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Genética de poblaciones de la langosta roja (*Panulirus penicillatus*), en la Reserva Marina de Galápagos

Proyecto de investigación

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Resumen

A medida que más áreas marinas protegidas (AMP) se implementan por todo el mundo, con la finalidad de asegurar el uso sostenible y la viabilidad de los recursos, se vuelve vital ampliar los conocimientos con respecto a la estructura poblacional y los patrones de dispersión de especies económicamente importantes, para una gestión eficaz. Existen limitaciones al momento de analizar el grado de dispersión de los individuos entre las poblaciones y subpoblaciones. La dispersión en poblaciones marinas ocurre durante su fase larvaria pelágica, que puede durar de entre varias horas hasta varios meses.

Los marcadores microsatélites son un método eficiente y rentable para responder a preguntas ecológicas importantes para la toma de decisiones y manejo de reservas, ya que son altamente polimórficos, además presentan una alta flexibilidad, ya que se puede trabajar con tejidos degradados o con bajas concentraciones de ADN, además estos marcadores trabajan con ADN nuclear, lo que mejora los estudios de migración. Por esto esta metodología brinda una visión actual de la estructura y composición de la población, al igual que los patrones de migración.

La langosta espinosa roja (*Panulirus penicillatus*) es una especie ampliamente distribuida en la región del Indo-Pacífico. En la Reserva Marina de Galápagos (RMG) se encuentra alrededor de todas las islas y desde 1960 adquirió un interés económico importante; lo que llevó a un uso insostenible de este recurso, por consiguiente se la incluyó en el calendario pesquero de la RMG, limitando así, sus actividades extractivas. Desde entonces, su gestión ha mejorado sustancialmente, sin embargo, pocos estudios se han realizado sobre la estructura genética y la conectividad de sus poblaciones dentro de la RMG.

Chow et al. (2011) y Abdullah et al. (2014) por medio de ADNmt y marcadores moleculares, encontraron estructura poblacional entre las poblaciones del Pacífico Occidental y Oriental. Sin embargo, Martínez (2006) analizó la estructura genética de *P. penicillatus* dentro de la RMG, y no encontró diferencias significativas entre las distintas poblaciones estudiadas.

En este estudio, se analizó la composición genética de *P. penicillatus* en la RMG, utilizando marcadores microsatélites desarrollados por Mulvihill et al. (*trabajo no publicado*) usando las lecturas de secuenciaminto Illumina provisto en la plataforma Galaxy. Al igual que estudios previos, no se encontró estructura poblacional entre sitios e islas dentro de la RMG. Los valores de Fst se encontraron entre 0.000-0.046. Sin embargo, se encontró un bajo número de alelos (promedio por sitios: 3.667, promedio por islas: 4.131); de igual manera, se encontraron bajos índices de riqueza alélica (por sitios: 2.174; por islas: 2.223). Finalmente, los análisis de migración revelaron un flujo del oeste hacia el este, al parecer Isabela es la fuente de larvas que abastecen al resto de las islas. Sin embargo, se necesitan más estudios para comprobar esta hipótesis, en caso de ser así, estos resultados pueden tener implicaciones importantes en el esquema de zonificación de la RMG.

Palabras clave: conectividad, alelo, riqueza alélica, pelágico, estructura poblacional, distancia genética, microsatélites.

Abstract

As more marine protected (MPAs) areas are implemented around the world, to ensure a sustainable use and viability of resources, it is vital to know about the population structure and dispersal patterns of important commercial species for effective management. Limitations are found when analyzing the degree of dispersal of individuals among populations and subpopulations. In marine populations, dispersal occurs during their pelagic larval phase and can last from several hours to several months.

Microsatellites markers appear to be an efficient and cost-effective method to answer important ecological questions, as they are nuclear DNA, highly polymorphic, and results can be obtained from degraded tissues or low concentration of DNA, providing a contemporary view of population structure and migration.

The Red Spiny Lobster (*Panulirus penicillatus*) is a widely distributed species, ranging throughout the Indo-Pacific region. It is found in all islands of the Galápagos Marine Reserve (GMR) and since 1960 it became of economic interest, however its intense harvest lead to an unsustainable use, so it was included in the fishing calendar of the GMR, limiting its extractive activities. Since then, its management has substantially improved, however little is known about the genetic structure and hence the connectivity of its distant populations within the GMR.

Chow et al. (2011) and Abdullah et al. (2014) using mtDNA and molecular markers, found genetic structure between populations of Western and Eastern Pacific regions. However Martinez (2006) analyzed *P. penicillatus* genetic structure inside the GMR, and found no significances differences among sites.

In this study the genetic composition of *P. penicillatus* in GMR was analyzed using microsatellite markers developed by Mulvihill et al. (*unpublished work*) using Illumina pair-end sequencing with a Galaxy-based pipeline. Like previous studies, no population structure was found among different sites or islands within the GMR. Fst-values ranged from 0.000-0.046. Nevertheless a low number of alleles (average sites: 3.667; average islands: 4.131) was found, as well as low average allelic richness (sites: 2.174; islands: 2.223). Finally, migration analyzes revealed an eastwardly flow, as Isabela appears to be the source of larvae supplying the rest of the islands. More studies need to be done to test this hypothesis, but if proven, these results can have important implications in the design of the GMR zoning scheme.

Keywords: connectivity, allele, allelic richness, pelagic, population structure, genetic distance, microsatellite.

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Introduction

Marine protected areas (MPAs) have been recognized to increase the biomass and density of heavily exploited species; therefore they are an optimal tool to protect marine environments, as well as endangered species (Botsford et al., 2009). MPAs are delimited locations under legal protection policies for conservation purposes, mainly to ensure the preservation of biodiversity, for which is common the application of a multiple use scheme.

Typical zoning categories are no take zones, fishing/extractive zones, touristic zones, among others, depending on the activities of the different stakeholders involved with the protected area. Authorities of MPAs center their effort on providing a sustainable use of resources. For instance, it is vital to have comprehensive information about the connectivity among distant populations within an MPA, which is determined by the number of adult individuals dispersing among populations, as well as by the frequency of larval dispersal and recruitment from other populations. All these factors will determine the adaptability potential of a particular species and its capacity to respond to drastic environmental changes.

Determining the connectivity of populations and subpopulations can be cost and time consuming with low chances of success, especially for benthic marine species, for which the majority of the dispersal occurs during their larval stages. Approximately 85% of benthic marine species have a spore, an egg or a larval phase, which constitute their primary dispersal form. Their long larval stage (up to several months) and its pelagic capability, have lead to the misconception of all marine populations as being highly connected, as marine currents are constantly circulating around the globe. But understanding larval dispersal implies knowing physical and chemical features of the environment, the biological traits of the larva and the interaction among them (Palumbi,

2004). With the progress and accessibility of different techniques, namely genetic, geochemical markers or biophysical models, now it is possible to infer more accurately the pathways of larval dispersal. For example, using mtDNA, Saunders et al. (1985) found for the Horseshoe crab (*Limulus polyphemus*) a major genetic break with no physical barrier between the north and the south populations of northeastern Florida, even though the crab presents a two-week larval stage. The same pattern was found by Reeb and Avise (1990) on the American Oyster (*Crassotrea virginica*), along the east North American coast, resulting in two divergent populations.

In addition, temporal variation in larval recruitment can also structure populations; this is the case of the Caribbean Spiny Lobster, *Panulirus argus*. Truelove et al. (2015) revealed that *P. argus* showed no genetic structure between two Mexican MPAs, the Sian Ka'an Biosphere Reserve and the Banco Chinchorro Biosphere Reserve, however individuals of different size classes showed genetic structure, suggesting seasonality in connectivity patterns (Truelove et al., 2015).

The Red Spiny Lobster (*Panulirus penicillatus*) is a transpacific Crustacean, distributed from the Indo-Pacific to the Tropical Eastern Pacific, including some oceanic islands like Revillagigedo and Galápagos (Hickman and Zimmerman, 2000; Cockcroft et al., 2013). Like most marine species, *P. penicillatus*, has an extended pelagic larval phase of ~10 months and as an adult it occupies a benthic ecological niche. It inhabits shallow rocky subtidal zones of coastal areas, from 1-16 m depth. As a nocturnal predator, it feeds on small invertebrates, like crabs and sea urchins (Hickman and Zimmerman, 2000; Hearn and Murillo, 2008), while during the day it remains in cracks or crevices. In addition of being a key ecological species, as it is a top-down regulator for smaller invertebrates, it is also an important commercial species, as it is being fished in more than 90 countries (Debevec et al., 2014). The Food Agency

Organization (FAO) reported catches of *P. penicillatus* greater than 300 000 tons of spiny lobster worldwide for 2016 (Debevec et al., 2014).

In the Galápagos, the fishery of *P. penicillatus* originally begun as of subsistence, but it became commercially important in the early 60's. The poorly controlled fishery intensified, and by 2005 and along the sea cucumber collapse, the lowest Catch Per Unit effort (CPUe) was recorded (4 kg per diver per day^{-1}; Hearn and Murillo, 2008), which was even lower than that recorded for any El Niño event (≈ 6 kg per diver per day^{-1}during El Niño of 1998; Hearn and Murillo, 2008). After this severe El Niño, additional events every 4-5 years, and a constant fishing pressure, CPUe for the Galápagos Red Spiny Lobster has been oscillating around its lowest values, however with a continuous trend of reduction (Hearn and Murillo, 2008; Reyes et al., 2013 and Buglass et al., 2018).

For these reasons, the Galápagos Marine Reserve (GMR) authorities decided to monitor the species and included it in the fishing calendar, which is a management tool that regulates all fishing activities within the GMR (Heylings et al., 2002). For *P. penicillatus* the following regulations are in place: an annual fishing of 4 to 5 months, landings of individuals with a minimum size (26 cm, total carapace length or 15 cm tail length), and the prohibition of landing of egged females (Toral, et al. 2002). In addition, fishing techniques are limited to the use of Hawaiian slings or by hand. Since these regulations were put in placed, CPUes have been increasing since 2008, with a peak of 8.7 kg per diver per day^{-1} in 2011 (Reyes et al., 2013). Despite of being a good sign of population recovery, intense fishery is still being applied to the lobsters in the GMR: 196 and 165 tons of lobster were captured for the years 2016 and 2017, respectively (Parque Nacional Galápagos, 2017; El Telégrafo, 2018). It is also important to note that fishing effort in the GMR can increase, since currently there is a 61% of inactive

fisherman that, given the favorable fisheries conditions, could come back and influence the recovery of the lobster population, making it susceptible to overexploitation (Hearn and Murillo, 2008; Reyes et al., 2013).

It is thus important to understand if the combined effects of El Niño event and the fishing pressure are impacting the populations of *P. penicillatus*, for example with a loss of genetic diversity, due to population decimation. In addition, understanding the degree of connectivity of distant *P. penicillatus* populations is key, because if highly connected, the transfer of genetic material can recover the loss of genetic variability due to harvesting (Botsford et al., 2009; Cowen and Sponaugle, 2009). Given that *P. penicillatus* larval stages are long lasting, their dispersal potential is high, and as a consequence, distant populations could remain connected. There have been various genetic studies done with *P. penicillatus*, both locally and regionally. Martinez (2006) in collaboration with the Galápagos National Park, carried out a study using mtDNA and RFLP, in which they included individuals from four main islands: Santa Cruz, Isabela, San Cristóbal and Floreana. Main results indicated a lack of genetic differentiation among islands, but with a slight differentiation from San Cristóbal's samples. Chow et al. (2011) and Abdullah et al. (2014) studied genetic diversity and connectivity of *P. penicillatus*, using mtDNA and found two distinct populations, one in the Western/Central Pacific and another one in the Eastern Pacific.

Despite the progress and accessibility of genetic methods like microsatellites, little effort has been done to understand the connectivity of the Galápagos Red Spiny Lobster among different islands of the Archipelago. The use of other genetic markers, such as microsatellites, has helped addressing important ecological questions, like the occurrence of cryptic species, gene flow, and population structure, among others. This technique is a much better method than mtDNA or RFLP, since microsatellites are

highly polymorphic, species-specific, cost-effective and are located in small segments of the genome that are inherited bipaternally (Selkoe and Toonen, 2006; Arif et al., 2011); therefore results have a better spatial and temporal resolution.

For this reason, the main objective of this study is to investigate population connectivity of the Galápagos Red Spiny Lobster using microsatellites that where designed for this species with the overarching goal of helping evaluate its genetic composition for future decision-making in the Galápagos Marine Reserve.

Objectives

Main objective:

- Characterize the genetic composition of *P. penicillatus* in the GMR, using next generation sequencing microsatellite markers

Specific objectives:

- Determine if there is a genetic structure among different sites or islands
- Determine migration patterns of individuals among different sites or islands

Methods

Study Area and Sample Collection

The GMR, is a multiuse reserve that encloses an area of 138 000 km^2 from which industrial fishing has been forbidden since 1998, but artisanal fishing is allowed in particular zones (Danulat and Edgar, 2002). The GMR is managed by the Galápagos National Park (GNP), which allows local fishermen to harvest protected marine species, such as the Galápagos Red Spiny Lobster (*P. penicillatus*).

The Archipelago is located on the equator were three major currents confluence and show a marked seasonality in terms of intensity and direction; these are: a) the Humbolt Current, with several months of duration (8-9 months), which brings cold and nutrient-rich waters from the Antarctic carried out by the south-east trading winds along the west coast of South America, then it turns with a westward direction and converges into the South Equatorial Current; b) the less nutrient-rich and warmer waters of the Panama Current with southward direction, which then turns west at the southwest from Central America; and c) the Cromwell undercurrent, with an eastward flow from the Central Pacific, that generates nutrient-rich upwelling in the west side of Galápagos (Fernandina and Isabela; Banks, 2002; Palacios, 2004).

In this study a total of 134 lobster samples were collected in 2015 from nine sites and six different islands (Table 1). The sites were: La Unión and San Pedro from Isabela Island; Rosa Blanca and Los Chorros from San Cristóbal Island; Punta Rocafuerte and Garrapatero from Santa Cruz Island; Punta del Miedo from Santa Fé Island; Bucanero form Santiago Island; and, Piedras Amarillas from Floreana Island (Table 1).

Samples were collected from fishermen landings at the three main fishing docks of Santa Cruz, San Cristóbal and Isabela Islands, thus the number of samples per site

was variable (9-20) and depended completely on the availability of fishermen catches. Samples consisted of fresh muscle tissue from the periopods of adult individuals. Tissues were preserved in 1.5 ml microtubes with 96% ethanol. Genomic DNA was obtained following the manufactures protocol from Qiagen® DNA Blood and Tissue Kit. Quality and quantity of the extracted DNA was determined using Nanodrop spectrophotometry (Nanodrop 2000 Thermo scientific).

Genotyping

Mulvihill et al. (*unpublished work*) developed 23 primers for *P. penicillatus*, using next-generation sequencing (NGS) and a bioinformatics pipeline in the Illumina paired-end sequencing package, available in the open source tool Galaxy (Griffiths et al., 2016). The process consisted of a series of filters that enhance optimal microsatellite reading outputs for primer designing. With Illumina and Trimmomatic v. 0.32, low quality reads were discarded. In addition, FastQC v0.11.4 generated reports with important information, like GC content, which identifies guanine-cytosine regions that indicates the primer melting temperature (Tm) and quality scores, among other things. Then, filtered information was analyzed using Pal_finder v.0.02.04 to identify repeat motifs and flanking regions. Lastly, with Pal_filter, microsatellites loci and primers were analyzed; loci with interrupted motifs, primers that appeared in the same read, and loci and primers that could not be designed by Prime3 were discarded. Then, PANDAseq filter was used to increase the chance of a successful PCR (Griffiths et al., 2016). After the 23 primers were developed, the Culley et al. (2013) 3-primer system was used. It consisted on a forward primer with a tail sequence attached to the 5' end, a labeled tail with a fluorescent dye attached to the 5' end, and, an unaltered reverse primer. This is an improved system to identify microsatellite sequences that during PCR phase's traceable amplifications are generated, therefore reducing time and costs.

After testing primers' PCR success, a total of 18 primers were selected to add the tail sequence (Mulvihill et al., *unpublished work*; Table 2). Selected primers were tested and attached with the universal primer M13(-21). For multiplexing procedures, two fluorescent dyes (6FAM® and HEX®) were added to the tail primer. Eleven forward primers (PEN: 4, 10, 13, 15, 16, 17, 20, 22, 27, 28 and 34) were labeled with 6-FAM® dye and the remaining seven forward primers were labeled with HEX® (PEN: 3, 18, 19, 21, 23, 24 and 33). With the Multiplex Manager Software (Hollele and Geerts, 2009), eight different multiplex PCRs were determined. Six mixes were duplex: PEN34 and PEN15, PEN17 and PEN27, PEN18 and PEN23, PEN21 and PEN3, PEN19 and PEN33, and lastly PEN16 and PEN24; while two other were triplex and had the following combinations: PEN22, PEN28 and PEN4; PEN13 PEN20 and PEN10.

Following Culley et al. (2013), primers were amplified with high annealing temperatures and only ¼ of the recommended concentration of the forward primer was used. Twenty-five µl PCR reactions were made according to Invitrogen™ Platinum™ *Taq* Polymerase protocol and PCR conditions were as follows: 94°C for 2 min initial denaturation, followed by two set of cycles. The first was of 30 cycles at 94°C for 30s, 60°C for 30s, and 72°C for 30s. The second was of 10 cycles at 94°C for 30s, 53°C for 42s and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplified products were sent to the Laboratory of Analytical Biology of the Smithsonian National Museum of Natural History in Washington DC, in order to be automatically genotyped by ABI PRISM 310. With the program GeneMapper® V. 3.0 (Applied Biosystems) scoring and binning of alleles were checked, from which a matrix was developed at the Laboratorio de Biotecnología Vegetal of San Francisco de Quito University.

Data Analysis

Data was analyzed with two different approaches: per population coming from nine different sites ("by Sites") and per island coming from six different islands ("by islands"). Basic population parameters among and within populations, such as allele richness, expected and observed heterozigocity, as well as Hardy-Weinberg equilibrium were calculated using Genodive v2.0b27 (Meirmans and van Tienderen, 2004). Likewise, for population differentiation analyses, a hierarchical AMOVA was performed using Weir and Cockerham's infinite allele model in Genodive with 50 000 permutations (Michalakis and Excoffier, 1994). Linkage disequilibrium was run in GENEPOP on the Web v4.2 (Raymond and Rousset, 1995). FreeNA at 5 000 replicates was run to check null alleles frequencies (Chapuis and Estoup, 2007). False Discovery Rate type of correction was applied in order to reduce type I error, when multiple analysis were done (Verhoeven et al., 2005).

Genetic Distances and Population Structure

Genetic distances were estimated in Genodive v2.0b27. Weir & Cockerham (1984) estimator was used to calculate genetic variability among populations. Fixation index ranged from 0-1, where values close to 0 indicate a freely interbred population, whereas values close to 1 could be explained by population structure. Bruvos' distance (Bruvo et al., 2004) was calculated among individuals; for this test values varied from 0-1, 0 meaning identical genetic composition and 1 different genotypes.

Principal Component Analysis (PCoA) was performed using the "hierfstat" R package to visualize relatedness or differences among populations (Goudet and Jombart, 2015). The genetic makeup among sites was determined in Structure V2.3.4, with a bayesian based-model with a clustering program (K), according to their multi-locus genotypes composition (Falush et al., 2003).

Inbreeding

With the R package “Adegenet”, the inbreeding coefficient (F) was calculated for each individual by computing its likelihood ratio. F represents the probability of an individual to inherit identical alleles from related ancestors; F values lower than 0.5 indicate lower chances of inbreeding events (Jombart and Ahmed, 2011).

Bottleneck Events

Bottleneck 1.2.02 (Cornuet and Luikart, 1997) was used to detect if the population has faced any drastic population reduction in the past generations; this program analyzes the mutation/drift equilibrium based on the number of alleles in a loci and its heterozygosity.

Migration

Finally and to understand more about the dynamics of the population, a migration network was created using information from genetic differentiation methods, with the package “diveRsity”(Keenan et al., 2013) in R. These analyses represent the probability of flow among populations using a distance matrix.

Results

Eighteen microsatellite were tested in 134 individuals. From the scoring process PEN10 and PEN22 were discarded, as they appeared monomorphic, therefore less informative. The rest of the analyses were done with the 16 remaining markers, however two pairs of loci were linked with the analyses done by Sites: PEN17 & PEN18 and PEN20 & PEN21 (Table 3a), whereas analyses done by Islands showed four linked loci: PEN17 & PEN18, PEN18 & PEN20, PEN18 & PEN21 and PEN20 & PEN21 (Table 3b). On the other hand, the frequency of null alleles ranged from 0.000-0.256 and from 0.000-0.240, when grouping the data by Site and by Island, respectively (Tables 4a & 4b). Given that PEN18 and PEN20 showed higher linkage frequencies and also relatively high null allele frequencies (Table 4), they were discarded. This resulted ending up with a total of 14 microsatellite markers for the rest of the analyses.

The 14 microsatellites presented a 4.96% of missing data. In addition, eight loci were out of H-W equilibrium, when analyses were done by Site (Table 5a) and seven when they were done by Island (Table 5b). PEN16 & PEN23 presented higher disequilibrium frequencies for both approaches (Table 5a & 5b).

Genetic Diversity

One hundred and three alleles were found in 14 loci. But on average a low number of alleles were found, being 3.667 and 4.131 alleles per loci by Sites and Islands, respectively (Tables 6a & 6b). On the other hand, the average number of alleles per locus was higher, with 7.429, and with PEN17 being the most polymorphic (Table 7).

Heterozygosity analyses presented mid-low values (Tables 6 & 7). Four Sites and three Islands had higher observed than expected heterozygosity, respectively (Table 6a & 6b), meanwhile average heterozygosity per Locus was equal between observed

and expected (Table 7). In addition, relatively low counts of private alleles were present: on average five private alleles when analyses were done by Site and eight alleles by Island. Garrapatero, Piedras Amarillas and La Unión were the Sites with higher counts, whereas Santa Cruz and Isabela had higher number of private alleles (Tables 6a & 6b). Lastly, a low allelic richness was encountered, both when analyses were done by Site or by Island (Tables 6a & 6b).

Genetic Distances and Population Structure

In general, genetic distances were very small (Tables 8a & 8b). Fst values ranged from 0.000-0.046. The greatest difference was between Rosa Blanca and Garrapatero (Table 8a), and between San Cristóbal and Santa Cruz (Table 8b). No significant differences were seen among Sites or among Islands; the majority of variability occurred within individuals and among individuals nested in Sites and in Islands (Table 9).

PCoA analyses were consistent for population structure: there was a lack of differentiation among Sites and among Islands, with very small genetic distances: visualization of individuals was all over the low dimensional space (Figures 1a & b). Nevertheless, genetic composition in Structure presented a K= 2, assuming that two populations explain better the variability of data, both by Site and by Island (Figures 2 a & b).

Inbreeding

Figure 3 plots the density of probability of F (the inbreeding coefficient), and reflects that less than the 5% of all samples could have high probabilities of suffering inbreeding events (hig F-values from 0.6-0.7, Figure 3), while the majority of data were located among a coefficient of 0.1-0.25, which represents low chances of inbreeding events.

Approximately 1-2 individuals presented a high inbreeding coefficient, for analyses arranged by Site and by Island, respectively (Figure 3). Either by Site or by Island, most of the individuals were located between 0.100-0.450, indicating less than a 50% chance of an inbreeding event (Figure 3a & 3b).

Bottleneck Events

Significance deviations from mutation/drift equilibrium were not consistent between the two-phase model (TPM) and the stepwise mutation model (SMM) in data arranged either by Site or by Island (Table 10a & 10b). Only one Site (La Unión) presented an excess of heterozygosity in both models (Tables 10a). As for the analyses performed by Island, the SMM model suggested a drastic population reduction in San Cristóbal, Floreana and Isabela islands (Table 10b).

Migration

Migration network analyses among Sites indicated that there is moderate migration among all Sites. However, San Pedro from Isabela is noticeable, as it shows higher probabilities of flow to other Sites like to Punta Rocafuerte in Santa Cruz, to Piedras Amarillas in Floreana and to La Unión in Isabela (Figure 4a). Rosa Blanca in San Cristóbal and Garrapatero in Santa Cruz, showed lower migration rates (less than the cutoff of 0.4) and appeared isolated form the network (figure 4a). Migration along the GMR is more asymmetrical when data is analyzed by Island, where a prominent flow results from Isabela towards the eastern islands of San Cristóbal, Santa Cruz, and Floreana (figure 4b).

Discussion

As more information has been published, a growing concern towards the genetic diversity and connectivity of marine populations has developed, especially for exploited species. Therefore the main purpose of this study was to improve our understanding and knowledge of the connectivity and gene flow of the Galápagos Red Spiny Lobster (*Panulirus penicillatus*) among different populations in the GMR with species-specific microsatellites markers.

In this study, a total of 23 primers were selected as candidates for amplifications, from which only 18 did amplified, and from those, four were discarded: PEN10 & PEN22, as they were monomorphic for all 134 individuals, therefore they did not contain much information. Likewise, primers PEN18 & PEN20 were discarded, as they presented a higher frequency of linked loci (Tables 3a & 3b) and relatively higher null allele values (Tables 4a & 4b). Loci in linkage disequilibrium were not included since all the programs used for the analyses assumed independent hereditability and high null allele values are known to reduce genetic distances among populations (Chaupuis and Estoup, 2007).

For Hardy-Weinberg (H-W) equilibrium analysis, eight primers were in disequilibrium when data was analyzed by Sites (Table 5a), while seven additional when analyzed by Islands (Table 5b). For Garrapatero in Santa Cruz, Punta del Miedo in Santa Fé, Piedras Amarillas in Floreana and Bucanero in Santiago, disequilibrium from H-W could be due to small sample sizes, which were the lowest with 9, 11, 15 and 13 individuals, respectively. For the rest of loci in disequilibrium with bigger sample sizes, deviations from H-W equilibrium implies that there are selective forces acting on the microsatellites regions or due to null alleles frequencies, as Delanghi et al. (2016)

and Perez-Enriquez et al. (2002) found for *Panulirus homarus* and *Panulirus interruptus*, respectively.

Genetic diversity indices by Site and by Island for *P. penicillatus* indicated low number of alleles as well as low allelic richness, and, a moderate heterozygosity (Tables 6a & 6b) when compared with similar species. Dao et al. (2013) found on average 5.3 alleles in *P. homarus* in the northern islands of Australia. Using the same markers, Dao et al. (2013) found in *Panulirus versicolor* and *Panulirus argus* 2.5 and 1 alleles on average, respectively. Years after, Dao et al. (2015) reported 14.3 alleles for *Panulirus ornatus* along the northern islands of Australia. Likewise, allelic richness found by Palero et al. (2010) for *Panulirus elephas* in the western Mediterranean, for *Panulirus mauritanus* in Morocco, Atlantic, for *Panulirus gilchristi* and *Panulirus delagoae* in South Africa, and for *Panulirus barbarae* in the Madagascar ridge, were higher than allelic richness found for *P. penicillatus* (highest average was 2.223, Tables 6a & 6b), with values of 9.87, 7.75, 8.87, 7.66 and 7.31, respectively. It is important to point out, however that the number and method of marker development varied among the mentioned studies, most of them had unspecific microsatellite markers for each species, while in this investigation we used specific *P. penicillatus* microsatellite markers.

Nevertheless it would be interesting to use our microsatellite markers with *P. penicillatus* in populations found on the western Pacific Ocean, and thus to compare migration and diversity indices between the two populations found by Chow et al. (2011) and Abdullah et al. (2014). Such a comparison was not possible in this study due to the differences between methodologies. This would help evaluate, however in real time, differences produced by their distinct evolutionary histories. Since Abdulla et al. (2014) suggested that the expansion time of *P. penicillatus* in the Western Pacific Ocean occurred earlier than in the Eastern population, and as migration happens among

these populations, this would suggest that the western populations are acting as a source population maintaining the diversity of the Eastern populations.

Allele richness was moderate (Tables 6a & 6b). Expected heterozygocity (0.405) was slightly higher than observed heterozygocity (0.410) when analyzed by Site, while by Island, the frequencies were equal (0.411). This indicates the existence of external forces, like overfishing or climate events, that are deleting low frequencies alleles (Masatoshi et al., 1975; Caballero and García-Dorado, 2013). It is important to mention that diversity indices, such as allelic richness or number of alleles are important aspects on the population dynamics, as they are the raw material for evolution and determine the adaptability potential of a population, therefore they are key elements for conservation and management (Leberg, 2002; Foulley and Ollivier, 2006; Greenbaum et al., 2014).

Panulirus penicillatus in the GMR did not present any population structure, as distance among individuals ranged from 0.0269-0.714, and Fst values were near 0, indicating a highly connected population. The same was true for the PCoA, Structure and AMOVA analyses, where there was a lack of genetic structure among Sites and Islands (Figs. 1 & 2, Table 9), and the majority of variance was explained by the differences within individuals (Table 9), which indicates complete panximia. These results are similar to those obtained by Chow et al. (2011) and Abdullah et al. (2014) using mtDNA, where no structure was found within each of the Western and Eastern Pacific populations. Similar results have been reported using mtDNA in the blue spiny lobster (*Panulirus inflatus*) along the Pacific Mexican coast, as well as reported by García-Rodríguez and Perez-Enriquez (2008), for *P. ornatus* along the Southern Asian archipelago, using mtDNA and microsatellites (Dao et al., 2015). The high connectivity of these populations can be due to their long lasting larval stages, as ocean currents drag

larvae all over the place. Biophysical factors also play an important role in the connectivity among populations, and the interaction of pelagic initial stages of marine species and its biophysical factors are still fields that need to be investigated (Matsuda et al., 2006; Sponaugle, 2009; Dao et al., 2015). For example a study carried out by Truelove et al. (2017) in the Greater Caribbean and Bermuda, found population structure for the Caribbean Spiny Lobster (*P. argus*) between neighboring basins and within basins, but not between most geographically distant basins, implying a close relationship between larval biology and a complex oceanographic circulation, which resulted in isolated populations.

In this study inbreeding events seemed very improbable, as only less than 1-2 individuals presented a higher probability of inheriting identical alleles from related ancestors (Figure 3). This suggests that Galapagos Red Spiny Lobsters has or is close to an optimal population size. As for bottleneck analyses only La Unión in Isabela appeared to have gone under a drastic reduction of its population size, as both mutational models yielded significant results (Table 10a). Los Chorros in San Cristóbal, Piedras Amarillas in Floreana and San Pedro in Isabela also presented significant results, however only with the SMM mutational model (Table 10a). Interestingly, these same islands also showed significant results when analyses were done by Island.

Although not all parameters of the Bottleneck program were met, these results coincide with Masatoshi et al. (1975) analysis, where he stated that a drastic reduction in the population size has greater impact on the number of alleles than the heterozygosity, since genetic drift deletes low frequency alleles, thus affecting the number of alleles found, while heterozyosity, instead, is also affected by the growth rate of the population, as the remaining alleles can maintain a moderate frequency, and with a high growth rate, mutation can act and allele frequencies can be recovered. As shown

by Palero et al. (2010) with *P. elephas* and *P. grilchristi* that despite of having gone through a bottleneck event, they present high allelic frequencies. Nevertheless it is important to mention that results can vary depending on the biology of each species, as Brooker et al. (2000) found three different genetic stocks among northern, western and eastern populations of the Giant Tiger Prawn (*Penaeus monodon*) but in western populations a low genetic diversity indices were evidenced due to previous bottleneck events. However, mutational models rarely mimic real life mutation dynamics.

Larval dispersal patterns of *P. penicillatus* within the GMR appears to be partially asymmetrical; flow from San Pedro and La Unión in Isabela to the rest of the Sites presented higher probabilities, but flow is still occurring among the different Sites at lower rates (Figure 4a). This observation is clearer when data is arranged by Island, in which a prominent migration of lobsters from the west to the rest of the archipelago appears (Figure 4b). This suggests that the main stock of lobster populations is supplied from the west side of the reserve. Buglass et al. (2018) evaluated the effectiveness of the GMR, by comparing the abundance and mean sizes of the Galápagos Red Spiny Lobster among No take zones and Fishing zones, and results did not show any significance difference among zones, thus implying a lack of effectiveness in the protection of the no-take zones. An important observation was the lack of information about larval supply, dispersal and recruitment patterns. Thus, this study can provide insights in order to reevaluate the GMR monitoring program and zoning scheme.

Conclusion

Previous studies done with Red Spiny Lobster (*P. pencillatus*) using mtDNA and molecular markers suggested a lack of population structure within the GMR, nevertheless they all concluded that more polymorphic markers were needed, therefore in this investigation, we analyzed the genetic composition of *P. penicillatus* using microsatellite markers developed by Mulvihill et al. (*unpublished work*) and designed specifically for this species. In spite of this, this study found similar results as previous investigations, where a lack of population structure and a highly connected network of populations was found. Nevertheless, results also showed a low number of alleles and allelic richness, representing a loss of genetic diversity that could eventually affect their capacity to respond against environmental changes. Results shown from bottleneck analyses are consistent with this, as some of the sites/islands seem to have passed through a drastic population reduction, and despite these results should be taken with caution, they are worth noting, as it is important to take precautions for the recovery of the *P. penicillatus* populations in the GMR, as they are subject to intense harvesting and to extreme oceanographic events, such as El Niño.

Gene flow among the selected populations of *P. penicillatus* of this study seems to be asymmetrical, with a major flow from Isabela to the east of the Archipelago. This suggests that the main stock of larvae is located in the West. This is consistent with findings by Buglass et al. (2018), who found bigger lobsters in Fernandina (west of Archipelago), and it is known that bigger females can produce up to 700 000 eggs, while medium-sized lobster produce up to 40 000 (Toral et al., 2002), thus there is a higher chance of larvae produced in the West to be dispersed to other regions of the Archipelago. If this hypothesis is verified by additional studies, these results may help

restructure the zoning scheme of the GMR, by closing more sites to fishing in the west of the Archipielago.

Work cited

Abdullah, M. F., Chow, S., Sakai, M., Cheng, J.-H., & Imai, H. 2014. Genetic Diversity and Population Structure of Pronghorn Spiny Lobster *Panulirus penicillatus* in the Pacific Region [1]. *Pacific Science*, *68*(2), 197–211. http://doi.org/10.2984/68.2.3

Arif, I. a., Khan, H. a., Bahkali, A. H., Al Homaidan, A. a., Al Farhan, A. H., Al Sadoon, M., & Shobrak, M. 2011. DNA marker technology for wildlife conservation. *Saudi Journal of Biological Sciences*, *18*(3), 219–225. http://doi.org/10.1016/j.sjbs.2011.03.002

Banks, S. 2002. Ambiente Físico. En: Reserva Marina de Galápagos. Línea Base de la Biodiversidad (Danulat E & GJ Edgar, eds.). pp 22-37. Fundación Charles Darwin/Servicio Parque Nacional Galápagos, Santa Cruz, Galápagos, Ecuador

Botsford, L. W., Brumbaugh, D. R., Grimes, C., Kellner, J. B., Largier, J., O'Farrell, M. R., Wespestad, V. 2009. Connectivity, sustainability, and yield: Bridging the gap between conventional fisheries management and marine protected areas. *Reviews in Fish Biology and Fisheries*, *19*(1), 69–95. http://doi.org/10.1007/s11160-008-9092-z

Bruvo, R, N.K. Michiels, T.G. D'Souza, and H. Schulenburg. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* 13:2101-2106

Buglass, S., Reyes, H., Ramirez-González, J., Eddy, T. D., Salinas-de-León, P., & Jarrin, J. M. 2018. Evaluating the effectiveness of coastal no-take zones of the Galapagos Marine Reserve for the red spiny lobster, Panulirus penicillatus. *Marine Policy*, *88* (November 2017), 204–212. http://doi.org/10.1016/j.marpol.2017.11.028

Caballero, A., and García-Dorado, A. 2013. Allelic diversity and its implications for the rate of adaptation. *Genetics*, *195*(4), 1373–1384. http://doi.org/10.1534/genetics.113.158410

Chapuis, M. P., and Estoup, A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, *24*(3), 621–631. http://doi.org/10.1093/molbev/msl191

Cockcroft, A., MacDiarmid, A. & Butler, M. 2013. Panulirus penicillatus. The IUCN Red List of Threatened Species 2013: e.T169951A6691002. http://dx.doi.org/10.2305/IUCN.UK.2011- 1.RLTS.T169951A6691002.en

Cornuet, J.M., and Luikart G. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144, 2001-2014.

Cowen, R. K., and Sponaugle, S. 2009. Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, *1*(1), 443–466. http://doi.org/10.1146/annurev.marine.010908.163757

Danulat, E. and GJ Edgar (eds.). 2002. Reserva Marina de Galápagos. Línea Base de la Biodiversidad. Fundación Charles Darwin/Servicio Parque Nacional Galápagos, Sanga Cruz, Galápagos, Ecuador. 484 pp.

Dao, H. T., Todd, E. V., & Jerry, D. R. 2013. Characterization of polymorphic microsatellite loci for the spiny lobster Panulirus spp. and their utility to be applied to other Panulirus lobsters. *Conservation Genetics Resources*, *5*(1), 43–46. http://doi.org/10.1007/s12686-012-9728-0

Dao, H. T., Smith-keune, C., Wolanski, E., & Jones, C. M. 2015. Oceanographic Currents and Local Ecological Knowledge Indicate, and Genetics Does Not Refute, a Contemporary Pattern of Larval Dispersal for The Ornate Spiny Lobster, Panulirus ornatus in the South-East Asian Archipelago, *PLoS ONE* 1–19. http://doi.org/10.5061/dryad.sp418

Debevec, T., Eddy, N., Johnson, L., Sim, J., & Westfall, K. 2014. Galápagos Lobster: Exploring innovative management for the red spiny lobster fishery in the Galapagos Islands. *Bren School Website*, 205.

Delghandi, M., Afzal, H., Al Hinai, M. S. N., Al-Breiki, R. D. G., Jerry, D. R., & Dao, H. T. 2016. Novel Polymorphic Microsatellite Markers for Panulirus ornatus and their Cross-species Primer Amplification in *Panulirus homarus*. *Animal Biotechnology*, *27*(4), 310–314. http://doi.org/10.1080/10495398.2016.1190372

El Telégrafo.(11 de enero de 2018). 165 toneladas de langosta fueron capturadas en Galápagos durante 2017 Recuperado de: https://www.eltelegrafo.com.ec/noticias/sociedad/6/165-toneladas-de-langosta-fueron-capturadas-durante-la-pesqueria-2017

Falush, D., Stephens, M., Pritchard, J. K. 2003. Inference of population structure using multilocus genetype data: linked loci and correlated allele frequencies. *Genetics*, 164: 1567-1587

FAO - Fisheries and Aquaculture Information and Statistics Branch - 27/03/2018

Foulley, J. L., and Ollivier, L. 2006. Estimating allelic richness and its diversity. *Livestock Science*, *101*(1-3), 150–158. http://doi.org/10.1016/j.livprodsci.2005.10.021

García-Rodríguez, F. J., and Perez-Enriquez, R. 2008. Lack of genetic differentiation of blue spiny lobster Panulirus inflatus along the Pacific coast of Mexico inferred from mtDNA sequences. *Marine Ecology Progress Series*, *361*(Skillman 1989), 203–212. http://doi.org/10.3354/meps07381

Goudet, J. and Jombart, T.2015. hierfstat: Estimation and Tests of Hierarchical F-Statistics. R package version 0.04-22. https://CRAN.R-project.org/package=hierfstat

Greenbaum, G., Templeton, A. R., Zarmi, Y., & Bar-David, S. (2014). Allelic richness following population founding events - A stochastic modeling framework

incorporating gene flow and genetic drift. *PLoS ONE*, *9*(12), 1–23. http://doi.org/10.1371/journal.pone.0115203

Griffiths, S. M., Fox, G., Briggs, P. J., Donaldson, I. J., Hood, S., Richardson, P., Preziosi, R. F. 2016. A Galaxy-based bioinformatics pipeline for optimised, streamlined microsatellite development from Illumina next-generation sequencing data. *Conservation Genetics Resources*, *8*(4), 481–486. http://doi.org/10.1007/s12686-016-0570-7

Hearn, A., and Murillo, J. C. 2008. Life History of the Red Spiny Lobster, Panulirus penicillatus (Decapoda: Palinuridae), in the Galápagos Marine Reserve, Ecuador. *Pacific Science*, *62*(2), 191–204. http://doi.org/10.2984/1534-6188(2008)62[191:LHOTRS]2.0.CO;2

Heylings, P., Bensted-Smith, R., and Altamirano, M.2002. Título del capítulo. En: Reserva Marina de Galápagos. Línea Base de la Biodiversidad (Danulat E & GJ Edgar, eds.). pp 10-21. Fundación Charles Darwin/Servicio Parque Nacional Galápagos, Santa Cruz, Galápagos, Ecuador

Hickman, C. P., and T. L. Zimmerman. 2000. A field guide to crustaceans of Galápagos. An illustrated guidebook to the common barnacles, shrimps, lobsters and crabs of the Gala´pagos Islands. Sugar Spring Press, Lexington, Virginia.

Jombart T. and Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics. doi: 10.1093/bioinformatics/btr521

Keenan, K., McGinnity, P., Cross, T.F. and Crozier, W.W. 2013. diveRsity: An R package for the estimation of population genetics parameters and their associated errors, *Methods in Ecology and Evolution*, doi: 10.1111/2041-210X.12067

Leberg, P. L. 2002. Estimating allelic richnes: Effects of sample size and bottlenecks. *Molecular Ecology*, *11*, 2445–2449. http://doi.org/10.1046/j.1365-294X.2002.01612.x

Martinez, L. 2006. Análisis de la variabilidad de secuencias de genes mitocondriales en diferentes poblaciones de *Panulirus penicillatus* (Olivier, 1791) (Decápoda: Palinuridae) de las Islas Galápagos. Memoria de Master 2 investigación en "Biología Marina y Ecología". UNIVERSIDAD D'AIX-MARSEILLE II.

Masatoshi, N., Maruyama, T., & Chakraborty, R. 1975. The Bottleneck Effect and Genetic Variability in Populations. *Society for the Study of Evolution*, *29*(1), 1–10. Retrieved from http://www.jstor.org/stable/2407137

Matsuda, Hirokazu. Takenouhci, Taisuke. Goldstein, J. S. 2006. The Complete Larval Development of the Pronghorn Spiny Lobster Panulirus Penicillatus (Decapoda : Palinuridae) in culture. *Journal of Crustacean Biology*, *26*(4), 579–600.

Meirmans, P.G., and van Tienderen, P.H., 2004. Genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology*, 4: 792–794, 792–794, http://dx.doi.org/10.1111/j.1471-8286.2004.00770.x

Michalakis, Y., and Excoffier, L., 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 142, 1061–1064

Mulvill, C. *unpublished work.* Isolating a set of microsatellite markers for red spiny lobster (*Panulirus penicillatus*) from the Galápagos Islands. University of Manchester.

Palacios, D. M. 2004. Seasonal patterns of sea-surface temperature and ocean color around the Galápagos: Regional and local influences. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *51*(1-3), 43–57. http://doi.org/10.1016/j.dsr2.2003.08.001

Palero, F., Abelló, P., Matthee, C. a., Macpherson, E., & Pascual, M. 2010. Genetic Diversity Levels in Fishery-Exploited Spiny Lobsters of the Genus Palinurus (Decapoda: Achelata). *Journal of Crustacean Biology*, *30*(4), 658–663. http://doi.org/10.1651/09-3192.1

Palumbi, S. R. 2004. Marine Reserves And Ocean Neighborhoods: The Spatial Scale of Marine Populations and Their Management. *Annual Review of Environment and Resources*, *29*(1), 31–68. http://doi.org/10.1146/annurev.energy.29.062403.102254

Parque Nacional Galápagos. 2017. Pesquería de langosta en Galápagos superó las 190 toneladas. Recuperado de: www.parquegalapagos.gob.ec

Perez-Enriquez, R., Vega, A., Avila, S. and Sandoval, J. L., 2002. Population genetics of Red Spiny Lobster (*Panulirus interruptus*) along the Baja California peninsula, Mexico. *Marine and Freshwater Research.* 52(8), 1541-1549. https://doi.org/10.1071/MF01104

Raymond M. and Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenisms. *Heredity*, 86, 248-249.

Reeb, C. A., and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, Crassotrea virginica. *Genetics* 124: 397-406.

Reyes, H., Ramirez, J., & Schuhbauer, A. 2013. Evaluación de la pesquería de langosta espinosa en la Reserva Marina de Galápagos. *Informe Galapagos 2011-2012*, 150–156.

Saunders, N. C., L. G. Kessler, and J. C. Avise. 1986. Genetic variation and geographic differentiation in mtDNA of the horseshoe crab Limulus polyphemus. *Genetics* 112:613-627.

Selkoe, K. a., and Toonen, R. J. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters*, *9*(5), 615–629. http://doi.org/10.1111/j.1461-0248.2006.00889.x

Toral, M. V., Espinosa, E., Hearn, A., and Martinez, C.2002. Langostas Espinosas. En: Reserva Marina de Galápagos. Línea Base de la Biodiversidad (Danulat E & GJ Edgar, eds.). pp 199-221. Fundación Charles Darwin/Servicio Parque Nacional Galápagos, Santa Cruz, Galápagos, Ecuador

Truelove, N. K., Ley-Cooper, K., Segura-García, I., Briones-Fourzán, P., Lozano-Álvarez, E., Phillips, B. F., … Preziosi, R. F. 2015. Genetic analysis reveals temporal population structure in Caribbean spiny lobster (Panulirus argus) within marine protected areas in Mexico. *Fisheries Research*, *172*(July), 44–49. http://doi.org/10.1016/j.fishres.2015.05.029

Truelove, N. K., Kough, A. S., Behringer, D. C., Paris, C. B., Box, S. J., Preziosi, R. F., & Butler, M. J. 2017. Biophysical connectivity explains population genetic structure in a highly dispersive marine species. *Coral Reefs*, *36*(1), 233–244. http://doi.org/10.1007/s00338-016-1

Verhoeven, K. J., Simonsen, K. L., and McIntyre, L. M. 2005. Implementing false discovery rate control: increasing your power. *Oikos*, *108*(September 2004), 643–647. http://doi.org/10.1111/j.0030-1299.2005.13727.x

TABLES

Table 1. Number of individuals collected per site and per island and coordinates.

Island	Site	Number of Individuals	Coordinates
Santiago	Bucanero	13	Lat.: 0° 9'40.76" S Lon.: 90°49'53.15" W
San Cristóbal	Chorros	17	Lat.: 0°57'7.76" S Lon.: 89°28'36.23" W
	Rosa Blanca	16	Lat.: 0°49'50.67"S Lon.:89°20'15.63"W
Santa Cruz	Garrapatero	9	Lat: 0°39'22.46"S Lon: 90°10'36.47"W
	Punta Rocafuerte	13	Lat: 0°40'20.87"S Lon: 90° 8'46.23"W
Floreana	Piedras Amarillas	15	Lat: 1°14'12.78"S Lon: 90°27'4.26"W
Santa Fé	Punta Miedo	11	Lat.: 0°50'15.67"S Lon.: 90° 1'42.35"W
Isabela	San Pedro	20	Lat: 1° 2'29.32"S Lon: 91°17'14.40"W
	La Unión	20	Lat: 1° 1'20.51"S Lon: 91° 6'6.47"W

Table 2. Primer sequences for the successfully amplified microsatellite loci. Source: Mulvihil et al. *unpublished work*.

Number of Primer	Primer (Fluorescent Dye)	Sequences(5'-3')	Motif	Size	# alleles per locus	Annealing temperature
1	PEN3 (HEX)	F: GGGCACGAGGACAGAAGTCAAAACG R: TAGGCGCTGACTTCTCGACCACTCTGG	TCTG	332	1	64
2	PEN4 (6-FAM)	F: GCGTACACAGAGGGATTGCTTCG R: AGTTTCCGCGAGTGAAACAAAAGCC	TCCG	387-413	3	64
3	PEN10 (6-FAM)	F: GATGTTGACACTGTTGCTGTTTCTGC R: ACAAGAGATGTTGTGACAGCGTTGC	TCTG	356	1	64
4	PEN13 (6-FAM)	F: CTGATTCCTTGGACTCGCACAAGC R: GGAAGGCATTTCCTACACCCTTTCC	TTC	208-222	3	64
5	PEN15 (6-FAM)	F: CATCTCCAGCCCAAGTATAGTGACC R: CTTCCACTGCTTACTGCACATGACG	ATT	332-338	2	60
6	PEN16 (6-FAM)	F: GCCACACTCTAGTATGAGGTTTATGAGG R: GTGATGACGATTCATGATCTGTTGC	ATT	269-291	3	60
7	PEN17 (6-FAM)	F: TACCGCGAAACACGATACATTCTCC R: GCTCATTGTTATAAGGTGCTTCTGACG	ATT	240-298	6	60
8	PEN18 (HEX)	F: ACGACCACCATGGCAGGAAAACC R: CTAGGATCGTGAAAGGAGCGAGAGG	TTC	230-255	6	60
9	PEN19 (HEX)	F: TCTCACTCTCTCTCACCACCAACTCC R: GGAAGCCAAGCTCGAGATGAATGG	TCC	222	1	60
10	PEN20 (6-FAM)	F: GTTGGGTAGGTGCTGGAGAAGC R: TTCTCAGTCCTTCAGATTAACATAGCC	TCC	282-285	2	60
11	PEN21 (HEX)	F: ACTAAAGCCGGGATCGCTTACACG R: CGAGTGAGGAGGATGCTGAAAACG	AGT	231-254	5	60
12	PEN22 (6-FAM)	F: CAAAAGATATTGTGAAGTCCTCTGC R: TCATGAGACTGACACATTTCAGAGC	TTC	228-235	2	60
13	PEN23 (HEX)	F: AAGGTGGTCTGAGTGGGGATGAGG R: TTGGACACAAGCAACGCTCATTAGG	TCC	318	1	60
14	PEN24 (HEX)	F: CCCAAAGAGCGAAGAGGAACAAGG R: CACACTCGGTTGAGAAATGGTCTCG	TCC	318-327	3	60
15	PEN27 (6-FAM)	F: GGTCTCACCCGTTGTGTTTTAAGGG R: GTTCGAAGTCGTGCTCAGGGAAGG	TCC	363-369	3	60
16	PEN28 (6-FAM)	F: CTTACTCTCCCTCCACGACGACACC R: CATTACCGCCTGGACATCACAACC	TCC	316-331	4	60
17	PEN33 (HEX)	F: CTCTGAGGGCATGTGTTGTGAAGG R: AGTCTGTACATCAGCAGCCCCTACC	ACC	292	1	64
18	PEN34 (6-FAM)	F: TCCATATGGGATCATGGTCTTGAAGG R: GAACAAGTTGTGCCAGTCTTTGCC	ATT	239	1	64

Table 3a. P-values from Linkage disequilibrium analysis by Site.

	PEN4	PEN13	PEN15	PEN16	PEN17	PEN18	PEN19	PEN20	PEN21	PEN23	PEN24	PEN27	PEN28	PEN33	PEN34
PEN3	0.800	0.727	0.531	0.999	1.000	0.853	0.905	0.659	0.974	0.981	0.641	0.691	0.604	0.913	0.673
PEN4		0.312	0.957	0.846	0.991	0.997	0.998	0.514	0.638	1.000	0.764	0.984	0.891	0.818	0.643
PEN13			0.245	0.520	0.930	0.726	0.597	0.411	0.073	0.929	0.709	0.321	0.595	0.191	0.880
PEN15				0.605	0.687	0.470	0.770	0.695	0.374	0.819	0.062	0.291	0.595	0.782	0.256
PEN16					0.884	0.923	0.431	1.000	0.614	0.382	0.480	0.281	0.158	0.628	1.000
PEN17						**0.002**	0.998	0.285	0.449	0.819	0.807	0.300	0.636	0.784	0.958
PEN18							0.985	0.020	0.014	0.871	0.601	0.511	0.094	0.987	0.804
PEN19								0.901	0.568	0.301	0.784	0.077	0.982	0.238	0.998
PEN20									**0.004**	0.452	0.959	0.882	0.814	0.868	0.190
PEN21										0.738	0.397	0.877	0.103	0.700	0.768
PEN23											0.853	0.992	0.977	1.000	1.000
PEN24												0.981	0.522	0.893	0.772
PEN27													0.988	0.362	0.618
PEN28														0.777	0.665
PEN33															0.562

Critical value = 0.0091. Numbers in bold represent loci in disequilibrium.

Table 3b. P-values from Linkage disequilibrium by Island.

	PEN4	PEN13	PEN15	PEN16	PEN17	PEN18	PEN19	PEN20	PEN21	PEN23	PEN24	PEN27	PEN28	PEN33	PEN34
PEN3	0.957	0.592	0.389	0.999	0.998	0.647	0.739	0.180	0.912	1.000	0.641	0.207	0.758	0.574	0.821
PEN4		0.322	0.864	0.876	0.979	0.950	0.951	0.439	0.080	1.000	0.819	0.996	0.964	0.370	0.519
PEN13			0.115	0.453	0.540	0.580	0.672	0.518	0.202	0.569	0.538	0.221	0.455	0.306	0.760
PEN15				0.557	0.091	0.658	0.328	0.596	0.117	0.688	0.391	0.101	0.061	0.473	0.023
PEN16					0.903	0.877	0.310	0.996	0.822	0.190	0.571	0.300	0.950	0.289	1.000
PEN17						**0.001**	0.990	0.146	0.075	0.640	0.863	0.243	0.091	0.126	0.687
PEN18							0.980	**0.003**	**0.009**	0.581	0.431	0.319	0.030	0.994	0.901
PEN19								0.817	0.994	0.293	0.580	0.123	0.743	0.215	0.977
PEN20									**0.001**	0.351	0.680	0.784	0.781	0.927	0.069
PEN21										0.795	0.290	0.775	0.023	0.794	0.459
PEN23											0.448	0.940	0.650	1.000	1.000
PEN24												0.982	0.375	0.593	0.624
PEN27													0.875	0.010	0.440
PEN28														0.861	0.382
PEN33															0.402

Critical value = 0.0091. Numbers in bold represent loci in disequilibrium.

Table 4a. Null allele frequencies by Site per Locus.

Site	PEN3	PEN4	PEN13	PEN15	PEN16	PEN17	PEN18	PEN19	PEN20	PEN21	PEN23	PEN24	PEN27	PEN28	PEN33	PEN34
Bucanero	0.119	0.000	0.000	0.000	0.000	0.004	0.032	0.000	0.052	0.067	0.001	0.000	0.000	0.000	0.001	0.001
Chorros	0.055	0.000	0.000	0.000	0.000	0.035	0.087	0.000	0.005	0.000	0.256	0.000	0.000	0.057	0.001	0.000
Garrapatero	0.001	0.000	0.098	0.000	0.001	0.000	0.034	0.107	0.000	0.000	0.001	0.000	0.079	0.178	0.145	0.224
Piedras Amarillas	0.000	0.000	0.116	0.000	0.000	0.000	0.000	0.000	0.070	0.077	0.000	0.001	0.000	0.012	0.240	0.001
Punta Miedo	0.000	0.001	0.000	0.165	0.001	0.000	0.156	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.196	0.000
Rosa Blanca	0.091	0.000	0.000	0.096	0.001	0.093	0.000	0.000	0.000	0.000	0.170	0.000	0.000	0.078	0.001	0.000
Punta Rocafuerte	0.108	0.124	0.000	0.000	0.001	0.000	0.000	0.000	0.156	0.080	0.001	0.000	0.021	0.000	0.099	0.000
San Pedro	0.001	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
La Unión	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.080	0.000	0.128	0.066	0.000	0.073	0.135	0.000

Table 4b. Null allele frequencies by Island per Locus.

Island	PEN3	PEN4	PEN13	PEN15	PEN16	PEN17	PEN18	PEN19	PEN20	PEN21	PEN23	PEN24	PEN27	PEN28	PEN33	PEN34
Santiago	0.119	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.052	0.067	0.001	0.000	0.000	0.000	0.001	0.001
San Cristóbal	0.106	0.000	0.000	0.035	0.000	0.066	0.020	0.000	0.000	0.000	0.217	0.000	0.000	0.071	0.001	0.000
Santa Cruz	0.001	0.000	0.098	0.000	0.001	0.000	0.034	0.107	0.000	0.000	0.001	0.000	0.079	0.178	0.145	0.223
Floreana	0.000	0.000	0.116	0.000	0.000	0.000	0.000	0.000	0.070	0.077	0.000	0.001	0.000	0.012	0.240	0.001
Santa Fé	0.000	0.001	0.000	0.165	0.001	0.000	0.156	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.195	0.000
Isabela	0.080	0.024	0.000	0.002	0.000	0.000	0.000	0.000	0.043	0.015	0.088	0.000	0.000	0.000	0.099	0.000

Table 5a. Hardy-Weinberg analysis by Site per Locus.

Site	PEN3	PEN4	PEN13	PEN15	PEN16	PEN17	PEN19	PEN21	PEN23	PEN24	PEN27	PEN28	PEN33	PEN34
Bucanero	0.187	1.000	0.209	0.299	0.882	0.368	0.200	0.199	0.000	0.249	0.562	0.18	**0.000**	**0.000**
Chorros	0.387	0.966	0.610	0.633	0.970	0.279	0.053	0.440	**0.002**	0.463	0.233	0.477	0.031	1.000
Garrapatero	**0.000**	1.000	0.160	0.927	**0.000**	0.471	0.076	0.389	**0.000**	0.596	0.439	0.021	0.122	0.067
Piedras Amarillas	0.625	0.780	0.122	0.704	1.000	0.580	0.055	0.102	1.000	0.423	0.411	0.278	**0.001**	**0.000**
Punta Miedo	0.945	**0.000**	0.694	0.072	**0.000**	0.348	0.018	0.416	**0.000**	**0.007**	0.240	0.134	0.045	0.947
Rosa Blanca	0.219	0.967	0.411	0.076	**0.000**	0.017	0.275	0.597	0.033	0.357	0.191	0.236	**0.000**	1.000
Punta Rocafuerte	0.346	0.121	0.419	0.653	**0.000**	0.307	0.056	0.180	**0.000**	0.07	0.272	0.048	0.204	1.000
San Pedro	**0.000**	0.653	0.508	0.140	1.000	0.583	0.052	0.413	**0.000**	0.37	0.307	0.348	0.977	0.976
La Unión	1.000	0.922	0.486	0.700	0.851	0.44	**0.002**	0.267	0.081	0.161	0.480	0.098	0.024	1.000

Critical value = 0.0091. Numbers in bold represent loci out of H-W equilibrium.

Table 5b. Hardy-Weinberg analysis by Island per Locus.

Island	PEN3	PEN4	PEN13	PEN15	PEN16	PEN17	PEN19	PEN21	PEN23	PEN24	PEN27	PEN28	PEN33	PEN34
Santiago	0.182	1.000	0.217	0.316	0.888	0.381	0.217	0.207	**0.000**	0.270	0.564	0.182	**0.000**	**0.000**
San Cristóbal	0.102	0.902	0.406	0.139	0.984	0.022	0.013	0.450	**0.000**	0.509	0.058	0.260	0.017	0.981
Santa Cruz	0.170	0.078	0.346	0.657	**0.000**	0.610	0.549	0.411	**0.000**	0.091	0.121	0.542	0.025	0.028
Floreana	0.628	0.782	0.123	0.708	1.000	0.583	0.059	0.111	1.000	0.402	0.417	0.276	**0.001**	**0.000**
Santa Fé	0.949	**0.000**	0.692	0.066	**0.000**	0.367	0.018	0.402	**0.000**	**0.004**	0.249	0.137	0.045	0.952
Isabela	1.000	0.602	0.533	0.226	0.870	0.407	**0.000**	0.469	0.042	0.413	0.446	0.344	0.026	0.958

Critical value = 0.0091. Numbers in bold represent loci out of H-W equilibrium.

Table 6a. Genetic Diversity Indices by Site

Sites	**Num**	**Ho**	**Hs**	**AR**	**PA**
Bucanero	3.500	**0.429**	**0.401**	2.180	3
Chorros	3.786	0.403	0.416	2.148	4
Garrapatero	2.857	0.334	0.417	2.157	8
Piedras Amarillas	4.214	0.399	0.426	2.276	6
Punta de Miedo	3.143	**0.446**	**0.399**	2.233	2
Rosa Blanca	3.571	0.380	0.403	2.152	4
Punta Rocafuerte	3.786	**0.456**	**0.445**	2.316	6
San Pedro	3.643	**0.404**	**0.376**	1.982	5
La Unión	4.500	0.399	0.410	2.121	7
Overall Mean	3.667	0.405	0.410	2.174	5

Num: number of alleles, Ho: observed heterozygosity, Hs: expected heterozygosity, AR: allelic richness and PA: private alleles. Numbers in bold represent situations when observed heterozygosity resulted greater than expected heterozygosity.

Table 6b. Genetic Diversity Indices by Island

Island	**Num**	**Ho**	**Hs**	**AR**	**PA**
Santiago	3.500	**429**	**401**	.180	3
San Cristóbal	4.571	390	412	.244	8
Santa Cruz	4.429	403	437	.342	14
Floreana	4.214	399	426	.276	6
Santa Fé	3.143	**446**	**399**	.233	2
Isabela	4.929	**399**	**391**	.065	16
Overall Mean	4.131	411	411	.223	167

Num: number of alleles, Ho: observed heterozygosity, Hs: expected heterozygosity, AR: allelic richness and PA: private alleles. Numbers in bold represent situations when observed heterozygosity resulted greater than expected heterozygosity.

Table 7. Genetic Diversity Indices per Locus

Locus	**Num**	**Ho**	**Hs**
PEN3	3	0.261	0.309
PEN4	5	0.143	0.150
PEN13	6	0.499	0.506
PEN15	10	0.427	0.473
PEN16	3	0.083	0.079
PEN17	19	0.878	0.886
PEN19	8	0.712	0.514
PEN21	10	0.594	0.671
PEN23	3	0.016	0.060
PEN24	5	0.712	0.642
PEN27	11	0.559	0.525
PEN28	7	0.712	0.690
PEN33	8	0.063	0.170
PEN34	5	0.067	0.083
Overall Mean	**7.429**	**0.411**	**0.411**

Num: number of alleles, Ho: observed heterozygosity, Hs: expected heterozygosity.

Table 8a. Matrix of genetic distances among Sites.

	Bucanero	Chorros	Garrapatero	Piedras Amarillas	Punta de Miedo	Rosa Blanca	Punta Rocafuerte	San Pedro	La Unión
Bucanero	0.000	-0.006	0.025	0.002	-0.001	-0.001	-0.010	0.030	0.001
Chorros	-0.006	0.000	0.042	-0.008	0.017	0.012	-0.002	0.035	0.010
Garrapatero	0.025	0.042	0.000	0.021	0.026	**0.046**	0.017	0.044	0.013
Piedras Amarillas	0.002	-0.008	0.021	0.000	-0.003	0.023	-0.016	0.003	-0.004
Punta de Miedo	-0.001	0.017	0.026	-0.003	0.000	0.011	-0.016	0.002	-0.010
Rosa Blanca	-0.001	0.012	0.046	0.023	0.011	0.000	0.004	0.030	0.010
Punta Rocafuerte	-0.010	-0.002	0.017	-0.016	-0.016	0.004	0.000	0.004	-0.010
San Pedro	0.030	0.035	0.044	0.003	0.002	0.030	0.004	0.000	-0.004
La Unión	0.001	0.010	0.013	-0.004	-0.010	0.010	-0.010	-0.004	0.000

Numbers in bold represent bigger differences among Sites, explained by population structure.

Table 8b. Matrix of genetic distances among Islands.

	Santiago	San Cristóbal	Santa Cruz	Floreana	Santa Fé	Isabela
Santiago	0.000	-0.008	-0.001	0.002	-0.001	0.018
San Cristóbal	-0.008	0.000	0.012	0.004	0.009	0.020
Santa Cruz	-0.001	**0.042**	0.000	-0.005	-0.006	0.005
Floreana	0.002	0.004	-0.005	0.000	-0.003	0.002
Santa Fé	-0.001	0.009	-0.006	-0.003	0.000	-0.003
Isabela	0.018	0.020	0.005	0.002	-0.003	0.000

Numbers in bold represent big difference among Islands, explained by population structure.

Table 9. Analysis of molecular variance (AMOVA) among sites and among individuals nested in Sites and Islands

Source of Variation	% variance	Variance Component	Fstat (Value)	P-value
Within Individuals	98.161	2.842	F_it (0.018)	0.455
Among Individuals nested in Sites	0.950	0.028	F_is (0.010)	0.091
Among Sites nested in Islands	0.834	0.024	F_sc (0.008)	0.300
Among Islands	0.055	0.002	F_ct (0.001)	0.156

C.v. = 0.005

Table 10. Bottleneck analyses using different mutation models a) by Site b) by Island.

a)

SITE	TPM	SMM
Bucanero	0.357	0.314
Chorros	0.239	**0.006**
Garrapatero	0.527	0.567
Piedras Amarillas	0.310	**0.002**
Punta de Miedo	0.569	0.509
Rosa Blanca	0.231	0.083
Punta Rocafuerte	0.426	0.078
San Pedro	0.214	**0.022**
La Unión	**0.008**	**0.000**

b)

ISLAND	TPM	SMM
Santiago	0.361	0.319
San Cristóbal	0.092	**0.001**
Santa Cruz	0.475	0.584
Floreana	0.326	**0.002**
Santa Fé	0.566	0.508
Isabela	0.032	**0.000**

TPM: *two phase mutation model*. SMM: *stepwise mutation model.* P-values lower than 0.050 mutation/drift disequilibrium, intense population reduction. Numbers in bold represent p-values lower than 0.050 mutation/drift disequilibrium, which suggest intense population reduction.

FIGURES

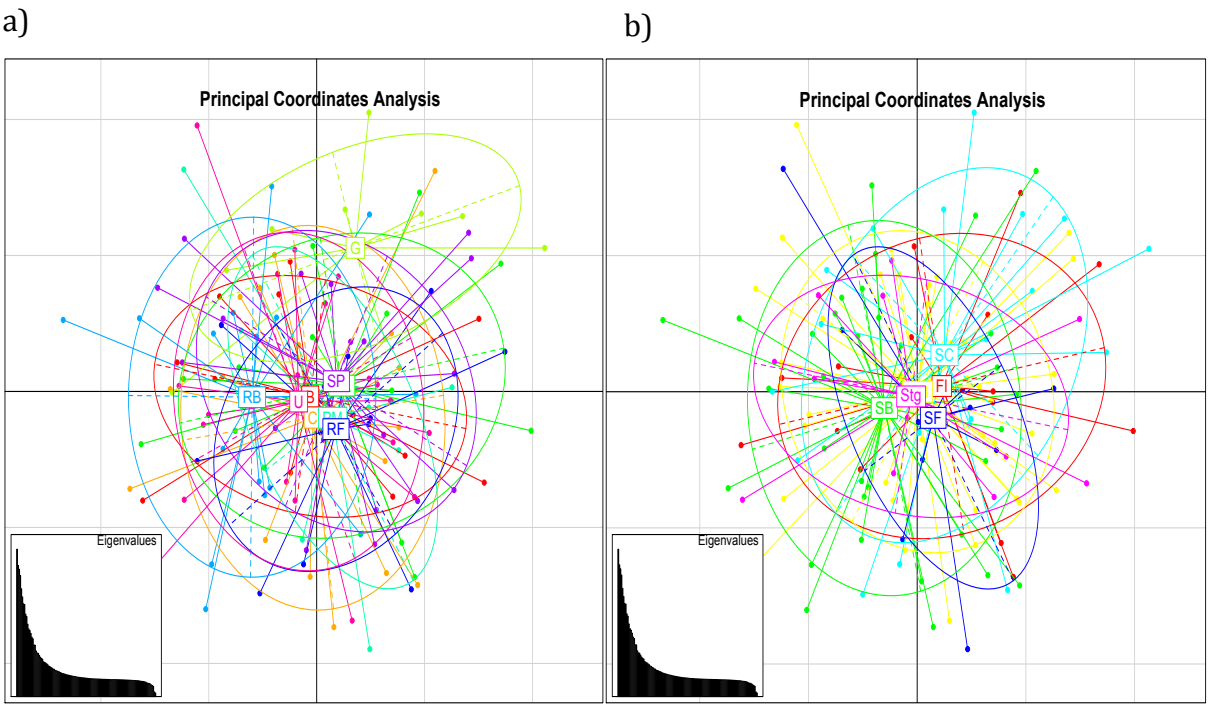

Figure 1. A) PCoA of data by Site, RB (Rosa Blanca in San Cristóbal), U (La Unión in Isabela), B (Bucanero in Santiago), RF (Punta Rocafuerte in Santa Cruz), SP (San Pedro in Isabela), C (Chorros in San Cristóbal), G (Garrapatero in Santa Cruz), PA (Piedras Amarillas in Floreana) and PM (Punta de Miedo in Santa Fé). **B)** PCoA of data by Islands: SB (San Cristóbal island), Stg (Santiago island), Fl (Floreana island), SC (Santa Cruz island), IS (Isabela island) and SF (Santa Fé island).

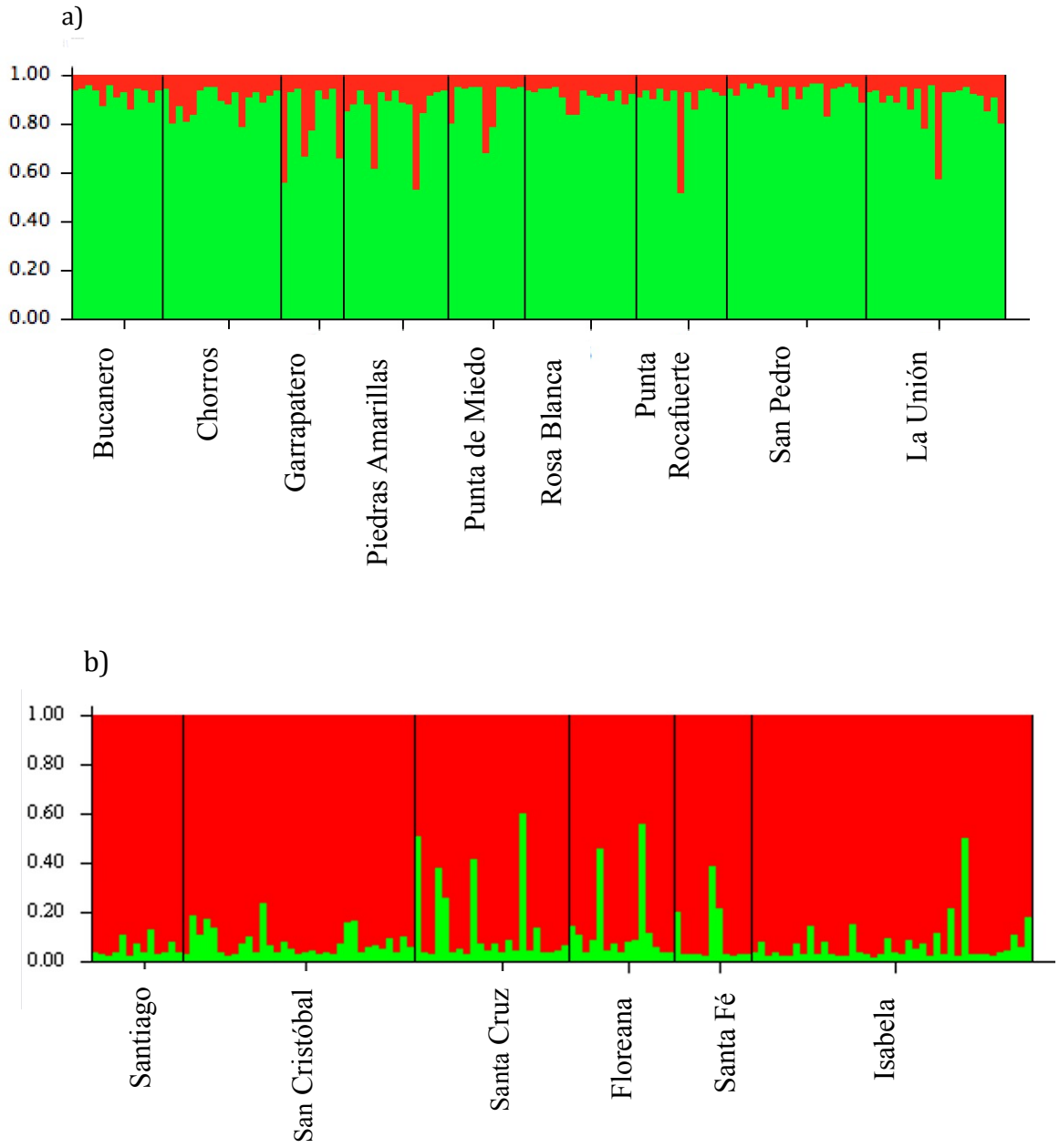

Figure 2. Plot chart with K=2 from the Structure analysis, a) shown per Site and b) per Island.

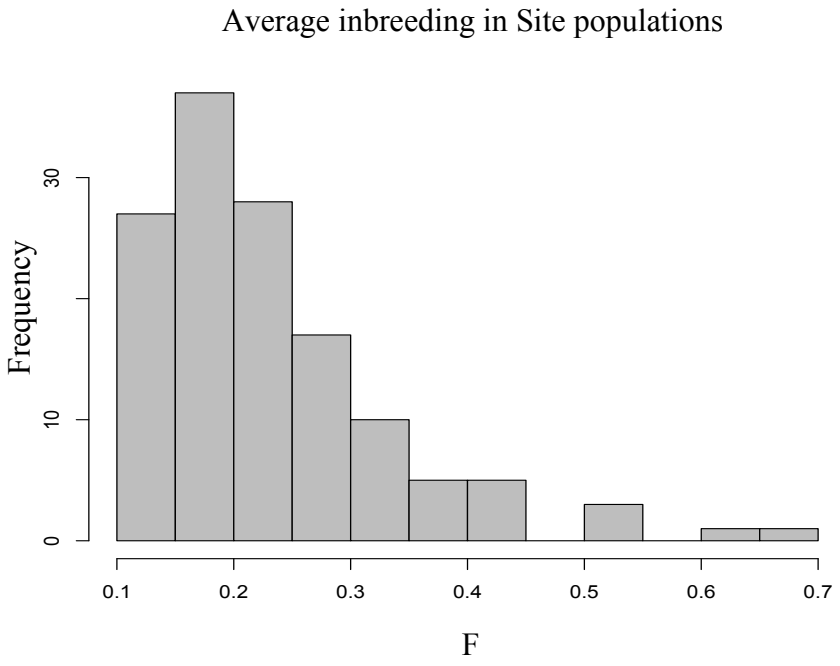

Figure 3a. Distribution of the mean inbreeding coefficient F, across all individuals by Sites

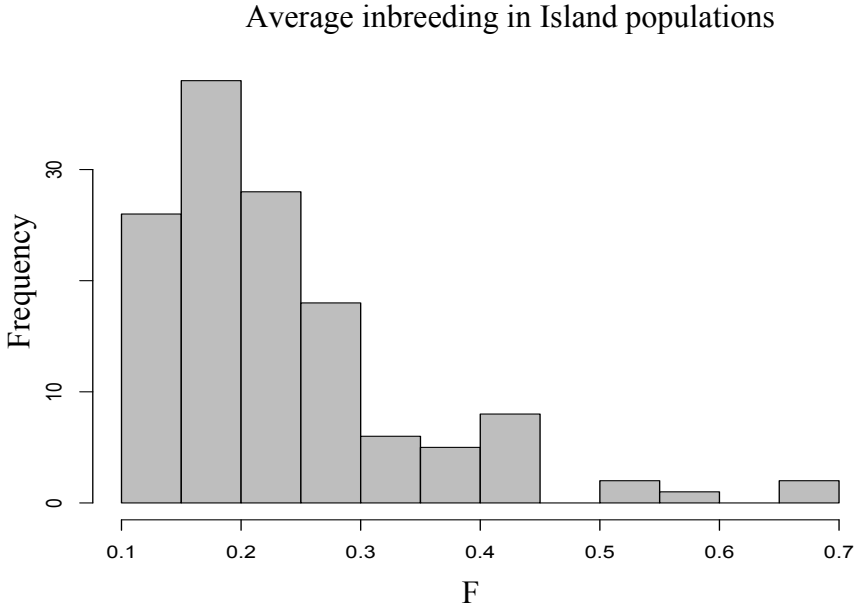

Figure 3b. Distribution of the mean inbreeding coefficient F, across all individuals by Islands

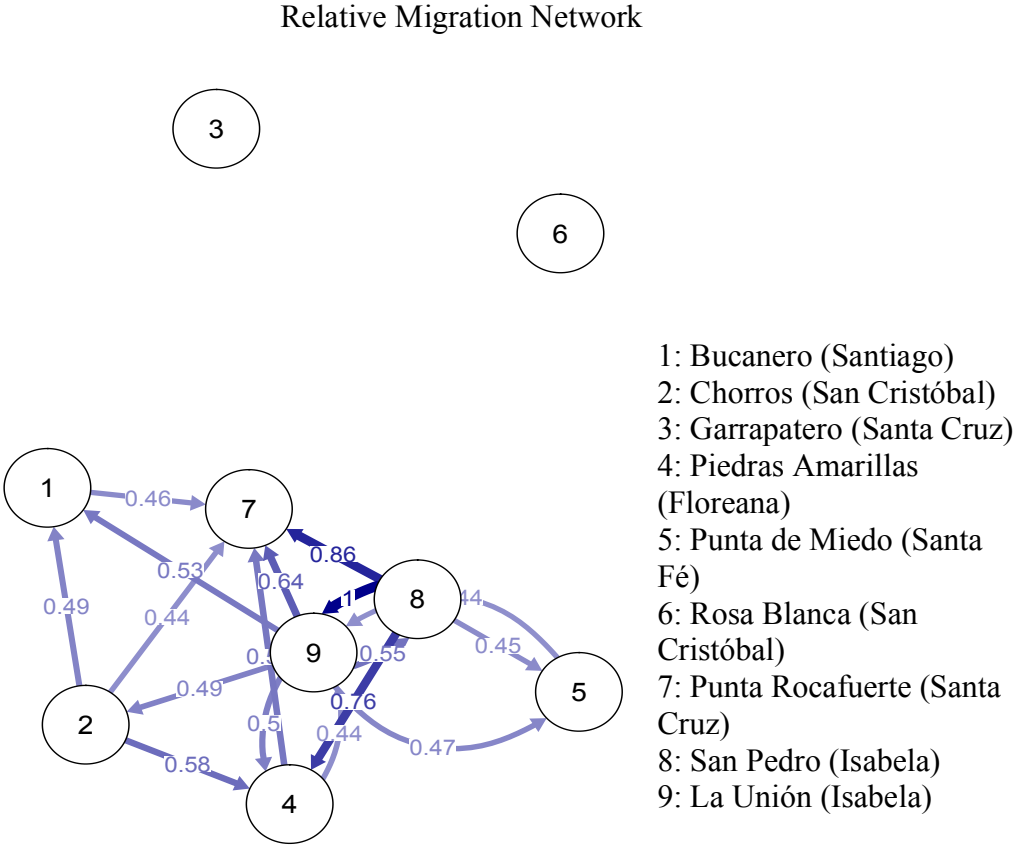

Figure 4a. Migration Network organized by Sites, with a threshold of 0.4. Dark blue represents higher migration.

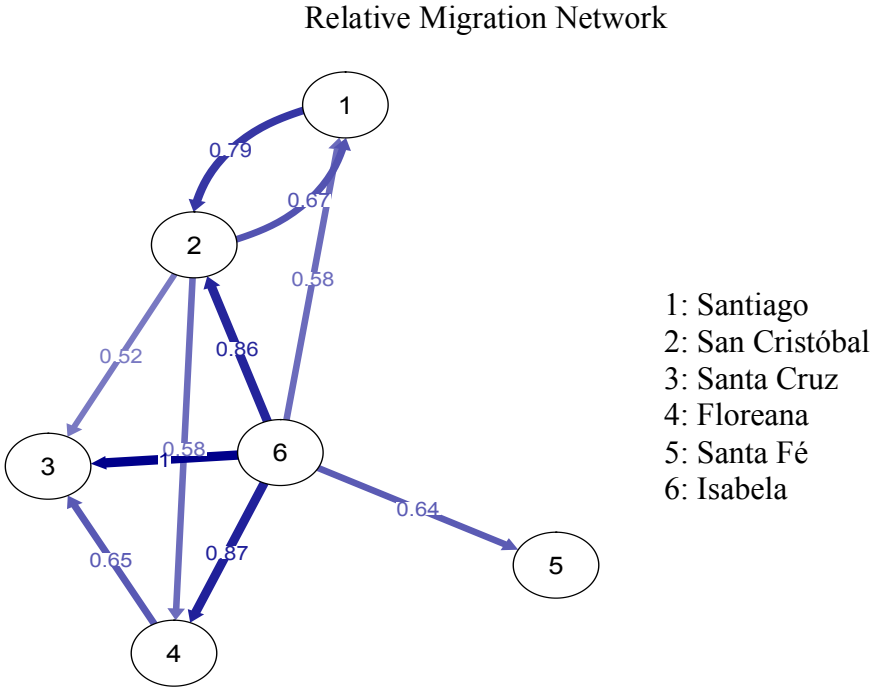

Figure 4b. Migration Network organized by Islands, with a threshold of 0.4.Dark blue represents higher migration.