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ARTICLE

Complex fine-scale spatial genetic structure in *Epidendrum rhopalostele*: an epiphytic orchid

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Abstract

Orchid seeds are presumably dispersed by wind due to their very small size and thus can potentially travel long distances. However, the few related studies indicate that seeds fall close to their mother plants. Because seed dispersal and colonization patterns can have relevant consequences for long-term species persistence, we assessed the fine-scale genetic structure of the epiphytic orchid *Epidendrum rhopalostele* to provide insight into these patterns. All individuals in the studied population were georeferenced and genotyped with AFLP-markers. Genetic structure was evaluated at two levels (forest and tree) using three approaches: principal coordinates analysis, model-based clustering, and spatial autocorrelation analysis. Results showed two genetic groups, composed of individuals from almost every tree with orchids. Spatial autocorrelation analysis at the forest level found no significant genetic structure when all individuals were considered, but a pattern of genetic patches was revealed when the analysis was performed separately for each group. Genetic patches had an estimated diameter of 4 m and were composed of individuals from more than one tree. A weak genetic structure was detected at the tree level at distances less than 1.5 m. These results suggest that many seeds fall close to the mother plant and become established in the same host tree. Additionally, a sequential colonization process seems to be the predominant mode of expansion, whereby progeny from orchids in one tree colonize neighboring trees. Thus, the existence of two distinct genetic groups and the presence of genetic patches should be considered when seed sampling for ex situ conservation.

Introduction

Fine-scale spatial genetic structure (SGS) is the non-random spatial distribution of genotypes within populations, which results from multiple evolutionary and ecological factors such as microenvironmental selection (Zhou and Chen 2010), plant density (Vekemans and Hardy 2004; Zeng et al. 2012), and limited pollen and seed dispersal (Torres et al. 2003) among others. Thus, the study of genetic diversity in a spatial context may provide insight into the underlying processes and population dynamics (Ennos 2001; Epperson 2003), which is essential for formulating conservation measures and selecting management options (Diniz-Filho et al. 2008).

Among the factors that shape SGS, seed dispersal is probably the most widely studied and, for many plant species, it has more influence on local patterns of gene flow and genetic structure than pollen dispersal (e.g., Tero et al. (2005) and Jordano (2010)). Two different scenarios are possible, regardless of whether pollen disperses over long or short distances. If seeds fall around the maternal plant, spatial clustering of adults will result in the development of significant fine-scale genetic structure as described by the isolation-by-distance model. Conversely, if seeds are widely dispersed, seed dispersal will effectively randomize the spatial distribution of genetic variation within populations (Hamrick and Nason 1996).

Because orchid seeds are extremely small and light, they can be dispersed by wind (Arditti and Ghani 2000). Only a very slight upwards air movement is required to lift the

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seeds away from the ground, allowing winds to potentially disperse them at long distances [tens or hundreds of kilometers have even been reported, see Arditti and Ghani (2000) and Collins and Brundrett (2015)]. Under this premise, a random spatial distribution of genotypes within populations is expected in the absence of prevailing winds or spatial heterogeneity. However, SGS research on terrestrial orchid populations has found significant genetic structure at distances less than 2 m (Chung et al. 2005a, 2005b, Jacquemyn et al. 2006, 2009; Helsen et al. 2016), which in most cases has been explained by limited seed dispersal.

For epiphytic orchids, the genetic structuring within populations is more difficult to predict due to the constraints inherent to this type of growth habit. First, differences between vertical and horizontal planes may exist depending on where the plants are growing (i.e., trunks or branches) (Trapnell et al. 2004). Secondly, the height from which seeds are released affects seed dispersal range. Even seeds dispersed from plants at the same height could reach different distances depending on air velocity and forest structure (Cousens et al. 2008). Lastly, another factor is the number and source of founders. If each tree is colonized by one or few founders, SGS would be detected at the tree level, and there would be a low genetic relationship between individuals of different trees. These factors, taken together, highlight a complex structure with at least two hierarchical levels (tree and forest) which may explain the scarce number of SGS studies on epiphytic orchids (Trapnell et al. 2004, 2013) –even though nearly 70% of all orchids are epiphytes (Zotz 2013) and they represent one of the most threatened plant groups in the world (IUCN 2017).

In the present study, we assessed the fine-scale genetic structure of *Epidendrum rhopalostele* Hágsater & Dodson, an epiphytic perennial orchid present in evergreen montane forests, to obtain insight into its seed dispersal pattern and colonization capability. Because sampling an insufficient number of individuals per tree or host trees could provide biased results, all individuals of one population of *E. rhopalostele* located in a forest south of the Ecuadorian Andes were georeferenced and genotyped by AFLPs (Vos et al. 1995). The relationship among individuals was assessed using three different approaches: principal coordinates analysis (PCoA), clustering based on Bayesian algorithm, and spatial autocorrelation analysis. Position within trees (i.e. trunk or branch) and height above ground of each plant was used to interpret the results in both planes. Specifically, we posed the following questions: (1) Is the population genetically structured?; (2) Are the genotypes randomly distributed within and between trees? If the seeds are dispersed mostly within trees, we expect to find SGS at the forest level; and (3) What are the patterns of seed dispersal and colonization? Finally, we discuss the implications of the results from a conservation point of view.

Material and methods

Study species

Epidendrum rhopalostele is a photosynthetic epiphytic perennial orchid native of Ecuador, Peru, and Bolivia that usually occurs in light gaps or along the forest edge in the evergreen montane forest. It is very similar to *Epidendrum dialychilum* Hágsater & Dodson, as they both have the lip free from the column, but it differs from the latter in its linear-lanceolate and acuminate lip, the long filiform acuminate petals and the stigmatic cavity only in the apical third of the column (Hágsater et al. 2001). *Epidendrum rhopalostele* plants are 20–40 cm long and have an inflorescence with 10–30 bright green flowers. Flowering takes place between January and March and June and August, and capsules ripen between the end of August and the beginning of November (personal observation). Reproduction occurs only by seeds of variable size (250 ± 350 µm long and 40 ± 55 µm wide). Other characteristics about its reproductive biology and seed dispersal mechanism have not been studied and are unknown.

Study site and mapping

The study site (~1 ha) was located on the eastern slope of Cordillera Real in the Andes of southern Ecuador on the border of Podocarpus National Park near the Loja-Zamora road in Zamora-Chinchipe province. It is an ~35-year-old regenerated forest classified as an evergreen montane forest (Báez et al. 2013) with more than 70 different genera of vascular plants (Riofrío et al. 2007).

All trees (dead and alive) with a diameter at breast height (DBH) > 1 cm were georeferenced. Their x, y, and z coordinates were obtained using a total station theodolite (Zeiss Elta 6). This device uses an electronic distance measurer (EDM) and an electronic angle scanning to measure distances and angles from the instrument to the points to be surveyed (the base of tree trunks in this case). An integrated microprocessor then calculates the relative coordinates of the target points using trigonometry and triangulation, and the data are recorded in a memory chip. Twelve base points were needed to cover the whole study area.

Each of the mapped trees was examined for the presence of orchids. All *E. rhopalostele* individuals (adults, juveniles, and seedlings) present at the time of the study were marked and georeferenced, including individuals located as close as 1 cm from each other. As the high density of

branches and trees prevented us from visualizing the orchids from the ground, we determined the coordinates of each orchid by laying a plumb line down to the ground and reading the resulting position with the total station. Next, the height at which orchids occurred on their phorophytes was added to the z coordinates measured with the total station.

Sampling for genetic analyses and DNA extraction

We collected young leaf samples of all *E. rhopalostele* individuals (adults, juveniles, and seedlings) present in phorophytes for DNA analysis. Leaf material was dried in silica gel and stored at room temperature until DNA extraction.

Total DNA was extracted from 30 mg of dried leaf material using PureLink Plant Total DNA Purification Kit (Invitrogen, Carlsbad, California, USA). After extraction, DNA concentration was estimated using a fluorospectrometer (NanoDrop 3300, Thermo Scientific, Wilmington, Delaware, USA). DNA samples were stored at −20 °C until use.

AFLP procedure

AFLP reactions were performed following the procedure of Vos et al. (1995) with minor modifications. Genomic DNA (~500 ng) was restricted with 0.1 units of *Mse*I (New England BioLabs, Ipswich, Massachusetts, USA) and 0.5 units of *Eco*RI (Takara Bio Inc., Otsu, Japan) endonucleases, and ligated to MseI and EcoRI adapters with 6 units of T4 DNA-ligase (Takara Bio Inc.). Samples were incubated in a thermocycler for 3 h at 37 °C and 1 h at 17 °C.

Preselective amplification was performed using the primers that complement the MseI and EcoRI adaptors plus one additional nucleotide i.e., MseI + C (5′ GAT GAG TCC TGA GTA AC 3') and EcoRI + A (5′-GAC TGC GTA CCA ATT CA-3′). PCR reactions were conducted in 12.5 µl reaction volume containing 2.5 µl of 10-fold diluted restriction-ligation product, 1× buffer (GeneAmp 10× PCR Buffer II, Applied Biosystems, California, USA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, and 0.5 units of Taq polymerase (AmpliTaq DNA Polymerase, Applied Biosystems). The thermocycler program used for amplifications was as follows: 72 °C for 2 min, then 30 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min, with a final extension at 72 °C for 10 min. The quality of undiluted preselective and restricted/ligation products was tested on 1% (w/v) agarose gels.

Selective amplifications were conducted in a reaction volume of 12.5 µl containing 2.5 µl of 10-fold diluted preselective product, 1× buffer (GeneAmp 10× PCR Buffer II, Applied Biosystems), 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.08 µM of EcoRI fluorescent primer (Applied Biosystems), 0.2 µM of MseI primer (Bonsai Advanced Technologies, Alcobendas, Madrid, Spain), and 0.5 units of Taq polymerase (AmpliTaq Gold DNA Polymerase, Applied Biosystems). PCR conditions were: 95 °C for 2 min, then 13 cycles at 94 °C for 30 s, 65 °C for 1 min (with a decrease gradient of 0.7 °C every cycle), and 72 °C for 2 min; and 24 cycles at 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 2 min, with a final extension at 72 °C for 10 min.

Four primer combinations producing clear bands were used for selective amplification: MseI-CTA/EcoRI-AGA (6FAM), MseI-CAC/EcoRI-AGA(6FAM), MseI-CAA/EcoRI-AAC(VIC), and MseI-CAT/EcoRI-AGG(VIC). The selection was based on a preliminary screening of 32 primer combinations in five individuals (three individuals from different trees and two from the same tree).

AFLP fragments were separated on an ABI 3730 sequencer (Applied Biosystems). A gene scan 500 Liz-labeled sizes standard (Applied Biosystems) was injected with each AFLP sample to allow sizing of the DNA fragments.

To assess reproducibility of the protocol, two independent DNA extractions were carried out for eight samples. The error rate for each locus was calculated as the ratio of the total number of mismatches (band presence vs. band absence) to the total number of replicated individuals (Bonin et al. 2007).

Data analysis

AFLP profiles were imported into GeneMarker v. 4.1 (Softgenetics) and scored manually. All bands between 80 and 500 bp were scored as present (1) or absent (0) excluding those that could not be decisively assigned. Monomorphic and polymorphic fragments were included in the analysis.

Detection of genetic structure

As a preliminary step, a principal coordinates analysis (PCoA) was carried out to provide a visual representation of the genetic distance relationships among the sampled individuals of *E. rhopalostele*. Following Bonin et al. (2007), a simple matching coefficient was used to calculate the distance matrix.

Next, population structure was inferred by applying model-based clustering methods. Unlike distance-based clustering, these methods use a model to identify genetic groups from the full set of genotype data and to probabilistically assign individuals (or a fraction of their genomes) to each group, representing the best fit for the variation patterns found. We used the approach implemented in Structure v. 2.3.4, which involves Bayesian inference and

parameter estimation through Markov Chain Monte Carlo (MCMC) in the modeling process (Pritchard et al. 2000; Falush et al. 2007). For analysis with Structure, we used the admixture model (which assumes individuals may have mixed ancestry) with correlated allele frequencies (Falush et al. 2003), and set the number of groups (K) from 1 to 10. For each value of K, 10 independent runs were carried out with a burn-in period of 10,000 followed by 300,000 MCMC iterations. The optimal number of groups was determined estimating the log-likelihood of the data for each K [ln(P(X|K)] as suggested by Pritchard et al. (2000), and the ΔK statistic proposed by Evanno et al. (2005), which is based on the rate of change in the log-likelihood of data between successive K values. Afterwards, the *Full-Search* algorithm of the software CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) was used to find the optimal alignment of 10 independent replicate cluster analyses and to compute the membership coefficient matrix (Q-matrix).

Once the optimal number of groups was determined, an AMOVA was used to test whether the genetic differentiation (measured by ϕ_{ST}) was significant. The analysis was performed using GenAlEx v. 6.5 (Peakall and Smouse 2012), with $n = 9999$ permutations. The values provided in Q-matrix were used to assign individuals to each group taking $q > 0.5$ as threshold value.

Detection of SGS

SGS was assessed at the forest level (i.e., to evaluate the relation between individuals of the same and different trees in the studied forest) and at the tree level (i.e., at a distance range that focuses on individuals within a tree). In both cases, we used the spatial autocorrelation method proposed by Smouse and Peakall (1999) and implemented in GenAlEx, which simultaneously assesses the signal generated by multiple loci. Briefly, this approach calculates an autocorrelation coefficient (r) between genetic and geographic distances for all pairs of individuals within user specified distance classes. Under isolation-by-distance, geographically close individuals are expected to be more genetically similar to each other than to other individuals occurring at greater distances. Therefore, $r > 0$ values are expected for short-distance lags and $r < 0$ for long-distance lags.

Genetic distances between all pairs of orchids were calculated using the method of Huff et al. (1993). Similarly, Euclidean distances of all pairs of orchids were computed from their x, y, and z coordinates. At the forest level, distance classes were defined at 1 m intervals, each including at least 30 pairs of points as recommended by Waser and Mitchell (1990). We also followed a classical rule of thumb and only considered pairs of points separated by less than half the maximum distance observed (Le Corre et al. 1998), which in our case was 15 m for the forest level. At the tree level, distance classes were defined at 0.25 m intervals following the above-mentioned sampling size considerations. In this case, the distance range covered in the analysis was 2 m, which included over 95% of the distances between individuals of the same tree and was lower than half the maximum distance observed.

Each value of r was tested for significant deviations from the expected value under the null hypothesis of no SGS by a permutation in which all AFLP phenotypes were randomly shuffled among the spatial positions occupied and r-values recalculated each time (up to a maximum of 9999 times) (see Smouse and Peakall (1999) and Smouse et al. (2008) for details). As we are interested in detecting positive autocorrelation in the short-distance classes, one-tailed probability values were calculated. The significance of r was also tested by generating bootstrap 95% confidence intervals around the mean value of r. Bootstrap values were obtained by sampling, with replacement, pairs of comparisons within a given distance class (Peakall et al. 2003). Bootstrap resampling was performed 9999 times and the significance of r inferred when the 95% confidence interval did not contain the zero value. Significance of the entire correlogram was tested using the heterogeneity test proposed by Smouse et al. (2008), considering a significance level of 0.01 (Banks and Peakall 2012).

The spatial autocorrelation analysis was repeated for each group identified by Structure because the individuals of the two genetic pools were located in the same trees. When this occurs, spatially close individuals are not necessarily more genetically similar than individuals which are farther apart. So, the presence of genetic neighborhoods could not be detected in the overall correlogram. The autocorrelation patterns generated for each group were compared (at both the distance class and whole correlogram levels) using the nonparametric heterogeneity test proposed by Smouse et al. (2008).

Finally, we built contour maps to help interprate correlograms at the forest level (Escudero et al. 2003). For this, a principal component analysis (PCA) was performed, and the first extracted axis was used to generate a genetic similarity map. The kriging algorithm implemented in Surfer v.8 was used as a gridding method.

Results

We found *E. rhopalostele* on only 25 of the 714 trees found in the studied forest fragment (Supplementary Figure 1). The number of orchids per phorophyte ranged from 1 to 32, with a total of 239 individuals located mainly on tree trunks.

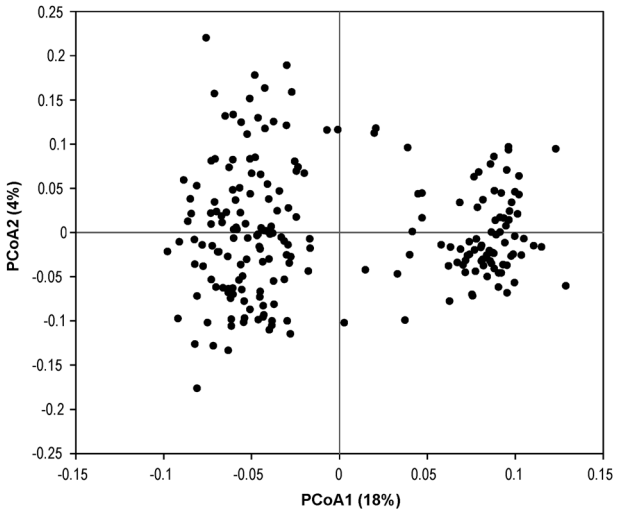


Fig. 1 Bi-dimensional plot of the principal coordinate analysis (PCoA) obtained from four AFLPs combinations in 216 *Epidendrum rhopalostele* individuals

AFLP analysis was successful in 216 of the 239 individuals, and provided a total of 621 bands between 80 and 500 bp, with a 2% average error rate. 100% of the studied loci were polymorphic, and most of them (64%) had a frequency lower than 0.23.

Population genetic structure

The scatter-plot of the first and the second axis of the PCoA showed two distinct groups (Fig. 1). Visualization of results considering the phorophytes on which orchids are located showed no relationship between the two genetic groups and orchid spatial distribution. Nor was any association found when the type of individual (adult or juvenile) was considered. The first two principal coordinates accounted for 22% of total variance (18% for PCoA1 and 4% for PCoA2).

The Bayesian clustering analysis conducted by Structure also identified two genetic groups (hereafter, called group 1 and group 2). As shown in Fig. 2, the first value of lnP(X) at which the curve reaches the plateau (−57513.6) and the highest ΔK value (714.6) were found for $K = 2$, indicating that the genetic structure produced by dividing all genotypes into two groups is the most likely. For $K = 2$, most individuals (152 of 216) had admixture coefficients over 0.8 (Supplementary Figure 2).

The two groups identified by Structure showed a low but statistically significant level of genetic differentiation according to AMOVA results ($\phi_{ST} = 0.076$, $P < 0.001$), and had similar values of genetic diversity estimated as percentage of polymorphic loci, Shannon's information index and expected heterozygosity (Supplementary Table 1).

It is also remarkable that 21 of 25 phorophytes had individuals of both groups (Table 1 and Supplementary Figure 2).

Detection of SGS

When all individuals were analyzed together, no genetic spatial autocorrelation was observed at the forest level ($\omega = 55.8$, $P = 0.014$; Fig. 3a) or at the tree level ($\omega = 30.6$, $P = 0.023$; Fig. 3b). However, when the data set was divided according to the genetic groups assigned by Structure, significant correlograms were obtained for lag-distances of 1 m ($\omega 1 = 149.6$, $P < 0.001$; $\omega 2 = 170.2$, $P < 0.001$) and 0.25 m ($\omega 1 = 102.3$, $P < 0.001$; $\omega 2 = 65.9$, $P < 0.001$) evidencing the presence of genetic structure within groups.

At the forest level, the correlogram for group 1 showed significant positive autocorrelation in the first three distance classes (1 m, $r = 0.063$, $P < 0.001$; 2 m, $r = 0.056$, $P < 0.001$; 3 m, $r = 0.033$, $P = 0.005$) and a first x-intercept of r at 4.1 m (Fig. 4c). Therefore, individuals below this threshold share higher genetic similarity than more spatially distant individuals. In group 2, we also found significant positive r-values for individuals in the first, third, and fourth distance classes (1 m, $r = 0.025$, $P < 0.001$; 3 m, $r = 0.016$, $P = 0.006$; 4 m, $r = 0.029$, $P = 0.005$) and an x-intercept at 4.6 m (Fig. 4c). Another remarkable result is the alternation from positive to negative autocorrelation values in both correlograms, which is characteristic of a pattern of patches. Although correlograms had a similar shape, they were statistically different according to the heterogeneity test ($\omega = 51.3$, $P < 0.001$). In particular, the distance classes 1 ($\omega = 11.1$, $P = 0.001$), 2 ($\omega = 24.2$, $P < 0.001$), and 14 ($\omega = 29.3$, $P < 0.001$) had significantly higher absolute autocorrelation values in group 1 than in group 2.

At the tree level, the correlogram for group 1 showed significant positive autocorrelation in almost all distance classes (0.25 m, $r = 0.098$; 0.50 m, $r = 0.068$; 0.75 m, $r = 0.064$; 1.25 m, $r = 0.079$; 2 m, $r = 0.074$) (Fig. 4d). In group 2, the correlogram also detected significant but weaker positive autocorrelation at very short distances (0.25 m, $r = 0.024$; 0.5 m, $r = 0.019$; 0.75, $r = 0.030$; 1 m, $r = 0.026$) and an x-intercept of r at 1.4 m (Fig. 4d).

Contour maps of the consensus genetic variable showed a pattern of irregular closed loops in both groups (Fig. 4b).

Discussion

This is one of the few studies so far reporting on the analysis of the SGS of an epiphytic orchid at the tree level (i.e., individuals within a tree) and forest level (i.e., individuals of different trees in the same forest). In this population with a total of 239 *E. rhopalostele* individuals located in only 25 of 714 possible phorophytes, we detected a complex genetic structure in which several ecological and evolutionary processes seem to be acting. Some aspects that need to be taken into account to adequately interpret the results are the

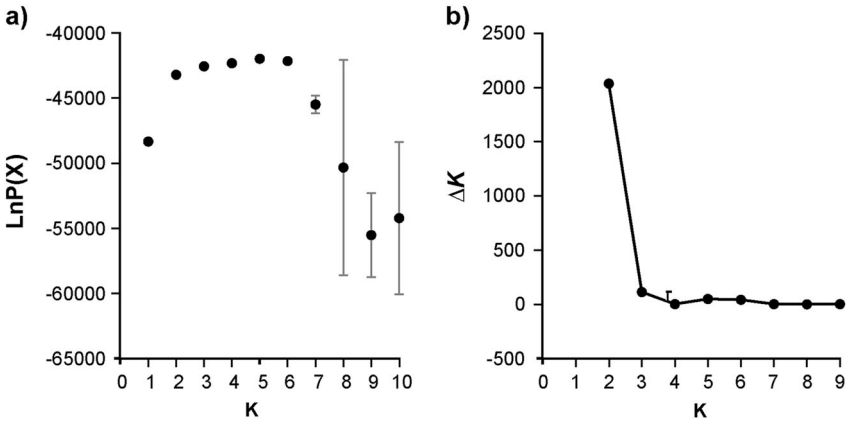


Fig. 2 Optimal number of genetic clusters in the studied *Epidendrum rhopalostele* population according to the admixture model with correlated alleles implemented in Structure. **a** The log-likelihood of the data [lnP(X)] averaged over 10 consecutive Structure runs for $K = 1$ to 10, with error bars representing ± standard deviation. **b** Evanno's ΔK statistic plotted against K

Table 1 Number of *Epidendrum rhopalostele* individuals per phorophyte belonging to group 1 or group 2 according to the admixture model with correlated alleles implemented in Structure

ID phorophyte	Group 1	Group 2	Total
Tree 1	11	18	29
Tree 2	1	2	3
Tree 3	1	2	3
Tree 4	6	11	17
Tree 5	9	11	20
Tree 6	2	5	7
Tree 7	3	5	8
Tree 8	2	2	4
Tree 9	1	1	2
Tree 10	1	0	1
Tree 11	10	9	19
Tree 12	1	5	6
Tree 13	4	6	10
Tree 14	3	6	9
Tree 15	2	2	4
Tree 16	1	3	4
Tree 17	12	20	32
Tree 18	1	3	4
Tree 19	0	3	3
Tree 20	6	4	10
Tree 21	2	6	8
Tree 22	3	4	7
Tree 23	0	1	1
Tree 24	0	2	2
Tree 25	2	1	3
Total	84	132	216

Assignment to groups is based on the admixture coefficient using a threshold value of 0.5

age and structure of the forest, and the patchy and dynamic character of the epiphyte habitat, as they influence genetic structure across time and space.

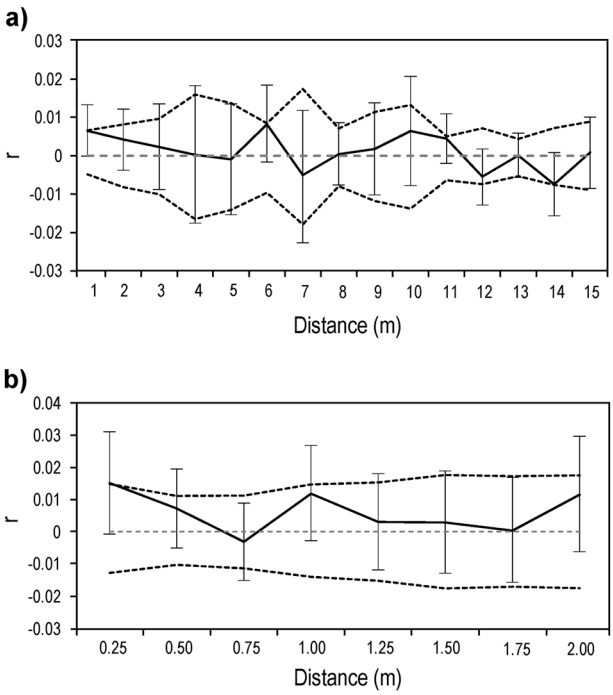
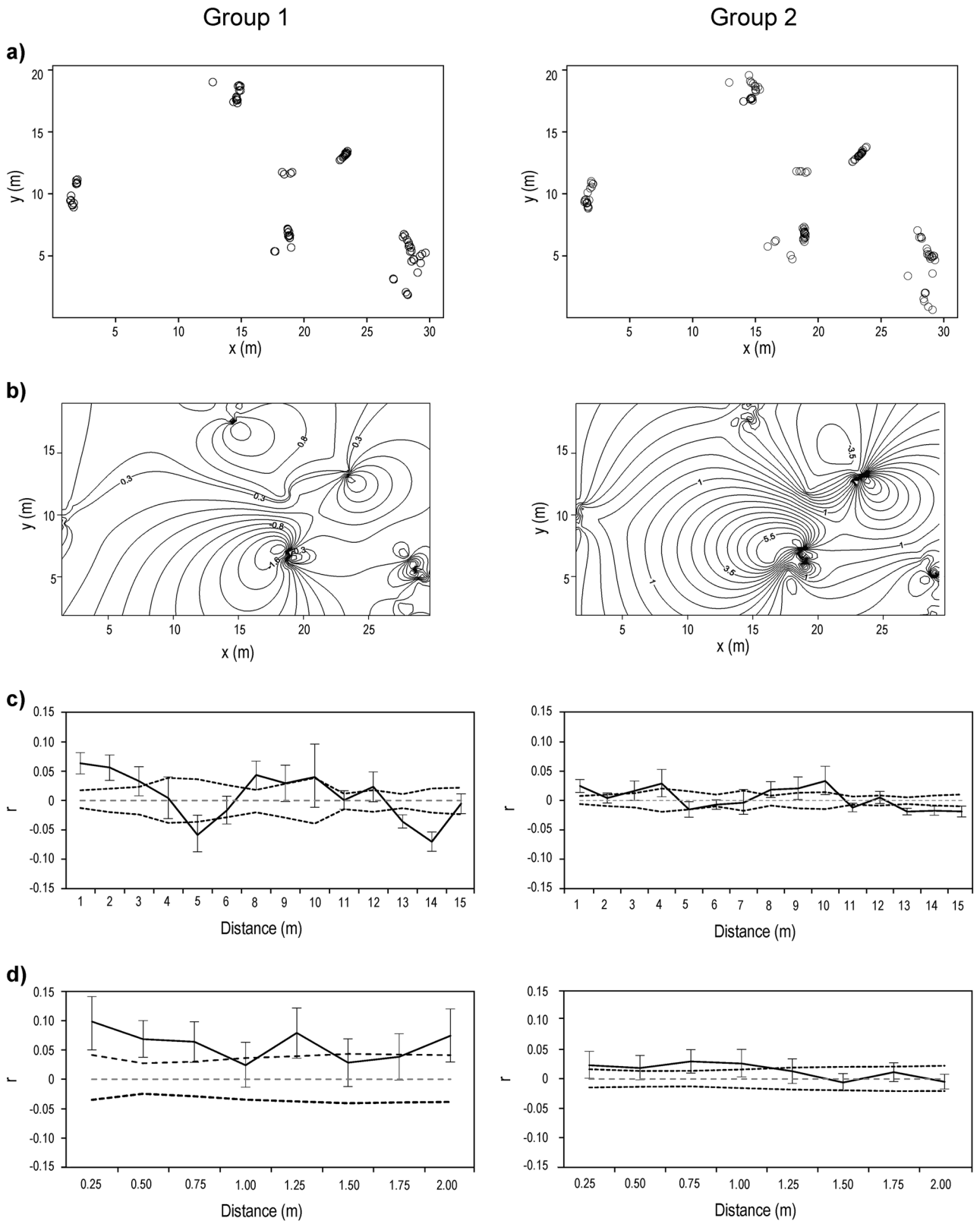


Fig. 3 Spatial genetic structure of 216 *Epidendrum rhopalostele* individuals located on 21 trees at two levels (**a** forest and **b** tree). Autocorrelograms are based on geographic distance classes of equal size: 1 m for forest level, and 0.25 m for tree level. Dashed lines represent the 95% confidence interval about the null hypothesis of $r = 0$ as determined by a permutation test; error bars represent 95% confidence intervals about r derived from bootstrapping

Population genetic structure

Two different genetic groups were found according to PCoA and Structure results. Although genetic structure can arise through spatial factors, for example, as consequence of environmental heterogeneity, examination of the orchid distribution on phorophytes clearly shows that this is not the cause because there were orchids of both groups in 21 of the 25 phorophytes (see Table 1). For this same reason, a scenario based on the "propagule-pool" colonization model



(Slatkin and Wade 1978) could not be considered either. Furthermore, it is very unlikely that founders from two different sources would have established in the same 21 trees considering the high tree-density in the study area and

◀ **Fig. 4** Results of the spatial genetic structure analysis at tree and forest levels for each group identified by Structure according to the admixture model with correlated alleles. **a** Spatial distribution of the *Epidendrum rhopalostele* individuals. **b** Contour maps depicting the isolines and the associated gray scale of the synthetic genetic variable obtained with the first extracted axis of a principal components analysis (PCA). **c** Autocorrelograms at the forest level showing the genetic correlation coefficient (r) as a function of geographical distance. Distance classes range from 0 to 15 with 1 m intervals. **d** Autocorrelograms at the tree level showing the genetic correlation coefficient (r) as a function of geographical distance. Distance classes range from 0 to 2 with 0.25 m intervals. Dashed lines represent the 95% confidence interval about the null hypothesis of $r = 0$ as determined by a permutation test; error bars represent 95% confidence intervals about r derived from bootstrapping. Graphics on the left side correspond to the 84 individuals from group 1, and graphics on the right side to the 132 individuals from group 2

the low phorophyte specificity of *E. rhopalostele* (Riofrío 2015). Neither can these two genetic groups be explained by differences between age-classes because adults, juveniles, and seedlings were found in both groups, suggesting that there is no differential selection between life-cycle stages.

Other possible explanations for the observed genetic structure are the presence of cryptic ecotypes or interspecific hybrids in the population. In some orchids with deceptive pollination, genetic differences have been related to ecotypes that exhibit different floral scents and attract different pollinator species (Menz et al. 2015; Phillips et al. 2015). Further studies to identify the pollinators of *E. rhopalostele* and the composition of floral scent are needed to test this hypothesis. Regarding the possible gene flow between *E. rhopalostele* and other closely related *Epidendrum* species, there is some evidence supporting this scenario: (1) a total of 51 individuals of *E. madsenii* Hágsater & Dodson were found in the study area, and most of them were located in the same trees as *E. rhopalostele* (Lorena Riofrío, Universidad de Loja, personal observation); (2) hybridization events have recently been reported in populations where *E. rhopalostele* and *E. madsenii* coexist (Marques et al. 2014); (3) a high degree of compatibility between these two species has been found, mainly when *E. rhopalostele* acts as an ovule donor (Marques et al. 2014); and (4) F1 hybrids between *E. rhopalostele* and *E. madsenii* are fertile, and in most cases they are morphologically indistinguishable from their maternal parents (Marques et al. 2014). Further studies using other molecular markers are also needed to test this second hypothesis.

Are the genotypes randomly distributed within and among trees?

Different genetic patterns can be detected according to the sign and direction of change in the spatial autocorrelation coefficient. In our study, when all individuals in the forest fragment were considered, a profile of random fluctuation of the autocorrelation coefficient around zero was obtained, suggesting no SGS. However, the significant correlograms obtained for distance classes of 1 m when the two genetic groups were considered separately, shows that genotypes in this population are not randomly distributed. The presence of individuals of both groups in the same trees would explain the lack of SGS when all individuals are analyzed together.

When positive SGS is found, the first x-intercept has often been considered an estimate of genetic patch size (Escudero et al. 2003; Peakall et al. 2003) and, therefore, of the scale at which the pattern occurs. However, some authors recommend interpreting this value with caution because spatial autocorrelation can be affected by the sampling design and the distance class sizes chosen (Vekemans and Hardy 2004). In our case, these limitations are not relevant because all individuals present in the study area were included in the analysis, and the 1-m lag size allowed us to detect genetic structure. Hence, we can assume the presence of a patchy genetic structure with a width of 4.1 m for group 1 and 4.6 m for group 2. Because these values are greater than the maximum distance between individuals of the same tree in 99% of the cases, we infer that the genetic patches include individuals of more than one phorophyte and, therefore, genotypes are not randomly distributed among trees. The shape of spatial-contour maps also supports this interpretation, as in most cases, isolines include orchids of neighboring trees.

At the tree level, inferences about the processes that are occurring need to be made with caution. The correlograms revealed a significant but weak SGS in both groups. This result is indicative of a family structure with genotypes randomly distributed within trees. However, the overlapping of different genetic patterns derived from differences in the demographic structure within each phorophyte or in the colonization pattern would be blurring the overall genetic pattern, leaving a weak signal of SGS.

What are the patterns of seed dispersal and colonization?

The presence of significant positive r-values at distances less than 2 m indicates genetic relatedness (i.e., family structure) between individuals of the same tree, supporting the idea that most *E. rhopalostele* seeds fall (and become established) in the immediate vicinity of their mother plants. This pattern of short-distance seed dispersal agrees with the results for other epiphytic orchids such as *Laelia rubescens* Lindley (Trapnell et al. 2004) and *Brassavola nodosa* (L.) Lindley (Trapnell et al. 2013) in which significant SGS was detected at the tree level.

On the other hand, the detection of genetic patches with a diameter of four meters suggests a sequential colonization process whereby progeny from orchids in one tree colonize neighboring trees. Additionally, the patchy genetic pattern inferred from the correlograms at the forest level supports the idea that *E. rhopalostele* seeds may occasionally travel longer distances. The positive *r*-values at distance classes of 8 and 9 meters could be attributed to the colonization of new trees by sporadic seed dispersal events to greater distances. In this sense, it is interesting to note that the colonization of distant trees must have occurred recently in the population and that the movement of pollinators among tree groups is limited; otherwise overlap of successive generations and gene flow would have increased genetic similarity between plants of different trees blurring this scenario. In any case, colonization events must be rare considering that *E. rhoplalostele* was present in only 3.5% of trees and the presence of suitable phorophytes was not a limiting factor (Riofrío 2015).

Conservation implications

Many epiphytic orchids are endangered by the high level of deforestation in recent decades (Laurance 2013). Under this scenario, knowing the fine-scale SGS is important because it provides useful information in conservation decision-making. In this *E. rhopalostele* population, the observation that seed dispersal occurs mostly within phorophytes increases the risk of local extinction because the fall of a phorophyte can imply a sharp reduction in population size and consequently reduce genetic diversity. In forests where colonization by the orchid is in its initial stages, the often-observed problem of fallen orchids due to the instability of the substrate (flaking bark or breaking branches) could be addressed by relocating the orchids in nearby trees in order to reduce the risk of local extinction and accelerate the process of expansion. Another consideration is the possible presence of hybrids in the population. If introgression is naturally occurring, *E. madsenii* should be taken into account to maintain evolutionary processes (Fitzpatrick et al. 2015). Finally, the seed collecting strategy for ex situ conservation should include both individuals of the same tree (to represent the two genetic groups) and individuals of several trees separated by at least 4 m (to capture intra-group genetic diversity).

Conclusion and future prospects

Analysis of genetic data using different approaches has allowed us to identify several genetic clusters that would otherwise have gone unnoticed. Similarly, spatial information has helped us to detect two genetic groups in this population. The interpretation of results considering both planes has provided insight into colonization processes at the tree level and forest level, which could be useful for the conservation of this species as well as other epiphytic orchids. Nevertheless, an extension of this study to other *E. rhopalostele* populations would be recommended to know how seed dispersal patterns vary among forests with different structures. Studies on population dynamics, seedling establishment, or reproductive biology should also be initiated to support in situ conservation actions.

Data archiving

The data sets used for this study, including the location of each orchid within the study forest and AFLP loci, are available in e-cienciaDatos (https://edatos.consorciomadrono.es/) under **doi:10.21950/SL9WLS**.

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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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