Urquia, Diego, Pozo, Gabriela, Gutierrez, Bernardo, Rowntree, Jennifer and de Lourdes Torres, Maria (2020) Understanding the genetic diversity of the guayabillo (Psidium galapageium), an endemic plant of the Galapagos Islands. Global Ecology and Conservation, 24. e01350-e01350. ISSN 2351-9894

DOI: https://doi.org/10.1016/j.gecco.2020.e01350

Publisher: Elsevier

Version: Accepted Version

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PII: S2351-9894(20)30891-X

DOI: https://doi.org/10.1016/j.gecco.2020.e01350

Reference: GECCO 1350

To appear in: *Global Ecology and Conservation*

Received Date: 20 March 2020

Revised Date: 29 October 2020

Accepted Date: 29 October 2020

Please cite this article as: Urquia, D., Pozo, G., Gutierrez, B., Rowntree, J.K., de Lourdes Torres, M., Understanding the genetic diversity of the guayabillo (*Psidium galapageium*), an endemic plant of the Galapagos Islands, *Global Ecology and Conservation*, https://doi.org/10.1016/j.gecco.2020.e01350.

Understanding the genetic diversity of the guayabillo (*Psidium galapageium*), an endemic plant of the Galapagos Islands

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ABSTRACT

Oceanic archipelagos are known to host a variety of endemic plant species. The genetic diversity and structure of these species are important indicators of their evolutionary history and can have consequences on the implementation of appropriate conservation strategies. A comprehensive consideration of the role of their natural history, as well as the landscape features and the geological history of the islands themselves are required to adequately understand the geographic patterns inferred from genetic data. Such is the case for guayabillo (*Psidium galapageium*), an understudied endemic plant from the Galapagos Islands with important ecological and economic roles. In this study we designed and evaluated 13 informative SSR markers and used them to investigate the genetic diversity, population structure and connectivity of the guayabillo populations from San Cristobal, Isabela and Santa Cruz islands. A total of 208 guayabillo individuals were analyzed, revealing a strong population structure between islands and two distinct genetic lineages for the Santa Cruz population. Overall, the relatively high genetic diversity of the species could be explained by different biological, demographic and environmental factors. For guayabillo populations such as the one in San Cristobal, the history of human disturbance in their habitats might play an important role in explaining their reduced genetic diversity. The coexistence of two distinct lineages in Santa Cruz, with one of them sharing genetic similarities with individuals from San Cristobal, could be attributed to limited, unidirectional gene flow from the latter island to the former. Our findings highlight the complex population dynamics that shape the genetic diversity of species like the guayabillo, and emphasize the importance of a species' evolution and natural history when interpreting its population genetics.

Keywords:
Galapagos Islands; endemic species; insular species; genetic diversity; *Psidium galapageium*; microsatellites

1. Introduction

Oceanic islands are home to unique species which have emerged as a product of their evolutionary histories being driven by geographical isolation and distinct topological and climatic conditions. This makes them ideal study cases for evolutionary and ecological processes (Carlquist, 1974; Emerson, 2002; Shaw and Gillespie, 2016). Studying these species has been an important step in addressing evolutionary biology questions about key processes such as adaptation, speciation, radiation, and the link between evolution and geography (Geist et al., 2014; Rumeu et al., 2016; Shaw and Gillespie, 2016). Among these, insular endemics are an interesting case of species that may comprise distinct gene pools compared to their counterparts in mainland ecosystems (e.g. Helenurm and Ganders, 1985; Wendel and Percival, 1990; Rumeu et al., 2016). The genetic diversity patterns observed for insular organisms are diverse and driven by multiple factors, ranging from founder events and genetic bottlenecks that constrain a species' gene pool (Mayr, 1954; Hagenblad et al., 2015; Stuessy et al., 2014) to long adaptive evolutionary processes and gene flow that contribute to the genetic makeup of different populations (Frankham, 1997; Stuessy et al., 2014). The geographical features of the islands inhabited by these organisms (e.g. size, age and habitat heterogeneity) also play major roles in shaping their population structures (MacArthur and Wilson, 1967; Stuessy et al., 2014).

The Galapagos Islands are a prime example of oceanic archipelagos; they are conformed by 13 main islands and more than 100 minor islets of volcanic origin. The archipelago is located in the Pacific Ocean, ~1000 km off the coast of South America. Thanks to their tropical location and oceanographic situation, the Galapagos harbor a great variety of unique species, as well as rich ecosystems which remain relatively undisturbed compared to other insular systems (Gillespie and Clague, 2009; Jaramillo et al., 2011). Moreover, the overall young age of the archipelago and the coexistence of islands of different ages make the Galapagos a fitting setting to observe evolutionary processes in action (Jaramillo et al., 2011).

The evolution and conservation of endemic species of the Galapagos have been extensively studied. However, most research has been focused on animal species (Geist et al., 2014; Shaw and Gillespie, 2016); few studies have explored the genetic diversity and population structure of endemic plant species, which are direct consequences of their evolutionary history and key indicators of their vulnerability and responsiveness to environmental change (Fridley et al., 2007; Jump et al., 2009; Stuessy et al., 2014). Moreover, insular endemic species are valuable genetic resources for bioprospection and plant breeding purposes (e.g. Guezennec et al., 2006; Pailles et al., 2017). Unfortunately, endemic insular species are intrinsically vulnerable to threats which include environmental change, disease, invasive species, human perturbation and habitat loss due to their isolation, relatively small population sizes and restricted distribution (Whittaker, 1998; Sakai et al., 2001). Thus, it is not surprising that in 2016, 40% of all recognized endangered species were found in island ecosystems (Island Conservation, 2016). The identification of factors that promote or negatively impact the genetic diversity of a species and the assessment of its population structure can help establish conservation areas, prioritize populations for conservation actions, and evaluate such strategies adequately (Bensted-Smith, 2002; Wallis and Trewick, 2009; Moritz, 2002; Gitzendanner et al., 2012).

Multiple driving forces have been associated with the evolution and genetic diversity of endemic species in the Galapagos Islands. For instance, *Scalesia affinis* presents a higher genetic diversity in Isabela island compared to Floreana island, partially explained by the former having a much larger landmass and a broader altitudinal gradient (Nielsen, 2004). Other factors pertaining to the evolutionary history of the species, including speciation mechanisms (anagenesis vs. cladogenesis) and other events such as past hybridization and polyploidization, should also be considered for interpreting genetic diversity patterns (Soltis and Soltis, 2000; Stuessy et al., 2006; Stuessy et al., 2014). It has been proposed, for example, that the Galapagos endemic shrub *Galvezia leucantha* harbors high levels of genetic diversity in part due to populations from different islands maintaining some gene flow (Guzmán et al., 2016); thus, all these populations still conform a single species (as observed in anagenesis; Stuessy et al., 2014; Takayama et al., 2015). Furthermore, the reproductive biology (outcrossing vs. selfing vs. clonal reproduction) and dispersal mechanisms of the species are also relevant factors that explain genetic diversity and structure (Crawford and Whitney, 2010). Species that inbreed, self-pollinize and/or reproduce clonally tend to show higher levels of genetic differentiation among populations, especially if they are weak dispersers (Ellstrand and Elam, 1993; Hamrick and Godt, 1996). For instance, the low heterozygosity and high between-population differentiation in the Galapagos endemics *Solanum cheesmaniae* and *Solanum galapagense* were partially attributed to their highly autogamous nature (Rick, 1983; Pailles et al., 2017). On the other hand, it is thought that gynodioecious dimorphism in *Lycium minumum* emerged as a mechanism to promote outcrossing and to maintain genetic diversity; in turn, this dimorphism would be linked with a tetraploidization event in the evolutionary history of the species (Sakai et al. 1995; Levin et al., 2015).

The recent geological history of the Galapagos Islands themselves must be considered when interpreting and understanding the genetic diversity and structure of an endemic plant species. Every island of the archipelago emerged progressively due to the eastward movement of the Nazca Plate over a mantle hotspot (Villagomez et al., 2007; Geist et al., 2014); thus, the older islands of the archipelago are located to the southeast, while the newer ones are located to the northwest (Geist et al., 2014). This movement of the Nazca Plate, in combination with historical changes in the sea level, lead to oceanic barriers that separated islands that emerged over the same hotspot and were initially close together (Christie et al., 1992; Geist et al., 2014). In consequence, populations from different islands are kept separated from each other by considerable stretches of ocean extending for several kilometers. Moreover, these isolated populations may be exposed to different environmental conditions and to different demographic events and genetic processes (e.g. population size changes, selection, genetic drift, mutations, etc.) (Lombaert et al., 2011; Shirk et al., 2014), establishing distinct patterns of genetic structure within a species and even triggering speciation (Rumeu et al., 2016; Pailles et al., 2017). This phenomenon has been observed in Galapagos endemic plants such as *S. cheesmaniae* and *L. minimum*, where a notorious genetic divergence arose between populations of the older eastern islands and the western younger islands (Levin et al., 2015; Pailles et al., 2017).

Guayabillo (*Psidium galapageium*; Myrtaceae) is one of the 241 endemic plant species in the Galapagos Islands (Jaramillo et al., 2014). Catalogued as *Near threatened* in the Red Book of endemic plants of Ecuador (Kawasaki et al., 2017), it is one of the few endemic tree-like plants in the archipelago, and hence a significant landscape component of the transition zones and *Scalesia* forests of several islands (San Cristobal, Santa Cruz, Santiago, southern Isabela, Fernandina, Pinta and Floreana); its distribution also includes drier lowland and humid highland sites (Porter, 1968; McMullen, 1999). Guayabillo serves as an anchoring substrate for nutrient-fixing lichen (Dal Forno et al., 2017), and chemical compounds produced by its leaves have been used as a natural repellent for parasitic and hematophagous insects by birds, including several species of endemic finches (Cimadom et al., 2016). Its hard and resistant wood is also used by the islanders for house and boat construction (Wiggins et al., 1971). Nevertheless, as many of the endemic plants of the Galapagos, guayabillo is threatened by human-induced disturbances including overexploitation of its wood, habitat loss, and the presence of invasive species (Wiggins et al., 1971; Adsersen et al., 1988; Frankham, 1995; Tye et al., 2007; Dal Forno et al., 2017). The direct competition between endemic and invasive species can cause a reduction and fragmentation in the populations of the former, as well as a loss of its genetic diversity (Nielsen, 2004; Jaramillo et al., 2011; Stuessy et al., 2014). For this reason, the introduction of exotic species is of great concern in insular ecosystems like the Galapagos (Whittaker, 1998; Tye et al., 2007). The common guava (*Psidium guajava*), for example, is an invasive species that shares some of the same ecosystems with guayabillo, raising the potential risk of guava populations outcompeting or forming interspecific hybrids with its endemic relative (which could cause genetic erosion) (Torres and Gutiérrez, 2018). Similarly, the Galapagos flora in general is threatened by destructive introduced grazers such as goats and feral livestock; these animals have already caused an impact for several endemic species in the islands such as *Calandrina galapagosa*, *S. affinis* and *G. leucantha* (Nielsen, 2004; Jaramillo et al., 2011; Guzmán et al., 2016).

Despite its economic and ecological importance and potential vulnerability as an island endemic, little is known about guayabillo’s natural history and its population genetics. In fact, the evolutionary history of this endemic species can serve as an important case study regarding the genetic diversity of endemic insular species. Until recently, the idea that island plant species were expected to present depauperated levels of genetic diversity was widely accepted, (DeJoode and Wendel, 1992; Barrett, 1996; Frankham, 1997). Although these patterns might still appear in species with narrow distributions as a consequence of bottlenecks and founder effects (Frankham, 1997; Garcia-Verdugo et al., 2015), this notion has been recurrently challenged for insular endemics with broader distributions and non-endemics. Increasingly cumulative evidence supports the idea that insular species or populations can show equivalent or even higher genetic diversity levels than their mainland relatives (e.g. Su et al., 2010; Rosas-Escobar et al. 2011; Desamore et al. 2012; Garcia-Verdugo et al. 2013; Garcia-Verdugo et al., 2015). Thus, considering the fairly broad distribution of guayabillo in the Galapagos archipelago (McMullen, 1999), a relatively high genetic diversity could be expected. A reduction of genetic diversity could be attributed to human disturbance (including invasive species) or abrupt natural events rather than the progressive evolution of the species (Whittaker, 1998; Geist et al., 2014; Garcia-Verdugo et al., 2015).

We present the design and evaluation of homologous SSR primers for *P. galapageium* in order to assess the genetic diversity, structure and connectivity of three populations of this species, in San Cristobal, Isabela and Santa Cruz Islands. The parameters inferred from the genetic data were used to describe the natural history of the species in the archipelago. Moreover, our results allowed us to discuss the effects of geographical distribution and human action on the expected genetic diversity of the species, further developing our understanding of the population structure patterns of endemic insular plants. This enhanced overview of guayabillo's evolutionary history and the factors driving it can be used to assess the current status of the guayabillo populations and to identify potential risks for the species, both relevant steps for the establishment and evaluation of conservation strategies.

2. Material and methods

2.1. Study sites and sample collection

In order to identify *P. galapageium* individuals, the morphological description by Porter (1968) was used. Guayabillo is a small tree or shrub of smooth, pinkish gray bark (Fig. 1a). Its branches are divaricate, its branchlets terete and gray. Its leaves are elliptic to ovate, equilateral and 1.8-5.5 cm long and 0.9-2.6 cm wide. Flowers are 1-1.5 cm in diameter, of a whitish color (Fig. 1b). Berries have a 2 cm diameter, they are globose to subglobose, glabrous, and of a pale yellow to yellow color (Fig. 1c).

Samples from *P. galapageium* individuals were collected from three islands: San Cristobal (seven sampling locations), Santa Cruz (six sampling locations) and Isabela (six sampling locations; Fig. 1d). For the selection of these sampling locations, sites were chosen based on previous reports of guayabillo populations, either documented in the literature or through personal communications with local inhabitants. From this pre-selection we chose sites close to roads or inhabited areas, since more remote locations in the Galapagos Islands are inaccessible for sampling.

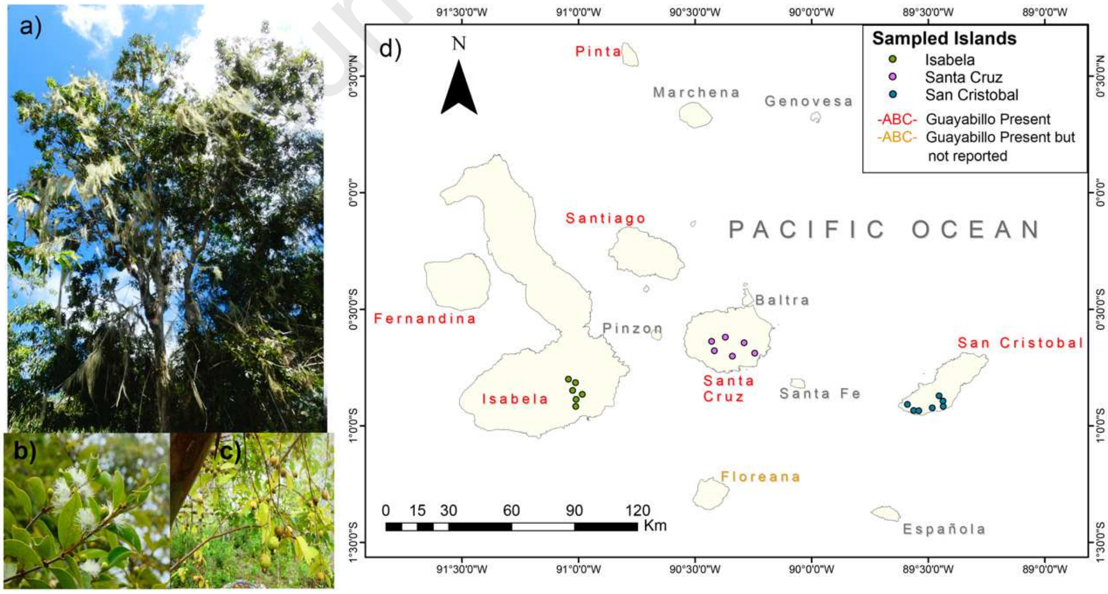

Fig. 1. a) A guayabillo tree, b) Details of the leaves and flowers of guayabillo. c) Details of the leaves and fruits of guayabillo. (Photos: Bryan Reatini, UNC-CH). d) Galapagos Islands map indicating the sampling sites of this study in Isabela, Santa Cruz and San Cristobal Islands. The islands where guayabillo is distributed are highlighted in red; note that although guayabillo is not officially reported as present in Floreana Island (orange label), it is actually distributed over there as well (Bryan Reatini, pers. comm.)

Two to five fresh leaves were taken from each sampled tree and stored in plastic bags, which were transported to the Galapagos Science Center (San Cristobal Island) for storage at -20ºC. A total of 208 individuals were sampled, ranging between 4 and 34 samples per location (Table A1). We collected the greatest possible number of individuals separated by a minimum distance of 100m to minimize the possibility of sampling genetically identical individuals.

2.2. Molecular Methods

2.2.1. Primer Design

Guayabillo specific primers for microsatellite regions were developed from a single genomic DNA extraction using the Galaxy-based bioinformatics pipeline reported by Griffiths et al. (2016). Sequencing was performed on an Illumina MiSeq platform at the University of Manchester genomics facility using shotgun 2x250 paired-end sequencing methodology (Nextera DNA Preparation Kit, Illumina, USA). The sample used 0.33 of a flow cell and primer design was optimized for use with Platinum *Taq* DNA polymerase (Invitrogen, USA) with an optimal T_m of 62ºC (Min 59ºC, Max 62ºC) and a maximum difference among primer pairs of 3ºC.

A total of 2 x 1,783,686 raw sequence reads were produced, with none flagged as poor quality. Sequence length ranged from 50 to 300 bp with a reported GC content of 40%. After screening, a total of 211 primer pairs were designed to amplify SSR regions with simple motifs of 2, 3, 4 and 6 base pairs. From this list, a total of 30 loci were selected as candidates and their respective primers were synthesized; all of them target SSR loci with trinucleotide, tetranucleotide and hexanucleotide motifs (Table 1). The Tail A sequence designed and reported by Blacket et al. (2012) was added to all forward primer sequences.

2.2.2. DNA Extraction, Amplification and Genotyping

DNA was extracted from 25mg of leaf tissue using the CTAB method described by Saghai-Maroof et al. (1984). Isolated DNA purity and concentration were measured using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, USA) and stored in TE buffer at -20ºC.

After testing the 30 candidate primer sets, we selected 15 markers that amplified successfully and were polymorphic (Table 1). Amplification conditions were standardized for these 15 SSR primer sets and all samples were amplified under the following cycling conditions: 15 min at 95°C; 35 cycles of 30 sec at 94°C, 90 sec at the standardized annealing temperature, 60 sec at 72°C; 5 min at 72°C. PCR products were labeled with a fluorescent dye incorporated in the universal Tail A primer using a three-primer system (Blacket et al., 2012). Amplification products were genotyped in an ABI 313 Genetic Analyzer (Applied Biosystems) by Macrogen (Seoul, South Korea). The resulting electropherograms were analyzed using GeneMarker v. 2.4.0 (SoftGenetics LLC).

Table 1. 30 microsatellite loci with forward and reverse primer sequences designed for *P. galapageium.*

Primers	**Forward Primer**	**F Primer Position**	**Reverse Primer**	**R Primer Position**	**Motifs(bases)**

GYB1	GTTAGGGTCGAAACAGTCCTAAACC	101	GTGATGGTCAACAGAGGGAAATAGG	333	AAAAAT(60)
GYB2	TTTGTCACCCTACATAATTCTTCCC	58	GGAGAGAGCGAGACAAGCATAGG	301	AAAGAG(54)
GYB3*	AAAAGTCAAATAAAGCCGCCTACG	28	AGGTTACTCTAATGCCCTTTCGG	460	AAAT(32)
GYB4*	GTAGGCTTTGAAAATTCTCATCCG	38	CTTATGGAGGCAAATTCGATCAGG	354	TTC(27)
GYB5*	CACAAGTATCGTGCTACCAAATCG	80	GGCGGATATGCAAATATATGATCG	370	TTC(27)
GYB6*	GCTACCTAAGTGTGGAAAGAGAACG	77	GGAAACCACTCGTGAGTATTACAGC	410	ATT(30)
GYB7*	GCAGGCAAGAACAGATAGAGATCG	60	TCTTCTCAACAACCAGATTCTCACC	322	TCC(33)
GYB8*	ATGGCCGGAAGAATCAAATCC	66	CATCTCTCATCTTGTTCCACATCC	347	ATT(39)
GYB9*	GCTCTGTTAATCTGGGCTTTGC	198	CTCCTTCACAAAATTCACACTGACC	442	TTC(30)
GYB10	ATGAGAGAAGTCAAAGCAAGGAACG	48	ATTCGCTCCCAAACTAATACACACG	311	ATT(39)
GYB11	AGTGAGAGTGGGTAAAAGTCAGTGC	29	GTGAGAAATTGGGGACTATATGGG	321	TTC(36)
GYB12	ACTATTGCTGCGACGTTCTTCC	29	ATGTATGCACCCTCTTGTTTTAACG	279	TTC(42)
GYB13	GATCAACCTGATCCTTGAAGTGG	81	TTATCGGTTAGTGCGTCTGAAAACC	272	ATT(27)
GYB14*	ATCCACTTTCATATCATGCAAGACC	37	TGCACAAATGTATCCTCTTAACTGC	280	ATT(36)
GYB15	TAAGCCTGGCCTTACTAAAATCACC	76	TATGACTTCGGAGGGACTGTGC	352	AAC(27)
GYB16	CCTGGTGCAGTACTTTCATTTATAAGC	52	CAATCATCAATTTTCGCTCTTACCC	452	TTC(33)
GYB17	AGTGAGTTCGTCAAGGCAAGG	74	GAAATTATTGACATGGACCTAACCG	329	ATT(36)
GYB18*	ACTGAGTTTCGATCGAGTCTATGGG	37	AGAGCCCTAAGGACTTAGAGAATGC	341	ATT(30)
GYB19	ACTGCTCAGCTCGTCTTCACC	24	TTAACAGAGGAGTTGAAGGCAAAGG	259	TGC(27)
GYB20	GAGGAAGCGATAGTGTATGTTGAGC	61	CACAAAGTCCCTTTGCTTTTGC	383	ATC(33)
GYB21*	CCGATTTCTGGTAAGGAGAGAGG	73	TGTTGTAGTTTGTAGGTCCATGTCG	286	TTC(33)
GYB22*	ATGAAGATCAACCTCTTCCATTGC	150	AGTAATGACTCCGGTCAGTCTTCC	388	TTC(27)
GYB23*	CTTAAATTTTCCGTCTCTCTCTTCG	102	ACAAATCCACGGTAATTGTATGAGC	381	ATT(30)
GYB24	GTGTTCAGAGACATTCTATCGTTGC	143	ATTCTAGAGCCGTGACTTGTTCTCG	370	TGC(30)
GYB25*	GCCAGCAAATCAAAATTATCCC	103	GGACCGATCAAATCTTCTAAACC	379	ATC(27)
GYB26	GGGGAATGAGCAAGAGAAAAGG	65	GTCATTCGTGGTAGAAGTTATTCGG	389	TTC(27)
GYB27*	TCTTGATGAACCAAGACCTACTGC	69	CCAAACACAAGATAACGAACTTTCC	330	ATT(36)
GYB28	GACCAGTGAATAAAAGAGTTTGTGC	50	TTGTTGAAGTGGAAAGAGAGAAACG	313	ATC(36)
GYB29*	TTTGTCGGGACTCTCTAATGGC	103	GAGGTTACGTAGAATTCTTGATGTGC	356	TTC(27)
GYB30	GGTCAAGCAAAAGAGAGAAATGC	30	TTTCTTGTCTTTCGTGATTCCG	197	TGC(27)

*Selected loci for present study

2.3. Statistical analyses

There is limited information regarding the ploidy of *P. galapageium*. Our observations from allele scoring suggests that up to four alleles can be found for any given locus in a single individual (Fig. A1). However, 3 out of the 15 loci analyzed presented two alleles, this leads us to assume an unbalanced polyploidy which can be an indicator of allopolyploidy (Singhal et al., 1985). Furthermore, hybridization has often been observed in the *Psidium* genus (Landrum, 2017). Given these observations and the reported polyploidies in several members of the *Psidium* genus (Tuler et al., 2019), we treated *P. galapageium* as an allotetraploid and used the *polysat* package (Clark and Jasieniuk, 2011) for R (R Core Development Team, 2015) to assign alleles to different isoloci (2 isoloci per locus), thereby allowing us to process the data as diploid (Clark and Schreier, 2017). Isoloci assignment in *polysat* was performed considering all the 15 amplified SSR loci, leading to a total of 30 potential isoloci.

Null allele frequencies for each isolocus were calculated through the De Silva method (De Silva et al., 2005) implemented in *polysat*. This method requires an estimated selfing rate which is unknown in guayabillo (although the frequent occurrence of perfect flowers in this species suggests its possibility; Porter, 1968). Therefore, we used two reported selfing rates (0.5 and 0.65) from the closely related *P. guajava* (Sitther et al., 2014). Monomorphic isoloci (isolocus GYB5_2), isoloci with null allele frequencies >>0.3 given both selfing rates employed (GYB14_1, GYB14_2, GYB18_1, GYB18_2, GYB27_2), and loci that were not assigned to isoloci with an acceptable clustering quality (GYB6, GYB25) were excluded for allele frequency calculations and downstream analyses that depend on allele frequencies. Thus, from the 15 SSR loci originally amplified, we used 13 loci (from which we derived 20 informative isoloci) to describe the population genetics of our data set.

GenoDive (Meirmans and Van Tienderen, 2004) was used to determine if the analyzed guayabillo populations deviated from Hardy-Weinberg Equilibrium (HWE). The *p*-values obtained from the HWE test were corrected using the B-Y correction.

We used the *adegenet* package in R (Jombart and Ahmed, 2011) to determine the total number of alleles for each isoloci. Private alleles were calculated with the *poppr* package (Kamvar et al., 2014), and allelic frequencies were obtained with *polysat* using the “simpleFreq” function. Allelic richness corrected through rarefaction for different sample sizes was performed with the *basicStats* function from the diveRsity package, assuming 35 individuals sampled for all populations (Keenan et al., 2013). Significant differences among the allelic richness of different island populations were assessed with Kruskal-Wallis and Pairwise Wilcoxon tests. The same *polysat* package was used to calculate the observed and expected heterozygosity, PIC, Lynch distances and pairwise F_{ST} between islands and between sampling locations on each island, from matrixes created after isoloci reassignments. For assessing inbreeding in guayabillo, we calculated F_{IS} for each population using the calculated H_o and H_e values. Pairwise F_{ST} between clusters found when we conducted PCoA and STRUCTURE analyses (below) were also estimated.

We also performed an analysis of molecular variance (AMOVA) to evaluate the population differentiation between island populations in GenAlEx (Peakall and Smouse, 2012), encoding all 15 SSR markers as binary data. A Principal Coordinates Analysis (PCoA) based on Lynch distances was also plotted using *ggplot2* (Wickham, 2009).

We performed an analysis of population structure using the STRUCTURE software (Pritchard et al., 2000) following the parameters described in Meirmans et al. (2018) for dealing with polyploid data. We estimated the population structure for both the complete data set and for each island individually, using the same parameters. We evaluated between 1 and 10 potential genetic clusters (*K*) and performed 10 independent replicates for each *K* value, consisting of $1x10^6$ MCMC steps with a $1x10^5$-step burn-in period. The STRUCTURE Harvester software (Earl and von Holdt, 2012) was employed to evaluate the optimum value of *K* using the Evanno method (Evanno et al., 2005). We used CLUMPP to estimate individual membership coefficients (Jakobsson and Rosenberg, 2007), and plotted them using the DISTRUCT software (Rosenberg, 2004). A plot of the relative migration levels between the three island populations was obtained by applying the Sundqvist et al. (2016) method implemented in the *divMigrate* function from the diveRsity package (Keenan et al., 2013).

Due to differences in the number of samples obtained from each island, we created subsamples for Isabela and Santa Cruz to match the San Cristobal sample size. To do so, we selected 35 individuals from Isabela and 35 from Santa Cruz (we included one random sample from each location) After this systematic downsampling, we repeated all the previously described analyses.

We used the following method in order to assigning and detecting clones: we calculated genetic distances assuming asexual reproduction under the SMM, as implemented in the GenoDive software. Missing data and unknown allele dosage were ignored. The genetic distance threshold used to classify individuals as clones (a distance of 7.0) was determined using the method suggested by Rogstad et al. (2002); note this threshold should not be equal to 0 due to the fact that mutations and genotyping errors may make identical individuals have slightly different genotypes (Meirmans and Van Tienderen, 2004). Specific clones per island were obtained. A test of clonal diversity was performed using Nei's corrected genetic distance as summary statistic, using 999 permutations and sorting alleles over individuals within populations. Finally, clonal diversity statistics were calculated in GenoDive and bootstrap tests were performed to detect significant differences among shc (sample size-corrected Shannon index values) in different islands (999 permutations); *p*-values were corrected using the B-Y method. Clonal richness (R) was also calculated for each island and overall, as R = (G−1)/(N−1) where G is the number of genotypes detected under the established genetic distance threshold, and N is the total number of samples.

3. Results

3.1. Marker information and genetic diversity

All 208 individuals in our sample set were genotyped and included in our analyses. All of the original 15 markers amplified deviated from HWE after B-Y correction, except for locus GYB25 in the Isabela population, and GYB04 in the Santa Cruz population. The information content for the 13 markers used for data analysis (parsed as 20 isoloci) was measured through their Polymorphic Information Content indices (PIC) and ranged between 0.006 and 0.808 (only two markers showed PIC values under 0.3), with low inferred null allele frequencies (with the exception of the excluded isoloci described in Materials and Methods; Table A2). Various descriptors of genetic diversity were estimated for the populations of each of the three sampled islands (Table 2), showing similar patterns between the populations of Isabela and Santa Cruz. Compared to the Isabela and Santa Cruz populations, the San Cristobal population shows a smaller number of alleles, of private alleles and both, lower observed and expected heterozygosities (H_O and H_E respectively), with a higher F_{ST} fixation index. While a smaller sample size for San Cristobal (N=35, compared to N=86 in Isabela and N=87 in Santa Cruz) may account for some of these lower observed values, our downsampled analyses (i.e. reducing the samples from Isabela and Santa Cruz to maintain a constant sample size for all three; see Materials and Methods and Table A3) show a consistent reduction in the numbers of alleles (A) and private alleles (PA) for the Santa Cruz and Isabela populations, and a reduction in the observed heterozygosity for the Isabela population, but not sufficient to match the San Cristobal population H_E estimates; this supports our finding of a lower genetic diversity on this island. The same trend is maintained when assessing allelic richness (AR) corrected thought rarefaction among the three island populations, with a higher richness in Isabela, followed by Santa Cruz and finally San Cristobal. The difference in AR was significant between Isabela and the other two islands, both Santa Cruz (B-Y corrected Pairwise Wilcoxon test, *p*=0.045) and San Cristobal (*p*=0.026); nevertheless, rarefaction-corrected AR did not show significant differences among Santa Cruz and San Cristobal. Inbreeding coefficients (F_{IS}) were high for the three island populations (especially Isabela and Santa Cruz) and overall for the whole dataset.

Table 2. Genetic diversity information of the analyzed *Psidium galapageium* populations from Isabela, Santa Cruz and San Cristobal islands: Number of individuals genotyped from each island (N), number of alleles found (A), number of private alleles (PA), rarefaction-corrected allelic richness (AR), observed heterozygosity (H_O), expected heterozygosity/gene diversity (H_E), F_{ST} global value for each island population, and inbreeding coefficient (F_{IS}). Overall results along the three islands are also shown.

Island	**N**	**A***	**PA***	**AR^s**	**H_O ^{a}**	**H_E ^{a}**	**F_{ST}**	**F_{IS}**
Isabela	86	142 (89)	77 (35)	12.29	0.147	0.570	-0.173	0.742
Santa Cruz	87	105 (67)	26 (8)	9.97	0.157	0.426	0.085	0.631
San Cristobal	35	70 (60)	5 (1)	8.20	0.119	0.275	0.286	0.567
Overall	208	174	-	10.15	0.141	0.482	0.230	0.708

* Values between brackets are the number of alleles or private alleles with a frequency >0.05 within the corresponding island population.

[a]Indicates average across all the SSR loci analyzed.

[s]Standardized through rarefation for N=35

3.2. Genetic differentiation of guayabillo populations

The genetic differentiation between islands, evaluated through pairwise F_{ST} genetic distances, shows a greater divergence between the San Cristobal and Santa Cruz populations, while Isabela remains equally divergent from both (Table 3). This pattern is observed with both the full and reduced data with normalized sample sizes (Table A4). Furthermore, the clustering of individuals based on Lynch genetic distances shows that the individuals from Santa Cruz are represented by two groups: a first group clearly separated from all the rest of individuals (henceforth referred to as Santa Cruz 1; Fig. 2, Fig. A2), and a second group clustering closely with individuals from Isabela and San Cristobal (henceforth referred to as Santa Cruz 2; Fig. 2, Fig. A2). This second group includes individuals from three different locations on Santa Cruz: Granillo Rojo, Garrapatero and Bellavista (Fig. A3). It should be noted, however, that the degree of population differentiation between islands appears to be limited: an AMOVA reveals that the majority of the genetic variation (72%) occurs within populations, and 28% of the variation occurs between Isabela, San Cristobal and Santa Cruz (Table 4).

Table 3. Pairwise and global F_{ST} values between the *Psidium galapageium* populations from the three islands.

	Isabela	**Santa Cruz**
Santa Cruz	0.164	-
San Cristobal	0.178	0.218

Global	0.230

Table 4. Analysis of molecular variance (AMOVA) between the three island populations of *Psidium galapageium*.

Source	DF	SS	MS	Est. Var.	%
Among Pops	2	691.79	345.90	5.12	28%
Within Pops	205	2653.82	12.945	12.95	72%
Total	207	3345.62	-	18.06	100%

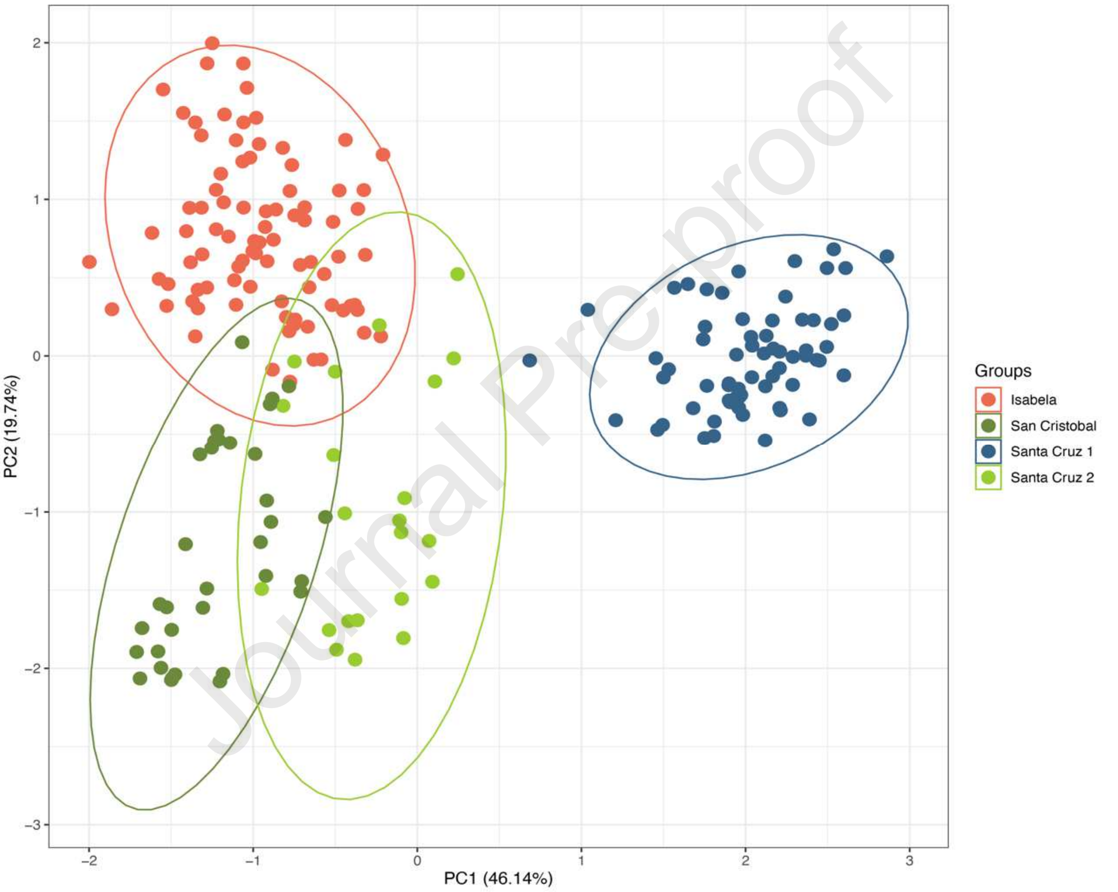

Fig. 2. PCoA based on the Lynch distances found between the *Psidium galapageium* individuals sampled in the three islands: Isabela, San Cristobal and Santa Cruz. For Santa Cruz, both genetic clusters are indicated (Santa Cruz 1 and Santa Cruz 2).

3.3. Population structure

To explore the population structure under an admixture model, the assignment coefficients for all individuals were estimated for different numbers of putative lineages, revealing higher similarities between individuals from Isabela and San Cristobal, in concordance with the clustering by genetic distances. The individuals from Santa Cruz display a greater contribution from a separate genetic stock, with some individuals showing similarities to the Isabela and San Cristobal populations (Fig. 3a). An evaluation of the optimum number of clusters that fit the data suggests that three putative lineages are observable in our data (*K*=3; Δ*K*=289.55), which highlight a closer resemblance between the genetic composition of the Santa Cruz outliers and the San Cristobal population (Fig. 3b). Overall, the three genetic lineages are determined by island, as would be expected given the physical separation and isolation between these populations. A similar analysis with the downsampled data (Fig. A4) reveals no observable differences when compared to the full data set.

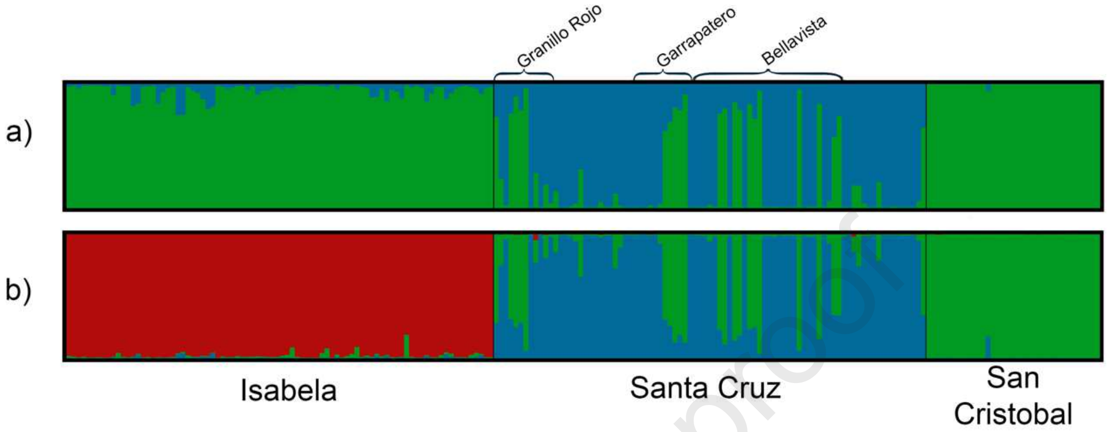

Fig. 3. Results of the Bayesian analysis of population structure (Software STRUCTURE) under the Admixture model. The results are indicated for a) K=2 (ΔK=134.51), and b) K=3 which is the optimum K value (ΔK=289.55). These values of K correspond to the clusters or lineages (represented by different colors) in which are grouped the *Psidium galapageium* individuals sampled in Isabela, Santa Cruz and San Cristobal islands. The Santa Cruz sampling sites of Granillo Rojo, Garrapatero and Bellavista (which mostly harbor individuals from the Santa Cruz 2 cluster) are marked as well.

We noted that the groupings observed through Bayesian inference in STRUCUTRE and the clusters observed in the PCoA were equivalent, reliably defining the main genetic groups in the guayabillo populations of the three islands. Pairwise F_{ST} values were calculated among these genetic groups, considering each island population individually, and including the Santa Cruz 1 and Santa Cruz 2 groups as separated entities as well. Here, the highest genetic differentiation was detected among the Santa Cruz 1 population and the populations of the other two islands: Isabela and San Cristobal. Furthermore, an important genetic differentiation was observed between the two Santa Cruz groups, comparable even to the values found among populations from different islands (Table A5).

Bayesian population structure analyses were conducted for each island. When analyzing the Isabela and Santa Cruz populations, no distinguishable population structure within each island was observed, suggesting widespread gene flow and an ancient shared history within each island (Figs. A5 and A6, respectively). The optimum K value (K=2; Fig. A6a) shows two lineages in Santa Cruz island, matching the Santa Cruz 1 and Santa Cruz 2 groups found in the PCoA (Fig. 2); however, this pattern is less clear at higher K values (Fig A6 b-d). Finally, a more distinguishable structure is observed in San Cristobal at K=2 and above, with individuals from any given sampling location tending to share their genetic background (Fig. A7).

Although limited, some migration could exist between the guayabillo populations from different islands. The relative migration analysis showed that most of the gene flow is directed towards Santa Cruz from both Isabela and San Cristobal. Outgoing migration from Santa Cruz and among Isabela and San Cristobal appears less prevalent, representing approximately half or less of that observed towards Santa Cruz (Fig. 4).

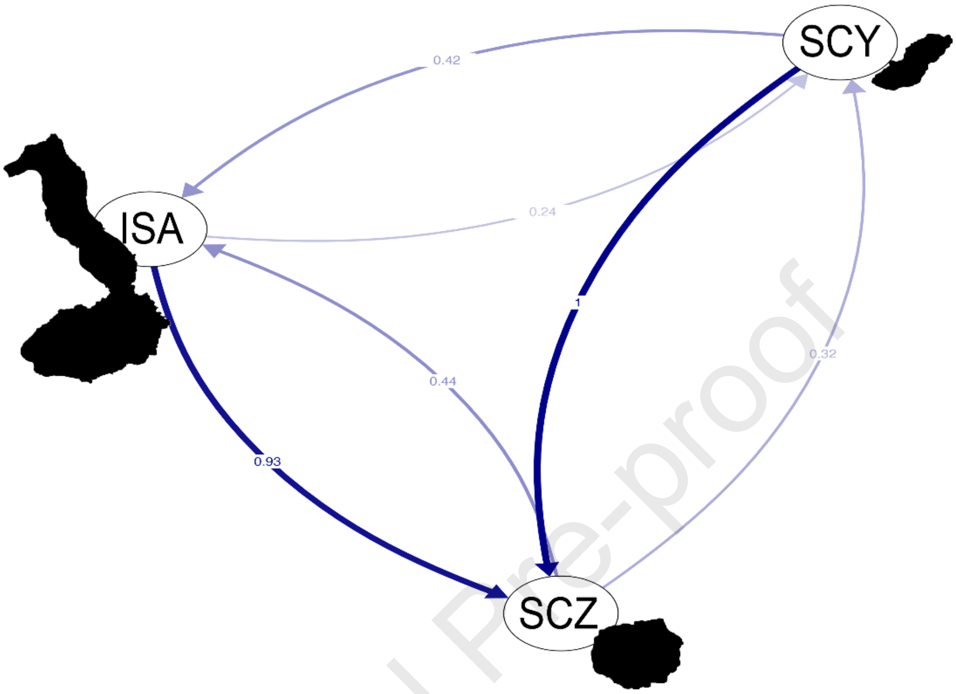

Fig. 4. Relative migration among the guayabillo populations from Isabela (ISA), Santa Cruz (SCZ) and San Cristobal (SCY) islands.

3.4. Clonal assignments and clonal diversity in guayabillo

A total of 201 different unique multilocus genotypes were identified in our dataset, and 11 of the 208 analyzed guayabillo individuals (5.28%) were identified as clones of another individual; a lower number of unique genotypes was obtained when considering the effective number of genotypes, nonetheless they are still considerable when taking into account the total number of individuals analyzed. Clonal richness and sch values over the three populations were relatively high in general terms. Nevertheless, the San Cristobal population had the highest number of individuals sharing the same multilocus genotype (with up to five individuals having the same genotype in one case); individuals assigned to the same clone in San Cristobal belonged to different sampling locations. On the other hand, only two individuals with the same genotype were found in Isabela, as well as in Santa Cruz (coexisting in the same sampling location in both cases). Similarly, the Isabela and Santa Cruz populations showed higher clonal richness and shc values than San Cristobal (Table 5); these differences in shc were significant (Isabela vs. San Cristobal: *p*=0.003; Santa Cruz vs. San Cristobal: *p*=0.003).

Table 5. Clonal diversity statistics for the three studied island populations, and overall values: Number of individuals genotyped (N), number of clones or unique genotypes detected under the established genetic distance threshold (G), clonal richness (R) and Shannon diversity index for

genotypes, corrected for sample size (shc). Calculations were performed twice: using the SSR genotyping directly without allele dosage correction for polyploids, and then using the genotypes corrected for allele dosage in polyploids.

Island	N	G (eff)*	R	shc
Isabela	86	85 (84.0)	0.988	3.564
Santa Cruz	87	86 (85.0)	0.988	3.574
San Cristobal	35	30 (21.5)	0.853	2.111
Overall	208	201 (190.5)	0.966	3.083

*Values between brackets correspond to the effective number of genotypes (G).

Finally, we found low statistical support for the hypothesis that the observed clonal diversity is explained by random mating in the three populations (Isabela: *p*=0.007; Santa Cruz: *p*=0.001; San Cristobal: *p*=0.001). This suggests that the observed clonal diversity patterns are not due to sexual reproduction; therefore, the occurrence of the same multilocus genotype in more than one individual is explained more likely by clonal or asexual reproduction rather than by sexual reproduction among related individuals.

4. Discussion

4.1. Genetic diversity in guayabillo and its contributing factors

With an overall H_E of 0.482, guayabillo showed a relatively high genetic diversity for a species endemic to an insular ecosystem (Table 2), as seen in other widespread insular plant species, such as the Galapagos endemic *Gossypium darwinii* (Wendel and Percy, 1990), the Hawaiian endemic *Metrosideros polymorpha* (Crawford et al., 2008), and *Periploca laevigata* from the Canary and some Mediterranean Islands (García-Verdugo et al., 2015). Care should be taken when comparing diversity levels among different species due to the numerous factors influencing them and the distinct molecular techniques used for analysis (Fernandez□Mazuecos et al., 2014; García-Verdugo et al., 2015; Guzmán et al., 2016). Even so, all the previously mentioned studies, along with many others (see García-Verdugo et al., 2015), set a trend in which insular plant species, including several endemics, show a relatively high genetic diversity, contrary to what is expected considering founder effects and genetic drift over small founding populations (Frankham, 1997; Whittaker and Fernández-Palacios, 2007). A widespread distribution, such as guayabillo's (occupying different islands and different vegetation zones within the Galapagos archipelago; McMullen, 1999; Valdebenito, 2018), could be a very important factor that contributes to this relatively high diversity level. A widespread distribution usually leads to populations with bigger effective sizes, which in turn tend to harbor more genetic variability while being more resilient to genetic drift (MacArthur and Wilson, 1967; Frankham, 1996; García-Verdugo et al., 2015; Costanzi and Steiffeten, 2019). Nevertheless, in the Galapagos there are endangered species with more restricted distributions such as *Galvezia leucantha* (Guzmán et al., 2016) and *Calandrina galapagosa* (Jaramillo et al., 2011), that still possess a relatively high genetic diversity. Then, other factors such as the relative absence of herbivores and competitors (Stuessy et al., 2014; García-Verdugo et al., 2015; but see Whittaker, 1998 and Nielsen, 2004 concerning introduced species), along with the tropical location and wide variety of climatic patterns and vegetation zones in the Galapagos (Kricher, 2006; Jaramillo et al., 2011), could also contribute to the maintenance of the genetic diversity of Galapagos' endemic plants regardless of their distribution range.

The physical characteristics of each island where guayabillo is found could be associated to its relatively high genetic diversity as well (Stuessy et al., 2014). Larger islands with broader altitudinal ranges host greater habitat heterogeneity (MacArthur and Wilson, 1967; Buckley, 1985; Geist et al., 2014), which in turn can favor genetic variability in a wide-distributed species as it adapts to new niches (MacArthur and Wilson, 1967; Stuessy et al., 2006; Chapman et al., 2013). In fact, morphological variation among guayabillo populations along the altitudinal gradient where the species is distributed has been observed (Valdebenito, 2018). Large island surface areas also translate into a greater capacity to host bigger populations with more genetic diversity (MacArthur and Wilson, 1967; Frankham, 1997; Costanzi and Steiffeten, 2019). In this regard, high levels of genetic diversity in the Hawaiian silverswords (Witter and Carr, 1988) and in *G. darwinii* (Wendel and Percy, 1990) were explained in part by their large population sizes. A similar scenario might be suggested for guayabillo, since the highest genetic diversity was found in Isabela island (Table 2; Table A3), which is the largest and most elevated island in the Galapagos archipelago (even if we only consider the southern part of the island where guayabillo is found) (Instituto Geofisico, n/d; Charles Darwin Foundation, 2012; Geist et al., 2014). Although our sampling covered a narrow range of the total altitudinal range (109-386 m.a.s.l.), this pattern is still observed. On the other hand, the San Cristobal population presented the lowest genetic diversity among our sampling sites (Table 2; Table A3), coinciding with the island's smaller size and narrower altitudinal range (Latorre, 1991; Charles Darwin Foundation, 2012); our guayabillo samples cover approximately half of this range (71-310 m.a.s.l.). Finally, Santa Cruz, where we obtained an intermediate H_E but not a higher AR than San Cristobal, constitutes an intermediate altitudinal and land mass range between Isabela and San Cristobal (Grenier, 2007; Charles Darwin Foundation, 2012). These general trends are not surprising and have also been observed in the Galapagos endemics *S. affinis* (Nielsen, 2004) and *G. darwinii* (Wendel and Percy, 1990), which showed greater genetic variation in Isabela compared to smaller and lower islands as Santa Cruz, Floreana (both species) and San Cristobal (*G. darwinii* only). A greater abundance and genetic diversity have also been reported for the endemic tomatoes *S. cheesmaniae* and *S. galapagense* in western islands like Isabela, something that was also tentatively attributed to the unusually high precipitation for this part of the archipelago (Rick and Fobes, 1975; Pailles et al., 2017). Knowing that plant richness is positively linked with precipitation in the tropics (Gentry, 1982), this could also explain the greater diversity observed in the Isabela guayabillo population, as well as in the case of other endemic plants. Thus, despite being one of the youngest islands in the Galapagos (Geist et al., 2014), Isabela would present certain conditions that favor diversity in endemic plant species, though other factors should also be considered when interpreting these genetic diversity patterns.

The limited available evidence suggests a complex evolutionary history for the guayabillo which may also partially explain the genetic diversity patterns observed in the species. Firstly, guayabillo could be a polyploid species, as suggested by our genotyping, where up to four different alleles were observed for several loci (Fig. A1). Furthermore, morphological studies point to guayabillo being very phenotypically similar to the hybrids between two mainland close relatives: *Psidium oligospermum* and

P. schenckianum (Landrum, 2017); Landrum (2017) also hypothesized that the hybrids of these two species were able to spread all over tropical America following the hybridization event, opening the possibility that they may have reached the Galapagos. With these antecedents, the Galapagos guayabillo could tentatively be an allopolyploid, with *P. oligospermum* and *P. schenckianum* as putative parental species, a hypothesis that could be confirmed through phylogenetic analyses of the *P. oligospermum* complex. In any case, hybridization is quite frequent in the *Psidium* genus (Machado-Marques et al., 2016; Landrum, 2017), and this potential allopolyploidy in guayabillo could also be one of the reasons behind its relatively high genetic diversity. Allopolyploids show a tendency towards higher heterozygosity and genetic variability levels compared to diploids as they draw from the gene pools of two separate species, which might be the case if guayabillo is in fact an allopolyploid (Soltis and Soltis, 2000; Chen and Ni, 2006). Moreover, previous hybridization and allopolyploidy have been tightly associated with the success of the colonization of oceanic islands by plants (Barrier et al., 1999; Wendel and Cronn, 2003; Madlung, 2013). These ideas could also be supported by the high genetic diversity found in the widespread tetraploid *G. darwinii*, for example (Wendel and Percy, 1990). However, polyploidy is not a requisite or a guarantee for high genetic diversity levels. For instance, the Galapagos endemic *Opuntia* cacti are hexaploid and still display low genetic diversity levels (Helsen et al., 2009). Likewise, there are diploid insular plant species that show moderate to high levels of genetic diversity (e.g. Crawford et al., 2008; Takayama et al., 2013; Takayama et al., 2015). Therefore, polyploidy is not the only aspect of evolutionary history that should be addressed for interpreting the genetic diversity observed in guayabillo.

The mechanism of speciation may also be important to explain the genetic diversity in insular plant populations (Stuessy et al., 2014; Takayama et al., 2015). Cladogenesis, for instance, generates several daughter species, each one with low levels of genetic diversity as observed in the endemic *Opuntia* cacti from different islands in the Galapagos archipelago (Helsen et al., 2009). On the other hand, guayabillo has not been reported to split into separate species in different islands (Porter, 1968; McMullen, 1999); this is also supported by our genetic data. Even though we observed a genetic structure between different islands and a limited inter-island gene flow (Fig. 3), we do not have evidence to claim the populations from Isabela, Santa Cruz and San Cristobal are distinct species. Firstly, the pairwise F_{ST} values among island populations, though high, are not high enough to reach that conclusion (Table 3; Table A5). Secondly, we would have expected a higher percentage of the total diversity explained by diversity among islands if they were different species (Table 4). Finally, most of the individuals, including samples from distinct islands, are clustered together in the PCoA (with the only exception of the Santa Cruz 1 group; Fig. 2). In consequence, the history of guayabillo aligns with the speciation mechanism of anagenesis, where different processes such as mutation accumulation, recombination, and local adaptation would have created more and new genetic diversity which was kept in a single species (Stuessy et al., 2006; Takayama et al., 2015; Stuessy et al., 2014). Other species in the Galapagos also continue to be a single species despite having populations separated in different islands; *G. leucantha* for example, keeps a moderate-low genetic differentiation even among islands, leading to a high genetic and morphological diversity within this species as a whole (Guzmán et al., 2016).

The reproductive biology of the species is also important to understand the genetic diversity patterns (Stuessy et al., 2014), yet it is poorly understood for guayabillo (Valdebenito, 2018). Complementary research on outcrossing-selfing rates, pollinization, seed dispersal and germination rates is required to determinate the effect of these factors on the genetic diversity of guayabillo. However, our genetic data could shed some light on these topics. To begin with, we found low H_O values compared to H_E, as well as high F_{IS} values in all the three islands (Table 2), which suggests that inbreeding and/or selfing (guayabillo has bisexual flowers and selfing is concurrent in the *Psidium* genus) in all guayabillo populations could be prevalent (Wright, 1951; Loeschcke et al., 1994; Frankham, 1998; Sitther et al., 2014). Guayabillo is also known to reproduce through root suckers which may lead to clones (Aldaz, 2008); however, we made sure to sample physically distant individuals to avoid the collection of this type of clones. No direct studies have been performed to test other kinds of clonal reproduction in guayabillo such as apomixis. In any case, our results do show a couple of individuals from distant locations sharing the same multilocus genotype. These cases appear to be sporadic, and most of the sampled individuals represent unique genotypes (Table 5). Similarly, guayabillo presents higher clonal diversity levels than other plants which are actually known to reproduce clonally such as *Ziziphus celata* (Gitzendanner et al., 2012) and *Trillium recurvatum* (Mandel et al., 2019). Finally, indirect evidence of outcrossing in guayabillo was obtained through the observation of flowers being visited by the Galapagos carpenter bee *Xylocopa darwinii* (Valdebenito, 2018), also an important pollinator for several other endemic plants of the Galapagos (see Jaramillo et al., 2014; Guzmán et al., 2016). Likewise, the higher within-island diversity compared to the between-island diversity (Table 4) aligns with what would be expected for a cross-pollinating plant species, despite the non-negligible 28% of among-island diversity. Thus, in light of previous descriptions of the ecology of the species (Aldaz, 2008; Valdebenito, 2018), our genetic data suggests that guayabillo might combine different reproductive mechanisms including selfing, outcrossing, and clonal reproduction in a lower extent; the common guava, a close relative of the guayabillo, shows similar reproductive mechanisms (Urquía et al., 2019). This combination could also explain the relatively high genetic diversity we found in guayabillo, as well as its success in colonizing the Galapagos archipelago. Clonal reproduction and selfing would have aided in the fast spread of the species over the islands during the first stages of the colonization, while the increasing population sizes solidified the endurance of high-fitness genotypes (Pluess and Stöcklin, 2004; Silvertown, 2008). In fact, self-compatibility would be the general rule for insular plants as it is essential for the establishment in new islands (Baker, 1955; Chamorro et al. 2012). However, this kind of reproduction is known to reduce genetic diversity and drive inbreeding depression (and its associated consequences such as disease susceptibility and low mate availability; Kwak and Bekker, 2006, Honnay and Jacquemyn, 2008), as seen in the highly autogamous endemic tomatoes *S. cheesmaniae* and *S. galapagense* (Rick, 1983; Pailles et al., 2017). Hence, thanks to its potential capacity for combining asexual reproduction and outcrossing, guayabillo would have also been able to maintain its genetic diversity and a wide variety of clones and genotypes, retaining the species' adaptive potential while keeping the advantages of clonal spread (Ward et al., 2008). This hybrid system would be beneficial in fluctuating and unpredictable environments, characteristics that recall the nature of the Galapagos Islands (Bengtsson, 2000; Silvertown, 2008; Capotondi et al., 2015). Thus, it is not surprising that other plants in the Galapagos such as *C. galapagosa* also hold considerable levels of genetic diversity, perhaps through outcrossing (Jaramillo et al., 2011), while *Lycium minumum* developed sexual dimorphism to equilibrate self-compatibility and outcrossing (Levin et al., 2015).

A case could also be made regarding the effects of human disturbance in the genetic diversity of the species, particularly for the San Cristobal population. The three studied islands host permanent human populations that in some cases use guayabillo as a source of wood. Moreover, these islands also sustain agricultural areas in their highlands, where local-scale cultivation and animal husbandry activities are developed. However, San Cristobal contains the largest agricultural area relative to its size, occupying the majority of its humid highlands (Rivas-Torres et al., 2018), and one of the oldest permanent settlements in the archipelago established during the second half of the XIX century (Latorre, 1997; Lundh, 2004). These events could have affected the guayabillo populations disproportionately when compared to the other islands. Santa Cruz was colonized more recently by humans, producing a milder historical disturbance (Kricher, 2006; PNG, 2016), although it currently hosts a larger human population (INEC, 2016). On the other hand, Isabela sustains the smallest agricultural area in proportion to island size (Rivas-Torres et al., 2018) and the smallest human population (INEC, 2016). Besides the direct impact on effective population size and genetic diversity decrease (Stuessy et al., 2014) as may be the case for San Cristobal, another direct effect of these anthropogenic activities is the fragmentation of habitats. Fragmentation can lead to genetic drift (Frankham et al., 2010), endogamy, and inbreeding depression (Wright, 1951; Frankham, 1998; Nielsen, 2004), and could partially explain the higher within-island F_{ST} value observed for the San Cristobal population (Table 2). Fragmented and decimated populations also experience a fast fixation of alleles, and populations within fragments risk differentiating to the point of sexual incompatibility (Gitzendanner et al., 2012). It is also noteworthy that the San Cristobal population presented a lower clonal diversity and a higher proportion of individuals sharing the same genotype compared to the other two islands (Table 5). This could be a consequence of the depauperated genetic diversity in this population, leading to less alleles and therefore, less possible genotypes (Jaramillo et al., 2011). *S. affinis* might represent a similar scenario to guayabillo in San Cristobal: habitat loss and intensive grazing by donkeys and goats has reduced the Floreana and Santa Cruz *S. affinis* populations leading to a genetic diversity decrease (Nielsen, 2004). Likewise, habitat loss and aggressive herbivory from introduced animals has fragmented and decimated the populations of *C. galapagosa* (Jaramillo et al., 2011) and *G. leucantha* (Guzmán et al., 2016).

Since sampling can be unfeasible in some of the smaller and more remote islands, this study could have excluded a segment of the guayabillo genetic diversity. The assessment of these unsampled populations remains to be performed in order to get a complete picture of the diversity and evolutionary history of the species across the entire archipelago. However, our sampling covers the islands with the biggest surface areas and that represent a considerable portion of the species distribution, illustrated by the fact that the surface area of the sampled islands exceeds the combined area of all the non-sampled islands were guayabillo populations have been reported. We are therefore confident that our results are representative of a major component of the genetic diversity of the species, and wouldn’t expect major changes in the diversity patterns observed if the smaller populations were included. Furthermore, the populations included in this study have direct contact with human settlements, and are hence interesting for conservation purposes. Regarding this latter point, our findings present a positive outlook for guayabillo in general. The relatively high levels of genetic diversity found in this species suggest that these populations show some potential resilience to environmental perturbations (Reusch et al., 2005; Jump et al., 2009). An increased adaptive potential would certainly be an asset for the species in face of threats such climate change and habitat alteration associated to human activities (Adsersen et al., 1988; Whittaker, 1998; Tye et al., 2007; Dal Forno et al,, 2017); however, the survivability of any species is not determined exclusively by its genetic adaptive potential, and other factors must be better studied to understand the conservation status of guayabillo in the archipelago. For instance, the interactions between guayabillo and multiple invasive plant species in the Galapagos, particularly those found in the highlands and transitional forests such as blackberries and Cuban cedars (*Cedrela odorata*) (Sakai et al., 2001; Tye et al., 2007), remain unknown. A particular emphasis should be placed on the invasive common guava due to its close relatedness to guayabillo and the fact that they share similar distributions, life history traits, pollinators and dispersers (Blake et al., 2012; Valdebenito, 2018). The high frequency of hybridization events in the *Psidium* genus (Landrum, 2017) should also be considered, as this combination of factors might facilitate the generation of (currently unreported) interspecific hybrids (Torres and Gutiérrez, 2018). This phenomenon can lead to genetic erosion, outbreeding depression, and genetic swamping in the guayabillo (López-Caamal et al., 2014; Ellstrand and Rieseberg, 2016; Chafin et al., 2019) while enriching the currently low genetic diversity of the guava populations of the Galapagos, further enhancing its invasive potential (Urquía et al., 2019). Such a case has already been reported in an insular *Psidium* endemic, *P. socorrense*, where hybridization with an introduced close relative took place in a particular zone of Socorro Island (López-Caamal et al., 2014).

4.2. Population structure and connectivity between islands

The observed patterns of genetic diversity do not necessarily match the population structures in different islands. Santa Cruz is the only island where two clearly separated genetic clusters were found (Figs. 2, 3, A2 and A4), while the populations in Isabela and San Cristobal behave as a single panmictic population. One of these Santa Cruz clusters, Santa Cruz 2, was exclusively made up of individuals from sampling sites within the transition zone (Granillo Rojo), the dry lowlands (Garrapatero) and some individuals from the Bellavista site (which is closely located to Garrapatero). On the other hand, Santa Cruz 1 predominantly included individuals from the humid highlands and the agricultural zone (Figs. 3, A3 and A6). These clusters may correspond to two different guayabillo ecotypes, a more generalist ecotype (Santa Cruz 1) and a dry climate ecotype (Santa Cruz 2) adapted to the transition zones and the lowlands. Interestingly, Valdebenito (2018) observed morphological differences among guayabillo individuals from the highlands and the lowlands in San Cristobal, (monopodial trees in the highlands, smaller shrubs in the lowlands); more significantly, lowland individuals would flower earlier, which could represent a temporal reproductive barrier between them and highland individuals. Although we did not identify different genetic groups in San Cristobal as we would have expected from previous observations, it highlights the possibility of two ecotypes in Santa Cruz; phenological and morphological studies of guayabillo in this island are currently being carried out (Valdebenito, pers. comm.), and they would certainly elucidate our hypothesis. This would entail a degree of genetic differentiation (observed as a high proportion of within-population variability in the AMOVA; Table 4) and adaptation to different climatic and ecological niches, phenomena which cannot be further explored with our current data. More in-depth research into the population genetics and ecology of the species in this island is essential to determine whether the concept of an ecotype might apply to this scenario. The emergence of different ecotypes and even parapatric speciation along environmental gradients have been previously reported in plants, such as the two sister species of the genus *Senecio* distributed along different altitudes at Mount Etna in Italy which may have arisen through these mechanisms (Chapman et al., 2013; Chapman et al., 2016).

It is also interesting to point out the genetic similarities observed in the PCoA between Santa Cruz 2 and the individuals from San Cristobal and Isabela (Figs. 2, A2 and A3). This could be interpreted as a link between Santa Cruz 2 and the populations on the other islands, particularly in San Cristobal (see Fig 3b). Under this scenario, the Santa Cruz 1 lineage would have naturally diverged from the other populations on different islands (Table A5), while the Santa Cruz 2 represents a more recent introduction. Given this possibility, Santa Cruz 2 (or its ancestors) could have adapted to the drier habitats before reaching Santa Cruz, helping it to settle into its current distribution (it would be expected that, upon arriving to a new island, plants would first encounter the more arid habitats in the lowlands near the coast; Kricher, 2006; Rivas-Torres et al., 2018). If this genetic connectivity between Santa Cruz 2 and the other islands is not spurious, the previously described population structure would be better explained by this rationale rather than a local adaptation to different environments, or through a combination of both scenarios. Note that Santa Cruz 1 appears surprisingly distinct, even compared to other individuals of Santa Cruz (Figs. 2 and A2; Table A5). Before, we supported the unification of guayabillo as a single species (Section 4.1), and this seems to be true even for this separated group, since it still maintains some (limited) gene flow with the rest of the Santa Cruz populations as seen in the STRUCTURE analysis (Fig. 3) and the pairwise F_{ST} values (Table A5). Nevertheless, the differentiation among the Santa Cruz 1 and Santa Cruz 2 groups is equivalent to the differentiation seen among different islands, and likewise, Santa Cruz 1 is the genetic group with the highest inter-island differentiation seen in guayabillo (Table A5). Therefore, this leads to either a strong (potentially early) divergence of the Santa Cruz 1 group from the rest of the species, or the possibility of two different colonization events of the ancestral guayabillo into the archipelago as an alternative hypothesis. The latter has been proposed for another Galapagos endemic, *Croton scouleri*, which also displays a notable genetic and morphological variability (Rumeu et al., 2016). In fact, multiple colonization events could be relatively common, since native plant species from oceanic islands are usually associated with high dispersion abilities, an important trait to overcome the sea barrier on repeated occasions (Rosas-Escobar et al. 2011; García-Verdugo et al. 2013). The current data we have for guayabillo is limited, and these hypotheses remain speculative; certainly. a broader sampling range across the archipelago and the use of more powerful molecular markers are necessary to solve the ancestry relations among different populations and lineages from different islands.

The degree of gene flow between islands is a key factor in explaining the previously described population structure. On a broader scale, there’s a clear genetic differentiation between the populations of the three islands, made evident by the high pairwise F_{ST} values observed (Table 3, Table A4) and by individuals clearly clustering according to their island of origin (Figs. 2, 3b, A2 and A4b). Furthermore, a good proportion of the alleles found in each guayabillo population were private alleles (Table 2), highlighting the independent evolutionary histories on each island. Selfing, inbreeding and clonal reproduction (to a lesser extent) in each island population would have led to the fast fixation of distinct alleles that, together with new mutations, could contribute to the current genetic structure and population differentiation (Rick, 1983; Hamrick and Godt, 1996). Moreover, this degree of differentiation suggests a limited gene flow between islands, similar to other endemic species such as *S. affinis* and the *Opuntia* cacti (Nielsen, 2004; Helsen et al., 2009). The oceanic waters that separate the islands are evidently an important barrier for inter-island gene flow in guayabillo and other endemic plants of the Galapagos, especially considering that its fruits and seeds are unlikely to be frequently dispersed through long distances over the ocean (Porter, 1968; Porter, 1976; Ward and Brookfield, 1992; McMullen, 1999). In addition, none of the known animal dispersers of guayabillo seeds -Giant Tortoises and possibly, small passerine birds (Blake et al., 2012; Guerrero and Tye, 2009; Heleno et al., 2013)- would frequently cross large expanses of ocean among islands (Petren et al., 2005; Gerlach et al., 2006; Smith, 2009). Nevertheless, we cannot exclude the possibility of occasional gene flow between guayabillo populations on different islands, potentially mediated by human beings transporting seeds or propagative material between islands as a trading activity (Wiggins et al., 1971), or by widespread and generalist pollinators like *X. darwini* which are also strong flyers that can be easily carried over the ocean by the wind (McMullen, 1990; Smith, 2009; Traveset et al., 2013; Valdebenito, 2018). In fact, our migration analysis shows that most of the limited inter-island migration is directed towards Santa Cruz, in the center of the archipelago (Fig. 4), matching the confluence of sea currents and winds acting upon the Galapagos (Merlen, 2014). Note also that this gene flow to Santa Cruz may also explain the presence of the Santa Cruz 2 group and its close relationship with the populations of the other two islands (Figs. 2 and 3). Despite the notoriety of the oceanic barrier, other plants as *L. minimum* (where a significant population structure among islands was also found; Levin et al., 2015) or *G. leucantha* (Guzmán et al., 2016) are also able to hold some inter-island gene flow, which has been attributed respectively to the action of bird dispersers and the long-range pollination by *X. darwinii*.

In other endemic plant species of the Galapagos, including *S. cheesmaniae* (Pailles et al., 2017), *L. minimum* (Levin et al., 2015) and *G. darwinii* (Wendel and Percy, 1990), a clear genetic structure pattern separating populations of the western and eastern islands was observed. Such pattern apparently follows the progression rule, separating populations from older and younger islands and suggesting an east-west migration (from old to young islands) following the movement of the archipelago with the Nazca plate (Geist et al., 2014; Merlen, 2014; Levin et al., 2015; Pailles et al., 2017). However, the natural history of guayabillo appears more complicated than that. Putting aside the possibility of a second introduction of guayabillo into Santa Cruz, we would expect a greater genetic similarity between closer islands (both temporally and geographically), a pattern that doesn’t hold true given the closer relation between the populations from Isabela and San Cristobal compared to the Santa Cruz individuals (Figs. 2 and 3a; Geist et al., 2014). The lack of an evident clustering of individuals from older and younger islands appears to refute the progression rule for guayabillo in the sampled islands. Note however that the compact spatial clustering of the archipelago in two-dimensional space (Geist et al., 2014; Shaw and Gillespie, 2016) make this observation not surprising. The ancestors of guayabillo, as several other endemic species, have not necessarily moved progressively from older to younger islands, instead moving through one or more of thousands of alternative pathways for spreading over the archipelago beginning from a single island (Geist et al., 2014). Hence, a movement of the guayabillo from Isabela to San Cristobal or vice-versa, is perfectly possible. The majority of the Galapagos endemic species, especially the most vagile organisms, did not follow the progression rule during their colonization (Shaw and Gillespie, 2016), including the endemic *Opuntia* cacti (where individuals from Isabela were contained in the same clade as the individuals of the oldest islands, Española and San Cristobal; Helsen et al., 2009) and several animals such as giant tortoises (Caccone et al., 2002), Darwin finches (Grant and Grant, 2008), land iguanas (Gentile et al., 2009), and various insect taxa (Schmitz et al., 2007; Sequeira et al., 2008). There are many other possibilities behind the biogeographic history of guayabillo, a task that could be better addressed through phylogenetic analyses using appropriate markers, and with the inclusion of samples from all the islands where guayabillo is distributed.

5. Conclusions

Our current data highlights some of the key questions that can be postulated about the history, evolution and future prospects of the guayabillo in the Galapagos Islands. Its relatively high genetic diversity could suggest an ancient history and extensive opportunities to differentiate through isolation from neighboring islands or through adaptation to new microclimates and niches. Several aspects would be promoting this genetic variability in guayabillo, including its widespread distribution in the archipelago, potential allopolyploid origin followed by anagenesis, and its capacity of holding outcrossing together with selfing and clonal reproduction; bigger and higher islands with less human impact as Isabela, also would be capable of harboring more genetic diversity on them. The relatively well-defined population structure we found in guayabillo between different islands, may also be reflecting the effects of reproductive mechanisms and oceanic barriers on the spread of this species, shedding some light into the main drivers of its range and mobility. However, finer details like a weak yet discernible differentiation process within Santa Cruz raise multiple hypotheses about the adaptive processes or potential gene flow between islands. It is likely that a combination of factors drives the population dynamics of guayabillo in the Galapagos, and the relatively recent human presence may play a more important role in its future.

Our results provide, for the first time, an insight into the population genetics of guayabillo while emphasizing the importance of using genetic tools to better understand the natural history of a species. Likewise, this genetic data can be informative for the implementation of conservation strategies. For instance, our data suggests that the San Cristobal population could be the most vulnerable among the ones analyzed in this study, prioritizing the implementation of management actions in this island. The possible fragmentation issue and its lower clonal diversity could be one of the biggest concerns in this case, since this may lead to more diversity loss due to genetic drift, and mate incompatibility among subpopulations (Scobie and Wilcock, 2009; Gitzendanner et al., 2012). Thus, multi-genotype populations should be promoted and established in this island, for example by translocating or outcrossing individuals from different fragments or by allowing corridors in the farming zone of San Cristobal to favor gene flow (Gitzendanner et al., 2012). The Isabela population on the other hand, thanks in part due to the lower human impact and big dimensions of the island, appears to harbor the highest genetic variability in the studied islands, making it a potential germplasm reservoir for the species. It must be also considered also that the populations of each island represent unique gene pools, and in particular Santa Cruz, counts on two very different genetic lineages (potentially different ecotypes). These genetic clusters need to be considered independently for conservation purposes and for ex-situ collections and potential breeding programs (Gitzendanner et al., 2012; Jaramillo et al., 2011; Guzmán et al., 2016). Note that maximizing genetic diversity is essential for restoring endangered plant species, as has already been observed with in the successful recovery of *C. galapagosa* in San Cristobal Island (Jaramillo et al., 2011). Finally, a holistic conservation approach is necessary in the Galapagos, not only to protect the guayabillo but all its flora and fauna (Atkinson et al., 2008; Carrion et al., 2011; DPNG, 2016). Finally, as basic biology questions (such as the ploidy of the species) are answered and new tools (such as genomic analysis pipelines) are developed, the current state of this endemic plant may be better understood for its adequate conservation.

Acknowledgements

We would like to thank Ricardo Campoverde, Carolina Cazco, Andrea Soria, Liseth Salazar, Gabriela Bruque and Sara Ponce for their contributions to the experimental phase of this project. We are grateful to Hugo Valdebenito, María José Pozo, Marcelo Loyola, Juan Delgado, Viviana Jaramillo and Daniel García for their assistance during the field work. We would also like to thank Hugo Valdebenito (USFQ), Todd Vision and Bryan Reatini (UNC) for the valuable conversations about the guayabillo throughout the execution of this project. We are also grateful for the support provided during the course of this investigation by the Galapagos Science Center staff and the Galapagos National Park.

In accordance with Ecuadorian regulations, plant material was obtained under the Genetic Resources Permit No. MAE-DNB-CM-2016-0041, granted by the Ministerio del Ambiente Ecuador to Universidad San Francisco de Quito.

Funding: This study was supported by the Galapagos Science Center and Universidad San Francisco de Quito.

Appendix A. Supplementary data

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Appendix A. Supplementary data

Understanding the genetic diversity of the guayabillo (*Psidium galapageium*), an endemic plant of the Galapagos Islands

Table A1. Sampling sites with its coordinates and altitude, and number of individuals collected there.

Island	**Sampling Site**		**Coordinates**		**Altitude (masl)**	**Number of individuals**	**Total**
Isabela	1	Ricardo García	0° 51.308'S	91° 00.023'W	148	15	86
	2	San Joaquín	0° 49.130'S	91° 01.304'W	379	18	
	3	El Basurero	0° 52.359'S	91° 00.137'W	125	8	
	4	Finca Morocho	0° 51.040'S	90° 59.442'W	139	20	
	5	El Mango	0° 53.135'S	91° 00.430'W	127	6	
	6	Cerro Grande	0° 49.506'S	91° 00.215'W	258	19	
Santa Cruz	1	Granillo Rojo	0° 36.931'S	90° 22.048'W	574	14	87
	2	Salasaca	0° 37.916'S	90° 26.188'W	382	5	
	3	Camote	0° 38.279'S	90° 17.448'W	442	9	
	4	Garrapatero	0° 40.367'S	90° 14.460'W	132	12	
	5	Bellavista	0° 41.558'S	90° 19.037'W	164	34	
	6	El Chato	0° 41.907'S	90° 24.118'W	228	13	
San Cristobal	1	Galapaguera	0° 54.893'S	89° 26.106'W	109	5	35
	2	Camino a Opuntias	0° 56.120'S	89° 32.819'W	124	5	
	3	Perimetral	0° 55.917'S	89° 32.923'W	150	4	
	4	Cerro Verde	0° 54.416'S	89° 26.513'W	206	5	
	5	Las Goteras	0° 53.058'S	89° 26.135'W	311	5	
	6	Cerro Gato	0° 55.452'S	89° 28.172'W	161	5	
	7	Centro de Reciclaje	0° 54.724'S	89° 34.794'W	138	6	
	Total						208

Table A2. Null allele frequencies (using both, a 0.5 and a 0.65 rate of selfing) and PICs for the two isoloci of each analyzed SSR locus.

	Null allele freq. (SELFING RATE=0.5)		**Null allele freq. (SELFING RATE=0.65)**		**PIC**	
Locus	**Isolocus 1**	**Isolocus 2**	**Isolocus 1**	**Isolocus 2**	**Isolocus 1**	**Isolocus 2**
GYB3	0.313	0.317	0.312	0.312	0.751	0.718
GYB4	0.225	0.175	0.229	0.174	0.411	0.545
GYB5	0.134	0.301**	0.146	0.281**	0.006	0.434**
GYB7	0.263	0.261	0.257	0.253	0.494	0.466
GYB8	0.286	0.197	0.271	0.194	0.726	0.623
GYB9	0.070	0.180	0.071	0.171	0.674	0.753
GYB14	0.337*	0.347*	0.325*	0.334*	0.646*	0.653*
GYB18	0.388*	0.372*	0.383*	0.367*	0.678*	0.749*
GYB21	0.279	0.306	0.264	0.293	0.708	0.620
GYB22	0.224	0.194	0.218	0.196	0.358	0.502
GYB23	0.183	0.234	0.187	0.227	0.150	0.613
GYB27	0.192	0.346*	0.189	0.333*	0.651	0.500*
GYB29	0.173	0.314	0.175	0.301	0.384	0.701

*Null allele frequency >>0.3 for both selfing rates, discarded from further analyses

**Discarded due to monomorphism.

Table A3. Genetic diversity information of the analyzed *Psidium galapageium* populations from Isabela, Santa Cruz and San Cristobal islands, after systematic downsampling in the Isabela and Santa Cruz samples: Number of individuals genotyped from each island (N), number of alleles found (A), number of private alleles (PA), mean allelic richness after rarefaction (AR), observed heterozygosity (H_O), expected heterozygosity/gene diversity (H_E) and F_{ST} global value for each island population. Overall results along the three islands are also shown.

Island	**N**	**A***	**PA***	**H_O** [a]	**H_E** [a]
Isabela	35	118 (97)	52 (38)	0.122	0.588
Santa Cruz	35	84 (59)	17 (9)	0.156	0.412
San Cristobal	35	70 (60)	12 (5)	0.119	0.283
Overall	105	161	-	0.141	0.465

* Values between brackets are the number of alleles or private alleles with a frequency >0.05 within the corresponding island population.

[a] indicates average across the 15 SSRs analyzed.

[s] standardized for N=35

Table A4. Pairwise and global F_{ST} values between the *Psidium galapageium* populations from the three islands, after systematic downsampling in the Isabela and Santa Cruz samples.

	Isabela	**Santa Cruz**
Santa Cruz	0.209	-
San Cristobal	0.212	0.328
Global	0.319	

Table A5. Pairwise and global F_{ST} values between the *Psidium galapageium* clusters defined from the STRUCTURE software and PCoA groupings.

	Isabela	**Santa Cruz 1**	**Santa Cruz 2**
Santa Cruz 1	0.228	-	
Santa Cruz 2	0.132	0.166	-
San Cristobal	0.180	0.290	0.222
Global	0.314		

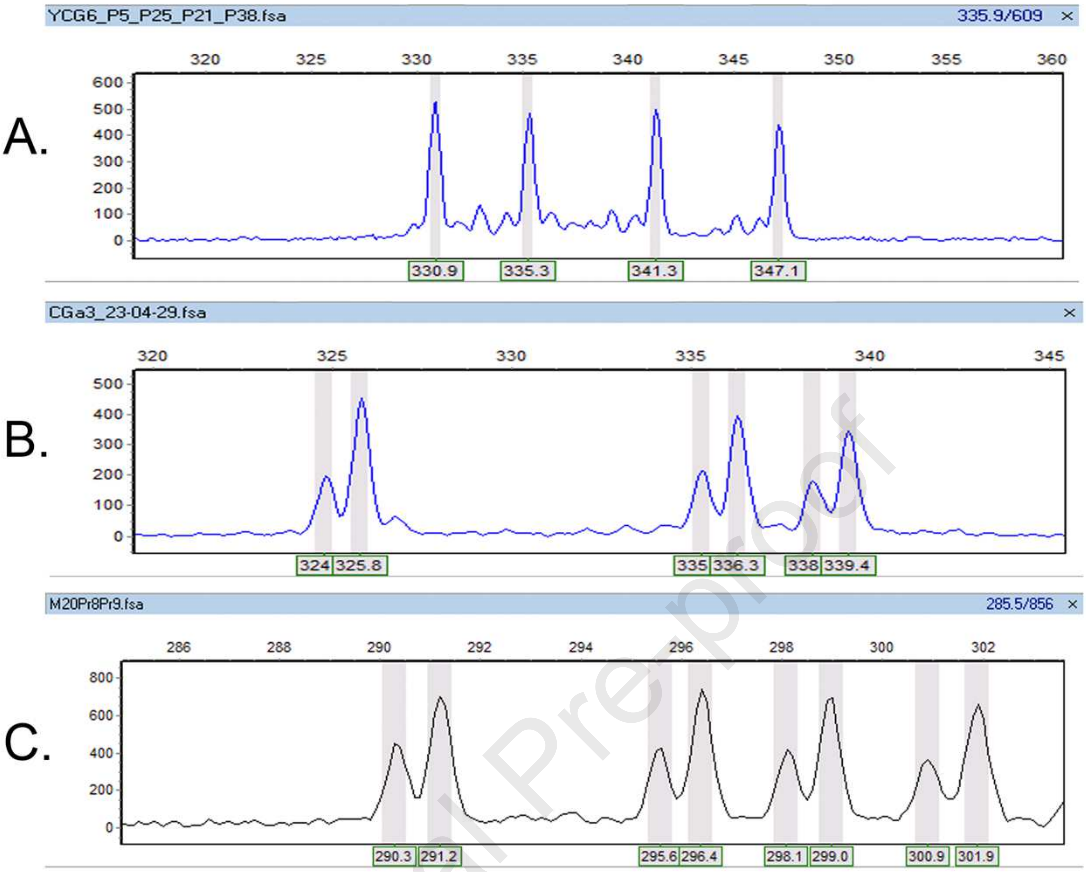


Fig. A1. Electropherograms obtained from the SSR amplification of three guayabillo samples showing up to four different alleles in a single individual, depicting potential polyploidy. A. Locus GYB25 amplified from sample YCG6 (Isabela). B. Locus GYB23 amplified from sample CGa3 (San Cristobal). C. Locus GYB09 amplified from sample YGE2 (Santa Cruz).

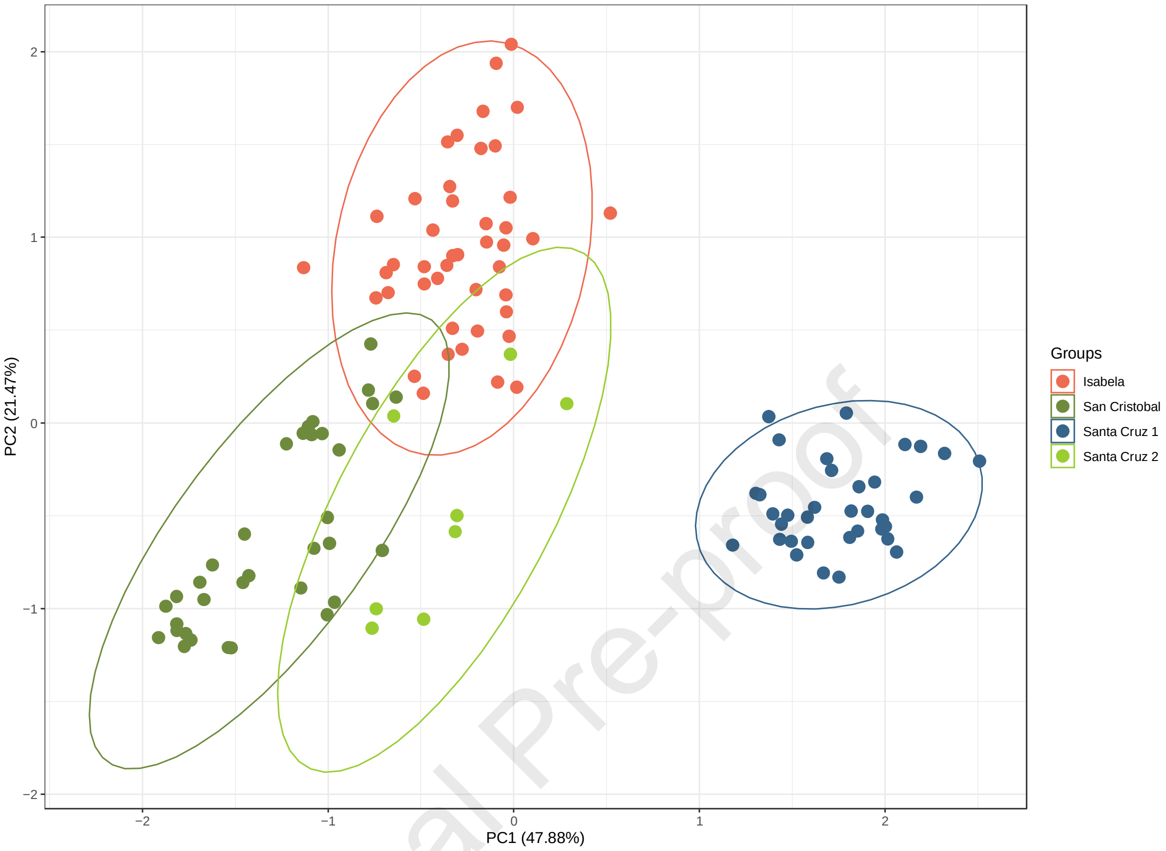


Fig. A2. PCoA based on the Lynch distances (after systematic downsampling in the Isabela and Santa Cruz samples) found between the *Psidium galapageium* individuals sampled in the three islands: Isabela, San Cristobal and Santa Cruz. For Santa Cruz, both genetic clusters are indicated (Santa Cruz 1 and Santa Cruz 2).

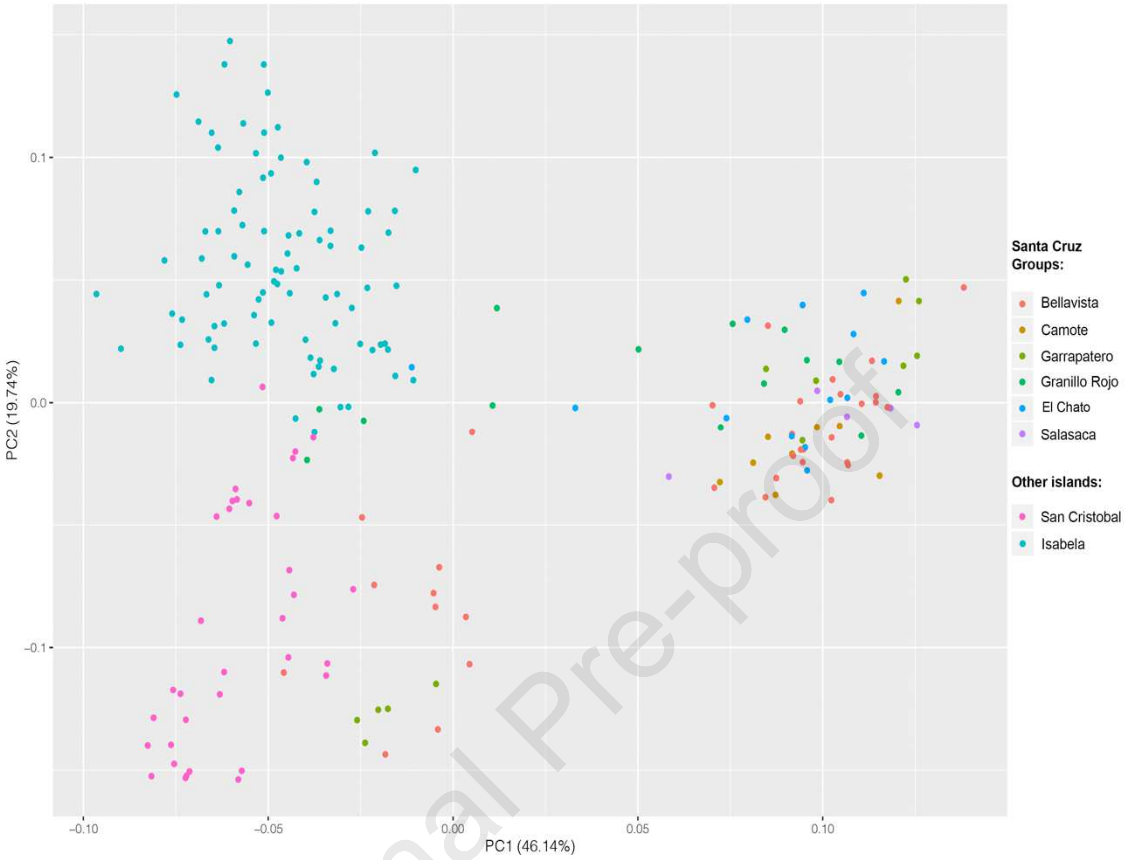


Fig. A3. PCoA based on the Lynch distances found between the *Psidium galapageium* individuals sampled in the three islands. Here, the different subpopulations of Santa Cruz are represented in different colors to show how some individuals (from Granillo Rojo, Garrapatero and Bellavista locations) are grouped with the individuals from Isabela and San Cristobal rather than with the other individuals from Santa Cruz.

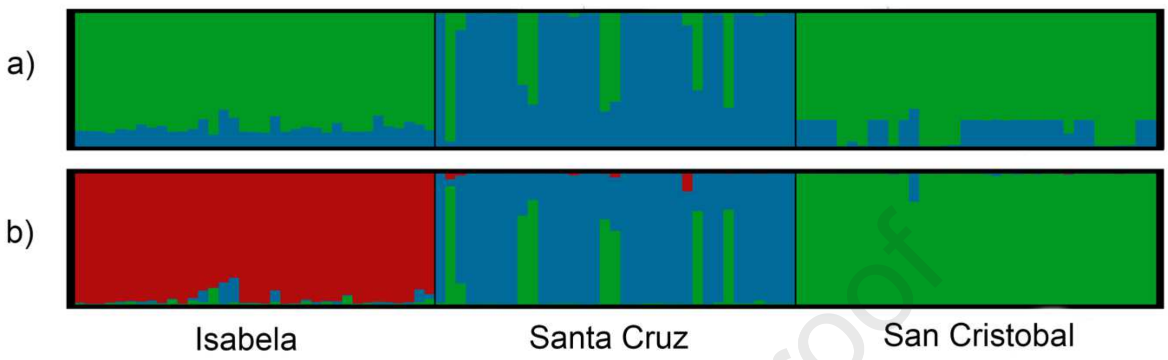


Fig. A4. Results of the Bayesian analysis of population structure (Software STRUCTURE) under the Admixture model, after systematic downsampling in the Isabela and Santa Cruz samples. The results are indicated for a) K=2, and b) K = 3 which is the optimum K value (ΔK = 250.69). These values of K correspond to the clusters or lineages (represented by different colors) in which are grouped the *Psidium galapageium* individuals sampled in Isabela, Santa Cruz and San Cristobal islands.

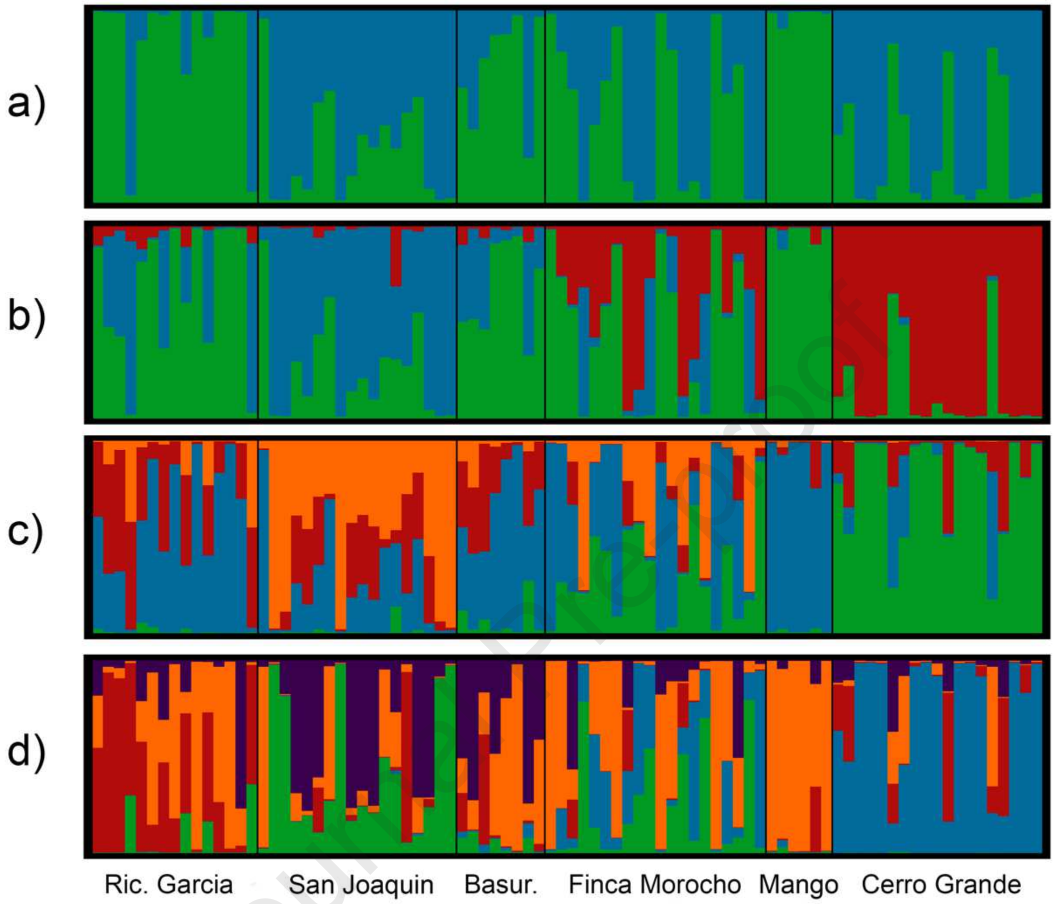


Fig. A5. Population structure Bayesian analysis results, among localities in Isabela Island (Admixture model). a) K=2, b) K=3, c) K=4, d) K=5. The optimum K value in this case was K=2 (ΔK=1195.71).

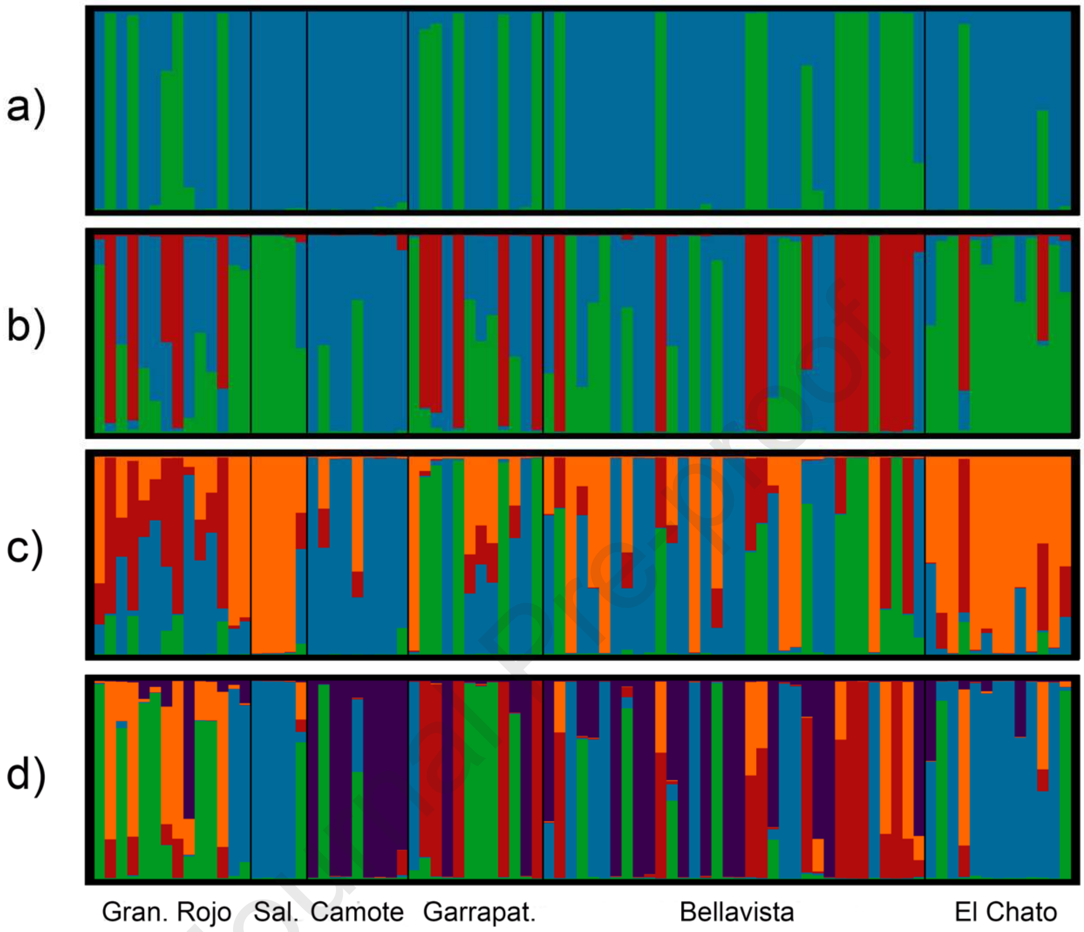


Fig. A6. Population structure Bayesian analysis results, among localities in Santa Cruz Island (Admixture model). a) K=2, b) K=3, c) K=4, d) K=5. The optimum K value in this case was K=2 (ΔK=1177.14).

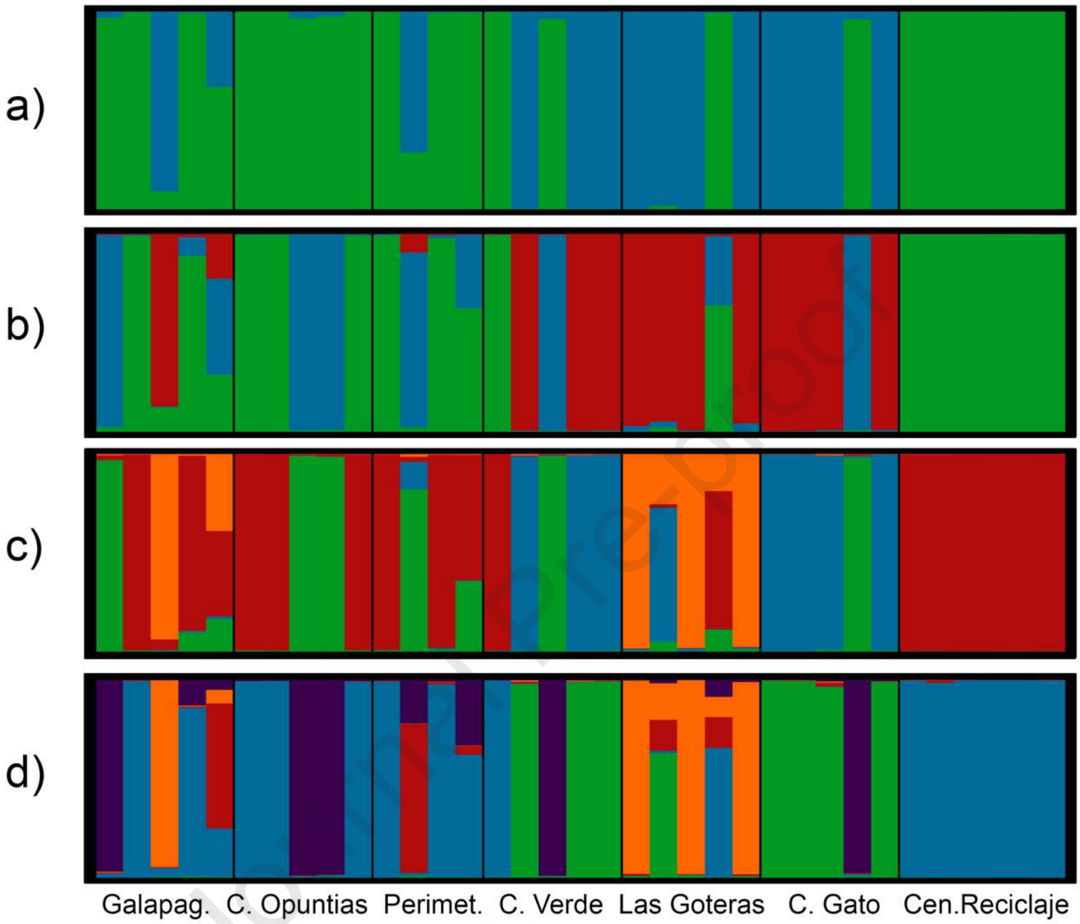
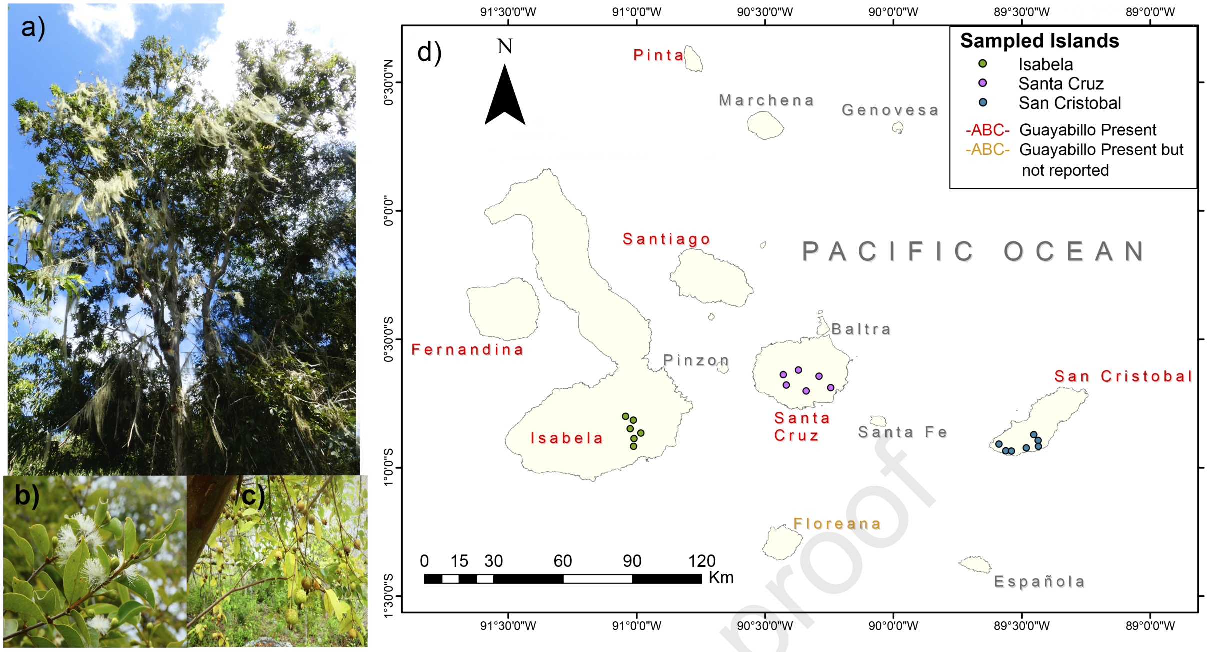
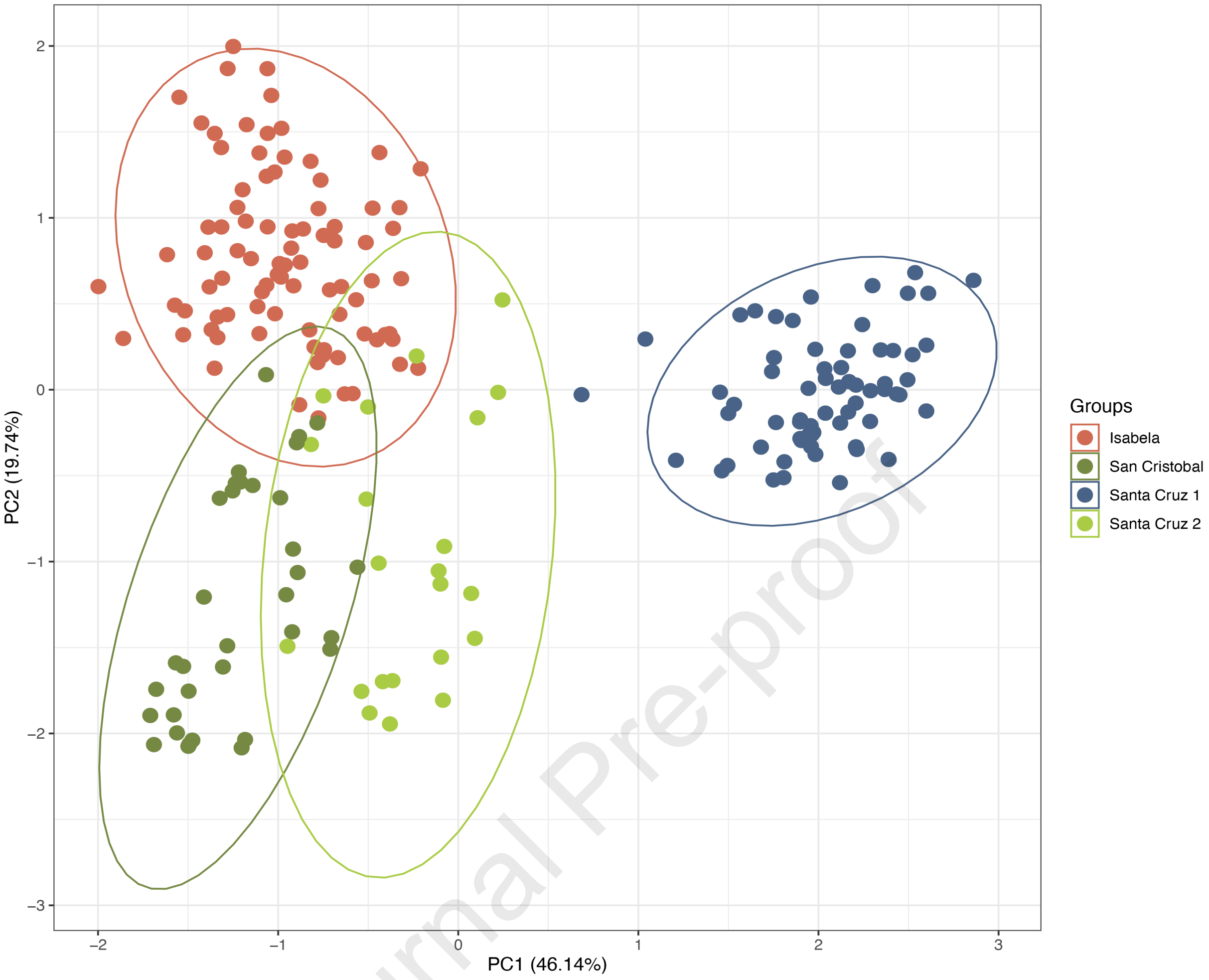
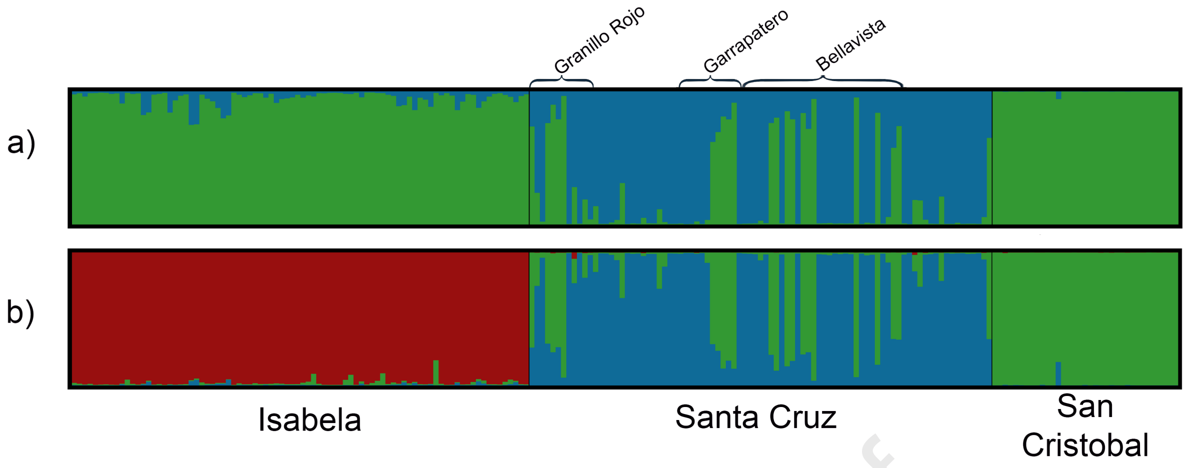


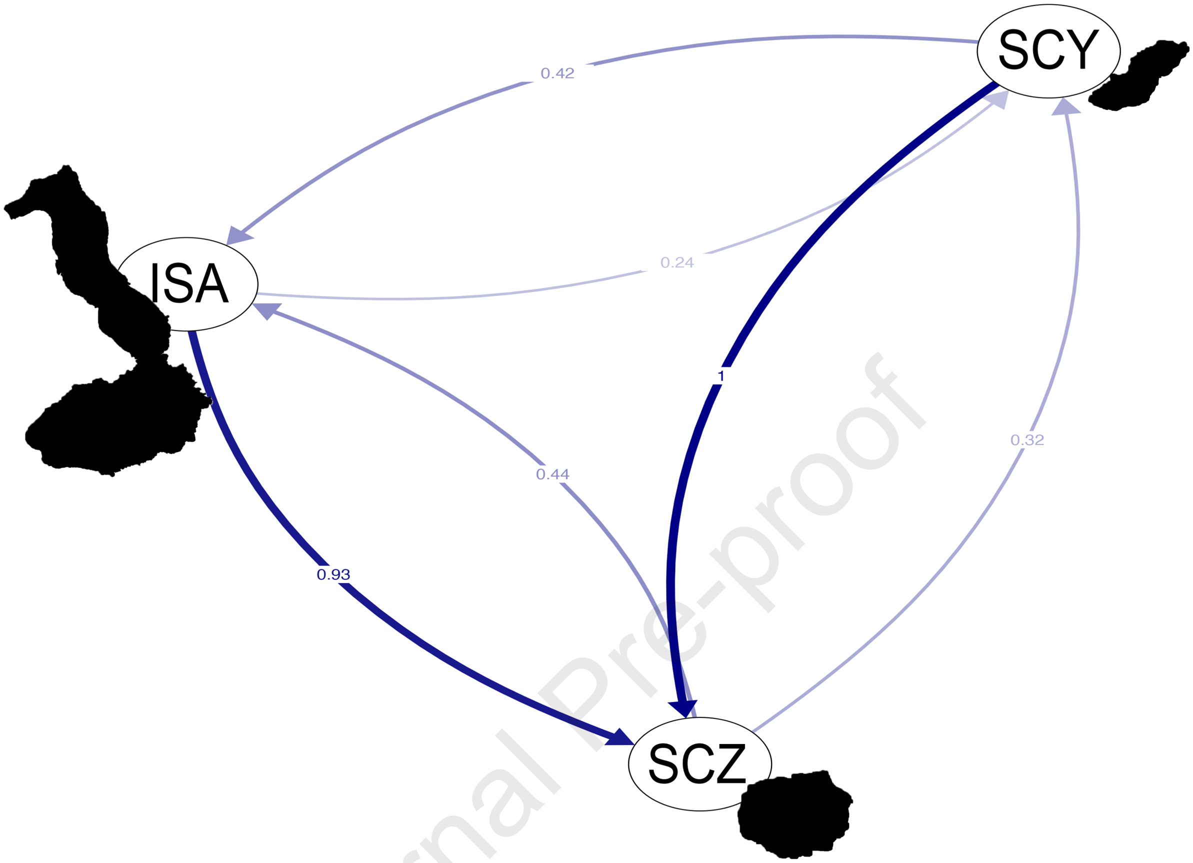
Fig. A7. Population structure Bayesian analysis results, among localities in San Cristobal Island (Admixture model). a) K=2, b) K=3, c) K=4, d) K=5. The optimum K value in this case was K=2 (ΔK= 533.70).

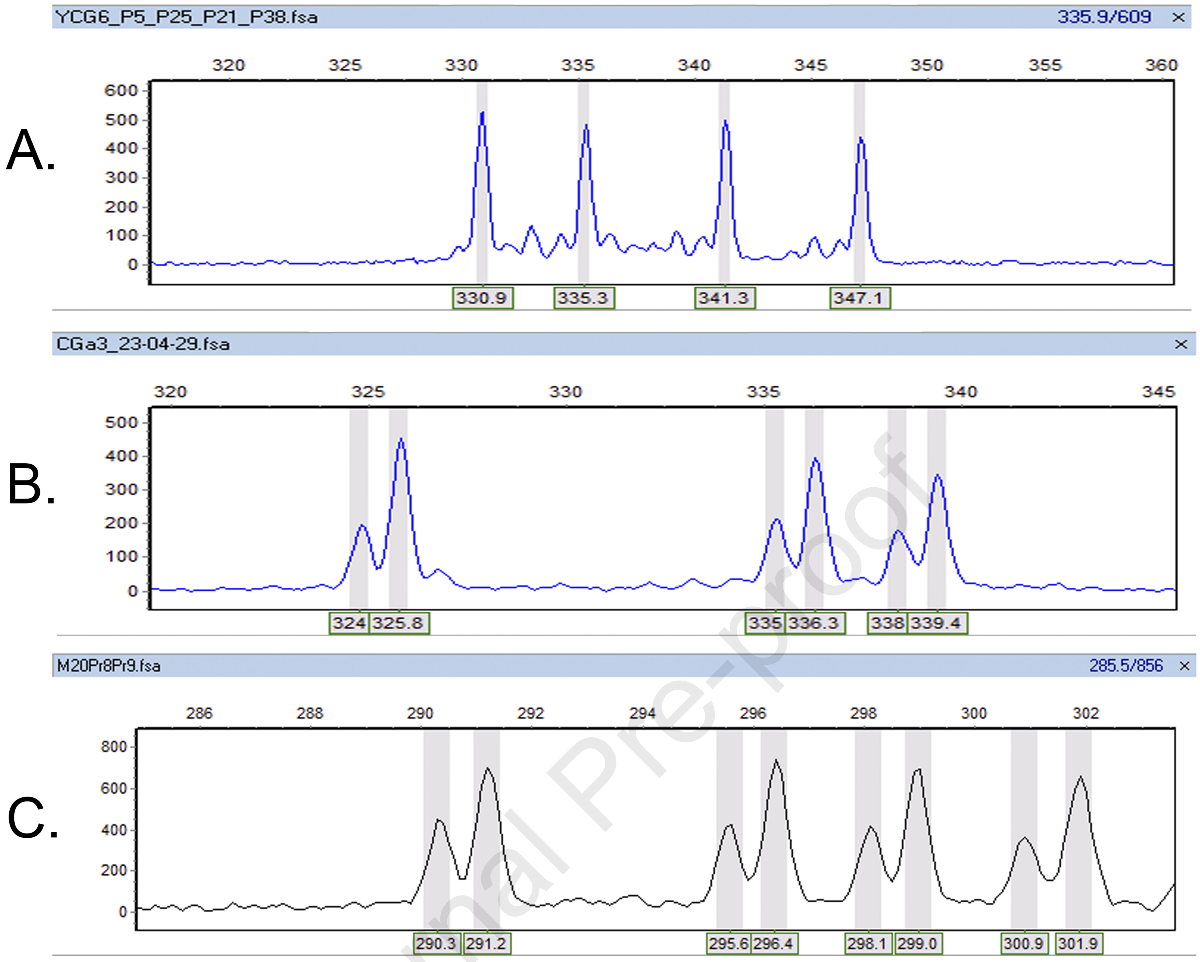

a)
b)
c)
d)
N
91°30'0"W
91°0'0"W
90°30'0"W
90°0'0"W
89°30'0"W
89°0'0"W
0°30'0"N
0°0'0"
0°30'0"S
1°0'0"S
1°30'0"S
Sampled Islands
● Isabela
● Santa Cruz
● San Cristobal
-ABC- Guayabillo Present
-ABC- Guayabillo Present but not reported
Pinta
Marchena
Genovesa
Santiago
PACIFIC OCEAN
Fernandina
Baltra
Pinzon
San Cristobal
Isabela
Santa Cruz
Santa Fe
Floreana
Española
0 15 30 60 90 120
Km

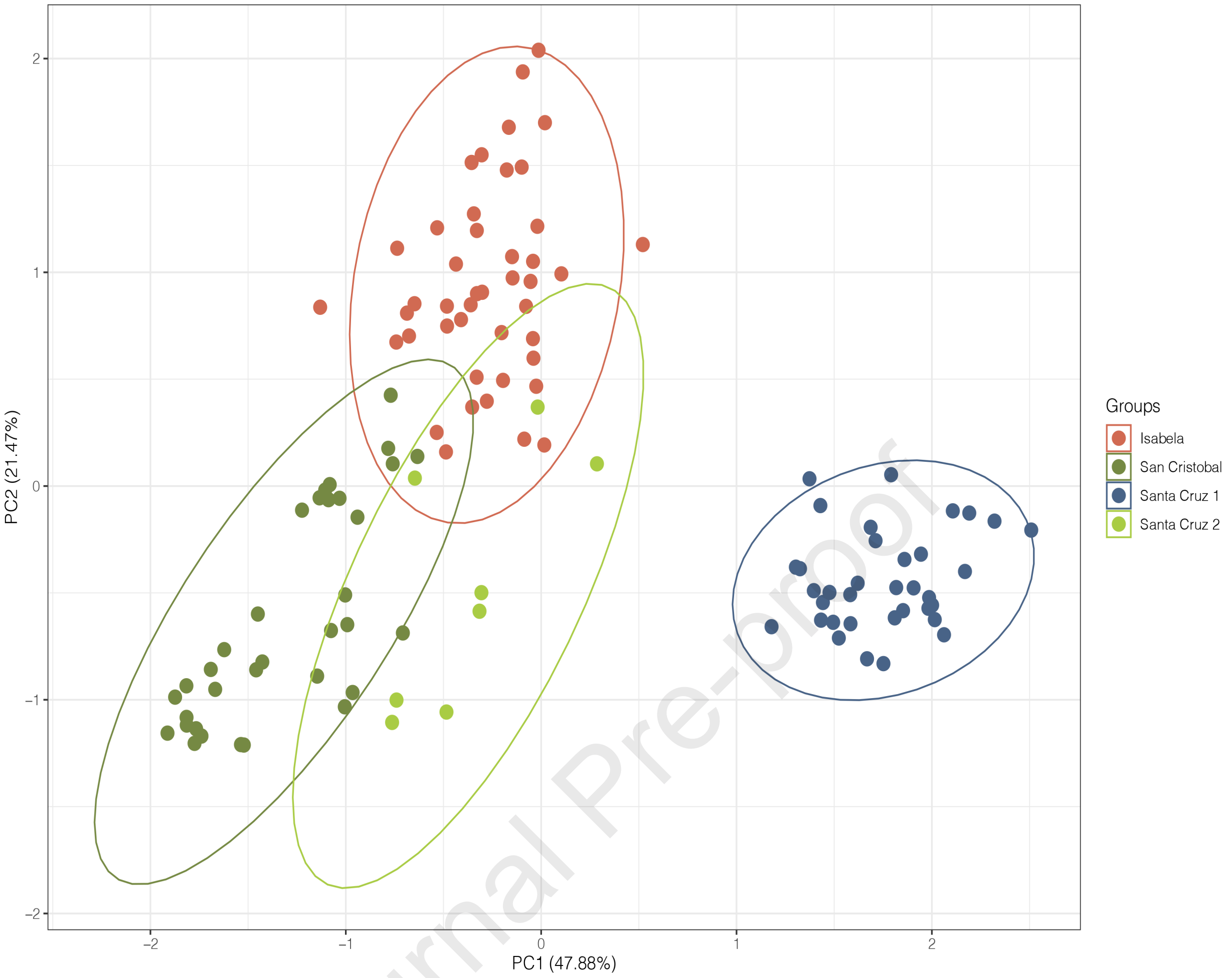


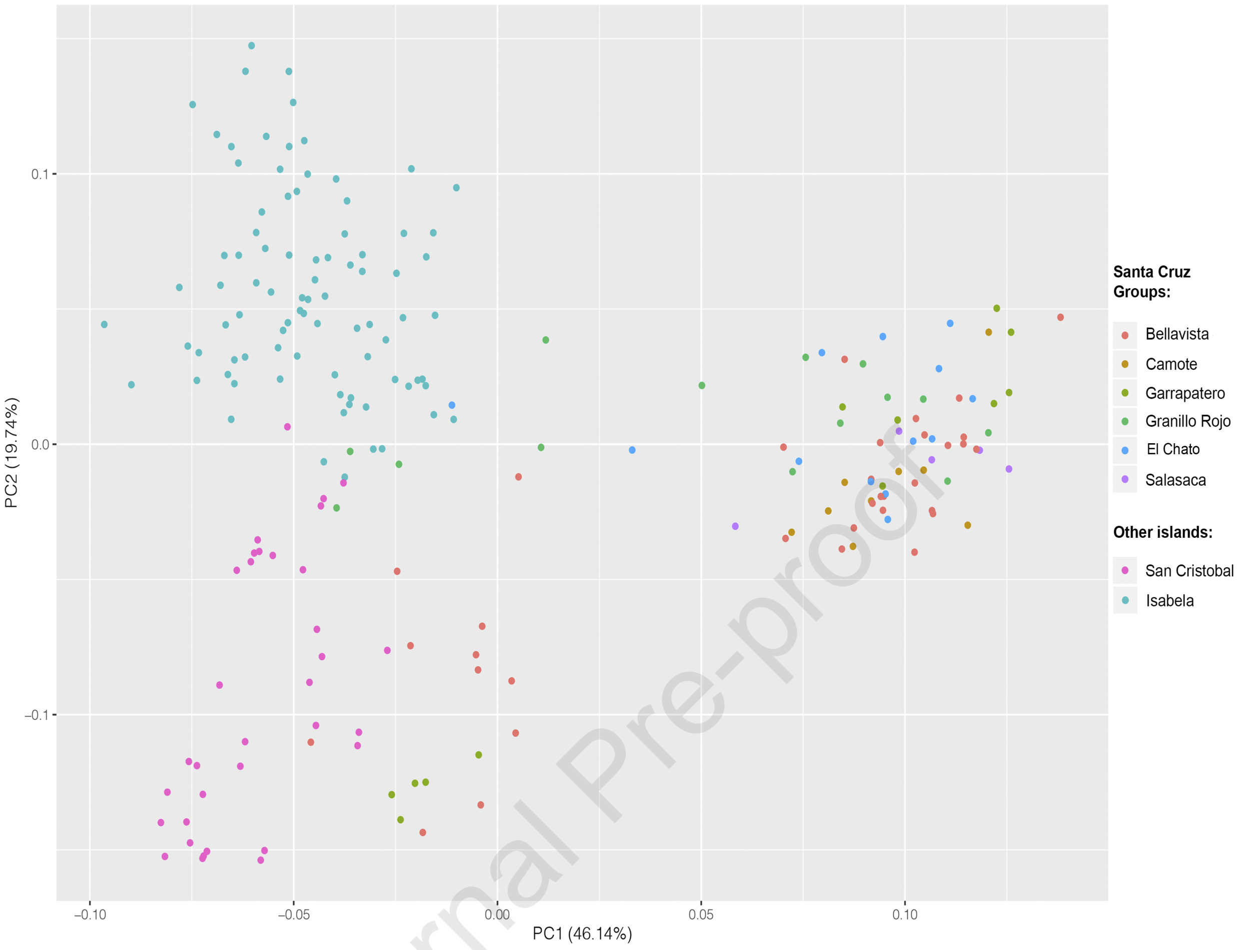
PC2 (19.74%)
2
1
0
−1
−2
−3
−2
−1
0
1
2
3
PC1 (46.14%)
Groups
Isabela
San Cristobal
Santa Cruz 1
Santa Cruz 2

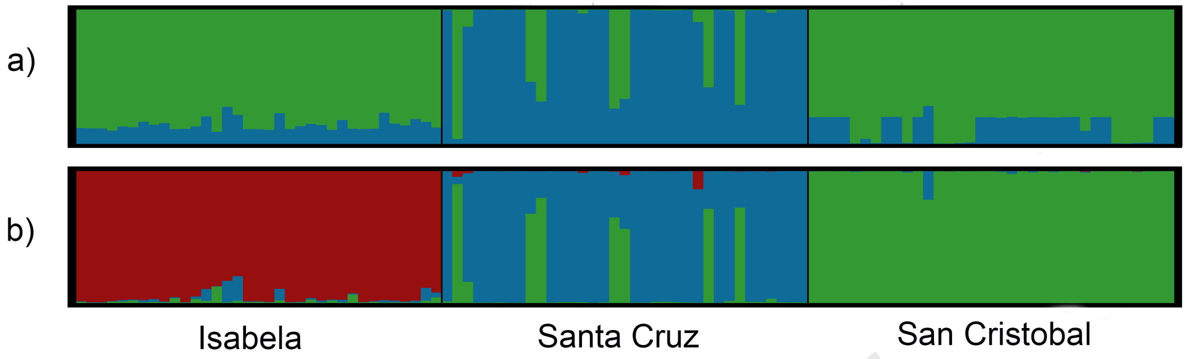

Granillo Rojo
Garrapatero
Bellavista
a)
b)
Isabela
Santa Cruz
San Cristobal

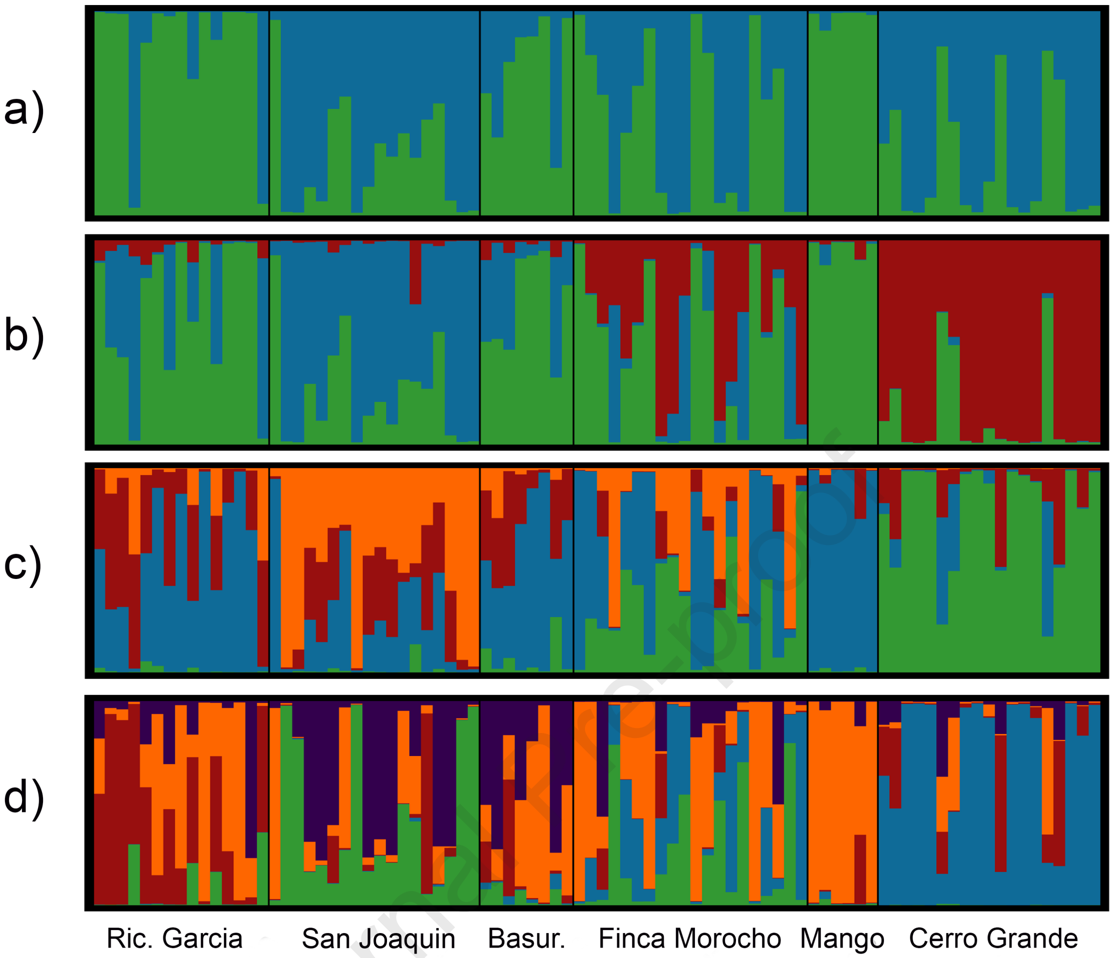

SCY
ISA
SCZ
0.42
0.24
1
0.44
0.32
0.93

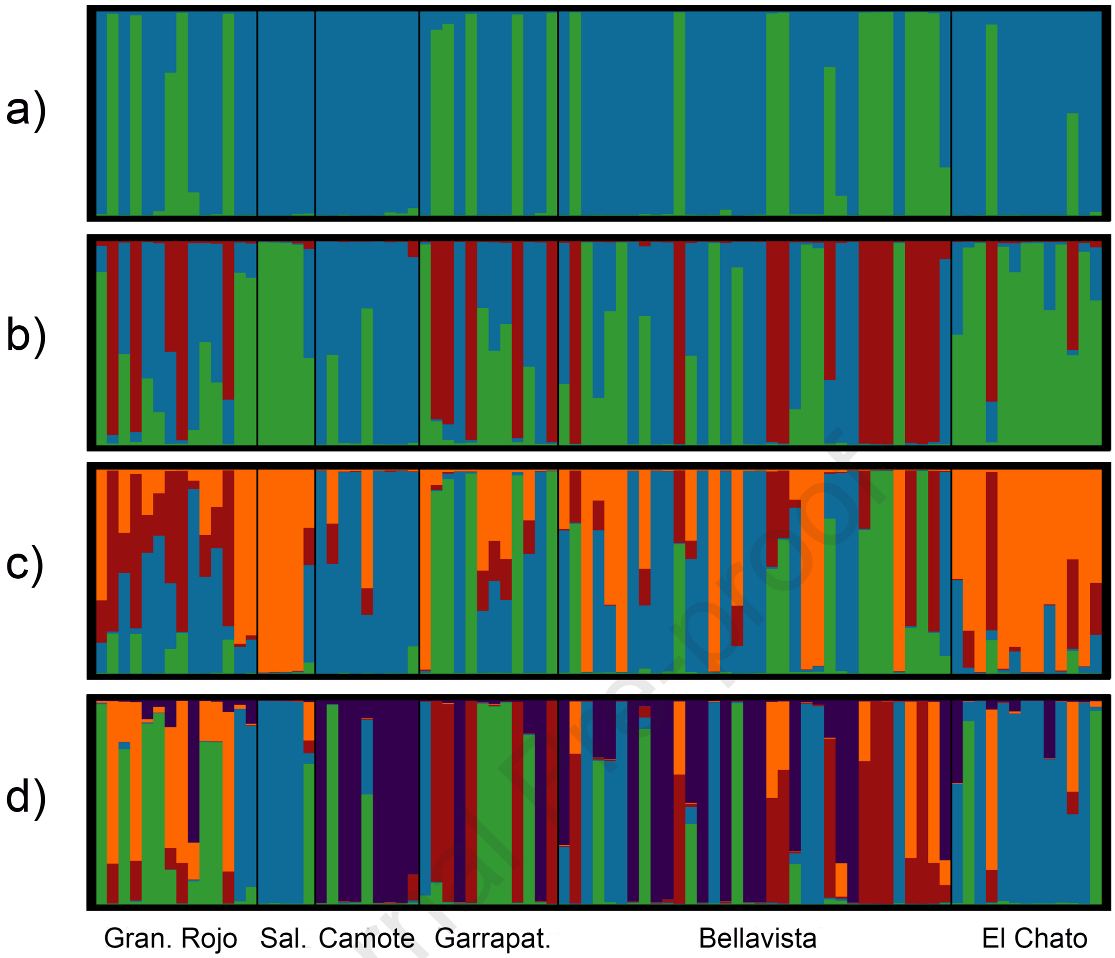

A.
YCG6_P5_P25_P21_P38.fsa
335.9/609
320
325
330
335
340
345
350
355
360
600
500
400
300
200
100
0
330.9
335.3
341.3
347.1
B.
CGa3_23-04-29.fsa
320
325
330
335
340
345
500
400
300
200
100
0
324
325.8
335
336.3
338
339.4
C.
M20Pr8Pr9.fsa
285.5/856
286
288
290
292
294
296
298
300
302
800
600
400
200
0
290.3
291.2
295.6
296.4
298.1
299.0
300.9
301.9

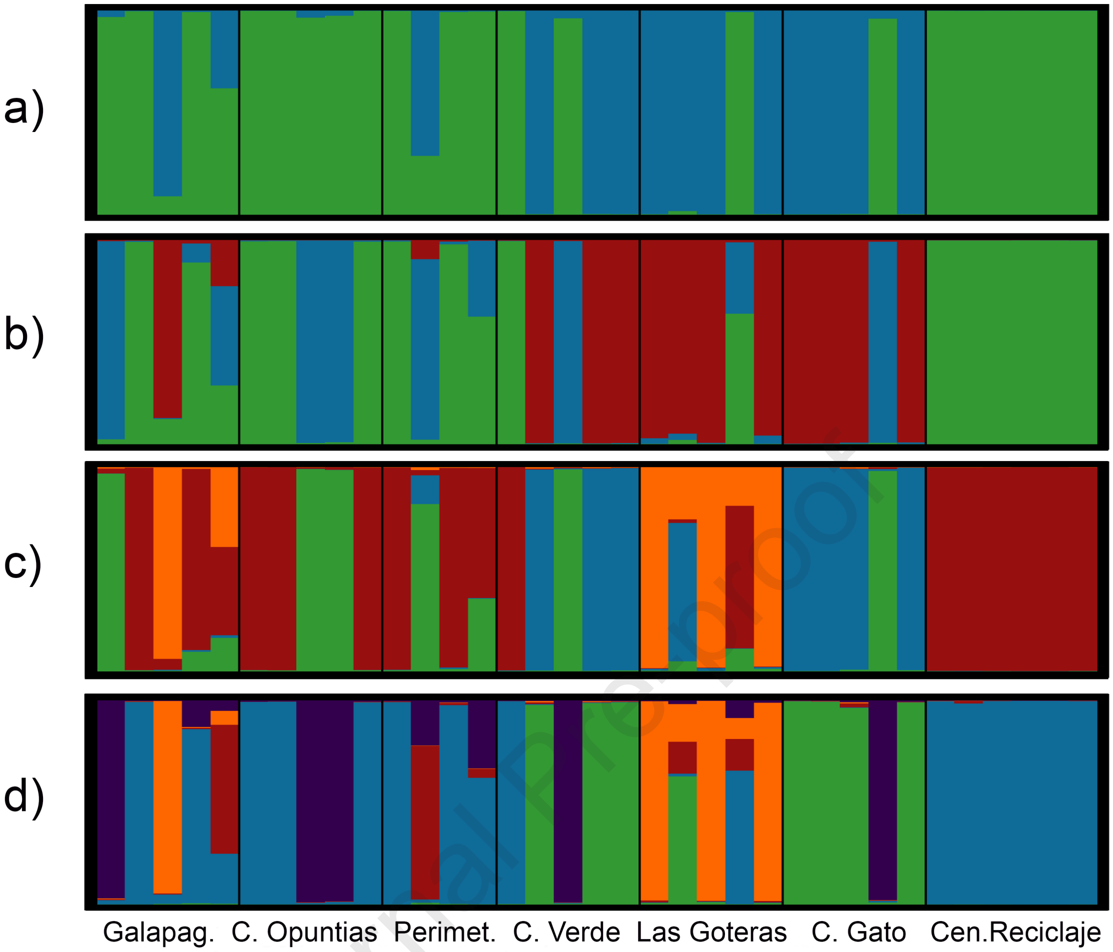

PC2 (21.47%)
2
1
0
−1
−2
PC1 (47.88%)
−2
−1
0
1
2
Groups
Isabela
San Cristobal
Santa Cruz 1
Santa Cruz 2


PC2 (19.74%)
PC1 (46.14%)
0.1
0.0
−0.1
−0.10
−0.05
0.00
0.05
0.10
Santa Cruz Groups:
Bellavista
Camote
Garrapatero
Granillo Rojo
El Chato
Salasaca
Other islands:
San Cristobal
Isabela


a)
b)
Isabela
Santa Cruz
San Cristobal


a)
b)
c)
d)
Ric. Garcia
San Joaquin
Basur.
Finca Morocho
Mango
Cerro Grande


a)
b)
c)
d)
Gran. Rojo
Sal.
Camote
Garrapat.
Bellavista
El Chato


a)
b)
c)
d)
Galapag.
C. Opuntias
Perimet.
C. Verde
Las Goteras
C. Gato
Cen.Reciclaje

Highlights

- Genetic diversity of *Psidium galapageium*, an endemic species of the Galapagos.
- The highest genetic diversity was found in the biggest, least disturbed island.
- Polyploidy, anagenesis and reproductive mechanisms could affect genetic diversity.
- The ocean could be an important barrier for inter-island gene flow in this species.
- Development of SSR markers specific for *Psidium galapageium.*

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: