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Alpine species in dynamic insular ecosystems through time: conservation genetics and niche shift estimates of the endemic and vulnerable *Viola cheiranthifolia*

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• **Background and Aims** Alpine oceanic ecosystems are considered amongst the most ephemeral and restricted habitats, with a biota highly vulnerable to climate changes and disturbances. As an example of an alpine insular endemic, the past and future population genetic structure and diversity, and the future distribution of *Viola cheiranthifolia* (Violaceae), endemic to Tenerife (Canary Islands), were estimated. The main goals were to predict distribution changes of this alpine oceanic plant under climate change, and to assist in actions for its conservation.
• **Methods** To perform population genetic analysis, 14 specific microsatellite markers and algorithms which considered the polyploid condition of *V. cheiranthifolia* were employed. The niche modelling approach incorporated temperature gradients, topography and snow cover maps. Models were projected into climate change scenarios to assess the extent of the altitudinal shifts of environmental suitability. Finally, simulations were performed to predict whether the environmental suitability loss will affect the genetic diversity of populations.
• **Key Results** *Viola cheiranthifolia* presents short dispersal capacity, moderate levels of genetic diversity and a clear population genetic structure divided into two main groups (Teide and Las Cañadas Wall), showing signs of recolonization dynamics after volcanic eruptions. Future estimates of the distribution of the study populations also showed that, despite being extremely vulnerable to climate change, the species will not lose all its potential area in the next decades. The simulations to estimate genetic diversity loss show that it is correlated to suitability loss, especially in Las Cañadas Wall.
• **Conclusions** The low dispersal capacity of *V. cheiranthifolia*, coupled with herbivory pressure, mainly from rabbits, will make its adaptation to future climate conditions in this fragile alpine ecosystem difficult. Conservation actions should be focused on herbivore control, population reinforcement and surveillance of niche shifts, especially in Guajara, which represents the oldest isolated population and a genetic reservoir for the species.

Key words: Alpine, Canary Islands, climate change, conservation genetics, genetic diversity loss, microsatellites, niche modelling, oceanic, polyploid, short-distance dispersal, *Viola cheiranthifolia*, volcanism.

INTRODUCTION

Alpine ecosystems located in oceanic islands can be considered among the rarest, most restricted and vulnerable habitats. They host a unique biota, which is especially vulnerable to climate change and disturbance due to their small and isolated distributions (Fernández-Palacios *et al.*, 2014; Harter *et al.*, 2015), leveraged to the extreme in the isolated summits of the highest islands. The ontology of high-altitude islands confers on the biota a complex and challenging biogeographic history. This is related to episodes of colonization, volcanism, subsidence, landslides, topographical complexity and particularities of the impoverished and unbalanced specific diversity of islands (Whittaker and Fernández-Palacios, 2007). Oceanic insular summits therefore constitute islands within islands, in the sense that they are scarcer, smaller and even more isolated than the islands to which they belong. This exacerbated insularity makes them hotspots of endemic biodiversity, which can be as ephemeral as their habitats, subjected to the dynamic geology of islands.

The colonization of newly emerging summits must occur either through colonization of summit-pre-adapted continental species (through long-distance dispersal) (Nogales *et al.*, 2012), as the result of dispersal from other high-altitude island sites (pre-adapted to alpine environments) or as the product of *in situ* diversification from lowland or mid-altitude ancestors that colonized and adapted to the ecological conditions prevailing on island summits (Trigas *et al.*, 2013). For example, Merckx *et al.* (2015) recently investigated the origins of species on a

mountain in tropical Borneo, and found two striking results: first, that most species are relatively young, starting to speciate after, or at the same time as the rise of the mountain they inhabit; and, secondly, that some of the endemics derive from distant immigrants that were pre-adapted to cool environments outside Borneo. Molecular studies also suggest that many of the Canarian endemics have diversified in the islands recently (Jones *et al.*, 2014; Saro *et al.*, 2014), and most originated from the Mediterranean region (Caujapé-Castells, 2011).

Apart from this, climate change is of particular importance among the threats to native and endemic island biota, especially on island summits. The effects of climate change on continental alpine ecosystems have been widely studied (Gottfried *et al.*, 2012; Morueta-Holme *et al.*, 2015). However, little has been addressed in oceanic islands in the same terms (Caujapé-Castells *et al.*, 2010; Courchamp *et al.*, 2014). In this regard, niche modelling predictions show that plant species in oceanic upland ecosystems are prone to decreasing or losing their range in the near future (Halloy and Mark, 2003; Upson *et al.*, 2016). Moreover, if species have a small range, are weakly dispersing and are in previously stable regions, but experience climatic changes, they will be at the greatest risk of extinction from anthropogenic climate change (Sandel *et al.*, 2011; Dullinger *et al.*, 2012). It is therefore of great importance to predict distribution changes in insular mountain species as indicators of the fate of other alpine species. However, to date, studies of the impact of climate change on the distribution of island species are scarce. Indeed, population genetic analysis and species distribution models with simulations for putative heterozygosity loss have rarely been used simultaneously to assess future population dynamics in alpine oceanic ecosystems.

The Teide mountain (3718 m) on the island of Tenerife is part of the complex Canarian archipelago and, due to its high isolation and altitude, may be a paradigmatic example of complex biogeographic history under geological change for predicting niche shifts of plant species. Tenerife is the tenth highest island worldwide and the highest peak in the Atlantic Ocean. It is occupied by an alpine endemic dry habitat and currently presents a rapid increase in temperature at the summit of the island [with an increase of 0.14 ± 0.04 °C per decade and a significant increase of maximum and minimum temperatures and changes in insolation (Sanroma *et al.*, 2010; Martín *et al.*, 2011)]. The Teide–Pico Viejo complex is now surrounded in the south by the vertical walls of the older series of volcanoes that used to form Las Cañadas (3.5–0.2 Ma) (Ancochea *et al.*, 1999). About 200 000 years ago, the Las Cañadas volcano suffered a lateral landslide, with subsequent episodes of massive landslides and eruptions that dramatically changed the morphology, topography, composition and age of the area (Carracedo, 2014). Later, the Teide–Pico Viejo complex was built on top of Las Cañadas caldera, whose construction culminated approx. 30 000 years ago, resulting in a 3500 m high stratovolcano with a large open crater. Finally, in recent geological times (Lavas negras; 1150 BP ± 140), the last eruption of the Teide stratovolcano occurred with the generation also of young peripherical lava domes (Roques Blancos and Montaña Blanca, 1714–2000 BP, respectively) (Carracedo *et al.*, 2007). Therefore, under these circumstances of rapid, violent events, the mountain insular biota must have undergone similarly dramatic biogeographical and genetic changes.

In this study, we focused on *Viola cheiranthifolia* as a model species, with a narrow distribution and short-range dispersal mechanisms, to infer its biogeographical history and to predict the impact of climate change on its genetic structure and diversity. *Viola cheiranthifolia* is a high mountain dwarf chamaephytic plant, endemic to Tenerife. It is the most dominant and structuring species within the summit vegetation of Teide. The largest populations are found around Teide and Pico Viejo stratovolcanoes at altitudes from approx. 2400 m to approx. 3700 m at the peak of Teide, although some small populations occur at the highest points of the caldera (Guajara, 2715 m; Pasajirón, 2531 m). The plant grows in poor soils on cinder flats amongst the volcanic rubble, mixed with pumice stones in some localities. The species is self-compatible, but cross-pollination by insects increases the seed production; it is visited by >20 pollinator species, especially hymenopterans (Seguí *et al.*, 2017). *Viola* species typically present diplochory with explosive ejection of seeds and subsequent myrmecochory (Beattie and Lyons, 1975). However, this double dispersal system by ants has not been observed in *V. cheiranthifolia*, and its seeds present a very small elaiosome. It is considered as Vulnerable (VU D2) in The Red List of Spanish Vascular Flora (Moreno-Saiz, 2008), and is especially threatened by the presence of non-native rabbits in the National Park, greatly reducing its plant fitness and abundance (Seguí *et al.*, 2017).

Viola cheiranthifolia is included within sect. *Melanium*, commonly called Pansies, which are distributed throughout frost-free regions of the world, mainly the Northern Hemisphere (Yockteng *et al.*, 2003). Hybridization and polyploidy are known to have played an important role in the evolutionary history of this clade within the *Viola* genus, with high variation in chromosome numbers and different ploidy levels (Ballard *et al.*, 1999; Marcussen *et al.*, 2010). The sect. *Melanium* has an allopolyploid origin and is derived from two diploid lineages. Indeed, further gene duplications have occurred in *Melanium*, resulting in species with higher ploidy levels (Marcussen *et al.*, 2010). According to Yockteng *et al.* (2003), *V. cheiranthifolia* has an approximate haploid chromosome number of 32, although exact estimations of the chromosome number and the ploidy level have not been determined yet.

Our main objectives were: (1) to infer the current genetic diversity and population structure of *V. cheiranthifolia*; (2) to track the recent demographic history using a Bayesian coalescent approach; (3) to estimate future environmental suitability by projecting species distribution models (SDMs) to different climatic scenarios; and (4) to project future fluctuations in genetic diversity (H_e), as well as setting out their conservation implications.

MATERIALS AND METHODS

Sample collection and genotyping

The distribution of *V. cheiranthifolia* covers extensive areas at the highest altitudes in the Teide National Park, with nine main localities described. A total of 266 individuals covering the whole distribution of the species were sampled during the spring and summer of 2013 and 2014 (Fig. 1D). All specimens were georeferenced when possible, and fresh leaves were collected and stored in silica gel.
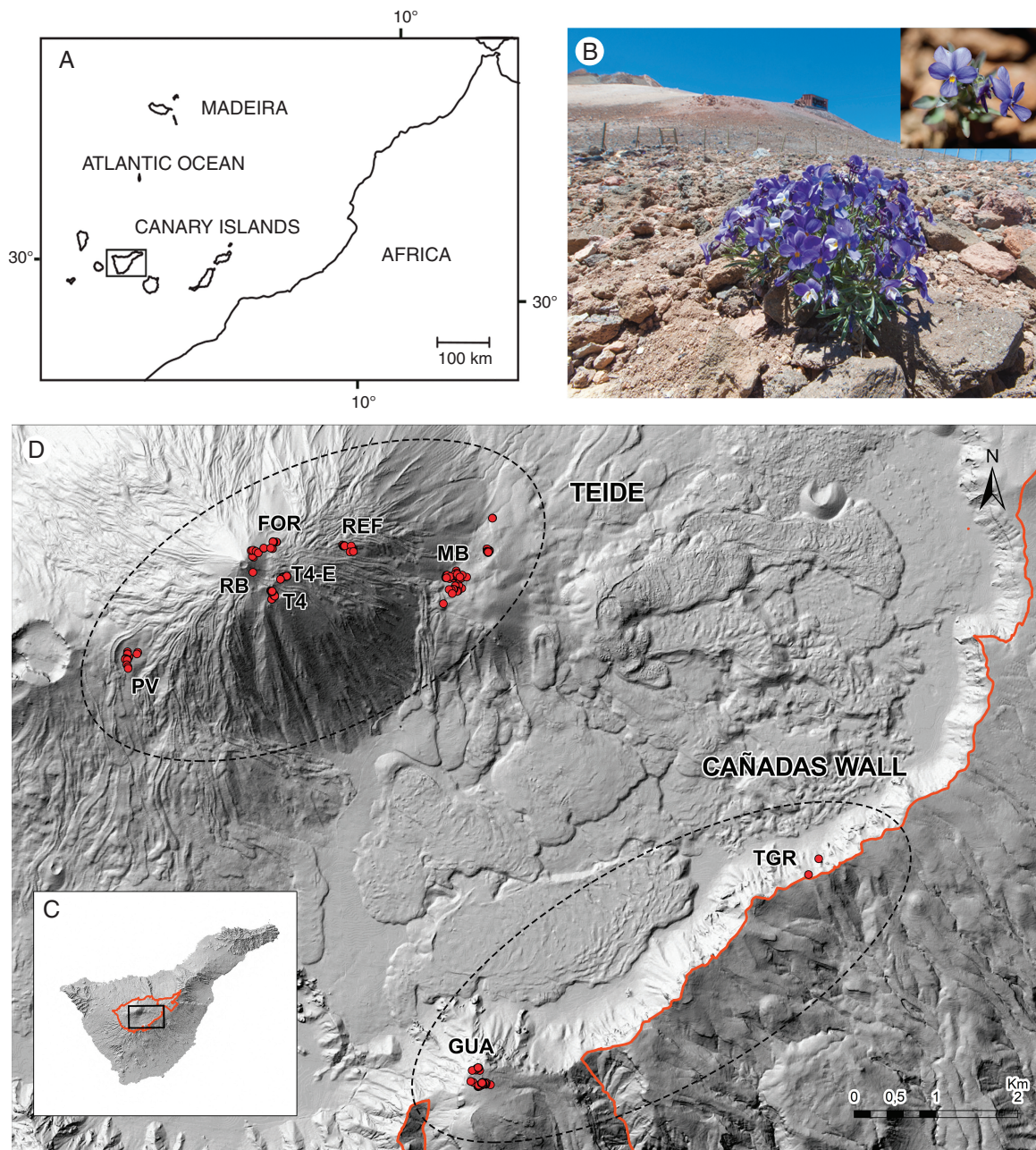
FIG. 1. (A) Geographical situation of the Canarian archipelago. (B) Habitat and aspect of *Viola cheiranthifolia*. (C) Tenerife island with the limits of Teide National Park (orange line) and (D) the *Viola cheiranthifolia* distribution divided in the groups Teide and the Cañadas Wall.

To perform the genetic analysis, 14 polymorphic microsatellite markers were used to genotype all the samples collected. Previously, 16 primer pairs for the amplification of microsatellite markers were newly developed and described for *V. cheiranthifolia* in Rodríguez-Rodríguez *et al.* (2015). Due to difficulties in the interpretation of two of the markers (VIOtri-6 and VIOtri-13), they were discarded.

DNA was extracted and amplified with the protocols implemented in Rodríguez-Rodríguez *et al.* (2015). We identified allele peak profiles at each locus and assigned a genotype to each individual. The polyploid status of *V. cheiranthifolia* was only determined after interpreting the peak profiles in all

the populations. In the aforementioned article, only Montaña Blanca was included for primer testing, and not many individuals presented more than two alleles per locus. Later on, with all the populations analysed, the electrophoretic profiles were consistent with a tetraploid species, since a high number of individuals for most loci presented up to four alleles. The allele copy number (allele dosage) could not be determined in partially heterozygous genotypes from the electrophoretic profiles. Therefore, the alleles were identified as single copies and the incomplete genotypes were exported to Polysat 1.4-1, which allows the data analysis of polyploid data sets with ambiguous allele copy numbers (Clark and Jasieniuk, 2011). The

overall ploidy was set to 4 and the allele dosage information was completed for homozygous (one allele in the electrophoretic profile) and fully heterozygous genotypes (four alleles in the electrophoretic profile). Polysat 1.4-1 was used to generate the input files for the following analysis.

Genetic data analysis

The statistics commonly used in polyploids assume a polysomic inheritance pattern (multiple homologous chromosomes pair between them, in autopolyploids), instead of a disomic inheritance pattern (two homeologous chromosome sets pair independently, in allopolyploids). It has been cited that sect. *Melanium* is allopolyploid (Marcussen *et al.*, 2010), but we did not detect either fixed heterozygosity or allele segregation in pairs of isoloci with the function 'allele.correlations' in Polysat. Furthermore, the distinction between allopolyploid and autotetraploid is not absolute, as there is usually a continuum between polysomic and disomic inheritance which depends on the divergence time between the progenitors (Comai, 2005). Meirmans and van Tienderen (2013) stated that some allele exchange between sub-genomes is enough to homogenize the allele frequencies between the two sub-genomes to an extent that biases associated with strict disomy are removed. Following this hypothesis, we proceeded to analyse our co-dominant data set as autotetraploid with statistics that allow genotypic ambiguity.

Genetic diversity. To examine the genetic diversity of populations of *V. cheiranthifolia*, we computed the average number of alleles (NA), the allelic richness expressed as the expected number of alleles among *k* gene copies [A_R ($k = 11$)] and the expected heterozygosity (gene diversity) corrected for sample size (H_e) (Nei, 1978) in each population using SPAGeDi version 1.5 (Hardy and Vekemans, 2002), a program which computes statistics and permutation tests of relatedness and differentiation among populations of organisms of any ploidy. In addition, selfing rate estimates were also obtained from SPAGeDi with a method suited to polyploids, which is based on phenotypes irrespective of the allele dosage (Hardy, 2015). The mean numbers of private alleles per locus were calculated using Microsoft Excel(R) based on SPAGeDi allelic frequency data.

Population genetic structure. First, an allele size permutation test (Hardy *et al.*, 2003) in the SPaGeDi v.1.5 program (Hardy and Vekemans, 2002) was used to assess if stepwise mutations on microsatellites affected genetic differentiation in our study populations. This test is based on the comparison of observed pairwise R_{ST} values with the distribution of R_{ST} values (pR_{ST}) obtained by 10 000 permutations of allele sizes among allelic states. A significant test (i.e. observed R_{ST} >95 % of the pR_{ST}) indicates that mutations have contributed to genetic differentiation between sub-populations.

Bayesian model-based clustering of microsatellite data was employed using the procedure implemented in STRUCTURE 2.3.3 (Pritchard *et al.*, 2000) to infer genetically distinguishable clusters in the sampled populations. Polysat 1.4-1 was used to generate the input data file for STRUCTURE computations, setting the overall ploidy to 4. The recessive alleles method was implemented to account for allele copy ambiguity in the co-dominant data set (Falush *et al.*, 2007). The program was run ten times from $K = 1$ to $K = 10$ with the burn-in length of 10^5 generations followed by 10^6 Monte Carlo Markov chain (MCMC) iterations, using the admixture model with correlated allele frequencies. The optimal K value for each analysis was estimated by the maximum value of ΔK following the Evanno method (Evanno *et al.*, 2005), implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Following a hierarchical approach, we continued the exploration within clusters at the highest ΔK using the same settings. Alignment of cluster assignments across replicate analyses was then conducted in CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007).

To examine genetic similarities and relationships between individuals, we performed a principal co-ordinates analysis (PCoA) in GenAlex (Peakall and Smouse, 2012). As input for the PcoA, a pairwise distance matrix for all samples was calculated using Bruvo distances (Bruvo *et al.*, 2004) as implemented in Polysat. This measure of genetic distance has been developed for polyploid organisms and takes distances between microsatellite alleles into account without knowledge of the allele copy number (Clark and Jasieniuk, 2011). In addition, a nested analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) was performed with the R packages 'adegenet' 2.0.1 (Jombart, 2008) and 'poppr' 2.3.0 (Kamvar *et al.*, 2014). The hierarchical level used for this analysis followed the clustering results obtained with STRUCTURE and the PCoA. Significance values were estimated over 999 permutations.

Finally, pairwise Rho statistics (Ronfort *et al.*, 1998) for all population pairs were calculated to infer the degree of population differentiation between the localities studied. Rho is an interclass relatedness coefficient permitting comparison between ploidy levels, and it was calculated using SPAGeDi version 1.5 (Hardy and Vekemans, 2002). The Rho statistic has shown to be more appropriate for polyploids, being independent of the ploidy level, the selfing rate and the rate of tetrasomic inheritance (Meirmans and Van Tienderen, 2013).

Fine-scale spatial genetic structure. Spatial genetic structure (SGS) of all the georeferenced individuals was assessed for the resulting Bayesian clusters from the whole database. The locality of Topo de La Grieta was excluded from this test due the low number of georeferenced individuals. Pairwise kinship coefficients (F_{ij}) between individuals and 95 % confidence intervals with jackknifing over loci were estimated at different distance classes every 20 m. After 250 m, the pairwise comparisons were unified into a final distance class. Significant SGS was evaluated by testing the regression slope on the logarithm of distance (b_{log}) with 10 000 randomizations. Estimates of the *Sp* statistics (a measure of SGS strength) were obtained for each group from the slope of the regression of F_{ij} on linear and logarithmic distance (b_{lin} and b_{log}) and the mean pairwise kinship coefficient measured at the first distance class (F_1) with the formula $Sp = -b (1 - F_1)$ following Vekemans and

Hardy (2004). All SGS analyses were perfomed on SPAGeDi version 1.5 (Hardy and Vekemans, 2002).

Inference of demographic history and effective population sizes. The approximate Bayesian computation (ABC) method (Beaumont, 2010) implemented in DIYABC v2.1 (Cornuet *et al.*, 2014) was used to explore the demographic history that may have generated the current genetic structure and to estimate associated demographic parameters. To simplify analysis and to respect the assumption of population isolation made by

DIYABC, populations were grouped according the structure results (Fig. 2A) and the Rho statistic. These groups are Teide (all localities in the Teide–Pico Viejo complex), Guajara and Topo de La Grieta.

As DIYABC does not allow tetraploid input data sets, we simulated a diploid data set with the same method implemented in Lepais *et al.* (2013). The function SimpleFreq in Polysat was used to calculate allele frequencies at each locus for each of the nine sampling sites. The obtained allele frequencies were then used with the function SAMPLE in R to resample alleles and generate diploid multilocus genotypes as input data
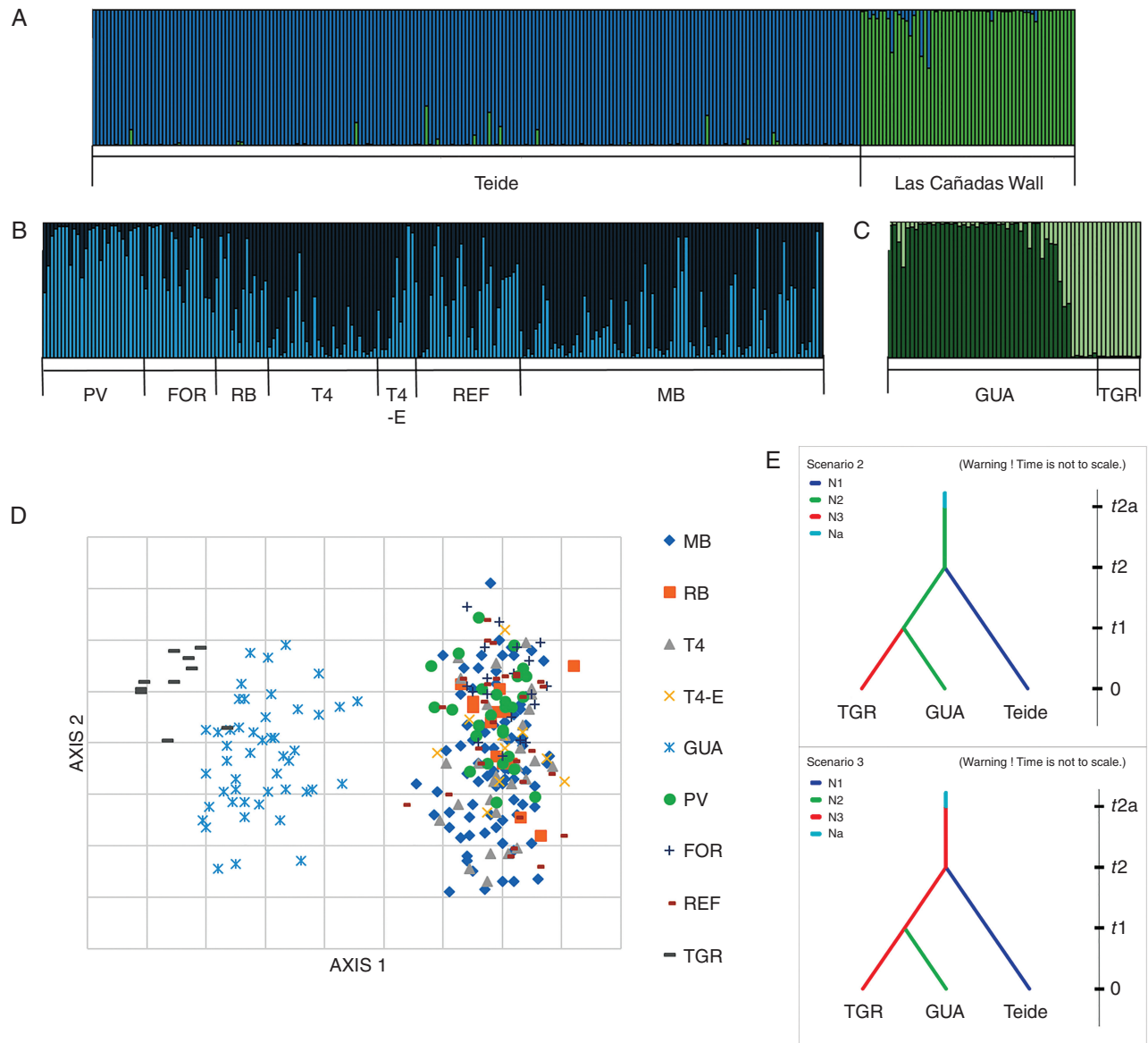

FIG. 2. (A) Bar plot of co-ancestry inferred from Bayesian cluster analysis implemented on STRUCTURE and CLUMPP, with the whole set of *Viola cheiranthifolia* sampled individuals and localities (*K* = 2). (B) Bar plot of the individuals within Teide. (C) Bar plot of the individuals within the Las Cañadas Wall. (D) Principal co-ordinate analysis (PCoA) with Bruvo genetic distances (Bruvo *et al.*, 2004) between individuals. (E) Representation of the most probable demographic scenario (Scenario 2) and the second most probable scenario (Scenario 3) with the ABC method implemented in DIYABC (Cornuet *et al.*, 2014). The populations studied were inferred from the STRUCTURE and pairwise Rho distance results. *t1*, *t2*, *t2a*: time scale of divergence times measured in generations, since the present (*t* = 0). N1, N2, N3 and Na refer to effective population sizes, respectively, of standing populations (Teide, Guajara, and Topo de la Grieta) and from a non-sampled ancestral population. See the tested demographic scenarios in Supplementary Data Fig. S1

for DIYABC. These simulated populations included the same number of individuals originally sampled. Before performing the DIYABC analysis, the diploidized data set was tested in the software STRUCTURE as the species was diploid, to ensure that it maintained the same population genetic structure as the polyploid one.

Six demographic scenarios characterized by divergence times in generations (t, $t1$, $t2$, $t2a$), effective population size of actual populations (N1, N2 and N3 for Teide, Guajara and Topo de La Grieta, respectively) and a putative ancestral population (Na) were compared (Supplementary Data Fig. S1). The scenarios and priors were chosen for computation after initial trials. In the simplest scenario, all three populations diverged simultaneously at time t from a common ancestor. Scenarios 2–5 considered all dichotomous combinations of population divergence, considering Guajara or Topo de La Grieta as the oldest populations that derived from the ancestor (of effective size Na) at time $t2a$. A second population took place at time $t2$ and the later divergence ($t1$) originated a third population. Scenario 6 showed an admixture between Guajara and Topo de La Grieta that originated the Teide population. A detailed methodology of the ABC analysis, such as the priors and settings for choosing the most probable scenario, the estimation of the demographic parameters and the confidence on the parameters and the scenario choice can be seen in Supplementary Data Material S1.

Species distribution modelling

Predictor variables. The high resolution of spatial environmental predictors improves the accuracy and performance of niche modelling procedures. For this purpose, we developed the assembly of predictor maps at the fine scale of 20 m per pixel grid based on the LIDAR Digital Elevation Model (DEM) of the Canarian Archipelago (Instituto Geográfico Nacional, Madrid, Spain), restricted to the island of Tenerife. We derived the topographic variables slope and topographic index from DEM using the 'raster' package (Hijmans and van Etten, 2014) implemented in R software.

We then derived layers for monthly predictors of minimum, average and maximum temperature and total precipitation with the procedure established by González Fernández de Castro (2016). The monthly data on precipitation and temperature from 275 climatic stations in the Tenerife agro-climatic network were used as response variables in a stepwise generalized additive model (GAM) using the following predictor variables: (1) for precipitation: altitude, northness, x and y co-ordinates; and (2) for temperature: altitude, northness, slope, and x and y co-ordinates, selecting models by the Akaike information criterion (AIC). The residuals of the values of meteorological stations were mapped and interpolated by fixed weighting splines in ArcGIS. The resulting 12 maps for monthly temperature variables were used to calculate bioclimatic variables following Hijmans *et al.* (2005). We developed potential evapotranspiration (PET) maps with package r2dRue in R (del Barrio *et al.*, 2013).

Finally, we developed, for the first time, a nival model for Tenerife following Carlson *et al.* (2015), based on snow cover data acquired either by Landsat images or aerial photography. Landsat 7 and Landsat 8 covered eight and five images, respectively.

Future climate variables. For each of the monthly variables of temperature and precipitation, we employed the Delta method technique REF to downscale anomalies to 20 m resolution. Downscaling was used with the downscaleR package in R (Santander Meteorology Group, 2017). We used General Circulation Models MIROC and CSIRO for the emissions scenarios 2.6, 6.0 and 8.5 developed by IPCCC 5 (IPCC Working Group I, 2013), for years 2030, 2050 and 2080.

Species distribution modelling. We used the BIOMOD package (Thuiller *et al.*, 2009) implemented in R software. We selected six algorithms: generalized linear models (GLMs) and GAMs, generalized boosted regression models (GBMs), random forest (RF), multiple adaptive regression splines (MARSs) and artificial neural networks. We performed ten runs for each method, randomly selecting five pseudoabsence locations in each run. Seventy-five per cent of the points in the data were randomly selected for model calibration, and the remaining 25 % were used for model evaluation, based on area under the curve (AUC) and true skill statistics (TSS). Models with AUC and TSS >0.8 were accounted for in the construction of an ensemble model based on the average suitability of each model. The ensemble map of topoclimatic suitability was converted to binary (presence–absence), by choosing the threshold maximizing TSS value. The ensemble model was finally projected to each future climate scenario combination of circulation model, year and emissions pathways.

Genetic diversity loss projections. The migclim package (Engler *et al.*, 2012) was used to project intermediate potential distribution areas for every year, assuming no migration limitations. Then, the suitable area for *V. cheiranthifolia* was divided in two, according to the population structure found by STRUCTURE (see the Results): one area comprised the Las Cañadas Wall population (Guajara and Topo de La Grieta) in the south, and the other area the localities in Teide. The microsatellite matrix was divided in two according to this split. The following simulation was then run: each iteration comprised a 5 year period. The probability of each individual in each population of becoming extinguished was 0 at present and varied according to the variation in the potential area. The migration rate was also used to calculate the probability of each individual migrating to the other population. With these two procedures, the resulting matrices for the populations were used again to compute allele frequencies and then expected heterozygosity as a measure of variation of genetic variation across time. The procedure was run until 2080 and repeated 5000 times for each combination of scenarios.

RESULTS

The scorability of the microsatellites included in this study was high for all the localities. Estimates of genetic diversity for all the sampling sites are presented in Table 1. A total of 126 alleles were found for *V. cheiranthifolia* with an average expected heterozygosity (H_e) of 0.550 and a total of 0.637. In total, the microsatellite loci revealed 9.0 alleles per locus ranging from 4 (loci Di-24 and Tri-1) to 30 (locusTet-8).

The localities showing the highest and lowest values for all diversity measurements were 'GUA' and 'TGR' respectively. Measurements of allelic richness (A_R) and H_e were similar for all

localities, except 'TGR', with a notably low diversity. The mean number of alleles ranged from 2.14 ('TGR') to 6.93 ('GUA') and the average of private alleles from 0.00 ('RB', 'FOR' and 'TGR') to 0.86 ('GUA'). Comparing the Las Cañadas Wall and Teide populations, the former presented higher diversity values in terms of allelic richness and heterozygosity. The results of the test for the selfing rate for every locality were quite similar and showed an autogamy rate of 50 % (Table 1).

The allele permutation test with SPaGeDi suggested that stepwise mutations at microsatellite loci did not significantly contribute to genetic differentiation between localities compared with genetic drift and migration (observed $R_{ST} = 0.181$, permuted $pR_{ST} = 0.105$, $P = 0.073$). Therefore, in our case study, allele identity-based estimators (F_{ST}/Rho) are preferable to R_{ST} for genetic differentiation.

Including the whole set of samples, the Bayesian structure analysis identified two genetic clusters according to ΔK ($K = 2$; Fig. 2A). In concordance with the PCoA, individuals are aggregated within the Teide and Las Cañadas Wall in well-assigned clusters with >80 % of assignation. Following a hierarchical approach, in the analysis carried out only with the Teide localities, the highest ΔK was found for $K = 2$. Pico Viejo showed a higher assignation to one of the clusters together with individuals from other localities, resulting in a high homogenization within Teide. However, it appears that there is a gradient of differentiation between the eastern and western part of Teide (Fig. 2B). In the third analysis, for the Las Cañadas Wall, the highest ΔK was also found for $K = 2$. In this case, there was a clear separation of Topo de La Grieta individuals into one cluster together with six individuals from the northern part of Guajara (Fig. 2C). All results from STRUCTURE HARVESTER for all the analysis are included in Supplementary Data Fig. S2.

The PCoA revealed two well-differentiated groups (Teide and Las Cañadas Wall) (Fig. 2D). The AMOVA indicated that most variation is within localities (83.9 %). The variation between Teide and Las Cañadas Wall (13.4 %) was higher than the variation between the sampled localities (2.7 % and 83.9 %, respectively) (Table 2). Rho statistic pairwise values (Table 3) ranged from 0.015 (MB-T4) to 0.558 (TGR-RB). All values were significant ($P < 0.001$) apart from MB-T4 ($P < 0.05$). The most differentiated population according to the Rho statistic was TGR, with the highest values (from 0.232 to 0.588). This is followed by GUA, with similar values in all the pairwise comparisons (from 0.232 to 0.291). All the localities within Teide showed lower values (from 0.015 to 0.119), with a higher degree of admixture.

The analysis of SGS in the populations analysed showed a negative linear relationship between kinship coefficients and the logarithm of distance (Fig. 3). The *Sp* statistics indicated signs of SGS in both analyses, but with higher values for Guajara than Teide (0.0112 and 0.0063, respectively) (Table 4). Permutation tests showed that regression slopes were significantly different from zero in both cases.

Demographic history and effective population sizes

Among the six tested scenarios, scenario 2 gave the highest posterior probability from the data [$P = 0.669$ (0.583, 0.754)] (Supplementary Data Fig. S3). However, confidence tests revealed moderate error probabilities for type I (P: 0.518) and type II (P: 0.486) calculated with the logistic regression method. This uncertainty could be due to the difficulties in distinguishing between scenarios 2 and 3 (Fig. 2E), as both scenarios

TABLE 1. *Genetic diversity estimates of the* V. cheiranthifolia *populations*

Group	Locality	Acronym	N	NA	PA	A_R	H_o	H_e	Sr
Teide	Pico Viejo	PV	27	5.14	0.14	3.39	0.655	0.556	0.521
	La Fortaleza	FOR	19	4.00	0.00	3.03	0.461	0.522	0.533
	La Rambleta	RB	14	4.07	0.00	3.09	0.540	0.547	0.536
	Torre 4	T4	30	5.57	0.07	3.32	0.560	0.566	0.527
	Torre 4–Este	T4-E	10	4.29	0.14	3.36	0.551	0.554	0.553
	El Refugio	REF	28	5.21	0.21	3.32	0.458	0.557	0.519
	Montaña Blanca	MB	80	6.64	0.21	3.50	0.577	0.592	0.530
	Total Teide		208	8.07	2.14	6.65	0.538	0.587	0.527
Cañadas Wall	Guajara	GUA	48	6.93	0.86	3.92	0.524	0.666	0.541
	Topo de la Grieta	TGR	10	2.14	0.00	2.08	0.471	0.391	0.553
	Total Cañadas Wall		58	7.00	0.93	6.89	0.624	0.645	0.543
Total			266	9.00	1.64	3.90	0.557	0.637	

NA, mean number of alleles per locus; PA, mean number of private alleles per locus; A_R, allelic richness based on the expected number of alleles among 11 gene copies; H_o, observed heterozygosity; H_e, Nei's 1978 gene diversity; Sr, selfing rate (0–1) values based on phenotypes and irrespective of allele dosage.

TABLE 2. *AMOVA for* V. cheiranthifolia*: hierarchical levels were implemented based on the clustering found by STRUCTURE (Teide vs. the Cañadas Wall)*

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage variation	Φ statistics
Teide vs. the Cañadas Wall	1	90.67	0.86	13.4	$\Phi_{CT} = 0.134$*
Between localities within populations	7	68.59	0.17	2.7	$\Phi_{SC} = 0.031$**
Within localities	257	1386.43	5.39	83.9	$\Phi_{ST} = 0.160$**
Total	265	1545.69	6.43	100.0	

*$P < 0.05$; ** $P < 0.01$.

TABLE 3. *Pairwise Rho statistics for all the population comparisons*

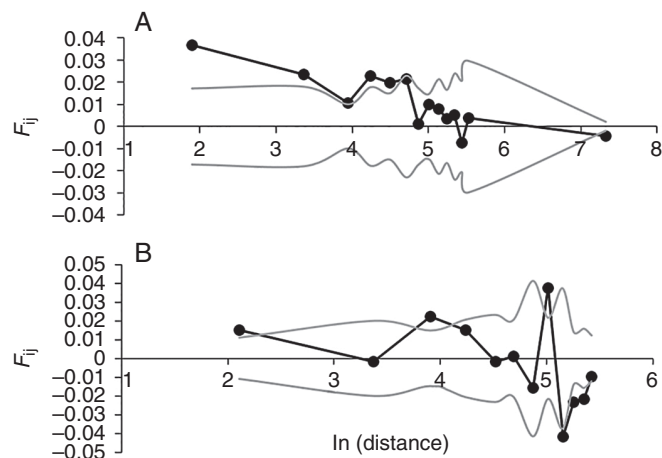|	PV	FOR	RB	T4	T4-E	REF	MB	GUA
PV								
FOR	**0.088**							
RB	**0.091**	**0.076**						
T4	**0.092**	**0.119**	**0.078**					
T4-E	**0.112**	**0.118**	**0.146**	**0.090**				
REF	**0.073**	**0.083**	**0.104**	**0.083**	**0.065**			
MB	**0.079**	**0.086**	**0.072**	0.015	**0.096**	**0.059**		
GUA	**0.265**	**0.266**	**0.274**	**0.274**	**0.291**	**0.263**	**0.253**	
PAS	**0.492**	**0.536**	**0.558**	**0.526**	**0.609**	**0.510**	**0.474**	**0.232**

Values in bold: $P < 0.001$.

FIG. 3. Average kinship coefficients F_{ij} between pairs of individuals at geographical distance intervals every 20 m (log scale) in Teide (A) and Guajara (B). Grey lines show the 95 % confidence intervals and the dots that are out of these margins indicate a significant deviation of the random spatial distribution ($P < 0.05$).

show an earlier divergence between the Las Cañadas Wall and Teide populations. Therefore, demographic parameters were calculated under scenario 2. In that scenario, the divergence between Las Cañadas Wall and Teide was again highlighted. The oldest current population was Guajara, which derived from an unknown ancestral population at the event *t*2a [4610 (2160, 19 100) generations; mode and 2.5–97.5 % quantiles of the posterior distribution, respectively]. From Guajara, two divergence times are presented, the earliest giving rise to Teide in *t*2 [857 (362, 4630) generations] and the latest one in time to Topo de La Grieta in *t*1 [134 (53.6, 657) generations] (Table 5). In general, mean bias estimates for the divergence times were low, with high factor 2 values (Supplementary Data Table S1). Following other estimates of perennial violet life spans, we have considered that *V. cheiranthifolia* may live for around 25 years (Solbrig, 1981; Batista and Sosa, 2002; Culley, 2002; Herrera and Bazaga, 2010). Given that generation time, Guajara would have derived from the ancestral population 115 250 (54 000, 477 500) years ago. The divergence times between GUA-TEIDE and GUA-TGR would be 21 425 (9050, 115 750) and 3350 (1340, 16 425) years ago, respectively. Nonetheless, the estimate of the generation time in *V. cheiranthifolia* is still uncertain, due to the difficulties in tracing the annual regeneration of the vegetative part of the plants.

Estimates of effective population size were contrasted across populations, with much bigger sizes in TEIDE [N1: 6820 (2420, 9680) diploid genomes, mode and 2.5–97.5 % quantiles of the posterior distribution, respectively; Table 5] compared with GUA [4040 (1290, 9160 diploid genomes) and TGR [N3: 115 (40.4, 1310) diploid genomes]. The ancestor population showed a higher effective population size [Na: 9810 (938, 9920) diploid genomes], although this last estimate is unreliable due to the high bias and error and low factor 2 (Supplementary Data Table S1).

Niche modelling

All algorithms performed well for the majority of runs and with an average of scoring >0.8 for both evaluation metrics (Supplementary Data Fig. S4). Snow cover was the most important variable in all models except GBMs, followed by annual precipitation (Bio12), mean annual temperature (Bio01) and slope (Supplementary Data Table S2). Conversely, topographic position and TPI showed very low values.

The total current present area encompassed 5.267 km² (Fig. 4); therefore, realized distribution is only 1.91 % of the total designated area. Climate suitability was declared exclusively within Teide stratovolcano and the top of the Las Cañadas Wall area, with no suitable areas between them. Climate change scenario combinations showed a general decrease in the species suitability (Fig. 4) but with strong variations between GCMs, RCPs and years (Fig. 5; Supplementary Data Table S3). For instance, in the MIROC model under RCPs 2.6 and 6.0, climate suitability showed an increase in the suitability in 2080 but a sharp decrease under the 8.5 RCP scenario. As shown in Fig. 4, suitable areas are prone to diminish in the Las Cañadas Wall and in the lower areas of the stratovolcano, under the scenario of climate change. Under the most pessimistic scenario (MIROC, RCP 8.5 year 2080), the remaining suitable areas persisted only at the summit of the Teide stratovolcano.

Regarding the simulations of heterozygosity loss, the Las Cañadas Wall cluster initially showed higher heterozygosity values than the Teide cluster (Fig. 6). Under all the combinations of scenarios, simulations of heterozygosity loss behaved similarly: values increased slightly for both genetic clusters, until year 2060, when values dropped sharply. In all scenarios except MIROC RCP = 8.5 and CSIRO RCP = 6.0, the decrease in heterozygosity was somewhat higher in the Las Cañadas Wall cluster than in the Teide cluster. Both year and RCP had

TABLE 4. *Spatial genetic structure (SGS) at a fine scale, analysed with 14 microsatellite markers in the two main groups detected with STRUCTURE: Teide and Guajara*

	F_1	b_{lin}	b_{log}	Sp_{lin}	Sp_{log}
Teide	0.0366 ± 0.0086	−0.0000051 ± 0.0000011	−0.0061 ± 0.0012	0.0000052 ± 0.0000011	0.0063 ± 0.0012
Guajara	0.0149 ± 0.0055	-0.0001746 ± 0.0000429	−0.0113 ± 0.0026	0.0002 ± 0.0000429	0.0112 ± -0.0026

F_1, mean co-ancestry coefficients (F_{ij}) in the first distance class; b_{lin} and b_{log}, regression slopes of F_{ij} values over the linear and logarithmic spatial distances, respectively; Sp statistics (Sp_{lin} and Sp_{log}), indicators of the SGS strength, calculated with the formula $–b (1 – F_1)$, and using both b_{lin} and b_{log}.

TABLE 5. *ABC demographic parameters estimates for scenario 2*

Demographic parameter	Mean	Median	Mode	Quantile 2.5 %	Quantile 97.5 %
Effective population size					
N1 (Teide)	6370	6470	6820	2420	9680
N2 (Guajara)	4690	4470	4040	1290	9160
N3 (Topo de la Grieta)	283	166	115	40	1320
Na	7090	7900	9810	9538	9920
Time scale in generations					
t1	244	208	134	54	657
t2	1530	1240	857	362	4630
t2a	9460	8740	4610	2160	19 100

The mean, median and mode for each parameter are given, along with 95 % credibility intervals. N1, N2, N3 and Na refer to effective population sizes in number of diploid genomes, respectively, of standing populations from 'Teide', 'Guajara' and 'Topo de La Grieta' and from a non-sampled ancestral population from which they have diverged as modelled in the most likely of the six tested scenarios (Supplementary Data Fig. S1). *t*1, *t*2 and *t*2a are divergence times of the standing populations from the populations from which they have derived.

a significant impact on the variation in heterozygosity in the simulations, but not the models or the interactions between factors (year, $F = 257.885$, $P < 0.001$, df = 1; RCP, $F = 39.562$, $P < 0.001$, df = 8).

DISCUSSION

Genetic diversity of an endemic alpine polyploid

The tetraploid pattern found in *V. cheiranthifolia* agrees with other studies of species of polyploid origin in the sect. Melanium (Marcussen *et al.*, 2010). Although we found a maximum of four alleles per sample and locus, a detailed chromosome count and exact estimation of the ploidy level would be of interest for the evolutionary history of the species within that section. Moreover, the selfing rate (approx. 50 %) estimated in this study agrees with the values of autonomous selfing obtained in a previous study (Seguí *et al.*, 2017). Nonetheless, they detected a significant increase in self-fertility with elevation and with intensity of herbivory.

Despite being an isolated endemic species with selfing capacity, *V. cheiranthifolia* did not show diminished levels of genetic diversity, as found with its relative *V. palmensis* at the summit of La Palma Island (Batista and Sosa, 2002). Furthermore, the expected heterozygosity levels detected in *V. cheiranthifolia* (average per population = 0.550; total = 0.637) were similar or even higher than those in diploid violets studied with microsatellites (Culley, 2005; Hirai *et al.*, 2012; Kang *et al.*, 2017*a*, *b*). In the Canary Islands, there are taxa with either lower or higher genetic variation than *V. cheiranthifolia* (Sosa *et al.*, 2011). For example, another Teide endemic *Silene nocteolens* (Sosa *et al.*,

2011), the Canarian olive tree *Olea europaea* ssp. *guanchica* (García-Verdugo *et al.*, 2010) and the narrow-ranged *Ruta oreojasme* (Meloni *et al.*, 2015) show surprisingly high values of genetic variation. In contrast, the species within the endemic genus *Bethencourtia* (Rodríguez-Rodríguez *et al.*, 2018), *Ilex perado* ssp. *lopezlilloi* (Sosa *et al.*, 2011) or *Limonium macrophyllum* (Jiménez *et al.*, 2017) present very low levels of diversity, typical of rare endemic species (Cole, 2003). However, the generalization that endemic species lack variability is not always certain, and in many cases their genetic diversity equals that of their widespread congeners (Gitzendanner and Soltis, 2000). It has also been pointed out that endemic plants in the Canary Islands show higher levels of genetic diversity than expected for oceanic archipelagos (Francisco-Ortega *et al.*, 2000), possibly due to their close proximity to the continent.

High genetic diversity levels in rare species can also be an indicator of a wider distribution in the past (Ellstrand and Elam, 1993; Sosa *et al.*, 2010), when all invasive herbivores were absent, and the climate was more favourable. The estimations of effective population sizes did not show a notable decline through time, although these estimates cannot be accurate due to the lack of prior knowledge of *V. cheiranthifolia*. Moreover, the species' biology makes demographic censuses difficult, as the emerging individuals depend highly on the annual precipitation and on the herbivore pressure level.

Therefore, the rapid radiation taking place in the section Melanium, coupled with polyploidization events (Yockteng *et al.*, 2003), might be the most feasible factors enhancing genetic diversity. It can be concluded that *V. cheiranthifolia* shows moderate levels of genetic variation possibly favoured by its polyploid origin, and counteracted by its isolated condition, and selfing capacity.
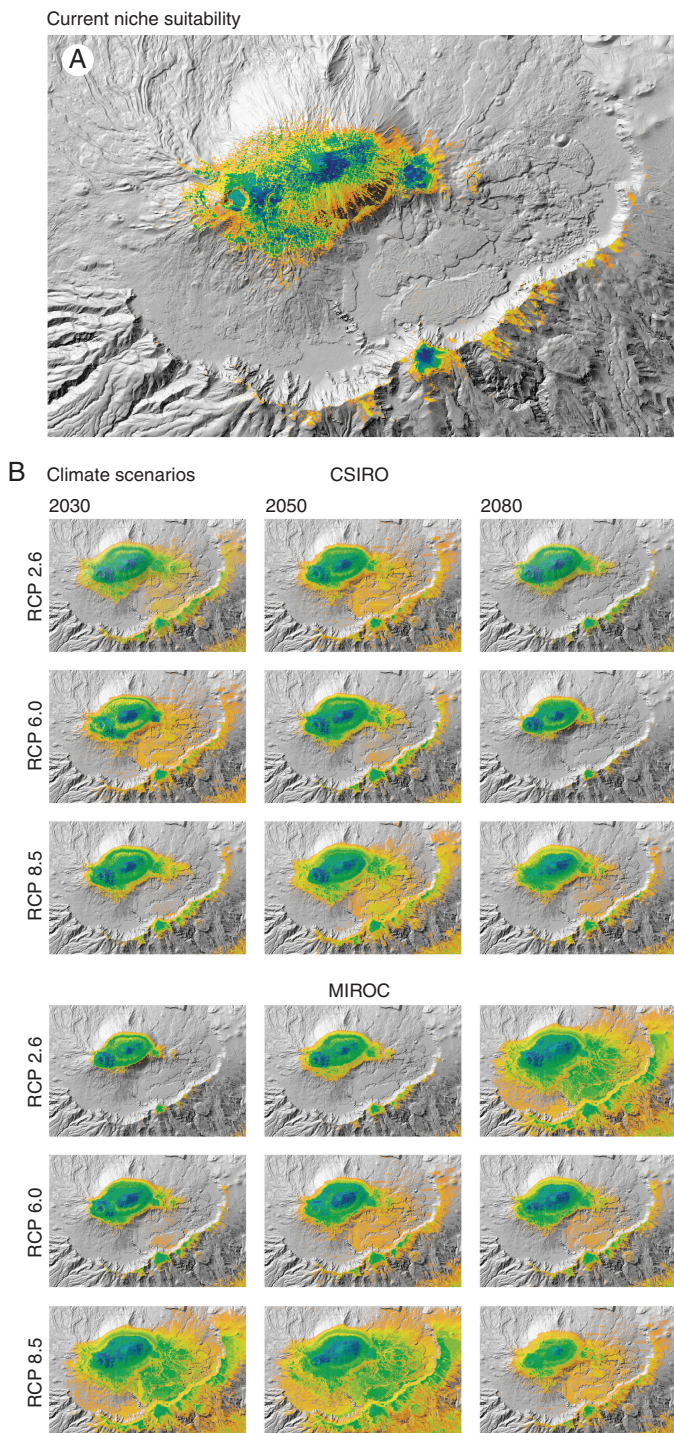
Current niche suitability

Fig. 4. (A) Current topoclimatic suitability of *Viola cheiranthifolia*. (B) Projections of topoclimatic suitability under climate change scenarios. Suitability is displayed in a continuous range for the present, and in presence/absence for climate change scenarios for a threshold of 650 according to the TSS score (see the Results).

Genetic structure and demographic history of populations

The population grouping analysis (STRUCTURE, PCoA, Rho statistic and AMOVA) showed a clear differentiation between the populations in Teide and in Las Cañadas Wall. A genetic barrier was expected since these two groups are separated by >6 km, and it is well known that solitary bees tend to forage within 2.0 km of their hive if there are attractive floral resources in the vicinity (Zurbuchen *et al.*, 2010). Nonetheless, periods of strong winds could promote the accidental displacement of bees, opening temporary windows for gene flow. The species distribution modelling approach also supports the existence of a geographical barrier. Since the models do not declare a central area as suitable, the hypothesis of the existence of a continuous population can be discarded. However, we did not detect a phylogeographic signal with the permutation tests described in Hardy *et al.* (2003). Lack of significance suggests high migration rates between populations and/or a relatively low number of generations in isolation (Hardy *et al.*, 2003). The second option is actually highly plausible, due to the young age of the Teide–Pico Viejo complex and the perennial trait of *V. cheiranthifolia*.

Although there is an apparent segregation within populations in the STRUCTURE results, the low percentage of variation between localities within groups shows evident gene flow within Teide. Between Guajara and Topo de La Grieta, this sub-population structure is more evident due to the greater geographical distance. Besides this, there was admixture found between the two clusters in Las Cañadas Wall, with some individuals on the north side of Guajara being clustered together with Topo de La Grieta individuals. A review analysis of genetic variation in plants has shown that long-lived, outcrossing, late successional taxa retain most of their genetic variability within populations (Hamrick and Godt, 1990; Nybom, 2004). The significant SGS found was also congruent with the sub-population structure findings and the high percentage of variation within localities, as the short-distance dispersal ability of *V. cheiranthifolia* seeds and the moderate to high levels of self-fertilization are promoting the genetic spatial structure among individuals. Nevertheless, following the review by Vekemans and Hardy (2004), our values for the Sp statistics are lower than their estimations according to the biotic traits of *V. cheiranthifolia*. Sp statistics are expected to be inversely proportional to the density (Heywood, 1991), and it has been found that the damage caused by rabbits in *V. cheiranthifolia* greatly decreases the plant population densities (Seguí *et al.*, 2017). Moreover, pollen dispersal by insects may be promoting higher outcrossing among individuals within populations, compensating for the selfing effects on the SGS. If the strong herbivory by rabbits continues and the species tends to shift upwards as expected, it could lead to an increase of selfing, and, therefore, a change in the spatial genetic structure of the populations.

According to ABC estimates, 115 250 (54 000, 477 500), the species was present in Guajara after the lateral collapse of the northern flank of the island of Tenerife which formed Las Cañadas Caldera. The hypothesis that the individuals in the Teide group were colonized from the older parts of Guajara was supported by the ABC estimates, 21 425 (9050, 115 750), since Teide stratovolcano reached its maximum size 32 000 BP. Moreover, Guajara showed higher levels of variation than Teide, which supports the idea of a founder effect from Guajara to Teide (Frankham *et al.*, 2002). Eruptive activity of the Teide cone seems to have declined over the past 30 000 years, with only some eruptions of peripheral lava domes
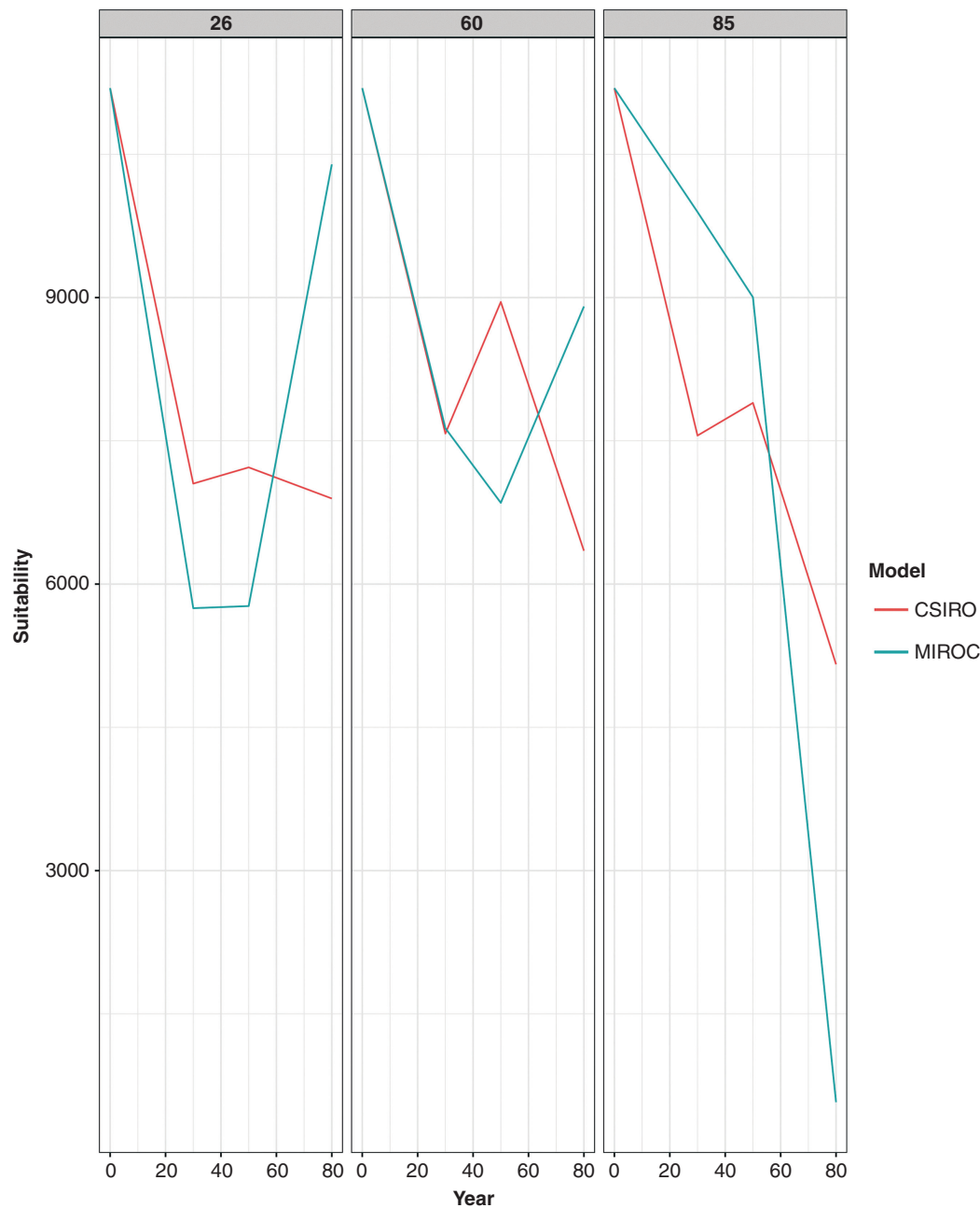
Fig. 5. Number of suitable cells of *Viola cheiranthola* (suitability >650) for each climate change scenario.

(10 000–2000 years BP) and the last prehistoric eruption of Lavas Negras (1150 years BP) in the Teide cone (Carracedo *et al.*, 2007).

The fate of Viola cheiranthifolia *populations and implications for conservation*

Projections of the fate of alpine species under climate change are considered among the most pessimistic due to the spatial limitation for upward migration to meet suitable climate conditions, especially accounting for the limited migration ability of most of these species (IPCC Working Group I, 2013). Here,

we used an accurate spatial scale to reflect the interplay between topographic and climate constraints, also allowing us to minimize the risk of overfitting models. SDMs and their use to simulate heterozygosity loss allow us to draw two conclusions for the conservation strategy of the species: one is the persistence of suitable areas and the second is the extent of heterozygosity loss.

Regarding the first, suitable habitats in the future will allow *in situ* conservation of the species. Contrary to the generalized expectation of extinction of mountain species, projections show that the climatic niche of *V. cheiranthifolia* will be able to persist under any scenario, thus facilitating the conservation approach for the species. We found, nevertheless, a great uncertainty
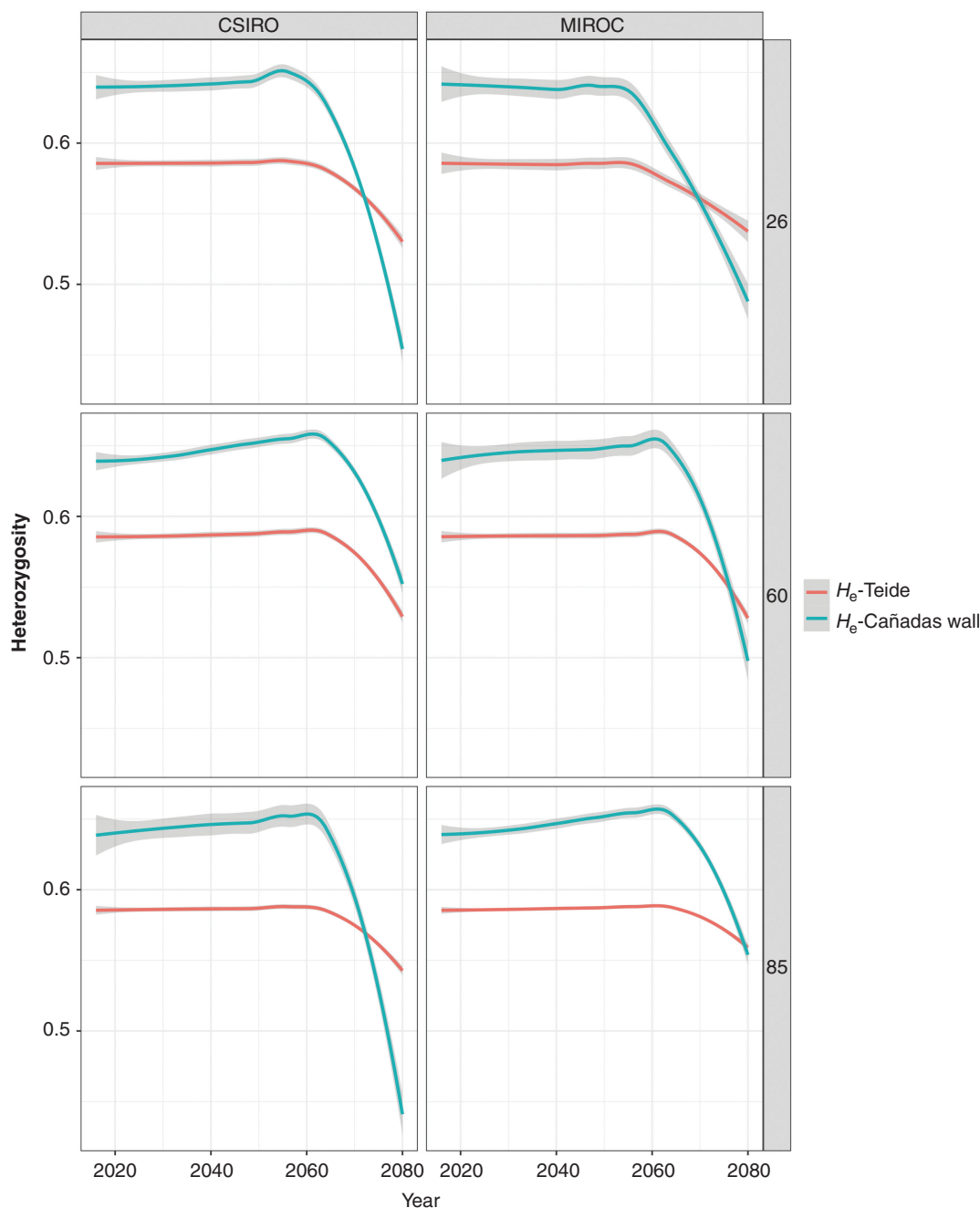
Fig. 6. Simulated levels of heterozygosity across time for the combinations of RCP and GCM for both populations, Las Cañadas Wall and Teide.

linked to the GCM used for climate change scenarios. We warn that three assumptions made in the modelling approach must be met to validate these forecasts. (1) reproductive success is high enough to maintain demographic viability of the species; therefore, the conservation strategy for the species has managed to prevent herbivory by introduced rabbits, as warned by Seguí *et al.* (2017). (2) *Viola cheiranthifolia* is fully able to migrate to new suitable areas. Seed dispersal occurs mostly by the explosive ejection of seeds at short distance, which indeed hinder species' ability to adapt to climate change. Nonetheless, SDMs show that in most scenarios, suitable areas do not increase towards the summit of Teide, but rather diminish within current

suitable areas. Therefore, conservation strategies may not need to take into account measures to facilitate seed dispersal, as populations are likely to be sufficiently static. Monitoring of distribution shifts must still be performed, as part of the conservation strategy. (3) No other environmental constraints related to human land occupation or soil properties are preventing suitable areas hosting *V. cheiranthifolia* populations. Regarding anthropogenic disturbances, since the summit of Teide has the maximum conservation status, further anthropogenic constraints affecting the distribution are unlikely.

The second conclusion – regarding the extent of heterozygosity loss – allows the linkage of genetic markers with climate

change scenarios, showing how heterozygosity values will only drop by the year 2060. This means that genetic diversity levels will remain stable for a long period before showing a decline consistent with the suitability loss forecast for the last time period. Whilst comparison with other organisms remains difficult given the number of factors affecting genetic diversity, our results were consistent with those of other studies predicting a loss of genetic diversity proportional to habitat loss (Blanco-Pastor *et al.*, 2013; Chala *et al.*, 2016). The main reason for concern is the loss of heterozygosity in the Las Cañadas Wall population, which needs to be supported *in situ* and *ex situ*, possibly by reinforcing/enriching populations.

CONCLUSIONS

This is the first study that combined population genetics with niche modelling and heterozygosity loss estimations in an alpine oceanic endemic. More studies like this could help to understand population dynamics and patterns of distribution of alpine plant species under current climate change conditions. In short, we have clarified the current genetic status of *V. cheiranthifolia*, together with its demographic history and future climate suitability. Overall, it is a resilient alpine species with moderate to high genetic diversity. However, herbivory pressure is seriously affecting its populations by reducing fitness and population densities, increasing self-fertilization and reducing their generation time (Seguí *et al.*, 2017). Although simulations show that the species will be able to sustain itself under climate change, there is a tendency to a shift in altitude of habitat, and the heterozygosity loss will increase by the year 2060. The localities in Las Cañadas Wall may be the most affected, as an upward migration is not possible. We suggest implementing the first conservation measures in Las Cañadas Wall localities, which are more vulnerable to the effects of climate change. On the one hand, Topo de La Grieta presents a small population size and variability. On the other, Guajara holds an important genetic source for the species, being the oldest present population. Therefore, the implementation of fenced areas, as suggested by Seguí *et al.* (2017), as well as the creation of a germplasm bank from specimens in these localities are urgent measures for the long-term survival of *V. cheiranthifoli*a. Moreover, monitoring of the upward shift in Las Cañadas Wall and Teide would help detect the decline in populations over time.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Supplementary Material S1: priors and settings for choosing the most probable scenario of demographic history implemented in DIYABC, the estimation of the demographic parameters and the confidence of the parameters and the scenario choice. Figure S1: the six scenarios of population demography of *V. cheiranthifolia* examined by ABC analysis. Teide (Pop1), Guajara (Pop2) and Topo de la Grieta (Pop3). Figure S2: output results from STRUCTURE HARVESTER. Figure S3: (A) Probability of each scenario over the 10 % of the closest simulated and observed data using the logistic regression method implemented in DIYABC. (B) The principal component analysis obtained by DIYABC to estimate

the goodness-of-fit of scenario choice. Figure S4: boxplots of the scores of the two evaluation metrics used in SDMs, TSS and ROC, for the ensemble of runs of each algorithm. Table S1: prior and posterior distributions of the ABC demographic parameters, including the confidence in parameter estimation (Bias, RMSE, coverage and Factor 2). Table S2: variables important for individual and ensemble models. Table S3: number of suitable cells for each combination of model, emissions scenario and temporal horizon.

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