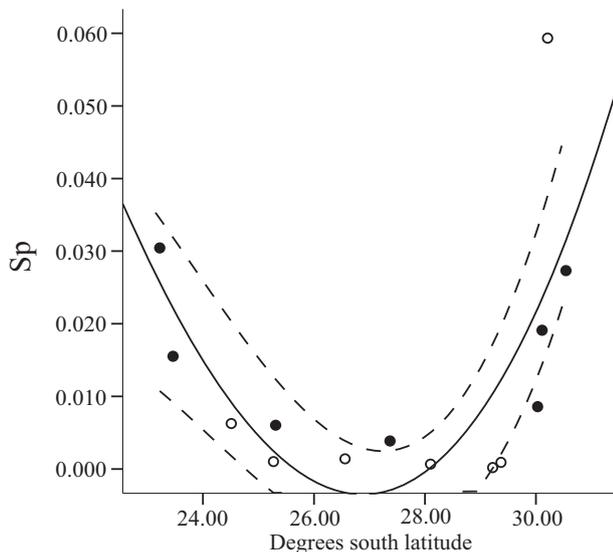


Fig. 3 Geographical trends of FSGS of *Epidendrum fulgens* populations in southern and south-eastern Brazil. Association between latitude and Sp statistic ($R^2 = 0.512$, $P < 0.05$) described by the cubic regression model, showing a significant increase in FSGS towards peripheral populations. Filled circles indicate populations with significant FSGS (see Table 2 for details). Dashed lines represent 95% confidence intervals calculated based on individual predicted y values from the regression line (as indicated in SPSS v.11.0)

peripheral populations, with a tendency to decrease in central populations (Fig. 3).

Discussion

By studying within-population FSGS in two coastal orchid species with distinct ecological preferences, we aimed to improve our understanding of the complex interactions between geography, hybridization, and introgression at local scales. Significant FSGS was found in nearly half of the populations of both species, indicating limited pollen and seed dispersal within populations. Inbreeding levels were very low for most populations, in agreement with the patterns expected for species pollinated by food deception (Scopece et al. 2010). When significant values were found, biparental inbreeding instead of selfing was identified as the main force shaping the kinship within populations. We detected a significant increase in FSGS towards peripheral populations, a pattern observed when adults are less abundant and offspring establishment predominantly occurs near maternal plants (Gapare and Aitken 2005; Chung et al. 2007). At sympatric sites where *E. fulgens* and *E. puniceoluteum* hybridize, the levels of FSGS of both parental species decrease when hybrids are analyzed together, suggesting that introgression shapes the FSGS of hybridizing species at local spatial scales. Our study suggests that both geographic distribution and hybridization are key components shaping the ecology and evolution of species ranges.

Ecological traits and FSGS in *Epidendrum*

The observed mean Sp statistics for populations of *E. fulgens* (0.014) and *E. puniceoluteum* (0.020) are within the range for species with a predominantly outcrossing breeding system (range: 0.0020–0.0393, Vekemans and Hardy 2004). Significant FSGS was detected in seven out of 14 populations of *E. fulgens* and in two out of five populations of *E. puniceoluteum*, suggesting that seed and pollen dispersal within populations are restricted to different degrees along the distribution range of both species (Table 2). In fact, as the species studied here were represented by several populations, our data can be interpreted in terms of variation in pollen and seed dispersal among populations in these coastal plant species. Significant FSGS structure followed by significant inbreeding was detected for only three populations of *E. fulgens* (Tables 2 and 3), and biparental

inbreeding instead of selfing was the main force shaping the restriction of gene exchange in these orchid populations (Table 2). Thus, significant FSGS followed by inbreeding is absent in most populations, suggesting higher levels of pollen dispersal compared to seeds (Barbará et al. 2008; Opedal et al. 2017).

Broad pollen dispersal is one of the most conspicuous traits of food-deceptive orchids, since pollinators avoid visiting plants in the same patch, resulting in pollen dispersal over long distances (Scopece et al. 2010). To date, high levels of gene exchange by pollen and low genetic differentiation have been found among populations of *E. fulgens* (Pinheiro et al. 2011) and *E. puniceoluteum* (Pinheiro et al. 2010). When significant FSGS is not followed by high inbreeding levels, restrictions in seed dispersal may increase the kinship of individuals over small distances. Most studies using orchids as models have detected significant FSGS due to restricted seed dispersal, in agreement with our results (Jacquemyn et al. 2006; Torres et al. 2019). Seed dispersal over long distances has been hypothesized for orchids (Tremblay et al. 2005) but not confirmed by empirical FSGS data. Only occasional long-distance dispersal events by seeds have been detected (Cozzolino et al. 2003). A detailed inspection of the significant FSGS values revealed that most cases were found in southern populations of *E. puniceoluteum*, and were more frequent in marginal populations of *E. fulgens* (Fig. 1, Table 2). Differences in population densities resulting from habitat disturbance, patchy occupancy, and specific cases of low pollen dispersal may account for the increase in FSGS in marginal populations, and this topic is discussed below.

Different patterns of FSGS in central versus marginal populations

Despite the low levels of FSGS found for both *Epidendrum* species, values of *Sp* statistics were variable among populations (Table 2). In fact, a significant latitudinal gradient of *Sp* values was found for the more widespread and abundant species, *E. fulgens*. Higher and significant levels of FSGS were found in marginal populations, when compared to the central distribution of the species range (Figs. 1 and 3, Table 2). The lack of spatial genetic structure in core populations is a logical outcome predicted by the central–marginal hypothesis, which also predicts lower population densities and higher inbreeding at peripheral populations (Eckert et al. 2008). Core populations usually have higher densities with a more continuous distribution of adult plants than peripheral populations, leading to overlapping seed shadows that would limit the development of genetic structure (Hamrick and Trapnell 2011). Vekemans and Hardy (2004) reviewed patterns of FSGS among plant populations differing in adult density, and detected a

significant increase in FSGS in populations with lower densities. Other predictions such as the decrease in genetic diversity and occurrence of bottlenecks at marginal populations were also previously confirmed for *E. fulgens* (Pinheiro et al. 2011). Historical and contemporary processes may shape the increase of FSGS towards peripheral populations (Eckert et al. 2008). More importantly, different ecological and evolutionary processes may affect dispersal and densities at northern and southern range limits (Samis and Eckert 2007, 2009).

Historical range contraction and expansion of tropical forests and the current demographic dynamics of sand dune vegetation communities may contribute to the increase in FSGS of northern *E. fulgens* populations. Sand dune vegetation among northern populations of *E. fulgens* (Ubatuba and Bertioga) is currently constrained by the advance of the Brazilian Atlantic Forest after the Last Glacial Maximum (Behling 2002). This pattern gradually changes towards the south, where sand dune vegetation becomes wider (1–10 km) and more connected (Seeliger 1992). Quaternary sand dunes are found in the central and southern ranges of the *E. fulgens* distribution, and potentially acted as refugia for coastal vegetation communities during climatic oscillation events (Pinheiro et al. 2011, 2015). In addition, current ecological dynamics found in coastal vegetation habitats, such as storms and dune movement, may increase disturbance and species turnover in coastal plant communities (Crawford 2008; Gornish and Miller 2010). This type of habitat disturbance would be intensified in narrow sand dune plant communities found at the northern range of *E. fulgens* (Pinheiro et al. 2011). Dispersal limitations, as observed in marginal *E. fulgens* populations, may destabilize population dynamics at range limits due to a reduction in patchy occupancy after extinction events (Holt et al. 2005). Previous results have shown an increase in FSGS in populations occurring in variable environments (Mosca et al. 2018). According to Chung et al. (2007), a combination of low genetic diversity and non-significant FSGS, as observed in northern *E. fulgens* populations, may indicate demographic decline. In contrast, according to the same authors, low genetic diversity but significant FSGS are signs of population expansion, as observed in southern *E. fulgens* populations. Indeed, phylogeographic studies have tracked shrinkage of northern populations and expansion of southern populations for species distributed in similar geographic ranges (Cardoso et al. 2015; Ramos-Fregonezi et al. 2015; Silva-Arias et al. 2017).

The restrictions of habitat size and availability observed for northern populations of *E. fulgens* are not observed at southern range limits. Sand dune plant communities are much wider and extend several kilometers inland (Seeliger 1992). Contrary to the patterns observed for most

populations, restrictions of pollen dispersal were confirmed in three *E. fulgens* populations at the southern range limit (Fig. 1a, Table 3), suggesting pollen exchange among neighbor plants. A number of ecological processes may explain restrictions of pollen dispersal in different plant populations, such as degree of habitat fragmentation (Opedal et al. 2017), changes in the fauna of pollinators, or changes in pollinators' behavior (see review by Barrett 2010; Wright et al. 2013). Several factors may thus act simultaneously to shape higher levels of FSGS in southern populations of *E. fulgens*. A change in butterfly behavior at southern populations of *E. fulgens* seems less probable because pollinator flight distances tend to increase at sites with lower plant densities (e.g., Levin and Kerster 1969; Fenster 1991). Studies have shown that different pollinator species visit *E. fulgens* at the Torres (Fuhro et al. 2010) and Ubatuba populations (Pansarin and Amaral 2008); distinct species showing shorter flight distances may thus occur at southern populations. Local pollinator assemblages may change patterns of FSGS across different plant populations (Meeus et al. 2013). Despite the restrictions of pollen dispersal, significant biparental inbreeding was detected for populations at Morro Santana and Morro São Pedro (Table 2), suggesting that pollination by deceit is an efficient strategy in avoiding self-pollination, even when plants occur at low densities. Future studies aiming to identify *E. fulgens* pollinators, mainly at southern populations, may clarify the spatial limits of pollen dispersal and differences in pollinator compositions, when compared to northern sites.

Hybridization links spatially and ecologically segregated species

The sympatric sites analyzed here are distributed in the central distribution range of *E. fulgens*, where the ecological and evolutionary forces shaping range margins are not expected to occur (Fig. 1a). In hybrid zones, *E. fulgens* and *E. puniceoluteum* occur in different but adjacent habitats, and hybrids are found intermingled with both parental species (Fig. 1b). The occurrence of introgression at different hybrid zones (Pinheiro et al. 2010) suggests that gene flow between parental species occurs, but the spatial impact of the interspecific gene exchange was never tested. Indeed, few studies have investigated hybrid zones using an FSGS approach (Cornman et al. 2004; Valbuena-Carabanã et al. 2007; Van Loo et al. 2008, Field et al. 2011). The role of clonality in the persistence of hybrids in time and space, and higher levels of FSGS in hybrids than in parental taxa, suggesting interspecific gene exchange and assortative mating, were found for poplar species (Van Loo et al. 2008). In contrast, hybrid persistence in an iris hybrid zone was due to sexual reproduction, also showing very low

levels of inbreeding (Cornman et al. 2004). Using oak species as models, Valbuena-Carabanã et al. (2007) showed hybrids acting as bridges for gene exchange between parental taxa, decreasing the levels of FSGS observed in hybrid zones. Similar patterns were found for three out of four replicate hybrid zones between *E. fulgens* and *E. puniceoluteum* (Fig. 2). Hybrids consistently decrease the levels of total FSGS observed in sympatric populations, suggesting that interspecific gene exchange dilutes the spatial segregation between *E. fulgens* and *E. puniceoluteum*. Our results are in agreement with the findings of Valbuena-Carabanã et al. (2007), suggesting that interspecific gene exchange may impact the spatial structure of populations. Hybridization may reduce FSGS through lowering kinship among parental species, because hybrids do not show specific preferences for different habitats (Fig. 1). At short distances, hybridization and introgression would result in spatial clustering of individuals bearing alleles from different species (Valbuena-Carabanã et al. 2007). More importantly, similar patterns were observed in oak (Valbuena-Carabanã et al. 2007) and orchid species (this study), suggesting that hybrids may change the FSGS of hybrid zones regardless of the life-history traits of parental taxa (short vs. long generation time, wind vs. animal pollen dispersal, wind vs. animal seed dispersal, etc.). Thus, a comparative analysis of hybrids and parental species is of particular relevance to our understanding of the evolution of hybrid zones in a local spatial context.

The observed Sp (0.005) in the hybrids was 2.8 and 4.0 times lower than that for *E. fulgens* and *E. puniceoluteum*, respectively (Table 2), indicating dispersal of pollen and seeds over longer distances when compared to parental species. In addition, low levels of inbreeding were found (mean $f = 0.003$), suggesting that selfing is almost absent among hybrid plants. Indeed, pollen sterility is very strong in hybrids between *E. fulgens* and *E. puniceoluteum*, and viable seeds are formed only when hybrids act as pollen receptors from parental species (Pinheiro et al. 2010). We draw two general conclusions from these results: (1) recombination among hybrids and parental species has occurred over large spatial distances because hybrid plants are less ecologically constrained, and (2) hybrid persistence relies mainly on sexual reproduction involving parental species producing F1 and backcrossed hybrids.

The consequences of hybridization between distinct lineages can result in a variety of outcomes (reviewed by Vallejo-Marín and Hiscock 2016; Goulet et al. 2017); here we explore two potential evolutionary pathways. Hybrids may become a new species when they colonize niches unavailable to parents, decreasing competition and increasing levels of reproductive isolation (Mallet 2007). In such cases, clonal reproduction, inbreeding, and assortative mating (Cornman et al. 2004; Van Loo et al. 2008; Field

et al. 2011) are mechanisms that increase hybrid persistence and may contribute to complete speciation. This possibility seems unlikely here, because in this case, hybrid persistence is still dependent on the parental species, with no evidence of clonal propagation or occurrence in an alternative habitat (Pinheiro et al. 2010). However, hybrid persistence may favor interspecific gene exchange, moving adaptive variation between parental species (Anderson and Stebbins 1954; Vekemans 2010; Cannon and Lerdau 2015). Adaptive gene introgression can modify several traits of the recipient species, increasing adaptation to both abiotic and biotic features of the environment (Vekemans 2010). The exchange of adaptive alleles may decrease the risk of extinction in populations occurring at low frequencies or in often-disturbed environments by increasing their diversity and adaptive potential (Cannon and Lerdau 2015). This scenario may apply for the hybrid zones studied here, where introgression expands the spatial frontiers of alleles from *E. fulgens* and *E. puniceoluteum*. In this case, introgression would increase dispersal and resilience of both species, and decrease their extinction risks in harsh coastal vegetation communities, which are prone to several natural and human-induced disturbances (Scarano et al. 2002). Indeed, high levels of gene exchange between populations and species occurring in different microhabitats have been documented in coastal plant communities (Silva-Arias et al. 2017; Meireles and Manos 2018). This hypothesis deserves future testing using genomic tools to demonstrate that alleles contributing to an adaptive phenotype in recipient species are in fact introgressed, as well as ecological experiments to identify whether modified traits are associated with adaptation to environmental pressures such as drought and salinity (Hoban et al. 2016). Such experiments would provide important information regarding the role of hybrid zones in the origin and maintenance of the high levels of biological diversity observed in tropical regions.

Conclusions and future prospects

This study provides a compelling argument for the importance of pollen and seed dispersal in shaping the FSGS among central versus marginal populations and within hybrid zones. In our study system, significantly higher levels of FSGS were found at peripheral populations when compared to the core distribution of the species range, supporting the perspective that seed and pollen dispersal change across species distribution ranges (Bridle and Vines 2007; Eckert et al. 2008). Differences in pollen movement, habitat availability, and inbreeding were found in northern and southern peripheral populations, suggesting distinct ecological and evolutionary forces acting at both marginal ranges, in agreement with previous studies using coastal

species as models (Samis and Eckert 2007). An opposite pattern was observed in the core distribution of *E. fulgens*, where *E. puniceoluteum* co-occurs in sympatry. Hybridization decreased the total FSGS in three out of four hybrid zones, expanding the spatial limits of interspecific allele recombination. In this context, hybrids act as bridges for gene exchange between parental taxa, which may benefit from the transfer of potential adaptive alleles. Given the frequent natural and human-induced disturbances occurring in coastal plant communities, the introgression of adaptive alleles may decrease extinction risks of populations (Cannon and Lerdau 2015), increasing the diversity and ecological resilience commonly found in these harsh communities (Scarano 2002). Further analysis combining specific genomic tools, such as Rad-seq and transcriptome sequencing will allow identification of genome signatures of selection and differential gene expression in populations distributed in core versus peripheral populations, as well the identification of specific introgressed alleles in recipient species. Integrated ecological experiments such as reciprocal transplants, reproductive biology experiments, and the analysis of traits associated with dispersal potential will cross-validate the mechanisms unveiled using genomic tools. This multidisciplinary approach will facilitate tests of the hypothesis raised by literature reviews (Bridle and Vines 2007; Hargreaves and Eckert 2014), as well as that identified here: (a) that southern and northern range margins are influenced by different biotic and abiotic pressures and (b) that introgressed alleles may have an adaptive value, increasing the resilience and ecological plasticity of hybridizing species. In closing, we suggest that future studies would benefit from including different species and populations, occurring at sympatric and allopatric sites, when attempting to characterize which ecological and evolutionary forces are shaping the patterns of FSGS.

Data archiving

The data sets used for this study, including the SSR genotypes for *E. fulgens*, *E. puniceoluteum*, and hybrids, are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.421dq43>.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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