The influence of environmental heterogeneity and sexual selection on the genome of the Brown Booby (Sula leucogaster)

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The influence of environmental heterogeneity and sexual selection on the genome of the Brown Booby (*Sula leucogaster*)

by

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TABLE OF CONTENTS

DECLARATION

The material contained within this thesis has not previously been submitted for a degree at Durham University or any other University. The research reported within this thesis has been conducted by the author unless indicated otherwise.

DEDICATION

I dedicate this work to my family, my mentors, and my friends. The foundations of who I am today.

" It pays to keep an open mind, but not so open your brains fall out."

- Carl Sagan

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ABSTRACT

Sexual selection has been a central theme in evolutionary biology since the time of Darwin, but even though field studies have shown that sexual selection on focal traits can vary across populations and over time, we rarely understand why. In some mating systems, ornamental traits (e.g., colorful displays) are thought to be condition-dependent signals that reveal the genetic characteristics and the current state of the bearer, acting as honest phenotypic cues through which the quality of potential mates can be assessed. In the present thesis, blood samples of brown boobies (*Sula leucogaster brewsteri*) were collected from males, females and offspring in three colonies with different environmental conditions in the Pacific Coast of Mexico. I used a customized ddRAD sequencing approach to generate genome-wide SNPs to investigate the genetic variability associated with phenotypes subject to sexual selection in brown boobies, and how selection of such traits might vary in the presence of environmental heterogeneity.

In chapter 2, I examined the mate choice and extra-pair paternity of brown boobies under different environments. A parentage analysis was performed to determine the rate of extra-pair paternity (EPP), and measurements of heterozygosity and genetic similarity were used to find correlations of genetic quality and ornaments used during courtship. Additionally, the genetic quality of the individuals was compared against measurements of colour and body mass which are traits that could be subject to sexual selection. The results suggest that variation in skin colour is an honest indicator that reflect the heterozygosity and dissimilarity of brown boobies, and that levels of EPP are very low and that it is potentially influenced by local environment.

In chapter 3, I investigated the distribution of the genetic diversity will and the correlation with geographic distance and to the environmental variables like sea surface temperature (SST) and primary productivity (PP) in each colony of brown boobies. I also measured the genomic structure, gene flow and demographic history of brown boobies and explore the possible implications in the context of mate choice. I found that brown booby populations in the Eastern Pacific seem to be highly differentiated and genetically isolated regardless of their relatively proximity. Additionally, a small but significant correlation between environmental variables and the genomic variation was found, which could be subject to natural or sexual selection.

In chapter 4, a specific sampling scheme of my genomic data was applied to characterize different signatures of selection (genome-wide selection component analysis framework). Two genome-wide selection scans were used to detect outlier loci under putative positive selection by comparing allele frequencies between males, females and offspring. The genomic regions near to such outlier loci were extracted to investigate the biological function (using Gene Ontology terms) associated with such regions. By comparing different groups of individuals based on their sex, living stage and geographical location, different types of selection were identified in brown boobies, though the caveats of the used framework are discussed. Various biological functions appear to be associated with different forms of selection simultaneously, like in the case of sex-specific viability, gametic and natural selection. However, the biological functions like growth, rhythmic processes and locomotion seems to be associated specifically to sexual selection.

Finally, I revisited the main results of my thesis in chapter 5 and discuss impact of my findings in a broader context.

1. GENERAL INTRODUCTION

1.1 Evolutionary processes in the dynamics of populations

1.1.1 Population genetics theory, evolutionary forces and change in genetic variation

Population genetics is a part of evolutionary biology that helps to investigate life-history, ecological traits and other essential components to explain evolution (Stearns, 1993; Travis, 2017). Population genetics deals with theoretical approaches to understand evolutionary forces interact with populations through time (Hartl & Clark, 1980), like the distribution of allele and genotype frequencies. The proportion of alleles at a specific locus in the population is known as allele frequency, whereas the genotype frequency is the proportion of individuals that share a specific genotype (Ridley, 1993).In fewer words, population genetics is the study of allele frequency changes through time. In past decades, population genetic has been considered more theoretical rather than experimental or observational (Gillespie, 2004), but with the technological advance in the field new interdisciplinary branches are emerging to answer complex evolutionary questions with the combination of observational and experimental approaches.

To understand better the dynamics of populations it is necessary to address several evolutionary forces that influence the allele frequencies like gene flow, genetic drift, mutations and natural selection (Ridley, 1993). Mutations introduce new genetic variation to the population that will be heritable, and they happen very slowly across large generational scales (Hartl & Clark, 1980). Migration can cause gene flow, moving changing allele frequencies among populations, and in a way, maintaining sub-populations “genetically connected” and preventing the divergence and differentiation of populations (Hartl & Clark, 1980; Ouborg et al., 2010). Random

genetic drift is the mechanism of the fluctuations on the frequencies of alleles from one generation to the next, which can affect randomly the allele frequencies specially when population is small (Ouborg et al., 2010). Natural selection is the force that allow specific alleles associated with survival and reproduction to be maintained, while those that are detrimental will tend to disappear leading to adaptation (Hartl & Clark, 1980). In summary, mutation increases genetic diversity, migration can prevent or promote the divergence of populations, while natural selection and genetic drift reduce the diversity of specific or random alleles (Frankham et al., 2004). The combination of several complex processes allows these forces to change the pattern of gene frequencies in populations or the new arrangement of previously existing patterns of variation within genomes or among subpopulations (Hartl & Clark, 1980). When talking at about populations, the level of genetic diversity can be affected by various factors. For instance, the effective population size (Ne), which is fundamental to determine how fast genetic drift is depleting the genetic diversity, (Frankham et al., 2004). For example, if the Ne is small, the genetic diversity will be lost faster and vice versa. In the present project, all these concepts are used in some way, from the postulation of hypotheses to the complex algorithms used in some of the most sophisticated software used in statistical analyses. Additionally, as the focus species of this thesis is the brown booby (*Sula leucogaster brewsteri*), special attention is given to aspects like the marine ecosystems, seabirds, and mating systems.

By using genetics and evolutionary theory, molecular tools have been developed to investigate the mechanisms that shaped genetic diversity of contemporary populations at a finer scale. Such tools usually help disciplines in biological sciences like taxonomy, phylogeography, biomedicine, genetic

engineering, etcetera. In the specific case of phylogeography, such approaches allow researchers to infer colonisation histories, positions of populations or species by tracking the patterns of molecular markers (Ouborg et al. 2010). Moreover, molecular biology in general can provide novel information about historical evolutionary forces affecting a species, especially when the morphological variation is absent or biogeographic history is unknown (Morrone & Crisci 1995). When the mentioned fields work together it is known as molecular biogeography, and it allows a way to answer evolutionary questions about the distribution of genetic variation based on morphological variation or historical influences (Weisrock & Janzen 2000).

The sequencing of nucleic acids has evolved greatly, but the principles and objectives remain the same; try to determine the exact number and order of base pairs in the DNA or RNA molecules. The use of sequencing tools has increased exponentially in the past decades, becoming every day more available to research (Grada & Weinbrecht 2013; 11 Shapiro et al. 2013). Since Edward Sanger developed the chain termination method in 1975 (Sanger sequencing), it became the primary sequencing technology (first generation) for almost three decades (Sanger et al. 1977) being implemented as the core technology for commercial and laboratory applications (Liu et al. 2012). The Human Genome Project was the first major attempt into sequencing a whole human genome, which took around 13 years and $3 billion to be completed with Sanger sequencing (Pettersson et al. 2009; Grada & Weinbrecht 2013). Shortly after the completion of this project, Life Sciences launched the 454 sequencer in 2005, allowing high-throughput sequencing at a low cost compared with Sanger's method. The following years, more companies lounched similar sequencing platforms like Genome Analyzer (Solexa) and SOLiD (ABI), becoming the first

sequencer systems in the newly called Next Generation Sequencing (NGS) (Liu et al. 2012). Presently, NGS technologies are opening new opportunities for research in different areas, since it has improved in precision and throughput, and have enabled the sequencing of entire genomes more easily (Lander et al. 2001; Walker et al. 2013).

The advance in sequencing technology has brought some revolutionary changes in some branches of evolutionary biology and conservation genetics. The changes enabled unprecedentedly data collection from genomes or subsets of genomes from many individuals but processing a high number of samples is still expensive enough to limit research to projects where funding opportunities are relatively substantial (Pettersson et al. 2009; Fumagalli et al. 2014). With the continuing improvement of NGS, the accessibility of this tool will continue to increase for research institutions in countries all around the world since it will be more cost effective, leading to progress in genomics and other areas (Liu et al. 2012; Snyder et al. 2015). Due to the current advance in sequencing, this provides critical timing for exploring the limitations and advantages of applying genomic tools to other disciplines that rely heavily in the interpretation of results, like for instance, conservation problems (Allendorf et al. 2010).

For many years, mitochondrial genes (mtDNA) were the preferred markers for population genetic studies because it presented clear advantages and proactivity. For instance, genetic drift will fix mutation faster in mtDNA than nuclear DNA due to low substitution rate and relatively low effective population size (Hickerson et al. 2010; Brown et al. 1979). Nuclear DNA markers that are useful for population are microsatellites (Selkoe & Toonen 2006), amplified fragment length polymorphisms

(AFLPs, Meudt & Clarke 2007), introns (Friesen et al. 1999), anonymous loci (Jennings & Edwards 2005), and single nucleotide polymorphisms (SNPs; Morin et al. 2004). Though, microsatellites have probably been the most commonly used nuclear marker for population genetic and phylogeographic research; however, due to frequent back mutation and homoplasy, they are less useful when population genetic divergence is deep (Selkoe & Toonen 2006).

As predicted by Moore's law, the growth in the number of transistors in an integrated circuit has doubled approximately every two years since 1975 (Schaller 1997). A similar tendency to this law can be applied to sequencing technology where current technological advancements are increasing the throughput even more, to the extent of analysing sequence-based expressions at individual cellular level (Simon et al. 2009). The growth of NGS studies is focusing nowadays on the history of selection, genetic architecture, regulation, and trying to relate this to conservation rather than only focusing on detecting signatures of selection (Ekblom & Galindo 2011). Bioinformatics is a fundamental part when dealing with genomic methodologies, given that is the primary tool to manage the output data, and which is adapting to every change in gathering techniques (Allendorf et al. 2010). The organisms being sequenced are increasing exponentially; thus, a vast amount of genetic data is being processed worldwide every day (Liu et al. 2012). If technology continues evolving at this rate, storage and sharing systems will need to be improved as well, in order to support the massive storage of genetic data (Ekblom & Galindo 2011).

1.1.2 Environmental variation

It is known that the environment has a major impact in evolutionary history of species, influencing adaptation or migration (May & McLean, 2007) (. Therefore, a

good understanding of the influence of environmental changes on species and to population level is necessary to assess and predict ecological events (Prost et al., 2010). Also, environmental variations can influence greatly the demographic dynamics of a species causing admixture or the drastic reduction of population size (Pyhäjärvi et al., 2013).

Drastic changes in species abundances will result in unpredictable response in reproduction and the survival of species (Harmon et al., 2009). Also, it can promote changes in foraging strategies and diet, which can allow species to survive in different environmental scenarios (Grémillet & Charmantier, 2010; Moseley et al., 2012). In marine ecosystem, some regions are in a delicate trophic balance and many species are sensitive to slight variations in environmental conditions (Botsford et al., 1997). Marine top predators like seabirds, are a good example of organisms that change their foraging behaviour with changes in food availability (Harding et al. 2007), adapting to new environments and prey species in order to survive (Burger & Piatt, 1990; Croxall et al. 1999). In such systems environmental heterogeneity promotes variation in phenotypic plasticity, changing the distribution of phenotypes and also influencing natural selection (Cornwallis & Uller, 2010).

1.1.3 Population differentiation, reproductive isolation and gene flow

A fundamental process that is responsible for the generation of biodiversity in the planet is speciation (Mallet 2008), and it has been studied since the publication of On the Origin of Species (Darwin, 1859). Speciation is the evolutionary process where populations differentiate from each other (genetic population differentiation) to become distinct species, and one of the main drivers of this process is through reproductive isolation, which can happen suddenly (Wood et al., 2009). However,

isolation between populations tends to develop over large timescales through reproductive barriers where geneflow between population can be prevented causing genetic differentiation (Coyne & Orr, 2004). While the formation of barriers is happening, gene flow between population can be restricted partially, promoting the accumulation of population genetic differentiation (Mayr, 1963). Geography in divergence has played a central theme in speciation research, and multiple theories have been proposed about different scenarios of speciation at different degrees of geographic isolation like when populations are complete isolated (allopatric speciation) or when populations partially or completely overlap (sympatric or parapatric) (Coyne & Orr 2004; Barluenga et al. 2006).

Seabirds have the ability to visit other islands in distant geographic locations given that they are greatly adapted to long distance flights, having the potential of disperse to other colonies for breeding (Reed et al. 1999; Weimerskirch et al. 2010). However, some studies propose a high fragmentation on seabird populations mainly because of elevated natal philopatry (Huyvaert & Anderson 2004). As seabirds depend on the marine environment at all life-stages and specific features like water nutrients and temperature can influence the breeding timings and the foraging habits (Schreiber & Burger 2002; Raymond et al. 2010; Weimerskirch et al. 2010), and consequentially in the genetic distribution among populations.

1.1.4 Sexual selection by mate choice and the impact of environment in selected traits

Sexual selection can be defined as the variation in direct fitness among different phenotypes caused by their ability to gain sexual partners, produce fertile eggs and generate offspring (Cornwallis & Uller 2010). It arises due to competition

for mates or their gametes when individuals with specific traits have an advantage over other members of the same sex, (Darwin 1871; Parker 1970). This sexual competition often leads to evolution of sexually selected traits on males, which can increase attractiveness in the form of vivid colorations, more vigour on courtship, exaggerated body modifications, or adaptations used to compete within the same sex, etc. (Andersson 1994). These modifications often differ among males within populations, and female preferences often vary simultaneously with them (Gray & Cade 2000; Brooks 2002; Grace & Shaw 2011), which could suggest that traits and preferences can evolve rapidly. However, the bias between males usually being the main competitor in sexual selection and females the choosers, has been debated extensively, though such question remains unresolved (Reeve & Pfennig, 2003).

In theory, the condition or quality of an individual is reflected in sexual traits which are usually phenotypically plastic (Nur & Hasson 1984; Grafen 1990; Qvarnström & Price 2001). According to this consideration, males showing these traits experience higher mating success, but only the fittest individuals can support exaggerated sexual traits (Price 2006). Several studies have demonstrated the importance in social interactions of plumage patterns in birds, where size and brightness of colour patches is often correlated with the condition and social status of the individual that bears such trait (Rohwer 1982; Andersson 1994; Johnstone 1995; Pryke et al. 2002; Jawor & Breitwisch 2003; Alonso-Alvarez et al. 2004).

Brighter colours in sexual traits can be acquired in some birds through a diet rich in carotenoid, which can cause feather colorations ranging from red, orange, yellow, blue and violet (Brush 1990; Völker, 1953). Manipulation of carotenoids also

affects the colour of pigmented bare parts, such as the beak of the zebra finch, *Taeniopygia guttata* (McGraw & Ardia 2003; Alonso-Alvarez et al., 2004). They also are important physiological modulators and so have a range of health-related functions (Lozano 1994; Rock, Jacob & Bowen 1996). Unlike the other main sexually selected trait in birds (like vocalizations), colour patterns have clearly identifiable environmental and genetic basis (Price 2006), which highlights the role of carotenoid pigments in sexual selection (Negro et al. 1998), and reflect genetic quality related with more efficiency in foraging. Even when most of studies have investigated carotenoids in plumage (Negro et al. 1998), carotenoids in bare parts, might reflect recent physiological events and therefore, indicate the current condition of the individual (Burley, Price & Zann 1992; Lozano 1994; Owens & Short 1995; Bortolotti et al. 1996).

Individuals that evaluate potential mates are likely to obtain direct or indirect benefits like high fecundity rates, higher offspring survival acquired by good genes or compatible genes (Rosenthal, 2017). In recent years, the several studies suggest that ornamental traits work as signals of male quality to help females to choose mates providing genetic benefits for their offspring (good genes) (Zahavi 1975; Andersson 1994; Tomkins et al. 2004; Cotton et al. 2004). Heterozygosity is linked to increased vigour (heterozygosity-as-good genes model) and, therefore, more-heterozygous males could more effectively provide direct benefits to females and their offspring (Mays & Hills 2004). Another pathway to acquire fitness for offspring is through dissimilarity of potential mates (compatible genes), where fitness is increased by certain alleles in a specific genetic context or by gene-to-gene interactions like epistasis (Kempenaers, 2007; Neff & Pitcher, 2005).There are several empirical

examples of how species choose mates based on good genes, compatible genes or heterozygosity. For instance, female blue tits, *Parus caeruleus*, increase the heterozygosity of their progeny through extra-pair mattings. Females thereby produce offspring of higher reproductive value, because less inbred individuals have increased survival chances (Foerster et al. 2003). Similarly, male attractiveness in guppies (*Poecilia reticulate*), based on visual cues for body size (Reynolds & Gross 1992) and colour (Houde & Torio 1992), have been cited as examples of selection by female choice based on "good genes", whereas olfactory cues in other fish have been shown to be reliable indicators of genetic dissimilarity (Aeschlimann et al. 2003).

A classical assumption is that in monogamous species, especially those with extended biparental care, sexual selection is relatively weak compared to highly polygamous species (Emlen & Oring 1977). Nevertheless, sexual selection can be strong in many monogamous species (Fisher 1930; O'Donald 1980b; Mock 1985; Kirkpatrick et al. 1990), and there can be competition in monogamous species over mates where individuals cannot have more than one mate, but some could have none if there are differences in the sex ratio (Andersson 1994). Molecular genetic studies of parentage have revolutionized the views of avian mating systems. The classic definition of monogamy is, "a prolonged association and essentially exclusive mating relationship between one male and one female" (Wittenberger, 1979). However, multiple mating by avian females is known to result in widespread and highly variable rates of extra-pair paternity (EPP) (Westneat & Stewart 2003), and among socially monogamous species, an average of 10% of offspring are the result of EPP (Griffith et al. 2002).

Temporal and spatial environmental heterogeneity can cause large fluctuations in both the strength and direction of selection, by changing the interactions between different selection pressures (Cornwallis & Uller 2010). Evolutionary processes can work differently depending on if they are present in a constant or a variable environment (Levins 1968; Schlichting & Pigliucci 1998; Lenormand et al. 2009), which can affect sexual or non-sexual traits. An example of where sexual selection has been influenced by environment heterogeneity is the case of a wild population of Soay sheep (*Ovis aries*), where phenotypic and genetic associations between male horn growth and lifetime reproductive success were positive under good environmental conditions (because of increased breeding success) and negative under poor environmental conditions (because of reduced survival) (Robinson et al. 2008). These kinds of studies shed light into the trade-offs of investing in the production of sexual ornaments and the association of environmental conditions.

1.2 Molecular tools to resolve evolutionary questions

1.2.3 Advantages of next generation sequencing technologies in molecular ecology

In the last decade, next generation sequencing (NGS) has become more widely used, allowing the sequence of thousands of loci at a relatively low cost, which are permitting the implementation of analysis that in the past were inaccessible. Some of these analyses that were benefited by NGS are the genome-wide association studies, which now allow for the investigation the association between genotype and phenotypes, their ecological relevance, and the discovery of locally selected loci (Stapley et al. 2010). Moreover, studies about gene flow, population history, demography, population history, inbreeding and genetic structure are aided from high-throughput sequencing (Ouborg et al. 2010; Ekblom & Galindo 2011). This has

allowed to ecologist to work in areas like transcriptome profiling, epigenetics, gene regulation and other disciplines that would have been inaccessible with traditional methods (Simon et al. 2009).

Thanks to the advance of sequencing technologies research about the changes in allele frequencies and the effect of natural selection, hybridization and genetic drift in wild populations can be used as tools for conservationist to develop better management strategies (Allendorf et al. 2010). These days, the use of AFLPS and microsatellites have been outperformed using Single Nucleotide polymorphisms (SNPs) discovered by genome sampling methods, which provide a better representation of the genetic variation of populations and at an individual level because it covers larger sections of the genome (Ouborg et al. 2010). One of the major advantages in ecology, is the small quantity of DNA that is needed for newer sequencing technologies. Studies about ancient DNA or those about endangered species received a major aid as tissue samples are usually difficult to obtain or is degraded (Ekblom & Galindo 2011).

Nowadays, all these advantages brought by NGS have become a standard practice in many fields and allowing to generate more sequences at a more affordable budget (Simon et al. 2009). The present study is benefited of the current improvement in protocols adapted to NGS data as it utilizes a customised double digest RAD sequencing approach (or ddRADseq), which outputs a reduced representation of the genome at an individual level. Sequences obtained by ddRADseq can be used for parentage assessment, evidence of philopatry to a particular population, and assessment of the outcome of mate selection. Microsatellites have been commonly

used for studies of parentage and kinship (Glaubitz et al. 2003; Jones et al. 2010), whereas the use of single nucleotide polymorphism (SNP) has increased recently in both model and non-model organisms (Weinman et al. 2015). However, the benefits of SNPs over microsatellites are that they are more easily genotyped on a per locus basis, have lower rates of genotyping error, and are cheaper to genotype per locus (Jones et al. 2010), and they are also emerging as a viable option for parentage analysis of wild populations (Weinman et al. 2015).

1.2.4. Advantages of molecular tools to investigate sexually selected traits

To understand sexual selection better, more research of the genetic variants that shape sexually selected traits is needed (Wilkinson et al. 2015). In this matter, molecular genetics and genomics allow a detailed characterization of genes and their effects on fitness (Andersson & Simmons 2006). The availability of lower costs of high-throughput sequencing methods has made genome-wide association studies (GWAS) a practical approach, which can identify regions of the whole-genome of multiple individuals that differ by phenotype and contain informative single nucleotide polymorphisms (SNPs) (Wilkinson et al. 2015). This can help to find loci that influence phenotypic traits of interest like are the candidate gene approaches (Fitzpatrick et al. 2005; Wayne & McIntyre 2002). It is important to note that SNPs found by GWAS are in Linkage Disequilibrium (LD) with statistically associated phenotypes, however, not the cause of the variation in such traits (Bush & Moore, 2012). Unfortunately, the underlying sequence variants that cause differences in sexually selected traits within or between the sexes remain largely unidentified (Wilkinson et al. 2015). One example where the genes underlying sexual phenotypes have been identified is Soay sheep. Johnston et al. (2011) also studied the wild Soay

sheep (*Ovis aries*) which have an inherited polymorphism for horn morphology in both sexes, controlled by a single autosomal locus, *Horns*. Individuals with this locus present deformed horns which provide reduced fitness compared with the ones presenting normal horns. Thanks to GWAS and using around 36 000 SNPs, it was possible to determine the main candidate for *Horn*s as *RXFP2*, an autosomal gene with a known involvement in determining primary sex characters in humans and mice.

Alternatively to the use of GWAS, the genome-wide selection scans try to detect regions of the genome under putative natural selection which later can be further investigated for biological functionality. Recently, an interesting new approach has been implemented to scan regions of the genome to detect different types of selection by using traditional genome-wide selection scans. In a study by Monnahan et al. (2015) they combined direct measurement of survival and reproduction with whole-genome genotyping of a plant species (*Mimulus guttatus*) that invaded a new habitat in recent years. The relevance of this study is that they adapted the theory of the classic selection component analysis (SCA) by Christiansen & Frydenberg (1973) in order to work with outlier SNPs detected by genome-wide scans. They were able to detect viability selection in a specific environment that presented high levels of divergence from neighbouring populations, which allowed them to make inferences about the local adaptation in that studied species. Similarly, Flanagan & Jones (2017) implemented a genome-wide selection components analysis in Gulf pipefish (*Syngnathus scovelli*), which present a reversed sex role through male pregnancy. By sequencing adult females (chooser sex), pregnant males, non-pregnant males and their offspring, they were able to detect 47 regions of the genome under putative sexual selection and 468 regions with signatures of sex-specific viability selection. They

found that these types of selection tend to benefit rare alleles in populations and concluded that the genome-wide selection component analysis can complement greatly other approaches to detect genome-level selection in non-model organisms.

1.3 Study system

1.3.1 Characteristics of brown boobies

The Sulidae family is a group formed by ten species of seabirds that are distributed around the world (Nelson 1978; Friesen et al. 2002; Chesser et al. 2010). Only three genera are recognized nowadays, *Papasula* (Abbott's booby), the *Sula* (known species of boobies), and *Morus* (gannets). Of these genera, Abbott's booby it is endemic of Christmas Island in the Indian Ocean, the boobies are restricted to the tropics, and the gannets breed in temperate regions in the Northern and Southern Hemisphere (Friesen & Anderson 1997; Friesen et al. 2002; Patterson et al. 2011). The red-footed, masked and brown boobies are part of the so-called blue water boobies, which share the same pantropical distribution, ecological attributes and breed in some of the same islands (Nelson 1978; Patterson et al. 2011).

Generally, the brown booby is a large seabird, which possesses sexual dimorphism among adults, where the face of males carries blue-grey skin, and the female has bright yellow skin (Bull & Farrand, 1984). Bill and feet (Figure 1.1) can vary in a range of colours like, bright yellow, bluish yellow, greenish yellow, and greyish (Schreiber & Norton 2002). This species has a pantropical distribution with multiple subspecies, however, in the present study I focus on *Sula leucogaster brewsteri*, which breeds on islands in the eastern tropical pacific from the northern Gulf of California south along the Pacific coast of Mexico (Bent 1922; Schreiber & Norton 2002). This subspecies is a ubiquitous seabird throughout the Gulf of California and north-western Mexico, where it nests on several islands (Everett & Anderson 1991). Brown boobies are a gregarious species and usually nest in colonies

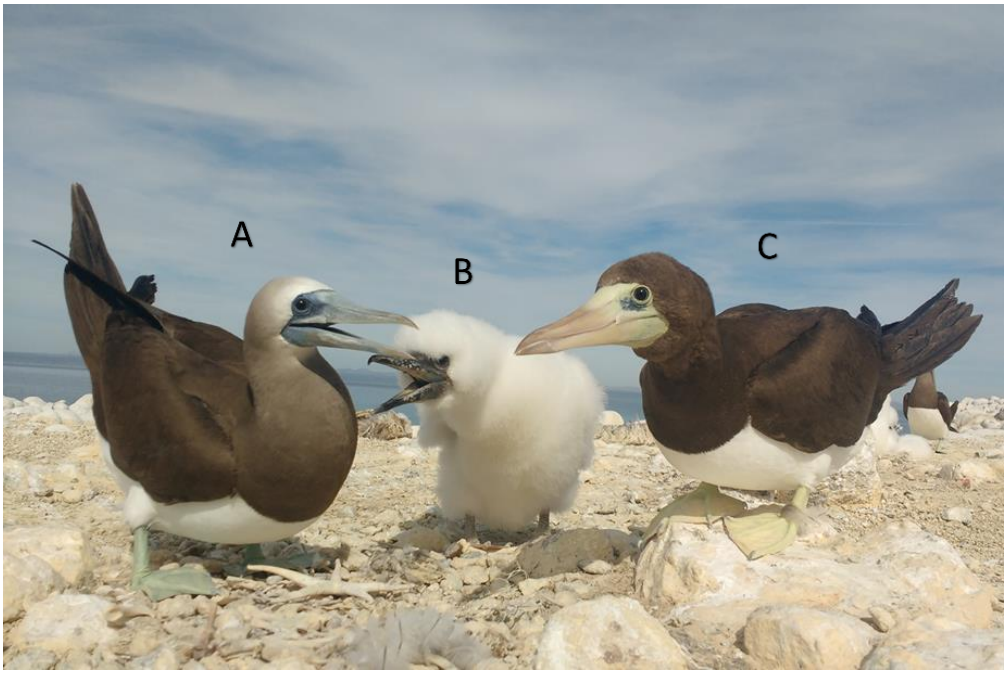


Fig 1.1. Typical brown booby nest. Brood usually conformed by (A) Male, (B) Chick, and (C) Female.

forming families consisting of a pair of parents, and in the breeding season, two eggs are laid but usually only one chick survives due to siblicide (Schreiber & Norton,

2002). Brown boobies are monogamous (Dorward, 1962), and when food is abundant thousands of pairs coexist, and when foraging, they tend to fly alone (Langteau, 2011).

It has been proposed that colourful integuments have genetic implications in the overall health of chicks as tested in a cross-fostering experiment on the closely related blue-footed booby (Velando et al. 2005). Genetic variation in the nanostructure of integumentary collagen creates heritable visible variations in the reflectance of ornaments that could become subject to natural, sexual or social selection for structural colour production (Prumm & Torres 2003). A study by Velando et al. (2006), showed that the blue colour in the closely related blue-footed booby is given by pigments in diet and collagen structures. They recorded that after 48 hours without any food the blue colour became less intense and brighter again when they were fed with fresh fish. Additionally, carotenoid intake influenced the immune system in a similar way as foot colour, which suggest these pigmentations reflect the immunological state of individuals and suggesting that pigments that modify foot colour is an honest signal that changes rapidly depending on current conditions (Alonso-Alvarez et al., 2004; Blount et al., 2003; Grether et al., 2004; Lozano, 1994). A more recently cross-fostering study carried by Montoya & Torres (2015) in brown boobies from Marietas showed that gular colour by rearing father was positively corelated to male parental care and chick body mass increase. However, genetic father gular colour was positively associated to chick structural growth, which suggest that the increase in size was directly related to the social father and to some extent to the genetic father, showing that colour in bare parts indicates both parental care and genetic quality.

Brown boobies typically forage very close to the breeding colony and appear to prefer inshore or coastal shelf habitat (Weimerskirch et al. 2009). Also, their primary prey is flying fish (Exocoetidae) and squid (Dorward 1962, Nelson 1978, Harrison et al. 1983), however this varies according to location and season. In a recent study carried by (Michael et al., 2018) was reported that males from Marietas island presented a positive correlation between ornament colour and longer foraging trips. Also, the individuals that consumed more pelagic prey presented more ornamented skin, suggesting that ornaments are an honest indicator of foraging strategies and probably influenced as well by the concentrations of carotenoids in the phytoplankton. In the present study, I propose to use genome-scale sequencing to study mate choice and genetic quality in brown boobies (*Sula leucogaster*) breeding in colonies along a gradient of environmental quality on the Mexican Pacific coast. This species responds to subtle changes in their marine environment temporally and spatially, which can affect the breeding success when combined with changes in abundance or in the prey species around (Ballance 2007). Also, as they are part of the marine ecosystem, they occupy a role as predators towards the top of the marine food chain; therefore, they can both influence the food chain and be influenced by it (Diamond & Devlin 2003; Tasker & Reid, 1997).

1.3.2. Distribution and studied colonies

I studied brown boobies on three islands on the Pacific coast of Mexico and in the Gulf of California. They differed in several features. In San Jorge Island, which has been estimated to have a population size of up to 6,000 individuals (Mellink 2003), they have a much longer breeding season than other colonies in the gulf (Mellink 2000). In this colony they do not abandon the island after the breeding season as they

do on other islands in the Gulf of California which suggest that food is sufficient throughout the year (Mellink et al. 2001). On the other hand, Isla San Pedro Mártir has an estimate of 74,000 and it is probably supporting the largest Brown Booby colony in the world (Tershy 1997). On this island, Tershy et al. (1992) found the breeding season to be from January to July, with hatching dates between 9 March and 20 May, with some variation through the year (Mellink 2000). Marietas Island is located just outside of the Gulf of California in triangular zone between Mazatlan, Cabo Corrientes, and Cabo San Lucas (Rojas 1984). The weather of this island is semi-warm and sub-humid with an average annual rain of 1,122.2 mm and an average temperature of 27° C on summer (García 1981). The abundance of brown boobies in this island has been estimated around 12,000 individuals (Rebon-Gallardo 1997). It has been reported that brown boobies from Marietas with greener skin often forage beyond the continental shelf, where food webs are based on phytoplankton, suggesting that longer and most energetically costly trips are needed in this colony (Michael et al., 2018).

The brown boobies are exposed to variable environmental conditions throughout their range (Nelson 1978, Schreiber & Norton 2002). They are abundant in the Gulf of California (Mellink 2000), where its local breeding distribution seems to be driven by food availability, as individuals are seen often in association with ocean currents and upwelling areas where prey is abundant (Schreiber & Norton 2002). Sea surface temperature (SST) and primary productivity (PP) has been used in a study by Castillo-Guerrero et al. (2016) as proxies of oceanographic conditions and its variability could influence foraging behaviour of brown boobies. In the study they measured the foraging strategies in San Jorge Island (SJI) (High productivity year-

round) and Farallón de San Ignacio Island (FSI) (Low productivity in summer and high in winter), showing adjustment in prey sizes and diving depth dependent of environmental variation. At SJI, Brown Boobies included more prey species in their diet and dove shallower than at FSI due to related lower SST and higher PP values, whereas deeper dives and larger prey items were related with higher SST and lower PP values. This means that the Gulf of California present a gradient of primary productivity, from higher concentrations in the northern area, to lower concentrations at the southernmost part of the gulf, while sea surface temperatures are higher in the south and lower in the north of the Gulf of California (Figure 1.2). If the productivity or the spatial or temporal distribution of a critical resource changes from year to year or from area to area, I should expect increased rates in EPP and honesty of ornaments used in mate choice if the primary productivity of the colony is low (Emlen & Oring 1977).

Relatively few empirical works have examined the genetic structure of brown boobies. Steeves et al. (2003) used mitochondrial cytochrome b variation to test the hypothesis that the Isthmus of Panama and Eastern Pacific Basin drove genetic divergence in brown boobies and found that population genetic structure was high between the Gulf of Mexico and the Eastern Pacific. Additionally, a study by Morris-Pocock, J. A. et al. (2011) sampled 215 individuals from all major breeding areas (including San Pedro) and genotyped them at eight microsatellite and three nuclear intron loci. They found that brown booby populations were highly differentiated and that colonies can be grouped into four major genetic populations. Finally, in a study by Nunes & Bugoni (2018), they studied the widely distributed brown booby and the potential role of isolation by distance (IBD) and isolation by environment (IBE) in the

current distribution of their genetic diversity. They used nine microsatellite loci to assess population structure and between-colony

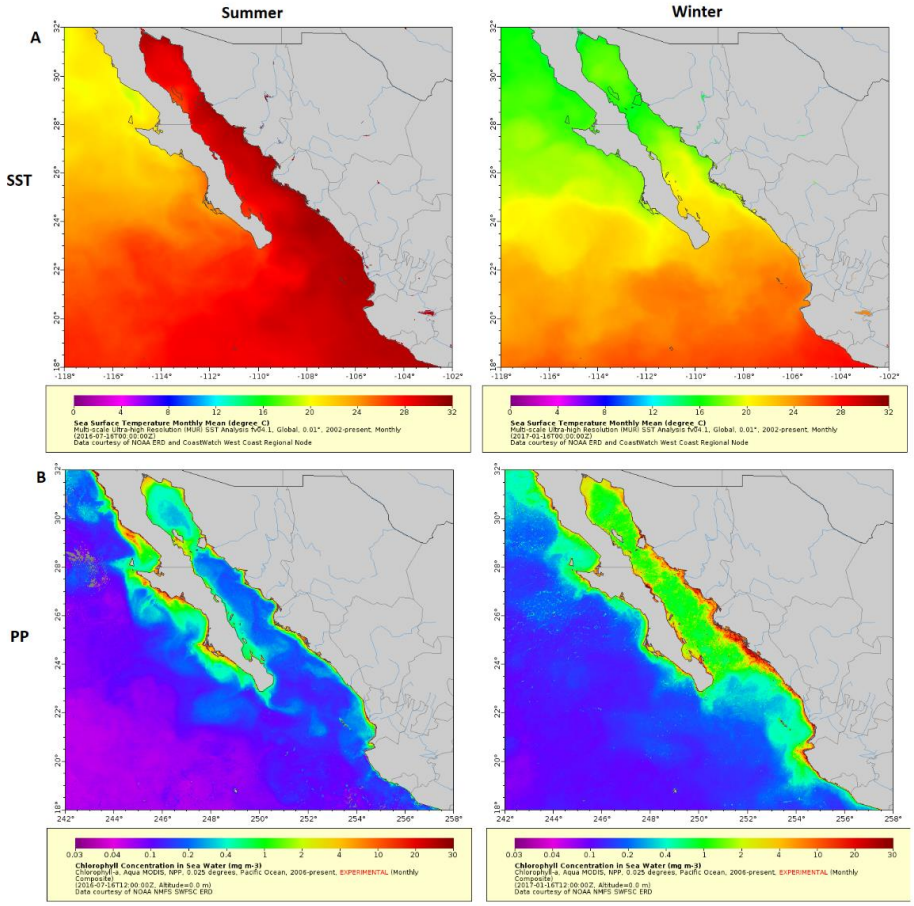


Fig 1.2. Maps with the variation in SST and PP during summer and winter. A). Sea surface temperature (SST) indicated in degrees Celsius B). Primary Productivity (PP) indicated in Chlorophyl concentration (mg m-3). Colours indicate the value of the measurements.

geographical distances using IBD. Moreover, variables like sea surface temperature, air temperature, chlorophyll a concentration, colony density and isotopic niche width were used as proxy to investigate IBE. They found that a remote island was genetically isolated, and it presented local adaptation driven by selective pressures in foraging areas and on land. Finally, they also found that the remaining colonies were part of the same cluster which was explained by seascape differences on oceanic conditions and

concluded that local adaptation by IBE explained greatly the patterns of genetic diversity in brown boobies.

The present study takes a similar approach to those mentioned before but with the implementation of genome-wide SNPSs in order to get a greater resolution about the processes involved in the distribution of genetic diversity based in environmental variables. The advancement in NGS technologies has made possible to investigate more in depth about the interaction of environmental variation and genetic background on mate preferences and sexual selection, and I investigate some of the genetic basis involved in mate selection of three islands in the Gulf of California that are under different levels of primary productivity and environmental conditions. Moreover, the colonies of brown boobies in these islands breed at different seasons, which could mean that the mating preferences could exhibit different honesty in sexual traits. Based in previous studies about the population genetics of brown boobies and the high natal philopatry reported in seabirds, I expect to find higher degree of population structure than previously reported using fewer markers, regardless of close distance between colonies. Similarly, the tendency to low extra-pair paternity in monogamous birds, allow me to predict similar estimates to those studies using microsatellites, though with a higher confidence on the certainty of parentage determination using hundreds of SNPs. Finally, given that the sampled colonies present different physical and ecological characteristics (e.g., sea surface temperature and primary productivity), I will find a significant proportion of the genetic diversity influenced by such environmental variables.

In brown boobies, sexual ornaments have both a genetic and environmental basis. The genetic aspect could be conveniently assessed by measurements of overall heterozygosity or similarity (and potentially be explained by the "good genes" & "compatible genes" models). The environmental aspect relates to their ability to forage and obtain prey items rich in carotenoid pigments, which may partially be due to genetics as well (e.g., health, ability to efficiently utilize energy resources). Moreover, ornaments may be providing different signals in environments that differ in quality (i.e., ornaments may not always be honest). Consequentially, any potential sexual mate assesses these dynamic variations by altering their patterns of copulation (Torres & Velando, 2003, 2005). Some studies propose a direct link between various fitness related traits (e.g., survival, fertilization, hatching, etc.), heterozygosity, and condition dependent phenotypic traits like colour (Ditchkoff et al., 2001; Foerster et al., 2003; Kempenaers, 2007). Therefore, heterozygosity could be a preferred "hidden" trait that is being evaluated by potential mates for quality (Brown, 1997; Li et al., 2016; Rosengrave et al., 2016). The compatible gene model proposes that fitness is increased by certain alleles in a specific genetic context or by gene-to-gene interactions (Kempenaers, 2007; Neff & Pitcher, 2005), and I could be alternatively measured as genomic-wide similarity between individuals in a pair-wise relatedness basis (Queller and Goodnight 1989). I hypothesize that ornament coloration (e.g., higher green chroma measurement) will be positively correlated to genetic quality in colonies in areas of low primary productivity, and extra pair mates will have higher genetic quality than social mates in such areas (chapter 2).

The brown Boobies have pantropical distribution and breed on islands from all ocean basins (Nelson, 1978), and do not perform true migration throughout the year

(Nelson 2005). Studies also show evidence of phenotypic population differentiation in brown boobies caused by environmental characteristics (Nunes et al., 2017) and trophic niche (Mancini, Hobson, & Bugoni, 2014). Colonies present different environmental pressures regardless of their near geographic location, such as differences in latitude, primary productivity, available area for nesting and sea surface and air temperatures (Nunes et al., 2017; Seeliger & Kjerfve, 2001). The Isolation by Distance (IBD) model propose that genetic differentiation among population increase with geographical distance, it can happen in the absence of selection, but is aided by genetic drift and low dispersal capacity (Wright, 1943; Meirmans, 2012). Alternatively, in the Isolation by Environment (IBE) the genetic differentiation of populations increases with environmental differences, regardless of geographical distance (Wang & Bradburd, 2014). Additionally, under seascape heterogeneity model there are some ecological processes known to promote population isolation like non-random gene flow (Edelaar & Bolnick, 2012), and sexual selection against migrants due to local adaptation (Hendry, 2004). Therefore, I hypothesize that there is a strong positive correlation between environmental variables and the distribution of genetic diversity in islands with higher SST and PP (chapter 3).

Natural selection in the wild is composed by different types, such as viability selection, sexual selection, and gametic selection (Flanagan & Jones, 2017). Some of the best examples of sexual selection have been documented in birds, where colourful ornaments are commonly used to attract mates (Catchpole, 1980; Irestedt et al., 2009; Loyau et al., 2005; Hosken and House, 2011). Under sexual selection, mate choice is not random, is based on an ornament trait that may bring benefits to offspring (and the chooser), or members of one sex that compete for access to mates using morphological

features that are secondary sexual characteristics (Miller et al., 2018). To measure how selection affects the genome, the Genomic Selection Component Analysis (GSCA) uses quantitative genetic theory, which focuses on individual episodes of selection (Arnold and Wade 1984b; Arnold and Wade 1984a), in combination with empirical work to identify signatures of selection in wild populations. According to this analysis, because sexual selection it is known to be strong in brown boobies and by collecting offspring and both parents, it is possible to detect sexual selection and other episodes of selection using genome-wide selection analyses. Finally, I expect to detect genomic regions under sexual selection when comparing adult individuals that successfully reproduced during the breeding season from colonies with contrasting environmental conditions (chapter 4).

1.4 Thesis objectives

1) Examine the relationship between ornament coloration and genetic quality, in terms of genome-wide heterozygosity and similarity, and compare the genetic diversity of males and females between different islands and relate that to sexual ornament coloration during rearing period, in nests that were able to secure the survival of offspring. Analysing heterozygosis (good genes) and similarity (compatible genes) on different colonies under different environmental conditions will provide insights about selection pressures and honesty of the ornament in these populations. By comparing heterozygosis and similarity, it can be determined if colonies with different environmental conditions are choosing mates based on compatible genes or good genes to provide benefits by increasing fitness on offspring.

Comparing mate choice between different islands could shed light into the reproductive strategies towards different environmental conditions.

2) Assess the population structure, genomic diversity and geneflow of three colonies of brown boobies under environmental heterogeneity. For this purpose, Sea Surface Temperature (SST) and Primary Productivity (PP) are used as proxy of environmentally variables to compare both isolation by distance and isolation by environment models. Also, potential impact in population isolation and gene flow in brown boobies will be investigated, if differences are found between colonies, this will be explained on terms of the environmental heterogeneity and mate choice.

3) Use genome-wide selection scans to characterize the signatures of natural and sexual selection by comparing allele frequencies in males and females from different islands. By comparing allele frequencies between males and females, sex-specific viability selection will be detected between sexes. Additional types of selection like gametic selection will be performed by comparing allele frequencies between adults and offspring. All the detected regions will be used to detect biological functions (Gene Ontology) and explained according to the type of selection involved in such function.

CHAPTER 2: MATE CHOICE IN BROWN BOOBIES (*SULA LEUCOGASTER*): ARE SEXUAL ORNAMENTS HONEST SIGNALS FOR GENETIC QUALITY?

2.1 Introduction

Why many organisms consider sexual ornaments during mate choice, is one of the oldest questions in evolutionary biology (Kokko et al., 2002). Since the times of Darwin, sexual selection and the preference of females for exaggerated male traits have puzzled scientist around the world (M. B. Andersson, 1994; M. Andersson & Simmons, 2006; Neff & Pitcher, 2005). Individuals that evaluate potential mates are likely to obtain direct or indirect benefits like high fecundity rates, offspring survival, good genes or compatible genes (Rosenthal, 2017). Some examples of direct benefits would be the capacity to provide nuptial gifts, a good territory or resources for offspring (Price et al. 1993), while indirect benefits would be elevated offspring fitness (Jennions & Petrie 2000). In organisms with biparental care such as birds, it is difficult for the female to directly assess the parenting and genetic qualities of males. In some groups of seabirds, carotenoid-dependent colour on gular and feet is an honest trait capable of dynamically update current changes in immune response, oxidative balance, and nutritional condition (Andersson 1994; Velando et al. 2006).Therefore, females might evaluate sexual ornaments that reflect honestly phenotypic or genetic characteristics that could bring an advantage for reproduction (Kodric-Brown & Brown, 1984). By investing in sexual traits, these dynamic signals could provide information about the current or recent condition of an individual, hence, the

individuals receiving the signals might increase their reproductive success (Grafen, 1990; Montoya et al., 2018).

Bright colours are usually a good example of sexual ornaments (M. B. Andersson, 1994; Hill et al., 2006). For instance, the brown boobies display sexual dichromatism in cheeks, feet and throat (bare skin parts), and during courtship, males exhibit colourful gular skin ranging from green to blue, while females display colours in the yellow spectrum (Nelson, 1978). These tones of colours are mainly gained through the consumption of carotenoids which are deposited in fleshy structures, and it has been proposed as an honest cue that reflects dynamically the current changes in quality of males (Alonso-Alvarez & Galván, 2011; M. B. Andersson, 1994; Bertrand et al., 2006; Faivre et al., 2003; Velando et al., 2006). Consequentially, any potential sexual mate assesses these dynamic variations by altering their patterns of copulation (Torres & Velando, 2003, 2005). In some models of sexual selection, mate preferences are rather assumed absolute, where potential pairs evaluate each other and assigning value to determined trait that would translate into a direct or indirect benefit (Jennions & Petrie, 1997; Real, 1990). Conversely, some studies propose a direct link between various fitness related traits (e.g., survival, fertilization, hatching, etc.), heterozygosity, and condition dependent phenotypic traits like colour (Ditchkoff et al., 2001; Foerster et al., 2003; Kempenaers, 2007). Therefore, heterozygosity could be a preferred "hidden" trait that is being evaluated by potential mates for quality (Brown, 1997; Li et al., 2016; Rosengrave et al., 2016).

The "good-genes-as-heterozygosity" hypothesis by Brown (1997) tries to explain the nature of male quality and proposes that, females will try to produce more

heterozygous offspring by copulating with mates presenting high heterozygosity. The previous statement is under the assumption that there is a selective advantage for heterozygous offspring and an underlying relationship with fitness (Brown, 1997). There are different hypotheses that sought to explain the link between mate choice and genetic quality, but most of them agree that genetic quality is "the contribution of alleles or a genotype to individual fitness" (Kempenaers, 2007). Additionally, the good genes model proposes that, if males with high genetic quality increases offspring fitness independent of genome architecture (by specific alleles or general allelic diversity), then females would evaluate mates through sexual ornaments or other cues reflect an indirect benefit (Barber et al., 2001; Eilertsen et al., 2009; Hamilton & Zuk, 1982). The other main model of genetic mate choice is the compatible gene or compatible allele model; and this one proposes that fitness is increased by certain alleles in a specific genetic context or by gene-to-gene interactions like epistasis (Kempenaers, 2007; Neff & Pitcher, 2005). Usually, studies that try to investigate compatibility in the wild use polymorphic gene region (e.g., Major Histocompability Complex) which bring specific fitness advantage to offspring when adults mate with dissimilar individuals in such genomic regions (Fulton et al. 2016). Genomic-wide similarity between individuals can be calculated as pair-wise relatedness values using the methods like the described by Queller & Goodnight (1989). It is important to note that these models that I have mentioned could bring indirect benefits by increasing the genetic diversity of the offspring; meaning they are not mutually exclusive as they influence two different attributes of the genetic variance (additive and nonadditive) (Colegrave et al., 2002; Neff & Pitcher, 2005). Additionally, species that present biparental care like the brown booby, they present limited opportunities of extra-pair

copulation or extra-pair paternity (EPP), therefore, sexual ornaments are expected to reflect the condition-dependent parental investment and genetic quality at the same time (Kokko, 1998; Pickett et al., 2013; Velando et al., 2006).

It has been proposed that females can modify their initial mate choice through EPP because they are limited to their initial choice of social mates (Albrecht et al., 2007; Moller, 1992; Zeh & Zeh, 2003). A tropical long-lived seabird such as the brown booby which is socially monogamous, has been reported to have low levels of EPP (Gañán et al., 2014; Nelson, 1978). Additionally, when the cost of mating is high in males, such as when males provide paternal care, it is predicted that there will be strong selection for mate assessment, hence strong selection for traits that signal genetic benefit in the opposite sex (Trivers, 1996). Male and females brown boobies incubate during 42 days approximately and feed the offspring with fish that is regurgitated directly into their mouths for a period of three months until chicks are ready to leave the nest (Nelson, 1978). The main hypothesised benefit that females might obtain from EPP is an indirect benefit to offspring fitness by inheriting compatible alleles or good alleles from a male of high quality (Brown, 1997; Johnsen et al., 2000; Løvlie et al., 2013; Mays & Hill, 2004). Thus, females that accurately assess mates through honest indicators of quality , are more prone to increase their own investment on offspring and preparing them for future rearing conditions (Montoya & Torres, 2015). Finally, many studies on EPP showed evidence for female preference towards males with high genetic quality (Griffith et al., 2002), which could suggest that the patterns of EPP in monogamous species may indicate an energetic trade-off between extra-pair engagement behaviour and female genetic benefits to offspring fitness (Jennions & Petrie, 2000; Lindstedt et al., 2007).

The notion, than seabirds have energetic limitations and therefore they only produce one slow growing chick per season, has been questioned more than once (Ashmole, 1963; Lack, 1954). Usually, Brown boobies practice siblicide ; which means that even when they lay two eggs, the first chick that hatches typically causes the death of the younger one during the first few days to get all the food resources and ensure survival (Drummond et al., 2003; Osorno & Drummond, 2003). However, cases like the colony on Johnston Atoll showing that adults have a high reproductive success around 70-85% and the observation that around 0.1-0.5% of families have the ability to raise two chicks, suggest no energetic restriction in this island (Schreiber & Norton, 2002). On the contrary, Isla Marietas in Mexico presents more challenging energetic restrictions, where Montoya & Torres (2015), conducted a cross-fostering experiment and showing that gular colour by rearing was positively corelated to male parental care and chick body mass increase, whereas genetic father gular colour was positively associated to chick structural growth. This suggest that, the increase in size was directly related to the social father and to some extent to the genetic father, showing that colour in bare parts indicates both parental care and genetic quality (Montoya & Torres, 2015). The breeding colonies in my study present different characteristics due their location in a productivity gradient and, they breed at different times of the year. Therefore, I hypothesise they will have different energetic requirements for biparental care; hence, the honesty of phenotypes and the proportion of extra-pair mates will be reflecting different levels of heterozygosity and similarity depending on the environment. I expect that females from lower primary productivity colonies will engage more often in EPP because they will seek out the relatively available high-quality males to sire higher quality chicks, while cuckolded males

would have lower quality than males in socially monogamous nests with one or two chicks.

In the present chapter, I examine the mate choice and extra-pair paternity of brown booby families that successfully ensured the survival of a chick (at least 40 days old) in three colonies in the Pacific coast of Mexico under different levels of primary productivity (PP) and sea surface temperature (SST). During courtship, males display green-blue coloration on the gular and feet, whereas females display yellow-green coloration on bare parts. Recently, it was found that gular and feet colour in brown boobies during courtship season in Marietas Island is carotenoid dependent suggesting that colour is an honest indicator of condition (Montoya & Torres, 2015). Therefore, I aim to investigate (1) if phenotype like colour in bare parts and body size honestly reflect genetic quality based in heterozygosity and dissimilarity in males and females, and if different islands with contrasting environmental features present the same patterns of honesty in ornaments. (2) Perform a parentage analysis to compare rates of extra-pair paternity in the different colonies; and use this information to distinguish the monogamy status of individual nest. (3) And to examine the relevance of phenotypic and genotypic characteristics and how they change across different colonies and between parentage status (i.e., those nest with extra-pair mates, within-pair mates with one chick and within pair nests with two chicks).

2.2 Methods

2.2.1 Collection of samples

I studied brown boobies inhabiting three different islands in the Pacific coast in Mexico (Figure 2.1). The study was conducted in different years and in different seasons depending on the breeding timing of each colony. In San Jorge Island (31°00'59.1"N 113°14'40.9"W), two expeditions were conducted between December and January of 2017 and 2018. Marietas (20°41'57.7"N 105°34'54.4"W) was visited during the summer months of July and August of 2016. Additional samples from a previous study in Marietas were also provided by our collaborators (Montoya et al., 2018). Finally, I managed to do a short expedition to San Pedro Island (28°22'56.8"N 112°17'53.1"W) during February 2018, where I obtained fewer samples because the weather conditions were difficult for navigation. All samples were collected during the rearing period, where families of brown boobies (male, female, and chick) were captured by night lighting (Velando et al. 2006), and each member was individually marked with a white polymethylmethacrylate numbered leg band (Interrex, Poland).

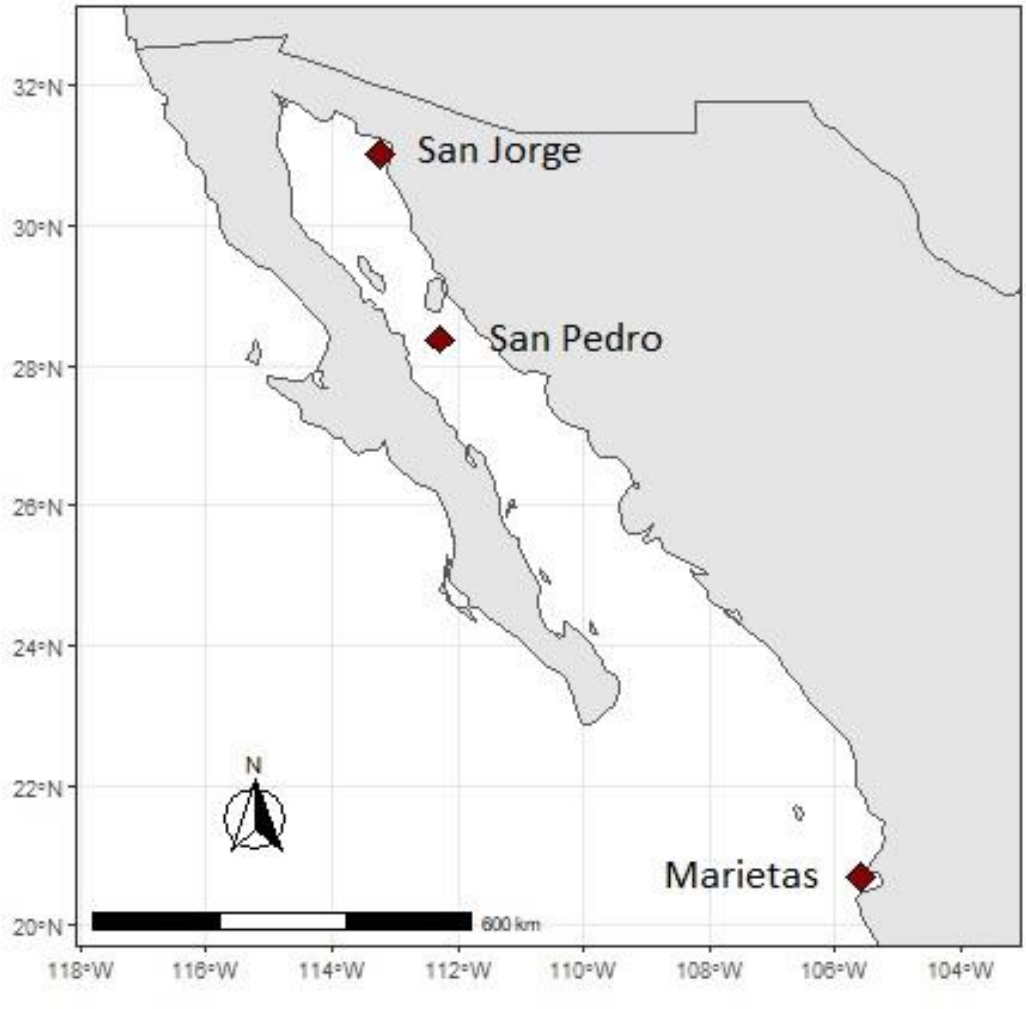
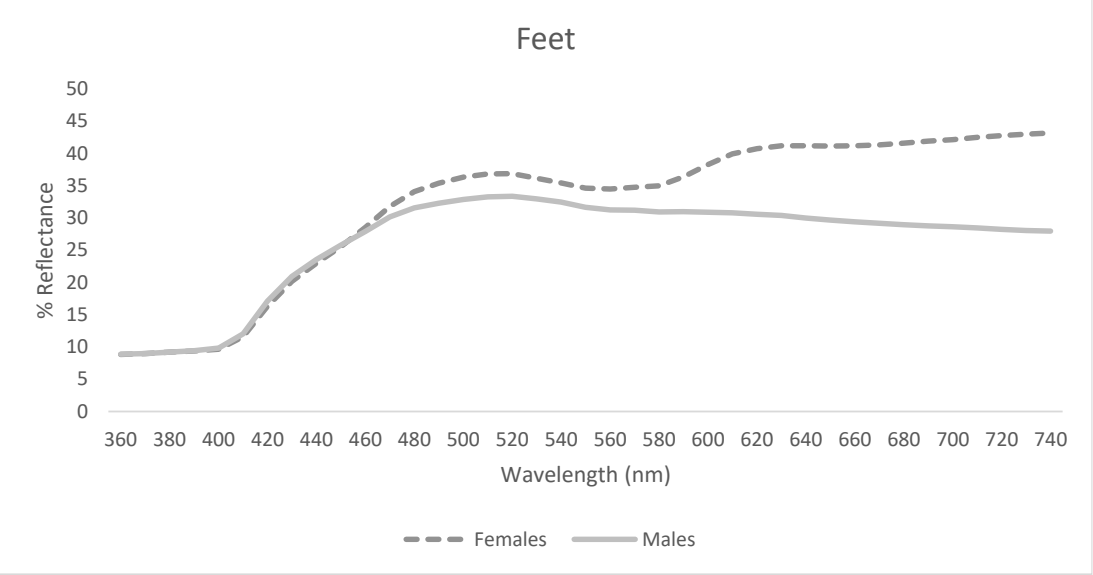


Fig 2.1. Geographic location of sampling areas. Brown booby colonies represented with a red square.

For captured birds, I measured the length of ulna, tarsus, and beak with vernier callipers and measuring tapes, while the body mass was recorded with the help of a portable scale and a restraining bag. The colours of the gular and feet were measured using a portable spectrophotometer that determines the reflectance at 10-nm intervals from 360 to 740 nm (MINOLTA CM 2600d, Osaka, Japan). The males display colours between blue and green with peaks at 360 (within the UV range) and the other at 540 nm (within the green range), whereas or the females, the gular colour has two peaks

at 520 and 620 nm (within the yellow range)(Montoya & Torres, 2015).. Previous studies about coloration throughout the year in bare parts and mate choice in brown bobbies, report that green coloration increases in the courtship season by the consumption of carotenoids and is maintained through out the incubation and rearing stages (Montoya et al., 2018). Carotenoid pigments reflect light at wavelengths longer than 500 nm, and absorb light at shorter wavelengths, therefore, is expected that males' and females' gular skin green chroma and stimulation to the green colour receptor (Hill et al., 2006). Consequently, in the present study I am using used the most important features of the gular reflectance spectra of males and females used during courtship for mate choice in feet and gular (wavelengths within the blue and green spectrum)(Figure 2.2).



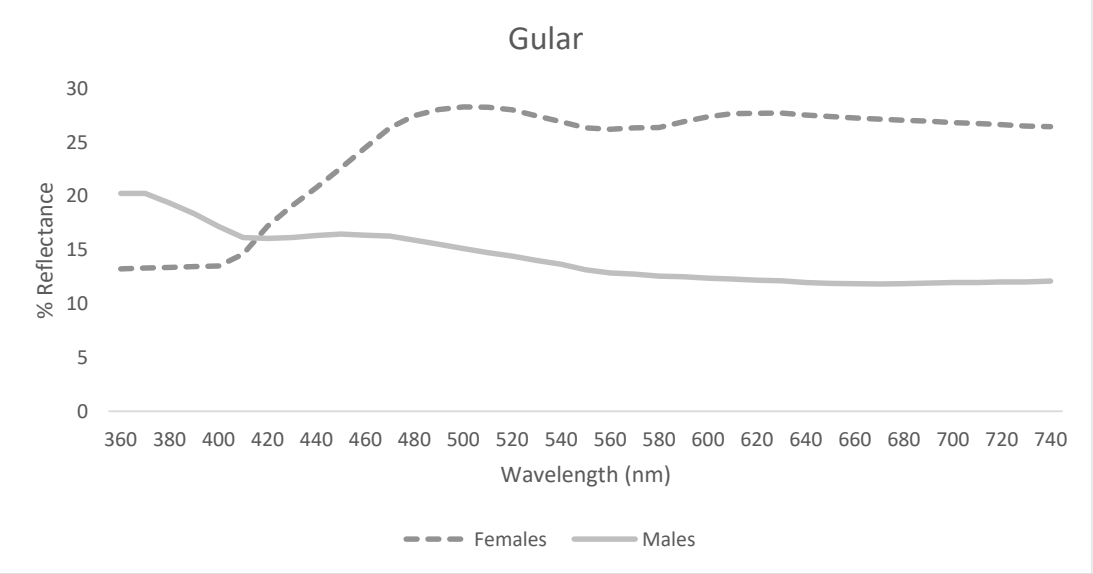


Fig 2.2. Gular and Feet skin reflectance of males and female Brown Boobies. Solid lines represent males. Doted lines represent Females. Values in the x axis represent the wavelength and values in the Y axis correspond to the reflectance readings by the spectrophotometer.

The colour measurements of each adult were obtained calculating the mean of three sequential readings roughly 2 cm apart placing the spectrophotometer at 90° from skin surface. The spectrophotometer was calibrated daily against a white target, and data were downloaded using the OnColor software (CyberChrome, Inc.)

according to the specifications of the manufacturer. The measurements of each individual were completed in 10 minutes, and then the birds were returned to their nests, which were previously marked with a flag and number for easy recognition (Montoya & Torres, 2015). Endler's segments and relative photon catches per visual receptor were calculated for each of the colours corresponding to the four visual receptors found in many bird species (Vorobyev & Osorio 1998, Endler & Mielke 2005). These visual receptors correspond to the violet (360–400 nm), blue (410–500 nm), green (510–600 nm), and red (610–700 nm). The segments or "chromas" are calculated as the sum of total reflectance of a colour segment of the spectrum divided by the total brightness or the sum of reflectance from 360 to 700 nm (Endler 1990).

For blood sample collection of parents and chicks were taken during parental care around 15 days after the first chick hatched, taking 2 ml of blood for adults and 0.5 ml for chicks from the brachial vein. All the samples collected in this study were conducted under ethics approval from Durham University. These blood samples were kept on ice until they were centrifuged at 10,000 g for 10 minutes to separate the red blood cells and plasma. I transferred blood samples to heparin-coated vials with 70% ethanol. The blood samples are used for DNA extractions whereas plasma samples are used by my collaborators in Akron University for isotope analyses which reflect the previous ca 10 days (Hobson & Clark, 1993). Additionally, I attached modified i-gotU GT-120 GPS loggers (Mobile Action Technologies, New Taipei City, Taiwan) to track the foraging behaviour on Brown boobies and I collected white breast feathers also for isotope analyses. For full details of the GPS loggers and Isotope methods, see (Michael et al., 2018).

2.2.2 Laboratory work

We extracted DNA from red blood cells using either a standard phenol/chloroform technique (Friesen et al. 1997) or the E.Z.N.A.® Tissue DNA Kit, following the manufacturer's protocol (OMEGA BIO-TEK). I used a modified 3RAD protocol (Bayona-Vásquez et al., 2019) which was optimised for low quality DNA (see supporting information for a detailed modified protocol). In summary, I normalized all DNA samples to 40 ng/µL, and then digested them with AseI and HindIII enzymes (NEB). The digested fragments were ligated to P1 (iTru7) and P2 (iTru5) adapters containing a unique combination of inline barcodes for identification. After digestion and ligation, these samples were pooled in groups of 32 samples (each with a unique P1-P2 adapter combination) and purified using 1.59 volumes of with Sera-Mag Magnetic Speed-beads (ThermoScientific) as described by (Rohland & Reich, 2012). Next, I proceeded to perform a 1 cycle PCR in the pools to add an iTru5 8N primer to detect PCR duplicates. After another bead clean up, I then performed a six cycle PCR by adding different unique iTru7 Primers to the pools and finished with a final bead clean up. Fragments of between 430 and 550 bp were selected using 2% DF marker L cassette (100-600 bp) with internal marker #CDF 2010 by sage science Pippin Prep. I used Tape station for assessing the quality of the pools and performed a qPCR quantification using a Kapa Illumina quantification Kit for an accurate final pooling of the final library and sequenced in the Illumina HiSeq2500 platform.

2.2.3 Data preparation, demultiplexing and SNP calling

Preliminary quality checks were carried using FastQC (Wingett & Andrews, 2018). For demultiplexing, initial filtering and trimming to (110 base pairs long), I used the process_radtags module from the STACKS version 1.37 pipeline (Catchen et

al., 2013), to obtain files with sequences that were specific to each sampled individual. Afterwards, SNPs were called using the populations module, where my samples were grouped in their respective populations, and a locus was 'exported' if it was present in 95% of the individuals in this population using the "r" parameter (-r 0.95) at a stack depth of at least 10 by using the "m" parameter (-m 10). I used the parameter "-write_single_snp" to avoid including SNPs in high linkage disequilibrium (LD), and I selected a minor allele frequency of at least 0.25 to process a nucleotide site using the "–min_maf" parameter (--min-maf 0.25). The reasoning to run populations with such strict parameters is to reduce the number of SNPs that was input to the COLONY v.2.0.6.2 (Jones & Wang, 2010) for parental analysis. A second run of the module population was perform using a more relaxed parameters appropriate for genomic diversity analyses (-r 0.55, -m 10, -write_single_snp, --min-maf 0.025).

PGD-Spider (Lischer and Excoffier, 2012), plink (Purcell et al., 2007) and vcftools (Danecek et al., 2011) were used to convert the SNP data called by STACKS into PED and MAP format. In vcftools, the flags --depth and --site-depth were used to calculate read depth per individual and per SNP, and Binary files (BED, RAW and BIM) were generated from PED and MAP files using PLINK with the flags --make-bed, --recode A, --chr-set 95, and allow-extra-chr. SNP data management and analyses were performed in R-4.0.5 (R Core Team, 2019) using wrapper functions of the R package SambaR (de Jong et al., 2021; github page: https://github.com/mennodejong1986/SambaR). Once the data was imported into R, it was then stored in a genlight object using the function 'read.PLINK' of the R package adegenet-2.1.3 (Jombart, 2008; Jombart & Ahmed, 2011). Two additional filtering pipelines were optimised for different analyses. The first optimised filtering was

performed using the function 'filterdata' of the R package SambaR, with the parameters indmiss=0.2, snpmiss=0.2, min_mac=2, dohefilter=TRUE and min_spacing=500 for the genetic distance and diversity analyses (hereafter ”genetic diversity dataset”). On the other hand, the parameters indmiss=0.58, snpmiss=0.2, min_mac=2, dohefilter=TRUE and min_spacing=500 were used for the “paternity dataset” to retain as many individuals as possible. The indmiss argument delimits the proportion of missing data per sample, the snpmiss argument allows the proportion of missing data per SNP, min_mac is for the minimum allowed number of minor allele copies per SNP, dohefilter it removes SNPs with heterozygosity levels which are potentially indicative of paralogs, and min_spacing delimits the minimum distance between adjacent SNPs in base pairs (bp) when thinning data.

2.2.4 Parentage analysis and genetic analyses

The paternity dataset was then used to assess family structure within populations by using the retained SNPs (207) into the software COLONY v.2.0.6.2 (Jones & Wang, 2010) following the developer recommendations with a random mating model (Wang, 2016). By using the genetic diversity dataset, I calculated similarity between mated individuals using the software PLINK and GCTA and plotted using SambaR functions, where kinship values (“kincoefficient”) are calculated following Waples et al, 2018. In other words, similarity is calculated as the degree of relatedness in a per -individual measurement relative to the social partner on a formed nest. Genome wide Heterozygosity (Multilocus Heterozygosity or MLH) and standardized Multilocus Heterozygosity (sMLH) were obtained using the inbreedR package (Stoffel et al., 2016). Moreover, Inbreeding coefficient (F), which is the probability that the two alleles at a locus are identical by descent (IBD), was calculated

according to Kardos et al, 2015. Finally, regression analyses were performed in the genetic diversity dataset by comparing heterozygosity and similarity with measurements of colour and phenotypic measurements. P-values, confidence intervals, Pearson, and Spearman corelations were calculated in R (cor function) using a custom script and using the package ggplot to generate the plots.

2.3 Results

2.3.1 Filtering datasets

We performed two independent filtering pipelines with different parameters depending on if the data were used for paternity assessment or for evaluation of genetic diversity. For the paternity dataset, we obtained 809 SNPs from the STACKS pipeline with very strict parameters to get a reduced number of high confidence SNPs. Moreover, I retained 436 out of 441 individuals (91-173 per population) after further filtering (Figure S2.1), of which, 207 were retained thinning (Figure S2.2). The GC-content of the retained dataset as 0.43 and the 'transversion vs transition'-ratio was 0.69.

For the genetic diversity dataset 267 out of 441 individuals (43-114 per population) passed my filters and were retained (Figure S2.3), of which, 16054 out of 26340 SNPs were retained after filtering and thinning (Figure S2.4). Moreover, the GC-content of this retained dataset was 0.59, and the 'transversion vs transition' ratio was 0.71.

2.3.2 Differences among islands

When comparing the mass of individuals across the three islands I found significant differences in males and females between different colonies. Individuals from San Jorge had the greatest mass, followed closely by San Pedro, and then

individuals from Marietas had the lowest mass of all islands (Figure 2.3A). There were also significant differences in the sMLH of the three islands. Individuals from Marietas (males and females) had greater heterozygosity than San Pedro and San Jorge (Figure 2.3B).

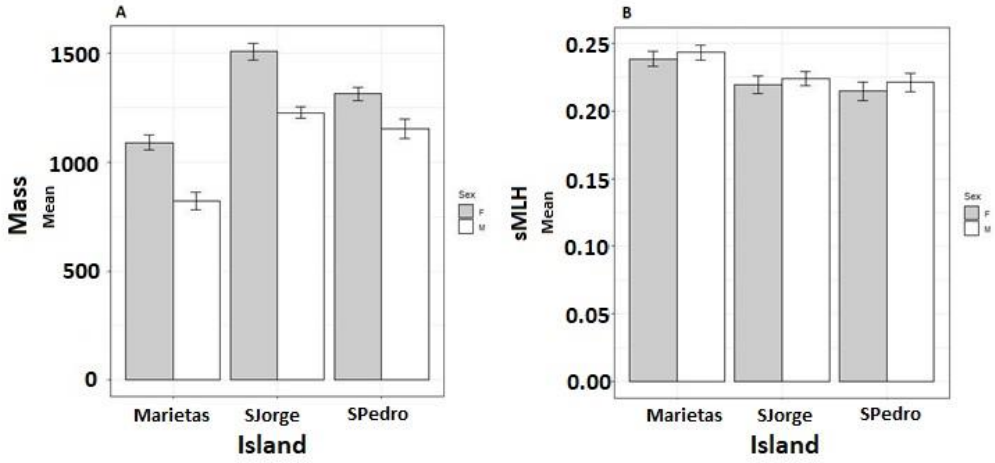


Fig 2.3. Comparison between islands. A). Total weight. B). Standardized Heterozygosity. Results are presented as upper-lower 95% confidence intervals for each group

Moreover, several positive and negative correlations between genotypic and phenotypic traits were identified. When considering individuals from all three analysed colonies, the colour green for gular and feet in females is positive correlated with heterozygosity and negative correlated with inbreeding (Figure 2.4). However, when assuming structured populations between islands there is no significant correlation between these traits (Figure S2.5). For males, I observed the opposite pattern, when grouped all populations there was a weak correlation between

heterozygosity-inbreeding values and greener feet-gular coloration. Green feet colour in males was positively correlated with sMLH in San Jorge, was negatively correlated in San Pedro, and there was no significant correlation in Marietas (Figure 2.5). Finally, I found that male green gular colouration was strongly correlated with sMLH in Marietas, but the other two islands were not significant (Figure 2.6). Body size was another important trait that showed strong correlation with both similarity (kinship coefficient between mated pairs as described by Waples et al, 2018) and heterozygosity. For instance, both males and females presented a strong corelation between body size and similarity, whereas sMLH showed the opposite patterns as smaller individuals were more heterozygous (Figure 2.7). Additionally, I found a negative corelation feet green colour with inbreeding in males from San Jorge, whereas in San Pedro I found a positive corelation for the same variables (Figure S2.6).

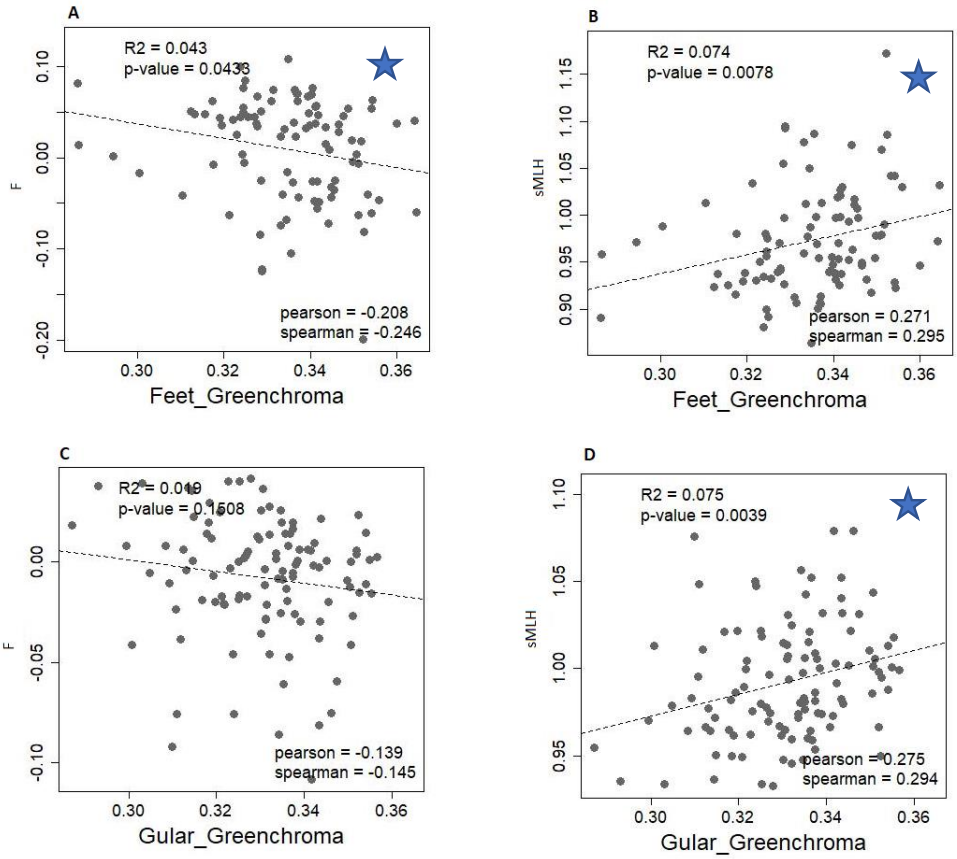


Fig 2.4. Correlation between standardized heterozygosity and inbreeding associated with green colour in bare parts in females. A-B). Correlation of genetic parameters with green chroma in Feet. C-D). Correlation of genetic parameters with green chroma in Gular when all females are grouped in a single metapopulation. Small dots represent one data point. Lines are based on regression analysis and indicate the direction of the correlation. R2, P-values, Pearson and Spearman coefficients are provided. Star indicates plots with significant correlations.

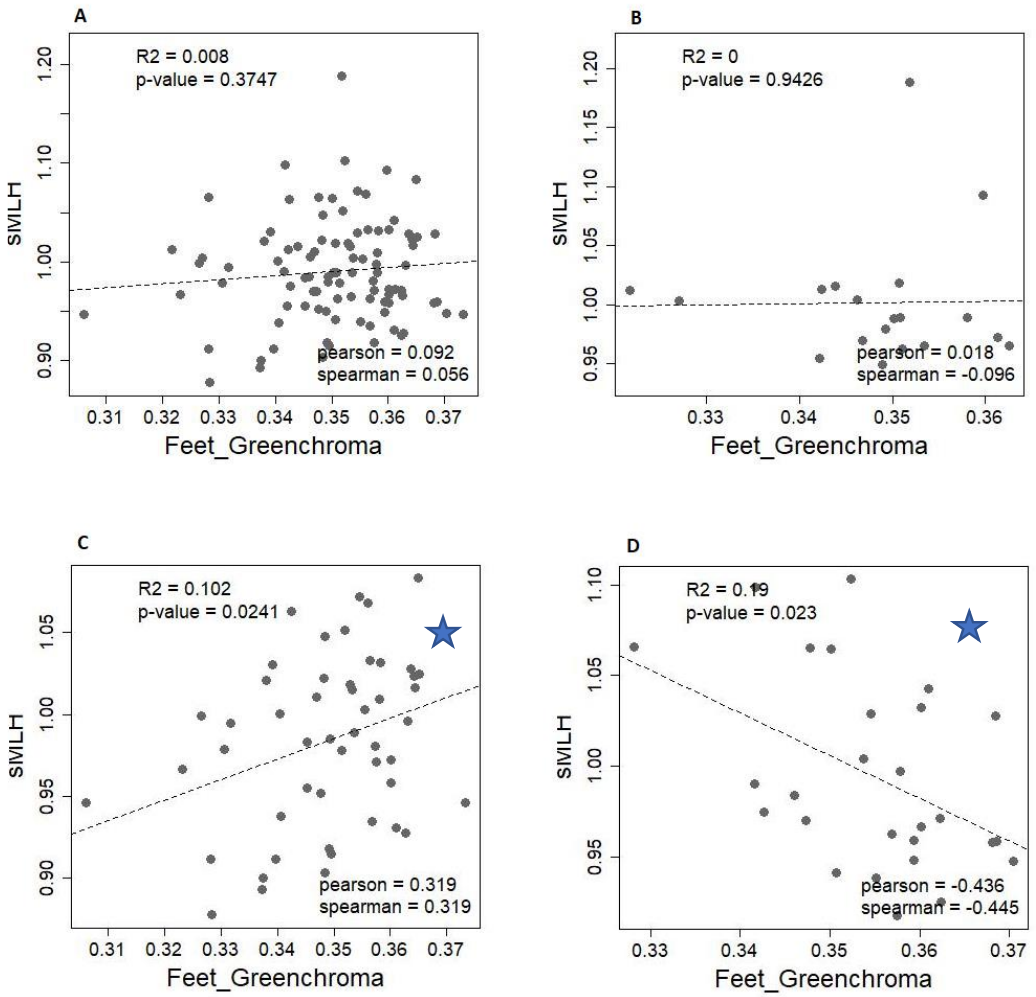


Fig 2.5. Correlation between Standardized Heterozygosity associated with green colour in feet (males). A) Metapopulation. B) Marietas. C) San Jorge. D) San Pedro. Star indicates plots with significant correlations.

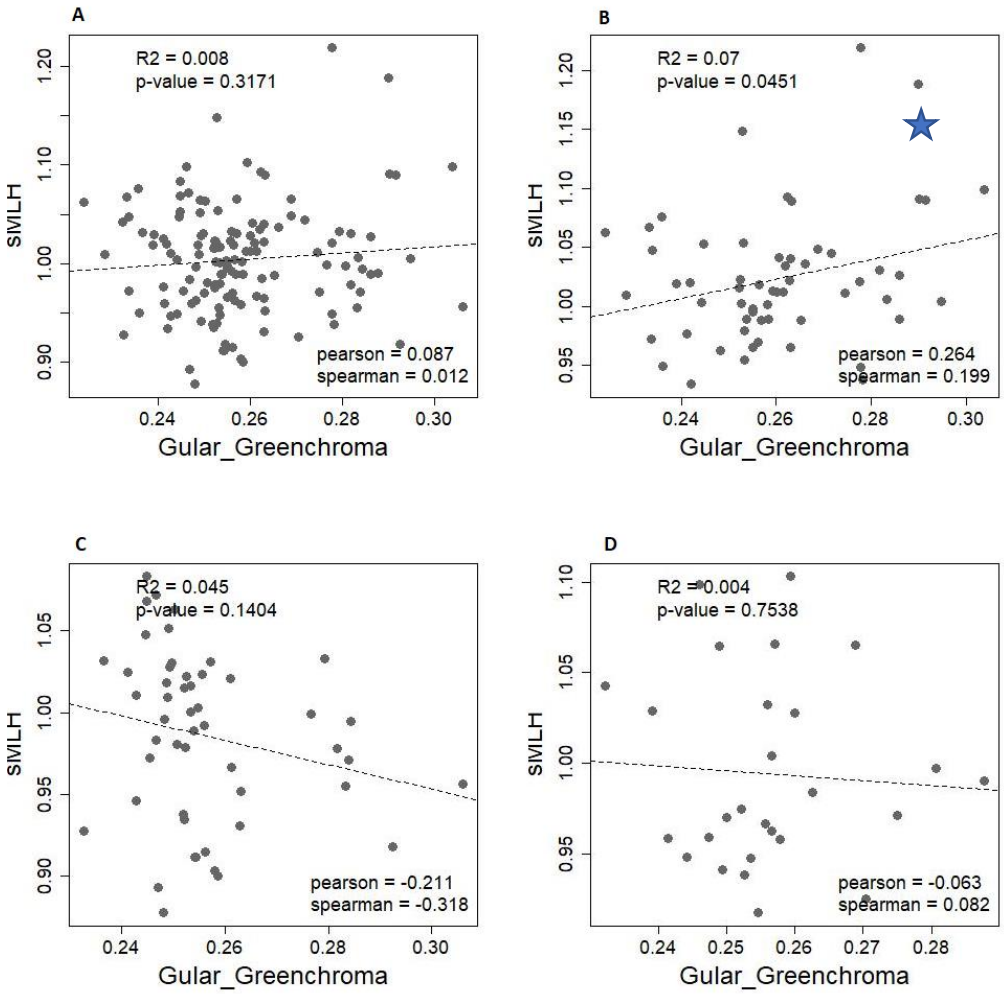


Fig 2.6. Correlation between Standardized Heterozygosity associated with green colour in Gular (males). A). Metapopulation. B). Marietas. C). San Jorge. D). Star indicates plots with significant correlations.

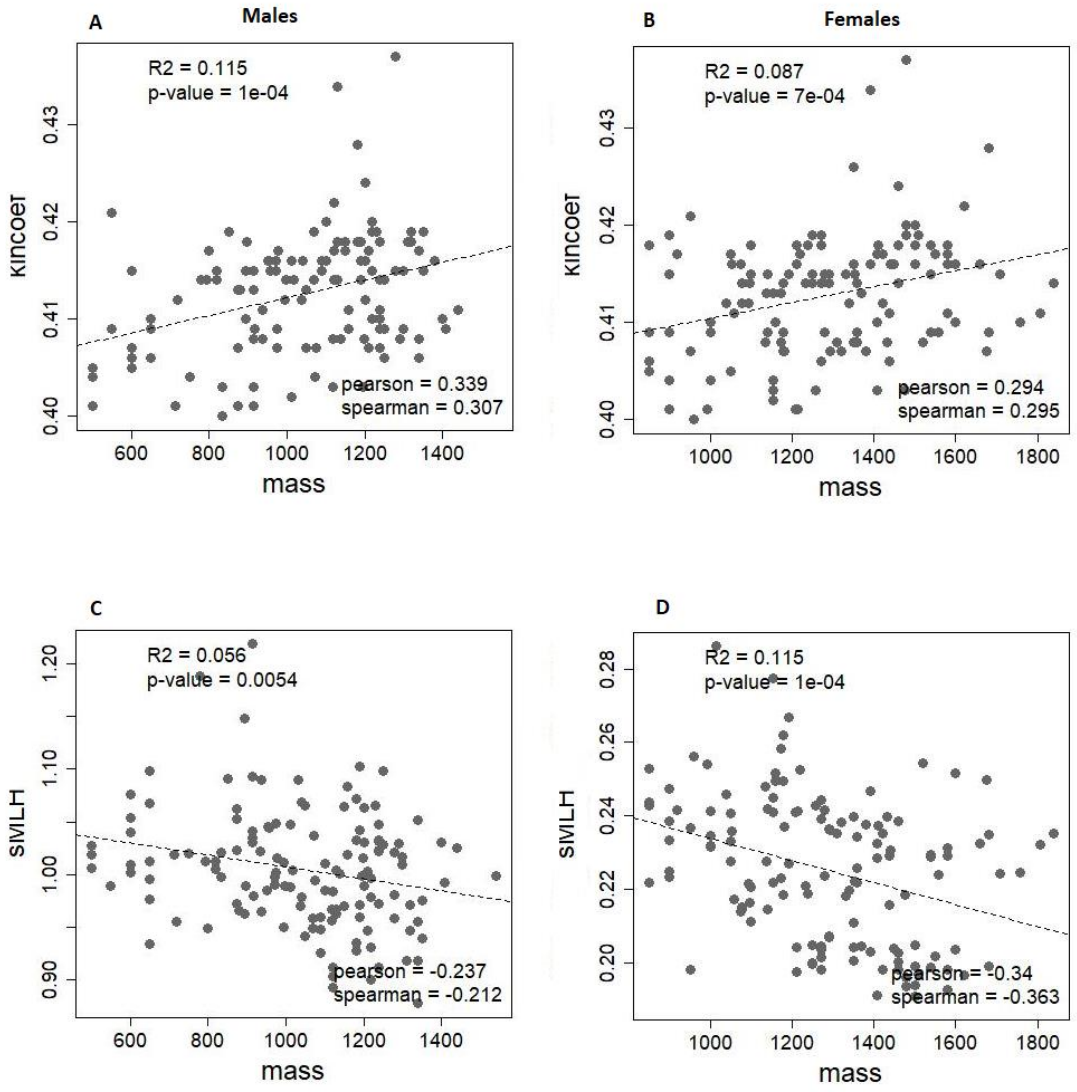


Fig 2.7. Significant correlation between Similarity and Standardized Heterozygosity associated with mass for both males and females. A). Metapopulation. B). Marietas. C). San Jorge. D). San Pedro.

As final remarks, I also got significant correlation for other traits like ulna, tarsus, and beak when compared against heterozygosity, however, they showed similar patterns as those compared against mass. As most traits related to body size were highly correlated between each other (see Figure S2.7), I am only showing mass in the

main text for simplicity. Similarly, the chromas in the spectrum of blue, red, and UV were also included in the correlation analyses which showed opposite patterns to those reported for green chroma, showing differences in the direction and strength of the correlation (Figure S2.8).

2.3.3 Pattern of extra-pair paternity

We analysed the paternity of 147 nestlings from 132 nests across the three different islands, and I found that 6.81% of nests (n = 9) contained at least one extra-pair young, 11.36% (n=15) contained two viable chicks, and 93.18% (n=123) were true monogamous families with only 1 surviving chick (Table 1.1). It is worth notice that I have different sampling size of families because of fieldwork limitations (mainly in San Pedro), however most of extra-pair young were found in San Jorge whereas all the nest with two surviving offspring were found in San Pedro and San Jorge, but none in Marietas (Table 1.1 & 1.2). In all cases the social mother was the biological mother of the chick, and none of the extra pair fathers were identified in the paternity data set. Similarly, all the nests that were able to raise two chicks were from the genetic father.

Table 2.1. Summary of the status assigned to each nest per island.

Status Nests	Marietas	San Pedro	San Jorge	Total
EPP	1	1	7	9
2 Chicks	0	9	6	15
Monogamous	63	17	43	123
All	64	27	56	147

Table 2.2. Summary of sequenced individuals

Islands	Males	Females	Chicks	Total
Marietas	64	63	50	177
San Pedro	27	27	38	92
San Jorge	56	52	64	172
All	147	142	152	441

The body mass of females that engaged in EPP was significantly higher than those females that were monogamous and with only one chick, while those females with two chicks were not significantly different to the other two groups (Figure 2.8 A). However, males in nests with 1 chick where significantly smaller than cuckolded males and males with two chicks. Similar patterns were detected when comparing the green feet chroma, where the females that raised two chicks were significantly greener than both females with monogamous nests and 1 chick and EPP nests, whereas the males did not differ regardless of their status (Figure 2.8 B). There was no significant difference for males or females regarding inbreeding or genetic similarity when comparing the status of the families (Figure 2.9 A-B). Although, it is worth notice that males from monogamous nests with only one chick had an inbreeding coefficient whose confidence intervals did not overlap zero. However, strictly monogamous nests with only one chick presented significantly higher heterozygosity than nests with two chicks (Figure 2.9 C).

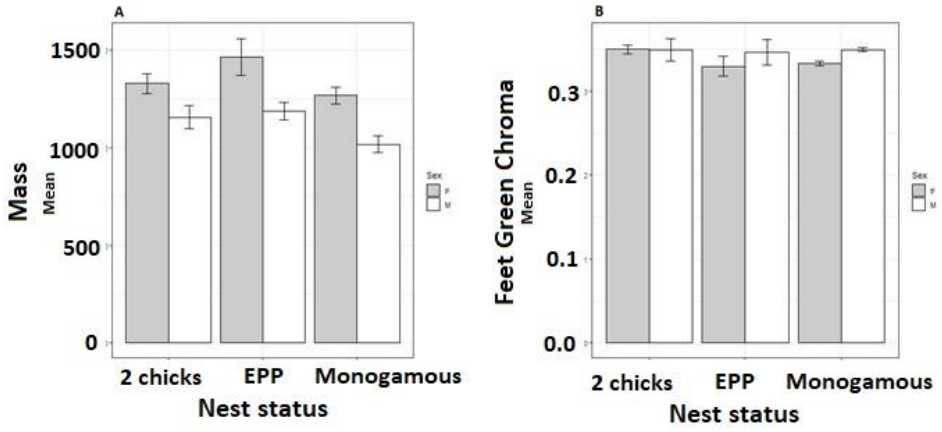


Fig 2.8. Comparison between nest types. A). Total weight of adults. B). Green chroma in feet. Results are presented as upper-lower 95% confidence intervals for each group. Males are represented by the white bars and females by the shaded bars.

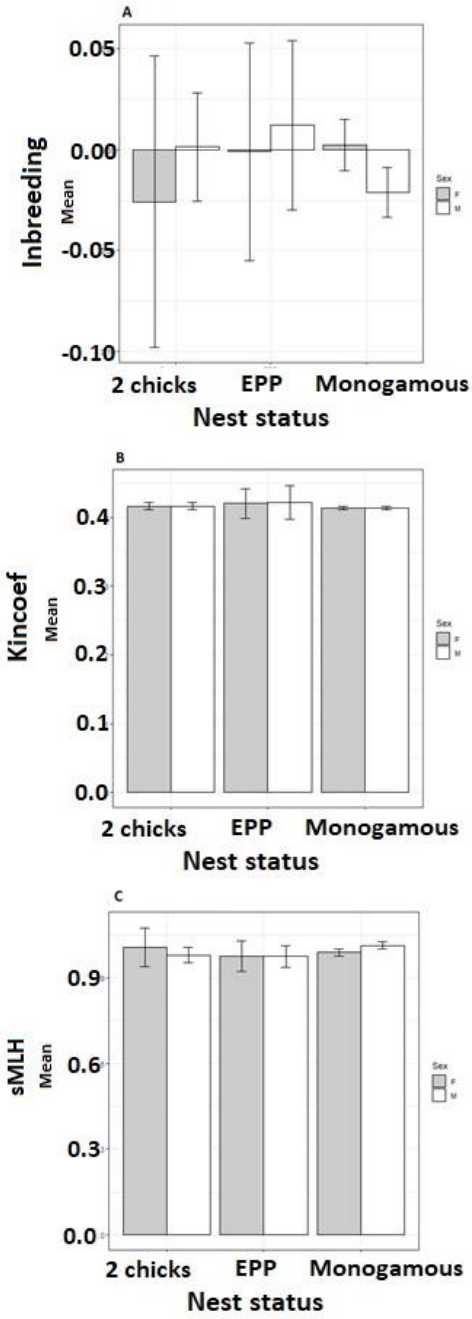


Fig 2.9. Comparison between nests under different status and genetic parameters. A). Inbreeding coefficient (higher positive values indicate grater inbreeding individuals). B). Genetic dissimilarity C). Standardized Heterozygosity. Results are presented as upper-lower 95%

2.4 Discussion

In this chapter I explored the association of two main variables in brown boobies: the association between the genetic quality and the relationship with coloration in sexual ornaments as an indicator of such quality. The relevance o mate choice and the genetic benefits has been discussed extensively (Roberts et al. 2006; Neff & Pitcher 2005; Seddon et al. 2004; Suter et al. 2007; Garcia-Navas et al. 2009), with a simplified version stating that females select males based in genetic quality that bring indirect benefits to the chooser (Ryder et al., 2010)**.** As the brown booby is a monogamous species with biparental care and low levels of EPP, it is expected that both indirect and direct benefits are reflected by sexual ornaments (Kokko 1998). In fact, a study carried in one of the islands included in my analysis (Marietas Island), suggested that paired with males with greener gulars may obtain indirect and direct benefits, given that, gular colour in rearing males is positive corelated to paternal care, chick body mass, and fast structural growth (Montoya & Torres, 2015). Similarly, in the present study I found that (1) colour in bare parts and body size honestly reflect genetic quality; (2) the rates of extra pair paternity are very low with different rates between colonies; and (3) the differences in the honesty of ornaments were found between colonies rather than between parentage status (i.e. nests with EPP, within pair mates and nests with two chicks).

To examine whether sexual ornaments are honest indicators of genetic quality, in the present study I compared the phenotypic and genetic characteristics of complete brown booby families (males and females that stablished a nest with a chick) from three colonies with different environmental conditions. Additionally, I performed a

parentage analysis to determine the percentage of nests with extra-pair paternity to compare the heterozygosity and similarity of individuals producing a within pair young (true monogamous) and some rare nests that were able to successfully raise two chicks. I predicted that if social mate of female brown boobies has low genetic quality, then females try to gain any benefit from EPP by engaging in extra-pair copulation with males that have higher genetic quality or higher dissimilarity.

The rates of EPP were very low (Table 1.1), where most of the nest engaging in in this behaviour were detected in San Jorge (12.5% n=7), followed by San Pedro (3.7% n=1) and Marietas (1.56% n=1).We took several considerations into account when studying brown booby colonies, like the fact that females might have fewer opportunities to engage in EPP depending on the location, the resources available, predators, the breeding densities, and how much energy they need to spend searching for food. Generally, monogamous seabirds have great energy limitations and thus produce only one offspring that requires longer parental care (Lack 1954, 1968; Ashmole 1963). Moreover, adults must stay at the nest at night and through much of the afternoon, and they take shifts to spend time searching for food to provide the chicks (Schreiber & Norton, 2002). Even when they lay two eggs per mating season, the second egg rarely results in a fledged chick because of the oldest chick almost always practice siblicide. However, in the present study I observed nests where parents were able to produce two chicks successfully in two of the three studied colonies (Table 1.1). Many factors have been hypothesized that might have an effect in the presence of extra pair paternity (Dunn et al. 1994; Stewart et al. 2009) or the capacity to raise two offspring (Schreiber & Norton, 2002; Dorward 1962), but research about

the positive interaction between density and synchrony will help to further better the occurrence of nests with two chicks and extra-pair paternity.

Through qualitative observations during fieldwork, I noticed that space for nesting is limited inside the islands, and some areas might represent an advantage for the overall survival of chicks, like areas that provide shade during the day or areas that offer some level of protection from floods or storms, like cliffs areas. Potentially, more dominant individuals or the ones breeding earlier would have access to these good quality areas as it has been reported in other birds (Forstmeier et al. 2002; Hasselquist 1998; Johansson & Jonzen 2012). Furthermore, there was a great variation in physical and ecological factors (e.g., habitat heterogeneity) between the studied colonies that could affect the occurrence of EEP or nests with two chicks. In San Jorge, there is no vegetation, birds establish their nests mainly in cliffs, there are no known important predators, the breeding season is during the late autumn and early winter when the weather conditions are better, and the abundance of a greater variety of prey species (Castillo-Guerrero et al. 2016). San Pedro in the other hand, is the biggest of the three islands and it contains an important density of Mexican Giant Cactus (*Pachycereus pringlei*) which provide extra shade during the day, areas are shared with other species like a big density of Blue Footed Boobies (*Sula nebouxii*) and rattlesnakes (*Crotalus sp.*), which can act as potential competitors and predators respectively; they also breed during winter when weather conditions are favourable, but they can encounter strong winds during those months.

Lastly, birds from Marietas are susceptible to very warm weather conditions during breeding season in summer and can encounter very strong storms; there is short

vegetation like grass but nothing that can provide shade or big cliffs. In Marietas I also observed a high predatory pressure of eggs and chicks by crustaceans specially during the night, so parents must be actively protecting their young at any time which means extra energy expenditure. Moreover, the brown boobies are exposed to variable environmental conditions throughout their range (Nelson 1978, Schreiber & Norton 2002) with different sea surface temperatures (SST) and primary productivity (PP), which in return cause an adjustment in foraging strategies defendant on prey sizes and abundances (Castillo-Guerrero et al. 2016). All the mentioned factors can potentially influence the honesty of the sexual ornaments or the patterns of extra-pair paternity (Emlen & Oring 1977). In some species, they guard mates as a strategy to prevent EPP by showing aggressive behaviours against potential competitors (Komdeur 2001). Contrary to my initial hypothesis, our results showed that island with good quality areas for nesting, no competitor species for niche, low predatory pressures, and higher productivity (San Jorge) presented higher rates of EPP than those islands with lower nesting quality areas for nesting (San Jorge and Marietas). An alternative explanation to my initial hypothesis could be that the occurrence of EPP in brown boobies depend on opportunistic factors provided by the environmental conditions to guard mates and offspring. For instance, birds in Marietas must spend most of their time guarding nests against predators and the heat to ensure the survival of the chicks, while in San Jorge the guarding of nests was not that critical for survival, creating more opportunities for extra-pair encounters.

2.4.1 Traits and genetic quality in extra-pair mates and monogamous pairs with one and two offspring

My results showed that extra-pair paternity in brown boobies is very low, given that only 9 out of 147 analysed nests presented extra-pair chicks and most of these where from San Jorge (Table 1.1).

We found partial support for the hypothesis that cuckolded males would have lower heterozygosity than males in socially monogamous nests. No difference was found between social males at EPP nests from males at other nests when comparing the colour and similarity of social males, whereas the heterozygosity of males with monogamous nests with one chick was significantly higher than EPP nests and those with two chicks (Figure 2.8 & 2.9). Is worth highlighting that, females did not show any difference in sMLH, inbreeding or similarity, but they showed that females raising two chicks had greener feet than those with only one chick or with EPP. When comparing the size of males and females, monogamous nests with one chick showed the smallest sizes followed by nests with two chicks and then EPP nests presented the highest mass overall. However, this could be biased given that most of the nests with two chicks and EPP nests were from the northern islands (San Jorge and San Pedro), where average body mass of the individuals is higher probably because cofounding factors of environmental conditions

When I compared the sMLH between islands, I found that Marietas have a higher heterozygosity than the other two islands, which suggest that some additional processes or historical events might be shaping these population for more heterozygous individuals. Early breeders or birds that arrive first to nesting sites are often considered high quality individuals, they are in better condition (Forstmeier et

al. 2002), they occupy relatively good territories and usually obtain higher quality mates (Forstmeier et al. 2002; Hasselquist 1998; Johansson & Jonzen 2012). Males that breed earlier are thought to pursue EP copulation after they ensure a social mate and the laying of an egg (Václav & Hoi, 2007) because they may have solved the conflict over paternity (Canal et al. 2012). Extra-pair paternity occurrence in a brood will depend on the efficiency and assurance strategies of males and the willingness of social mate to engage in extra-pair copulations (Kokko & Morrell 2005). I observed that each island has different characteristics that could represent challenges to reproduction, like competition for better nesting areas or high-quality mates. These islands represent different habitats, and the breeding season is dependent on environmental conditions, and it usually happens at different times of the year in each colony. Therefore, the timing for breeding, the efficiency of mating strategies and the interaction with the environmental conditions of a colony could be influencing the EPP rates and heterozygosity values presented in this chapter.

When comparing the overall mass of the individuals, I found that the biggest individuals were habitants of San Jorge, followed by San Pedro and then Marietas (Figure 2.3). This makes sense if I consider the different levels of Primary productivity and Sea surface temperature (Figure 1.2), suggesting that San Jorge can support more availability of food year-round (Mellink et al. 2001), whereas Marietas have less food specially during the breeding season (summer), making it energetically costly to maintain bigger bodies.

San Pedro somehow, share conditions more similar to San Jorge and when I look at the distribution of EPP and nests with two chicks these two have the highest occurrence. This contrasts with my initially hypothesis that females from lower primary productivity colonies will engage more often in EPP because they will seek out the relatively few available high-quality males to sire higher quality chicks. My results suggest that abundance of EPP and nests with two chicks might be associated with better conditions that allow to engage in these practices and where food availability is enough to ensure the survival of two offspring. Some variation in EPP rates can be explained by phylogenetic history (Griffith et al. 2002), whereas many additional hypotheses attribute the remaining variation to life-history differences that affect the costs and benefits of engaging in EP copulations (Cramer et al. 2011). In previous studies about EPP variation has been proposed to be influenced by phylogenetic history (Griffith et al. 2002) and life-history differences affecting the advantages and negative consequences of engaging in EP behavior (Cramer et al. 2011). In summary, even though my results do not completely explain the variation of EPP, an alternative explanation could be that the occurrence of extra pair-mates is influenced by the habitat (Tables 2.1 & 2.2), and to a lesser extent, to the heterozygosity of the males (Figure 2.9).

2.4.2 Association of sexual traits with heterozygosity and dissimilarity

My results showed that sexual ornaments like color in bare parts and body size are correlated with heterozygosity or the level of dissimilarity between individuals (Figure 2.5, Figure 2.6 & Figure 2.7), however, in some cases such correlation was different depending on the breeding colony suggesting that mate choice vary as well in each population. Two main models have been proposed that try to explain the

indirect benefits that females could gain by evaluating mates. The model by which "good genes" will benefit females by copulating with high-quality males (social or EPP mates) and producing offspring with enhanced genetic viability, assuming that females have the ability to evaluate the quality of potential mates through traits that are honest and heritable, such as colourful ornaments (likely condition-dependant traits), increased vigour or body size (Houtman 1992; Hamilton 1990; Ryder et al., 2010; Hamilton and Zuk 1982). Such traits could reflect general allelic diversity (heterozygosity hypothesis) or specific superior alleles (traditional good genes), and the model assumes that genetic diversity and the chosen characteristic positively covary (Brown 1997; Kempenaers 2007). On the other hand, the "genetic compatibility" model stablishes those females choose social mates (or EPP) based on genetic dissimilarity (Brown 1997; Mays et al. 2008; Mays and Hill 2004), if such choice is positively correlated with offspring's fitness (Kempenaers 2007). The effects of the model can happen in the form of female preferences for dissimilar males (Zeh & Zeh 1997) or by inbreeding avoidance. In this model, male attractiveness largely depends on the interaction of male and female genotypes, like in the case of the Major Histocompatibility Complex (Ryder et al., 2010; Lovlie et al. 2013; Ditchkoff et al. 2009). Nevertheless, both hypotheses assume that females preferences are likely driven by heritable phenotypic attributes and that have the capacity to evaluate males for genetic quality by using cues like morphology or behavioural; and to assess genotypes of potential mates with respect to their own (Ryder et al., 2010). Therefore, my results are concordant with both the good genes and compatible genes hypotheses since I obtained strong correlations between colour, body size, heterozygosity, and similarity.

Green colour in bare parts from males was positively or negatively correlated with heterozygosity depending on the sampled population, whereas female skin colour showed a positive corelation with heterozygosity for all islands (Figure. 2.4) Condition-dependant models for the evolution of signals in animals propose that if colour honestly indicate genetic quality, females can benefit by mating with colourful males (Hamilton & Zuk 1982; Kodric-Brown & Brown 1984). In Brown boobies, gular and feet coloration is carotenoid dependant and acts as an important condition-dependant signalling in sexual selection, because carotenoids are acquired through diet and might be indicating a nutritional state and foraging efficiency (Hill et al. 2002; McGraw et al. 2003; Casagrande et al. 2006; García-Navas et al. 2012). In males, skin coloration displayed during courtship becomes more yellowish after carotenoid ingestion, which suggests an interaction between the deposition of yellow carotenoids and the structural colour of the skin (Velando et al. 2006, Torres & Velando 2010). Most likely, this variation in skin colour has the potential to have evolved as an honest sexual signal of condition suggests that mate choice may be an important force favouring the evolution of sexual signals by reflecting overall quality and current condition (Montoya et al., 2018).

Moreover, many studies have found positive correlations between genome-wide heterozygosity with wide range of fitness-related traits (Chapman et al., 2009) like condition dependent phenotypic traits (Foerster et al. 2003; Ditchkoff & Lochmiller 2009), song repertoire size, clutch size, fertilization, fledging success (Cohas et al. 2009; Annavi et al. 2014), territory quality (Kempenaers 2007), increased disease resistance (Whiteman et al. 2006; Coltman et al. 1999; Whiteman et al. 2006; Reid et al. 2007), survival (Chapman et al., 2009; Daniels and Walters 2000; Cohas

et al. 2009; Mainguy et al. 2009), increased reproductive success (Kruuk et al. 2002; Slate et al. 2004), reduced expression of deleterious recessive alleles, increased developmental stability (Kempenaers 2007). Therefore, heterozygosity preferences could be used as a quality trait in mate choice (Brown 1997; Li et al. 2016; Rosengrave et al. 2016) and mating with a heterozygous partner is thought to give both direct (Foerster et al., 2003; García-Navas, et al., 2009) and indirect benefits (Kempenaers, 2007; Mitton et al., 1993) to choosers.

In the present study I found that traits such as green colour in feet and gular of females was positive correlated with heterozygosity in all the colonies (Figure 2.4). The males in the other hand, show a more specific and unique pattern depending on their geographic location. For instance, green colour in the gular was positively correlated with heterozygosity in Marietas, but not in the other two islands (Figure 2.6). However, the green colour of male's feet seems to be positively correlated with heterozygosity in San Jorge, negatively correlated in San Pedro, and no correlation in Marietas (Figure 2.5). The opposite pattern seems to be true when comparing inbreeding (Figure S2.6). In my expeditions, I observed that the nesting sites in Marietas have grass which is absent in the other colonies. An alternative explanation for my results could be that grass impedes the visibility of feet in some degree making other traits like gular colour a more important trait subject to sexual selection.

My results about gular coloration in the individuals from Marietas (Figure 2.6) show some level of concordance with those reported in a cross-fostering experiment carried in Marietas by (Montoya & Torres, 2015)**,** where they found that males with greener gulars provided better parental care and genetic quality to offspring;

supporting the idea that in species with biparental care and low levels of extrapair copulations, sexual traits may signal direct and indirect benefits. In the other two studied islands, San Jorge and San Pedro, similar cross-fostering experiments have not been carried out, so I do not have a direct comparison to my results. However, these colonies present very opposing results to the ones from Marietas regarding the signalling of heterozygosity through condition dependant ornaments like gular and feet in males. There are several explanations that could fit into my results, like the possibility that in islands with abundance of food resources the colour signalling is less important for mate choice (less honest trait), or maybe there are fewer negative consequences of choosing a poor-quality male. This poses more interesting questions about the local conditions for mate choice in these colonies, where genetic structure and other evolutionary processes might be playing a bigger role than I previously predicted.

Finally, I found a negative association with mass and heterozygosity of both males and females from all the islands; more Interestingly, I also found a positive correlation between mass and dissimilarity between individuals (Figure 2.7). The negative association between heterozygosity and size does not necessarily mean the good genes hypothesis should be rejected, as bigger bodies does not automatically translate in better fitness. Another way to look at this could be that bigger bodies are energetically more costly than smaller bodies to maintain in an environment with restricted resources. Therefore, this would mean that both the good genes-by-heterozygosity and the compatible gene models can be influencing the selection of this trait, suggesting both provide some level of benefit. Importantly to note, both models could potentially provide indirect benefits, so their contributions are not mutually

exclusive because they contribute to different components of genetic variance (additive and nonadditive) (Colgrave et al. 2002; Neff & Pitcher 2005). Traditionally, compatibility has been described as females selecting dissimilar mates to maximize the genetic diversity in offspring (Mays & Hill 2004). Nevertheless, mate choice for dissimilarity would not necessarily be favouring genetic diversity if gene on gene interactions is providing the benefit, instead, what is important is the adequate combination of female and male genotype (Ryder et al., 2010). This means that, under specific circumstances as those sometimes found under locally adapted populations, dissimilarity can be not so evident because sometimes female choice for more related males can provide the more compatible genes (Lehmann & Perrin 2003).

In conclusion, my results suggest that variation in skin colour has the potential to reflect the heterozygosity and dissimilarity of brown boobies, which may be promoting simultaneously the diversification and evolution of sexual signals in different environments. Additional research needs to be done on male phenotypic traits and their genetic mechanisms and how they are used as cues by females. For instance, the study of the separate roles on the structural colour of bare parts and the interaction of carotenoids to gain a more attractive coloration for courtship. This will help to understand better the mate choice, their evolutionary processes and how genetic diversity is gained in certain systems. Studies about the genetic structure and the distribution genes under selection would be especially beneficial to complement my views on mate choice in heterogenous habitats. In conclusion, my results open the door to even more questions on how populations under different environmental conditions interact with sexual selection and what effect does it has in the maintenance of overall genetic diversity.

CHAPTER 3: DISTRIBUTION OF GENOMIC DIVERSITY, STRUCTURE AND GENE FLOW IN BROWN BOOBY COLONIES UNDER ENVIRONMENTAL HETEROGENEITY.

3.1 Introduction

In the modern world, where human impact is changing the environment, understanding the genetic basis of local diversity has become more fundamental than ever for predicting evolutionary responses of species (Savolainen et al. 2013). With the advance of new genomic techniques, abundant genomic-wide data is now more affordable (Ekblom & Galindo 2011; McCormack et al. 2013) for researchers focusing on evolutionary processes like local adaptation to ecological variables (Grummer et al., 2019; Luikart et al., 2018). The development of restriction site-associated DNA sequencing protocols (RADseq, Baird et al. 2008) permits the identification of candidate loci across the entire genome, which might be subject to local adaptation, and it can be used in non-model species (e.g. Guo et al. 2016; Pujolar 2018). Additionally, when considering local adaptation in novel and diverse environments (Colosimo et al. 2005; Nosil 2007), genomic data can be used to investigate divergent evolutionary lineages (Gompert et al. 2010), and the effect of climate change on diversification within species (Papadopoulou & Knowles 2015). Even though RAD-seq only sample a small fraction of the entire genome (Lowry 2016), it is one of the most used methods to investigate differentiation and adaptation under environmental change (Yakub & Tiffin 2016; Munshi-South 2016). These methods are specially valuable on less studied taxa where evolutionary and ecological histories are not

known beforehand (Massatti et al., 2018). Under several models of speciation (Gavrilets, 2003; Coyne & Orr, 2004), genetic differentiation is the first step before reproductive isolation of emerging species, so investigating patterns of genetic differentiation within the genomic landscape can provide insight onto the genomic basis of divergence (Gompert et al., 2012; Teeter et al., 2010). Consequently, by understanding such mechanisms that have an influence in population differentiation will shed light into the speciation processes.

Understanding how biodiversity associates with environmental variation is crucial when studying adaptation in geographically close ecosystems (Richardson et al., 2014). For instance, at different latitudes the species richness is different, so intraspecific diversity might have a more important role depending on the geographic location (Pamilo & Savolainen, 1999). In the context of a rapid changing climate, identifying how selection pressures and biological diversity correlate might help to understand better microevolutionary processes and to predict how wild populations respond to changes in the environment (Hoffmann & Sgrò 2011). Traditional ecogeographical models generally explain the phenotypic variation of a wide range of species, but empirical phylogeographic research about the mechanisms shaping biodiversity, suggest that processes that influence intraspecific differentiation are probably more complex than previously assumed (Carling et al. 2010; Berkeet al., 2013; Fisher; Nunes, Mancini, & Bugoni, 2017). Identifying these processes would help to understand the first steps of emerging sub-species (Rensch 1938), and also could be useful for successful conservation strategies towards species that are sensitive to environmental change (Quillfeldt & Masello 2013, Brommer et al. 2015).

It is well known that local adaptation is one factor that prevents gene flow in wild populations (Sexton et al. 2014), because under different environmental conditions an organism will experience different selection pressures, causing a disadvantage towards immigrants entering such new environment (Wright 1943, Kawecki & Ebert, 2004; Williams, 1966). Many studies have investigated the genomic footprints of divergent selection implicated in local adaptation in a wide range of species and systems (Savolainen, et al., 2013; Tigano & Friesen, 2016; Hohenlohe et al., 2010; Nielsen et al., 2009; Nunes, Beaumont, Butlin, & Paulo, 2010). Many of these species exhibit high natal philopatry (e.g., Huyvaert & Anderson 2004; Quinn, 1993), and such behaviour usually reduce further the gene flow amongst populations, therefore, it also promotes local adaptation and genetic differentiation even at fine spatial scales (reviewed in Fraser et al., 2011). In summary, local adaptation and high philopatry, have the potential to be a major driver of population differentiation in wild populations (Sexton et al., 2014).

In marine ecosystems, local primary productivity tends to influence energy transfer pattens in larger top predators, whereas more trophic levels occur on higher productivity environments because there is less energy loss along the trophic chain (Pinet 2009). Under these conditions, slight variations in the use of local resources may be sufficient to isolate populations (Mallet et al., 2014; Noguerales et al, 2016). In terrestrial ecosystems, barriers are known to generate genetic discontinuity in species with limited dispersal capabilities (Rocha et al., 2005; Pinera et al., 2008), however, the demography and genetic differentiation will be proportional to the dispersal capacity of species (Perrier et al., 2018). While fragmentation and physical barriers can exacerbate genetic differentiation in terrestrial organisms (Gortat et al.,

2012; Lourenço et al., 2017), bird species in general are not affected the same way because their grater dispersal capacity to suitable habitats (Partecke et al., 2006 ; Björklund et al., 2010). Even though marine organisms can cross most barriers, heterogenous seascapes promotes local adaptations and population structure (Amaral et al., 2012; Selkoe et al., 2016). Therefore, local isolation influenced by environmental pressures promotes the population structure of species (Mayr 1956), and colonies of seabirds are a good example as they usually present highly structured populations regardless of high dispersal capacity (Friesen 2015).

Seabirds also tend to show phenotypic diversity along climatic gradients, with larger body sizes at higher latitudes, which is described as the Bergmann's effect (James 1970). Thus, they are an interesting model to study the relationship between spatial ecology and the genetic diversity, because there are few obvious physical barriers to dispersal yet populations and species breeding on different islands or archipelagos are often genetically distinct, suggesting that natal philopatry or local adaptation are important (Schreiber & Burger, 2001). Additionally, it is possible to compare some seabird populations across environmental gradients because they are widely distributed (Nunes & Bugoni, 2018). Some of the differences that have been reported in seabird populations are in foraging behaviour, non-breeding distribution, isotopic niche, phenotypic, and genotypic variation (Hailer et al. 2010; Morris-Pocock et al. 2011; Grémillet et al. 2004; Wakefield et al. 2013; Wiley et al. 2012; Mancini et al. 2014; Le Corre & Jouventin 1999; Bertellotti et al. 2002). Most tropical seabird species usually have complex evolutionary histories due to a strong site fidelity (Huyvaert & Anderson 2004; Schreiber & Burger 2001), and their fragmented distributions of nesting habitats on remote islands which might be separated by long

distances (Nelson 1978). In organisms with strong flight? capacity that can disperse long distances, high levels of gene flow would be expected, however, a wide range of seabird species exhibit strong population structure (reviewed in Friesen et al., 2007). The influence of ecological factors like local adaptation on dispersal costs (Weatherhead & Forbes 1994), is evident in the fact that resident birds present stronger philopatry than migratory species, hence population structure in tropical birds is usually high (Friesen et al. 2007).

The brown Boobies (*Sula leucogaster*) are strictly marine birds, members of the Sulidae family (Aves: Pelecaniformes), which have pantropical distribution and breed on islands from all ocean basins (Nelson, 1978), and do not perform true migration remaining around their colonies throughout the year (Nelson 2005). Genetic analyses using mitochondrial DNA (Steeves et al. 2003; Morris-Pocock et al. 2010) have suggested that brown boobies have a relatively simple evolutionary history when compared with other species, and that gene flow among colonies seems to be low or zero, involving fragmentation through their breeding range (Morris-Pocock et al., 2011). Other studies also show evidence of phenotypic population differentiation in brown boobies caused by environmental characteristics (Nunes et al., 2017) and trophic niche (Mancini, Hobson, & Bugoni, 2014). In the present study, I sampled three brown booby colonies in the Pacific Coast of Mexico which are exposed to very different environmental conditions (see chapter 1 and 2 of this thesis for more information). Colonies present different environmental pressures regardless of their near geographic location, such as differences in latitude, primary productivity, available area for nesting and sea surface and air temperatures (Nunes et al., 2017; Seeliger & Kjerfve, 2001).

The Isolation by Distance (IBD) model propose that genetic differentiation among population increase with geographical distance, it can happen in the absence of selection, but is aided by genetic drift and low dispersal capacity (Wright, 1943; Meirmans, 2012). However, it would be rather simplistic to explain population isolation only by geographical distance, because usually distinct geographic regions present environmental heterogeneity (Shafer & Wolf, 2013; Wang & Bradburd, 2014). Alternatively, in the Isolation by Environment (IBE) the genetic differentiation of populations increases with environmental differences, regardless of geographical distance (Wang & Bradburd, 2014). Additionally, under seascape heterogeneity model there are some ecological processes known to promote population isolation like non-random gene flow (Edelaar & Bolnick, 2012), and sexual selection against migrants due to local adaptation (Hendry, 2004). I hypothesise that the degree of differentiation and distribution of the genetic diversity will be correlated with geographic distance and to the environmental variables like sea surface temperature (SST) and primary productivity (PP) in each colony of brown boobies. Given that the studied colonies in the Gulf of California and the Pacific Coast of Mexico present environmental heterogeneity, I expect that the influence of such environmental variables will be different in each colony.

I used ddRAD sequencing data from three different colonies of brown boobies (*Sula leucogaster*) which present different environmental conditions and breed at different times of the year. For this chapter, the main objectives are to use Sea Surface Temperature (SST) and Primary Productivity (PP) as proxy of environmental variables to investigate (1) the population structure and differentiation in brown boobies under

environmental heterogeneity, (2) direction of gene flow among colonies, and (3) the potential impact in genomic diversity by comparing both IBD and IBE models.

3.2 Methods

For sampling methods and laboratory methods see chapter 2.

3.2.1 Data preparation, demultiplexing and SNP calling

For demultiplexing, I used process_radtags module from the STACKS version 1.37 pipeline (Catchen et al., 2013), to identify files with sequences that were specific to each sampled individual. Afterwards, SNPs were identified and exported using the populations module from STACKS, where the samples were grouped in their respective populations, and a locus was exported if it was present in 65% of the individuals in each population using the "r" parameter (-r 0.65) at a stack depth of at least 10 by using the "m" parameter (-m 10) to obtain a balanced number of shared SNPs between all populations, as well as SNPs that were unique to a specific colony. I used the parameter "-write_single_snp" to avoid including SNPs in high linkage disequilibrium (LD), and I settled a minor allele frequency of at least 0.025 to process a nucleotide site using the "–min_maf" parameter (--min-maf 0.025).

We used the output files generated by STACKS, to convert them to the appropriate formats for the following analyses. PGD-Spider (Lischer & Excoffier, 2012), plink (Purcell et al., 2007) and vcftools (Danecek et al., 2011) were used to convert the SNP data into PED and MAP format. In vcftools, the flags --depth and --site-depth were used to calculate read depth per individual and per SNP, and Binary files (BED, RAW and BIM) were generated from PED and MAP files using PLINK

with the flags --make-bed, --recode A, --chr-set 95, and allow-extra-chr. SNP data management and analyses were performed in R-4.0.5 (R Core Team, 2019) using wrapper functions of the R package SambaR (github page: https://github.com/mennodejong1986/SambaR). Once the data was imported into R, it was then stored in a genlight object using the function 'read.PLINK' of the R package adegenet-2.1.3 (Jombart, 2008; Jombart & Ahmed, 2011).

Two additional filtering pipelines were used to generate two targeted datasets, one to investigate the genetic structure related questions, and another one to investigate the genetic diversity questions of this project. Therefore, the additional "structure" filtering was performed using the function 'filterdata' of the R package SambaR, with the parameters indmiss=0.3, snpmiss=0.02, min_mac=2, dohefilter=TRUE and min_spacing=500 for the genetic distance and diversity analyses. The reason of these strict snpmiss parameters is to get a relatively low number of high-quality SNPs (with almost no missing data) to get a more accurate picture of the true population structure. On the other hand, the parameters indmiss=0.2, snpmiss=0.2, min_mac=2, dohefilter=TRUE and min_spacing=500 were used for the "diversity dataset" to retain a relative small number of high quality individuals with almost no missing data that could bias heterozygosity estimations.

3.2.2 Population structure

Correspondence analyses (CA) were performed using the function 'dudi.coa' of the R package ade4-1.7.16 (Dray and Dufour, 2007; Bougeard and Dray, 2018). Data was imputed per SNP/individual by calculating genotype probabilities from population specific minor allele frequencies. Principal coordinate analyses (PCoA) were performed using the function 'pcoa' of the R package ape-5.4.1 (Paradis &

Schliep, 2018) on distance matrices containing 3 different measures of genetic distance. The first was Nei's genetic distance, which is calculated with the function 'stamppNeisD' of the R package StAMPP-1.6.1 (Pembleton et al., 2013). The second was the Hamming's genetic distance, which is calculated with the function 'bitwise.dist' of the R package poppr-2.9.1 (Kamvar et al., 2014). And finally, pi (pairwise sequence dissimilarity) was calculated with the function 'calcpi' of the R package SambaR.

Principal component analyses (PCA) were also performed using the function 'snpgdsPCA' of the R package SNPRelate-1.24.0 (Zheng et al., 2012). DAPC analyses were performed using the function 'dapc' of the R package adegenet-2.1.3 (Jombart, 2008; Jombart & Ahmed, 2011), both with and without prior population assignment. Multi-dimensional scaling (MDS) was performed using the function 'cmdscale' (metric MDS) of the R package stats-4.0.5 (R Core Team, 2019) and the function 'isoMDS' (non-metric MDS) of the R package MASS-7.3.53.1 (Venables & Ripley, 2002), on a Euclidean distance matrix generated with the function 'dist' of the R package stats-4.0.5 (R Core Team, 2019). ADMIXTURE coefficients were calculated with the functions 'obj.snmf' and 'Q' of the R package LEA-3.2.0 (Frichot & Francois, 2014). Alpha was set to 10, tolerance to 0.00001, and number of iterations to 200. Finally, the ancestry coefficients were calculated with the software Admixture-1.3 (Alexander et al., 2009) and plotted using the 'plotstructure'-function of SambaR.

3.2.3 Genetic diversity and differentiation

Linkage disequilibrium (LD) estimates were calculated using PLINK (--genome --r2 --ld-window-kb 1000000 --ld-window -r2 0). HWE, (2D) folded site frequency spectra (SFS), Tajima's D and genome wide heterozygosity analyses were

executed using the function 'calcdiversity' of the R package SambaR. More specifically, population specific SFS vectors were generated with the function 'getfoldedsfs' of the R package SambaR, which bins SNPs in classes based on their number of copies of the minor allele, and which subsequently calculated the size of each bin (i.e., number of SNPs within each bin). Genome wide Heterozygosity (Multilocus Heterozygosity or MLH) and standardized Multilocus Heterozygosity (sMLH) per population were obtained using the inbreedR package (Stoffel et al., 2016) using the same dataset and parameters as the ones presented in chapter 2 in a per-individual basis.

Genome wide 'Weir & Cockerham 1984' Fst estimates (for all pairwise population comparisons) were calculated with the function 'stamppFst' of the R package StAMPP-1.6.1 (Pembleton et al., 2013). Locus specific Fst estimates (according to Wright 1943; Nei 1977; and Cockerham & Weir 1987) for all pairwise population comparisons were calculated with the functions 'runWrightFst', 'locusNeiFst', and 'locusWCFst' of the R package SambaR. Relatedness between samples was calculated using the software PLINK and/or GCTA and plotted using SambaR functions.

The neighbour-joining (NJ) and UPGMA trees were based in Hamming's genetic distance using the bitwise.dist function of poppr package. These trees show genetic difference between populations, for instance, if a recently diverged founder population can be genetically more distant from the source population than a bigger population which diverged earlier.

3.2.4 Demography, gene flow, isolation by distance and isolation by environment

Historical Ne estimates were inferred from the folded SFS vectors with the software stairway_plot_v2 (Liu & Fu, 2015). The mutation rate was set to $\mu = 3.55.e^{-8}$ (Cristofari et al., 2019) and, the generation time to 6 years (Ancona et al., 2015)**.** Gene flow was estimated by performing a recent migration analysis using BayesAss3-SNPs (Mussmann et al., 2019). The number of iterations was set to 1000000, burn-in to 100000 and delta values to 0.1. Output matrices were converted into gene flow plots with the function 'plotmigration' of the R package SambaR, with use of the R package circlize-0.4.12.

For the isolation by distance model, geographical coordinates of each colony were used to calculate pairwise geographical distances (km), and I applied a Mantel test for correlation between genetic and geographical distance (Slatkin, 1995). The Mantel test was performed with Pearson's corelation, and p-values were calculated using 10,000 permutations in the "vegan" package (Oksanen et al., 2016) R package SambaR (de Jong et al., 2021)). Sea surface temperature (SST; °C) and Primary Productivity (PP; chlorophyll a concentration; Chla; mg mm^{-3}) for each colony were obtained from the environmental data catalog of NOAA (https://polarwatch.noaa.gov/catalog/), considering average values from 2011 to 2018. I downloaded the data series with a resolution of 4 km/pixel and calculated the average chlorophyll a value within a 40-km radius surrounding each colony, following previously published maximum foraging range of brown boobies (Weimerskirch et al. 2009). I calculated two average values per parameter: average values during the summer months (from June to September) and during the breeding months (rearing period of each island). Given that each colony breed in different times of the year and

it can vary from year to year slightly, I calculated average values from the month that have been reported for each island. For Marietas I calculated the average monthly values from June to September, for San Pedro from February to April, and San Jorge from November to January. The logic behind this was to test how much of the genetic variability is explained by the lowest environmental values of each colony (which occur in the summer), and also during the most critical months for reproduction and survival (breeding season).

In addition, isolation by environment (IBE) is a pattern of isolation in which genetic differentiation increases with environmental differences, independent of geographic distance (Wang & Bradburd, 2014). In order to calculate IBE, a redundancy analysis (RDA) was used to identify the effect of each environmental variable on genetic variation by running the ordistep function in the "vegan" package using a backward stepwise procedure, and Akaike's information criterion (AIC) was used to select the best model. p- values were calculated based on 10,000 permutations.

3.3 Results

3.3.1 SNP calling and filtering datasets

We performed two independent filtering pipelines with different parameters depending on the purpose of my main objectives. For the structure dataset, I retained 348 out of 441 individuals (64-151 per population) after filtering (Figure S3.1), of which, 3753 SNPs out of 26340 were retained after filtering and thinning (Figure S3.2). Also, the GC-content of the retained dataset equalled 0.62 and the 'transversion vs transition'-ratio equalled 0.72.

For the genetic diversity dataset 267 out of 441 individuals (43-114 per population) passed the filters and were retained (Figure S2.3 in chapter 2), of which, 16054 out of 26340 SNPs were retained after filtering and thinning (Figure S2.4 in chapter 2). Moreover, the GC-content of this retained dataset equalled 0.59, and the 'transversion vs transition'-ratio equalled 0.71. Therefore, the structure dataset was used for the principal component and ancestry analyses, whereas the diversity dataset was used for the genetic distance and derived analyses from such measurements.

3.3.2 Population structure

The PCoA plot based in Nei's standardised data was built from coordinates 1 and 2, which explained 9.8% and 2.7% of the genetic variance respectively (Figure 3.1). Coordinate 1 separated the Marietas colony from the remaining colonies, while Coordinate 2 separated San Pedro from the other two colonies (Figure S3.3). The PCoA plots base in Hamming's genetic distance and pi (pairwise sequence dissimilarity) showed the same pattern and can be found in Figure S3.4. Additionally, Correspondence analyses (CA), Principal component analyses (PCA), DAPC analysis, multi-dimensional scaling (MDS) showed the same separation between colonies (Figure S3.5).

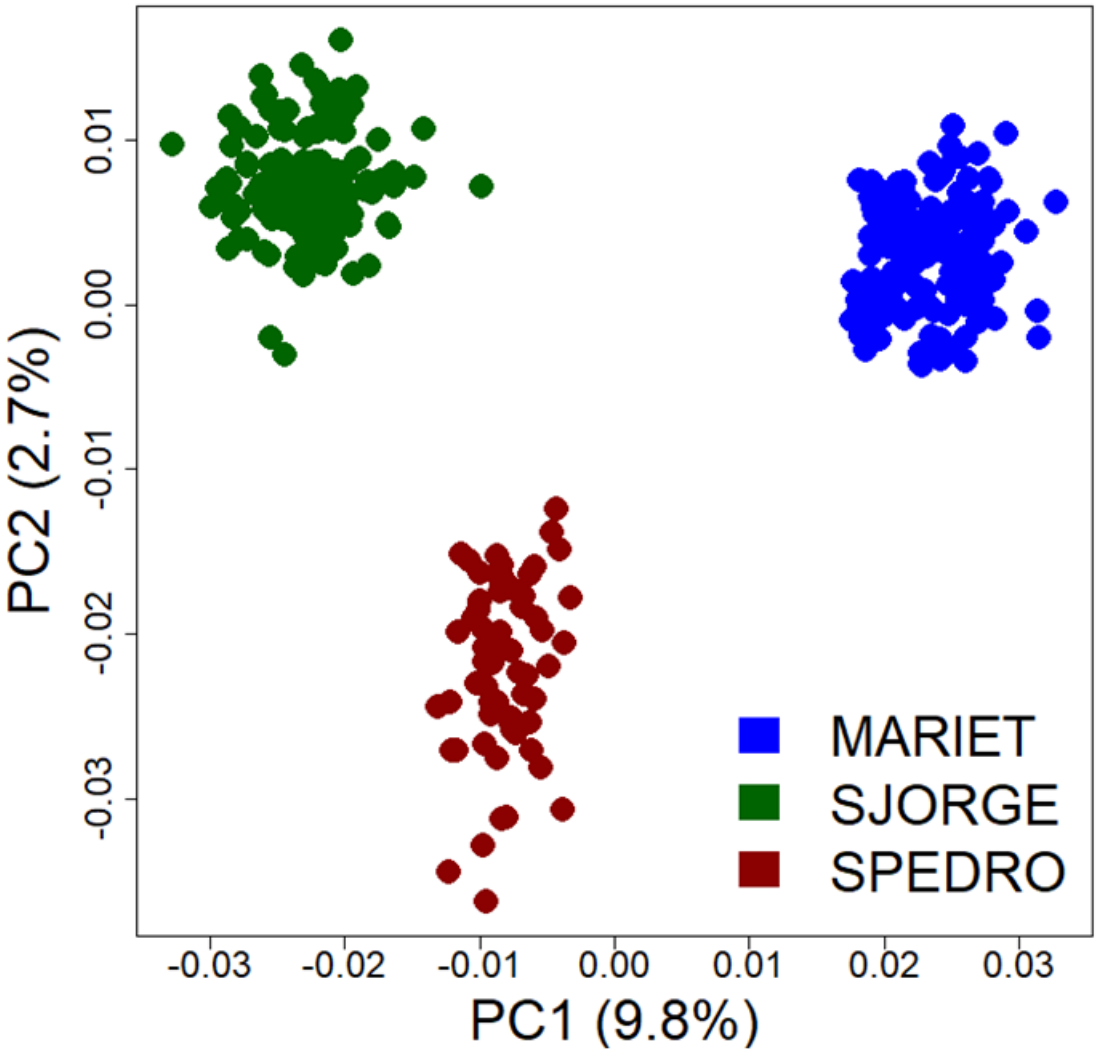


Fig 3.1. Principal Coordinate Analysis (PCoA) plot of the three sampled colonies. Dots are standardized data based on Nei's genetic distance carried out in ape 5.4.1 (Paradis and Schliep, 2018) to explore similarities and groupings among colonies.

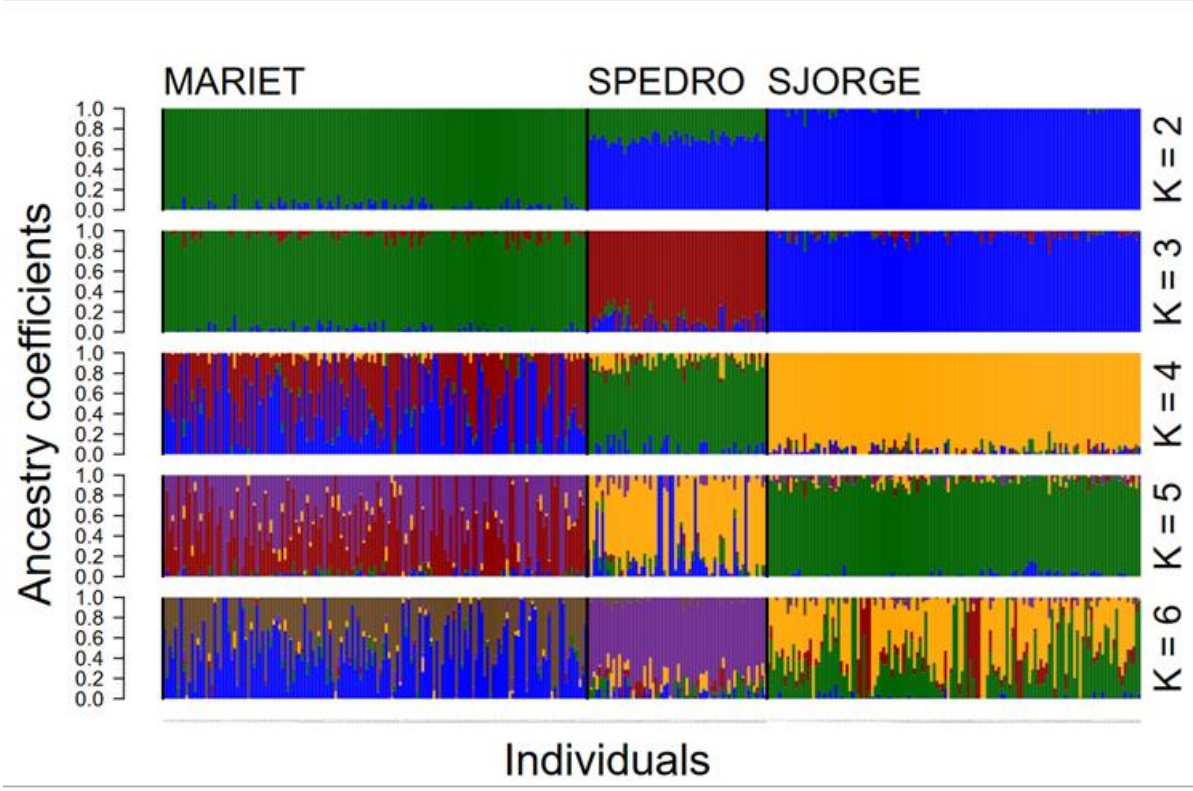


Fig 3.2. ADMIXTURE plot and ancestry coefficients. K represent the number of clusters for structured populations.

A population structure based on three clusters (K = 3) was most strongly supported by the DAPC statistics (Figure S3.6), and the ADMIXTURE plot showed a clear differentiation of all colonies (Figure 3.2). The phylogenetic trees reconstructed using the neighbour-joining (NJ) and UPGMA methods based on genetic distance also demonstrated a strong hierarchical differentiation in all colonies (Figure S3.7). To evaluate genetic differentiation between the three clusters, I computed pairwise FST and Nei's genetic distances (Figure 3.3). My results show that Marietas and San Jorge are the most differentiated colonies, followed by San Pedro and Marietas, and finally San Jorge and San Pedro as the most similar colonies (Figure 3.3). Additionally, I also calculated Nei's genetic distance of individuals among the three populations and showing a similar differentiation pattern (Figure S3.8).

3.3.3 Distribution of genetic diversity, gene flow and demographic reconstruction

The rates of gene flow in general were very low between any of the colonies, with slightly higher percentage of migrants from San Pedro to the other two islands where the numbers of migrants per generation were very close to 0 and the direction of migration is shown in Figure 3.4. Additionally, Watterson estimator and Tajima's D showed that San Pedro have higher genetic diversity and excess of rare alleles followed by Marietas and San Jorge respectively. However, Marietas presented a higher nucleotide diversity (pi) compared to the other islands (Figure 3.5). Additionally, Marietas showed significant higher heterozygosity and dissimilarity than San Pedro and San Jorge respectively (Figure 3.6). The negative values of Tajima's D suggest the abundance of rare alleles or a recent selective sweep and population expansion after a recent bottleneck or founder event (Tajima 1989). However, my demographic reconstruction showed a stable effective population size (Ne) of 2-2.5 thousand individuals from 50 to 15 thousand of years ago (kya) for all colonies (Figure 3.7). This followed by a sudden rise in Ne that peaked at 4-5 thousand individuals, and then, an ongoing reduction that started 500 years ago (Figure S3.9).

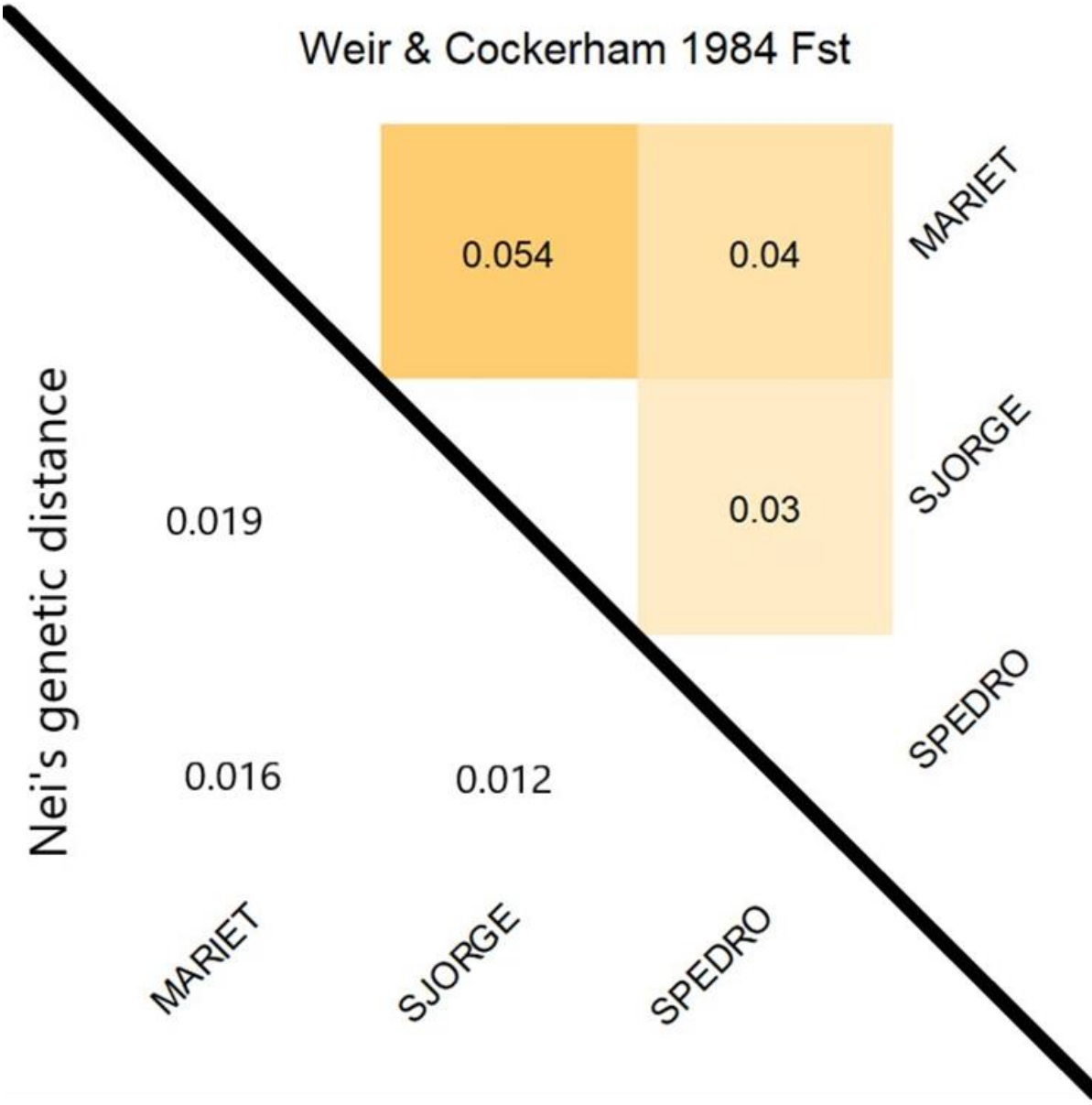


Fig 3.3. Genetic differentiation between three genetic clusters. Fst (upper) and Nei's genetic distance (lower) estimates.

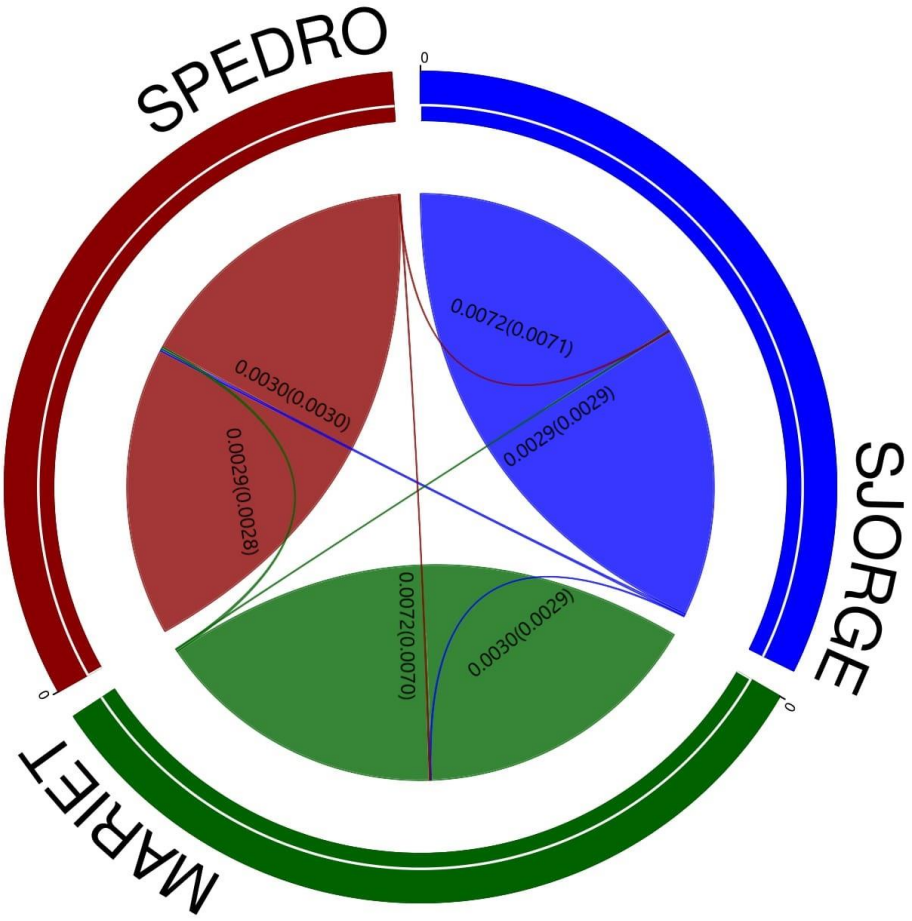


Fig 3.4. Gene flow between genetic clusters. The direction of migration and strength of migration is represented in the coloured lines. The values next to the line indicate the migration rate with the confidence intervals in parentheses.

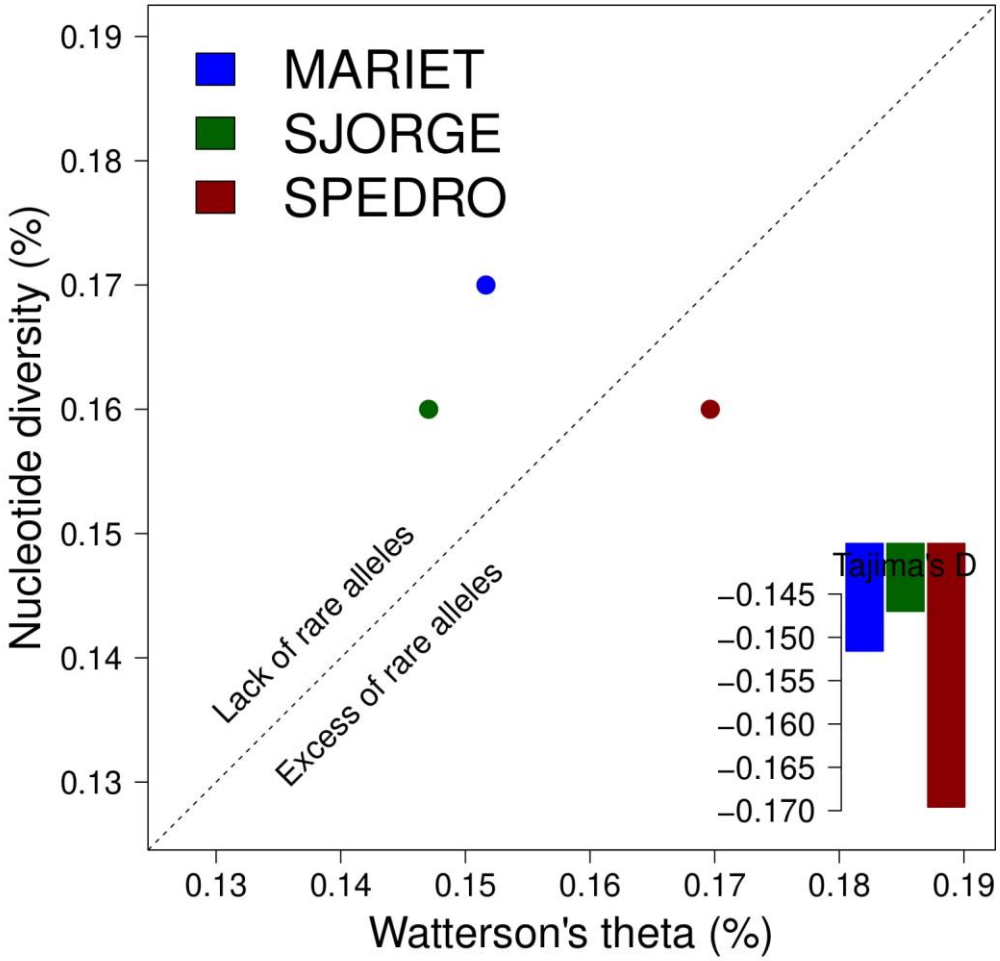


Fig 3.5. Distribution of nucleotide and genetic diversity. Tajima's D, Nucleotide diversity (Pi), and Watterson's theta indicate the proportion of rare alleles in the studied populations.

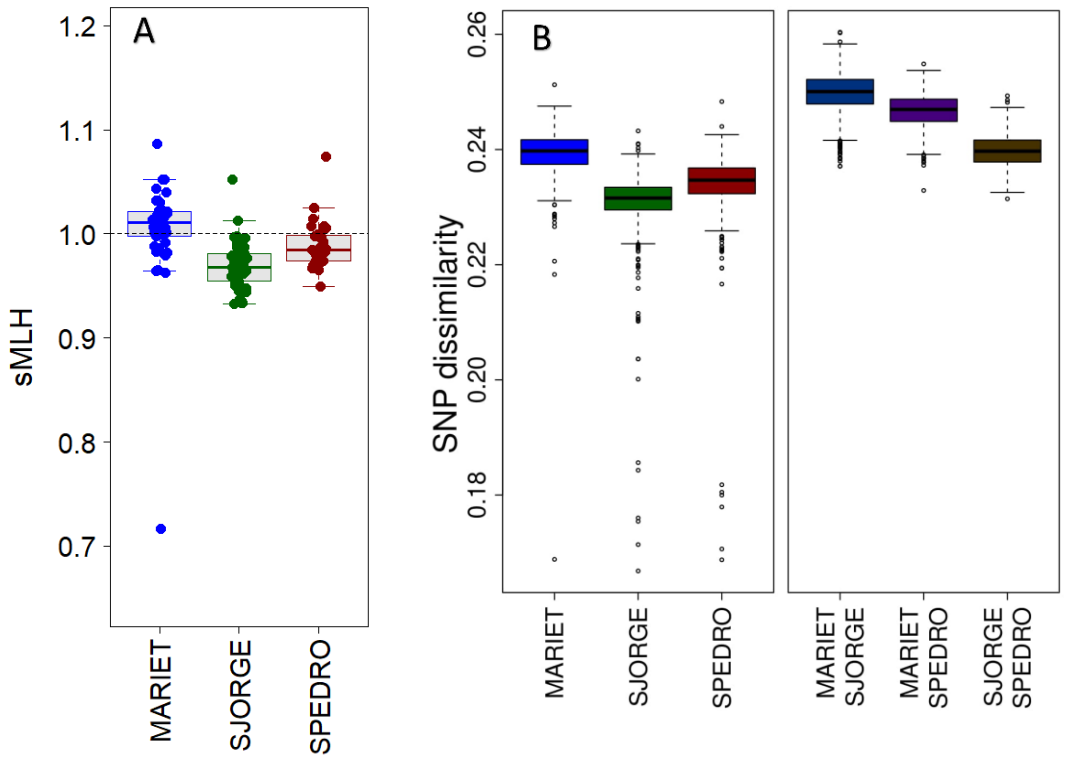


Fig 3.6. Distribution of heterozygosity and dissimilarity among populations. A. Standardized Multilocus Heterozygosity (sMLH). B. SNP dissimilarity per population and between populations.

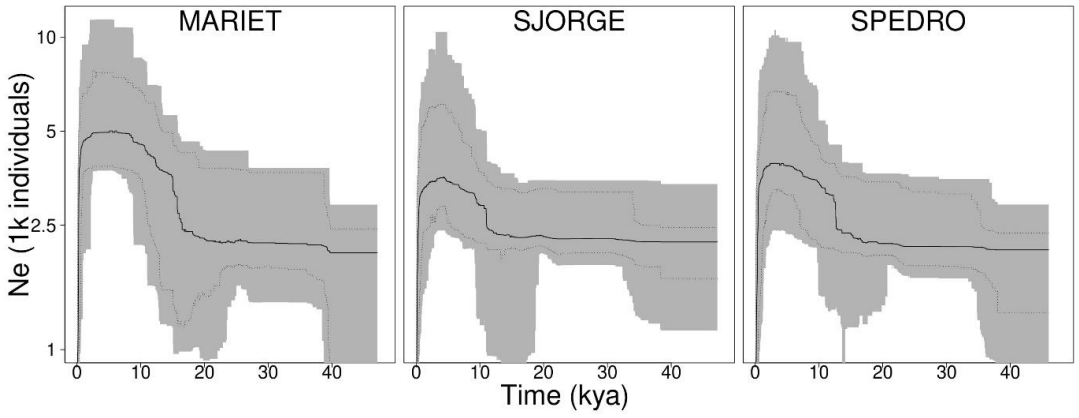


Fig 3.7. Demographic reconstruction. Stairway plot showing the demographic history of the three colonies. Time is shown in thousands of years ago. Shaded areas show the 95% conficence intervals.

3.3.4. Isolation by distance and isolation by environment

The Mantel test showed a moderate-strong correlation between geographic distance and genetic distance (pi), where Marietas and San Jorge show the highest geographic and genetic distance (Figure 3.8). For the environmental variables, I generated maps showing the average values of SST and PP for each colony (e.g., Figure S3.10) and for the entire range (Figure 1.2 in chapter 1) of the sampling area and I calculated the average values for the summer months and for the breeding months.

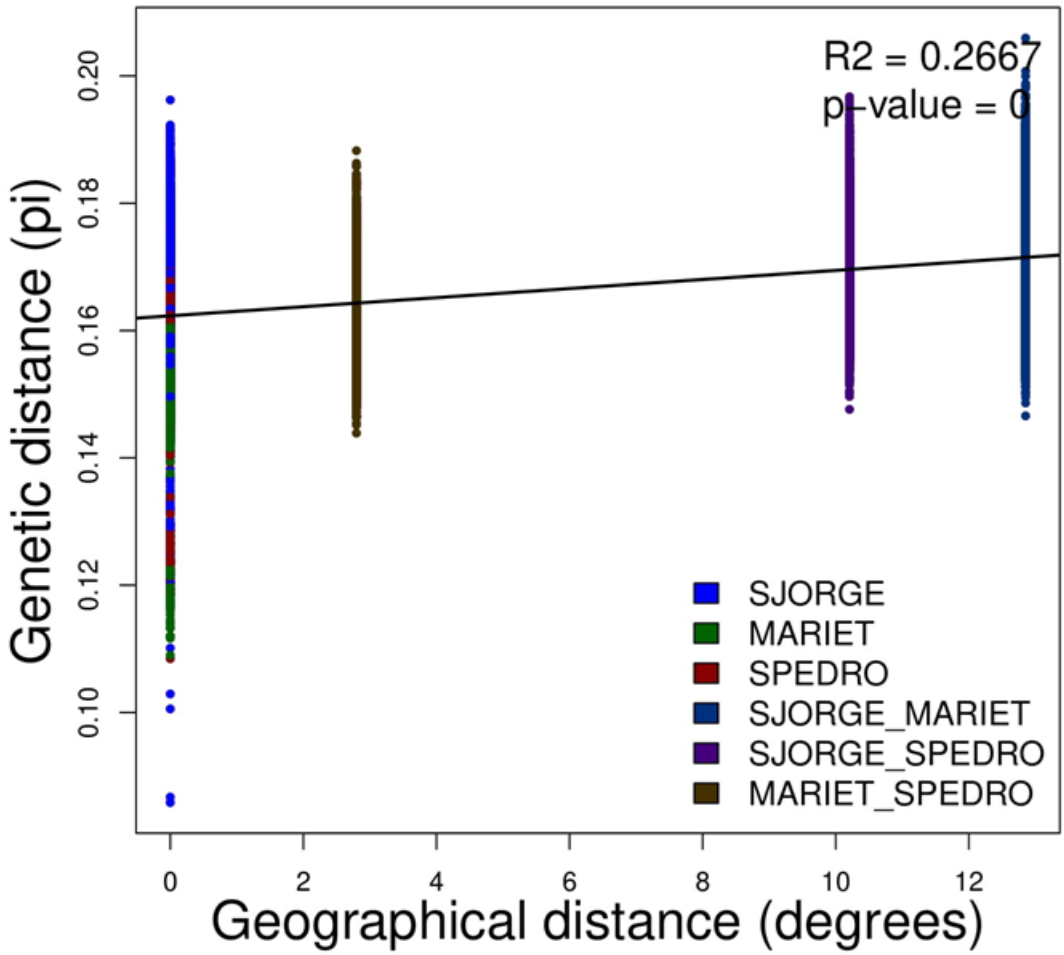


Fig 3.8. Isolation by Distance (IBD) plot. Correlation between geographic and genetic distance (in terms of nucleotide diversity).

To quantify relative contributions of environment an RDA was performed using the "summer" variables and the "breeding" variables. PP and SST explained 5.92% of the total variance (F = 8.3124; df = 2; p-value = .001) according to the RDA, while genetic diversity of individual variables was different depending on the time of the year as shown in Table 3.1. It seems that the individual variable that better explained the total variance was the primary productivity during summer with 4.33%

(F = 12.006; df = 1; p-value = .001), whereas the SST during summer explained only 1.42% of the variation (F = 3.8226; df = 1; p-value = .001). The RDA triplots show the significance of SNPs (dark grey points), individuals (coloured circles) and environmental variables (Figure 3.9), where their relative arrangement in the ordination space reflects their relationship with the ordination axes, which are linear combinations of the predictor variables (Brena et al).

For the breeding season, the RDA plot showed a positive correlation between PP and the colonies of San Jorge and San Pedro, while Marietas showed a negative association with PP. Also, Marietas seems to be strongly correlated with high temperatures while San Jorge and San Pedro were negatively correlated with SST. For the summer season, San Pedro had a negative correlation with SST whereas San Jorge presented a positive correlation with PP, but Marietas had a negative correlation with productivity during the breeding season (Figure 3.9).

Table. 3.1. Effect of environmental variables estimated by RDA models. P-values are computed by permutation tests with 10,000 replicates. Var(%) show the percentage of variance composed by the environmental variables.

Description	Environmental variable	F	Var	Var (%)	p-value
Individual effect of environmental variables during Breeding Season	Primary Productivity (E1:PP)	10.551	614.7	3.83E-02	0.001
	Sea Surface Temperature (E2:SST)	11.278	655.3	4.08E-02	0.001
Individual effect of environmental variables During Summer	Primary Productivity (E1:PP)	12.006	695.8	4.33E-02	0.001
	Sea Surface Temperature (E2:SST)	3.8226	228.3	1.42E-02	0.001
Combined effect of environmental variables	Sea Surface Temperature (E2:SST)	8.3124	951.1	5.92E-02	0.001

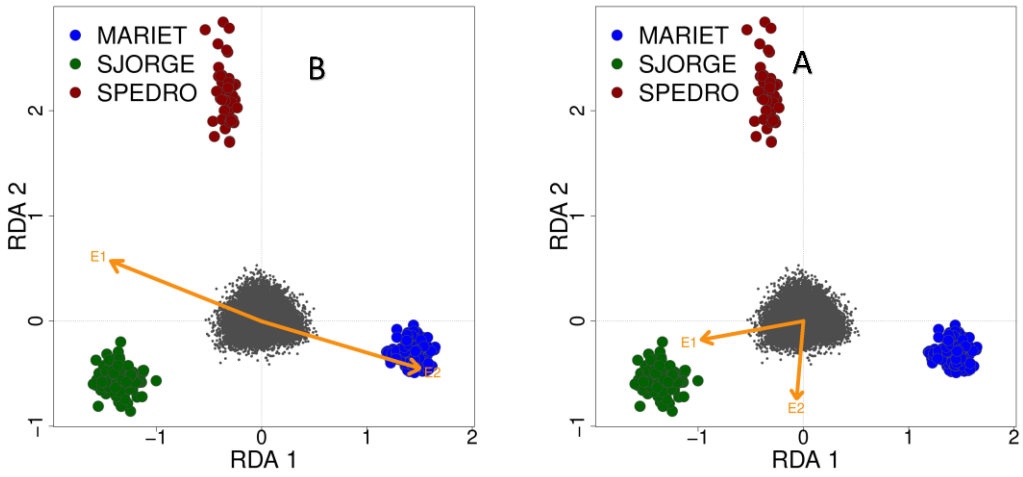


Fig 3.9. Isolation by Environment (IBE) demonstrated by Redundancy analyses (RDA). Correlation between genetic variation and environmental variables in Brown booby colonies during Summer (A) and the breeding seasons (B). Angles between arrows are defined by Pearson's correlation and direction of a projected arrow indicates where the highest values are. Yellow arrows represent which environmental variables better explain variations in allele frequencies among colonies. E1= Primary Productivity. E2=Sea Surface Temperature. Triplot scaling is symmetrical (both SNP and individual scores are scaled symmetrically by the square root of the eigenvalues)

3.4 Discussion

In the present chapter I explored the genomic-wide characteristics likely to be affected by historical or environmental of three breeding colonies of brown boobies. By performing two independent filtering pipelines of all genomic sequences, I balanced the trade-offs between numbers of individuals and levels of missing data. For the "structure" pipeline, the selection of fewer individuals and SNPS that contained almost no missing data was a priority in order to avoid artifact structure caused by the stochasticity of different sequencing runs, low quality samples, or the abundance of rare alleles. In the other hand the "diversity" pipeline focused in getting a balanced number of high quality SNPs and individuals to enhance the power of my analyses related to genetic diversity (de Jong et al., 2021).

3.4.1 Population structure

I found strong evidence for genetic structure between the studied colonies according to the PCoA, Principal Component (PC), DAPC analysis, and multi-dimensional scaling (MDS) were performed in order to test the structure patterns of the different sampled colonies. Such analyses were based in Nei's standardised values, Hamming's genetic distance, and pi (pairwise sequence dissimilarity), however, all these analyses showed a similar level of structure like the one showed in Figure 3.1. From the PCoA, the coordinate 1, 2 and 3 explained 2.7%, 9.8%, and 1.4 % of the genetic variance respectively, where coordinate 1 separated the Marietas colony from the remaining colonies, coordinate 2 separated San Pedro from the other two colonies, and coordinate 3 didn't showed a clear separation between the three colonies (Figure S3.3.) Is interesting to note that San Pedro remained as an independent cluster in all PCoA coordinates, even when San Jorge and Marietas were grouped in the same cluster by the coordinate 2 regardless of its geographic distance. This suggests some sort of additional historical events reflecting such structure pattern or an ongoing barrier to gene flow, specially between San Pedro and Marietas.

Several admixture analyses were performed under different population structure assumptions (K=2- K=6), to analyse population structure and ancestry (Figure 3.2). When considering K=2, Marietas and San Jorge appeared as two distinct populations sharing almost no ancestry, whereas San Pedro was more genetically similar to San Jorge than to Marietas. When considering K=3 it seems that all three colonies are very different from each other, with San Pedro showing some higher percentage of mixed ancestry from the other 2 colonies. The plots showing K=4, 5 and 6, it seems that Marietas is the one colony showing more levels of mixed ancestry.

Additionally, the DAPC strongly supported a population structure based in 3 clusters (Figure S3.6), which is consistent with the observed PCA plots assuming no prior knowledge of the genetic structure. These result support even further the idea of three genetically distinct populations, where K=3 suggest that San Pedro and Marietas have individuals with a small percentage of recent mixed ancestry coming from San Jorge. However, it seems that both San Jorge and Marietas have small levels of ancestral admixture descending from San Pedro. This could indicate that San Pedro does not have any ancestral admixture coming from Marietas and San Jorge, but it does present a recent admixture coming from these islands. Moreover, it seems that the recent admixture in San Pedro and Marietas coming from San Jorge could be the effect of some individuals moving to southern islands. However, I appreciate that admixture analyses should be complemented with other higher resolution phylogenetic analyses to confidently confirm the historical founder populations.

Additionally, the phylogenetic reconstruction of the sampled individuals further supported this pattern of population structure (Figure S3.7), suggesting that San Pedro and San Jorge were founded by Marieta's descendants. The Fst and Nei's genetic distance showed that Marietas and San Jorge were the most differentiated colonies, followed by San Pedro and Marietas, and finally San Jorge and San Pedro were the least differentiated, though still significant (Figure 3.3). When comparing pairwise Nei's genetic distance to an individual level, I found that most individuals between San Jorge and Marietas presented great genetic distance (0.16-0.2), San Pedro and Marietas presented fewer individuals with high genetic distance (0.12-0.16), and finally, most of individuals in San Jorge and San Pedro presented moderate genetic distance (0.08-0.12) (Figure S3.8).

According to the BayesAss3-SNPs analysis, it seems that the high levels of structure in the studied colonies happen in the absence of gene flow. The number of migrants per generation are very close to 0 with a marginally higher migration rate happening from San Pedro to the other two islands (Figure 3.4). Brown boobies can travel long distances and no physical barriers are recognized between the sampled colonies, suggesting that non-physical barriers like natal philopatry (Friesen et al. 2007), different timings of breeding of each colony or habitat preferences are preventing gene flow and promoting their isolation. Some studies propose that elevated natal philopatry is the main cause of high fragmentation on seabird populations (Huyvaert & Anderson 2004). However, seabirds also tend to show phenotypic diversity along climatic gradients, with larger body sizes at higher latitudes, which is described as the Bergmann's effect (James 1970). Thus, they are an interesting model to study the relationship between spatial ecology and the genetic diversity, because there are few obvious physical barriers to dispersal yet populations and species breeding on different islands or archipelagos are often genetically distinct, suggesting that both natal philopatry and local adaptation to environmental variables are important (Schreiber & Burger, 2001).

Since early studies about the genetic structure of Sulids, the role of local adaptation, behavioural and geographical isolation has been proposed as main drivers of genetic differentiation in the absence of physical barriers. For instance, Steeves et al. (2003) used mitochondrial cytochrome b variation to test the hypothesis that the Isthmus of Panama and Eastern Pacific Basin drove genetic divergence in brown boobies and found that population genetic structure was high between the Gulf of Mexico and the Eastern Pacific. Shortly after, Steeves et al., (2005) found low levels

of gene flow within Indo-Pacific and Atlantic populations mainly attributed to geographic distance, whereas, populations withing these regions diverged despite not having physical barriers. They concluded that their results could be explained by limited natal dispersal combined with local adaptation and genetic drift, though the influence of these was not measured at that time. In another study carried by Morris-Pocock et al., (2010) about the genetic structure and divergence of brown booby populations, they suggested that San Pedro diverged from colonies outside of the Gulf of California 130,000 years before the present in the absence of gene flow, yet they are separated by only 500 km. They also suggested that factors such as salinity and sea surface temperature could act as cryptic barriers to dispersal. Additionally, a similar study by Morris-Pocock, J. A. et al. (2011) sampled 215 individuals from all major breeding areas (including San Pedro) and genotyped them at eight microsatellite and three nuclear intron loci. They found that brown booby populations were highly differentiated and that colonies can be grouped into four major genetic populations. Even when all the mentioned studies have mentioned local adaptation and environment as potential drivers of population differentiation, to my knowledge, none of them have tried to measure the real influence of environmental variables to explain such differentiation, thus highlighting the importance of my current study for the general knowledge of brown boobies and other seabird species.

Additionally to seascape heterogeneity, the availability of nesting areas and density, could be factors influencing gene flow between islands (Nunes & Bugoni, 2018). Generally, brown boobies can build their nests on plain surfaces slopes or cliffs, and colonies are formed by groups of these nests with some spacing between them, ranging from 0.6 to 27 m in between (Nelson, 2005). Nevertheless, different studies

have shown that nesting preferences changes in different islands (Branco et al., 2013; Alveset al., 2004; Alves et al., 2000). I So, if colonies present different nesting qualities and preferences, it could be promoting the isolation of such colonies and the breeding success of the individuals. When a colony has a high density of nests (~1m separation), some aggressive behaviour starts to show between neighbours (Kohlrausch, 2003), generating strong competition for space causing combats between adults, the killing of chicks, and in the more extreme cases even cannibalism (Neves et al., 2015). Therefore, living in colonies with such high densities would require the ability to compete for nesting areas, promoting even more isolation by local adaptation and selection against migrants (Nunes & Bugoni, 2018). In my sampling expeditions, I observed some of these high-density areas where nests were separated by around one meter in all three islands, but more research would be required about nesting densities to evaluate the impact of nesting sites as driver of structure. San Pedro Martir in particular, showed a high density of both brown boobies and blue-footed boobies in it was described by Tershy (1997) several decades ago as the biggest populations of these seabirds in the world. If this is still true nowadays, it could pose interesting demographic questions about the dynamics the populations in the East Pacific Ocean.

Male coloration is usually subject to sexual selection, and it has been cited numerous times as a reproductive isolation barrier in birds (Price 2008). For instance, natural selection, selection against migrants, divergent mate choice, and divergent sexual selection, can be considered environmental variations (Wang & Bradburd, 2014) that can prevent gene flow are not adapted to new environments (Morris-Pocock et al., 2010). It is also known that morphological characteristics that are different among populations of brown boobies like the bare part, are important sexual

ornaments that signal quality (Pierotti, 1987). In chapter 2 I found that each colony present differences in size and coloration of bare parts (feet and gular), which are most likely driven by environmental factors and play an important role in mate choice. For instance, gular green colour in males from Marietas are positive corelated with heterozygosity, San Pedro presented males negatively correlated with green colour in feet, and males from San Jorge were positively corelated with also green colour in feet. Additionally, each island presented significant differences in body size, with larger individuals nesting at higher latitudes as expected by the Bergmann's effect (James 1970). This could cause negative selection against migrants which might present differences in sexual ornaments, or they might be subject to divergent natural selection based on habitat differences and breeding sites.

3.4.2 Isolation by distance and isolation by environment

In an attempt to explain the possible causes of such strong structure and isolation in relatively close breeding colonies, I performed Isolation by Distance (IBD) and Isolation by Environments (IBE) analyses. As expected, the Mantel test showed a moderate-strong correlation between geographic distance and genetic distance (pi), where Marietas and San Jorge show the highest geographic and genetic distance (Figure 3.8).

I also assessed the correlation between the distribution of the genetic diversity and the environment in the three breeding colonies, where I found a significant association between both variables. As mentioned before, I calculated average environmental values in the summer months where the lowest productivity and the highest temperatures present a challenge for breeding and survival, and then also calculated the environmental values for the breeding season which happen at different

times of the year depending on the colony (see chapter 1 and chapter 2). When I first postulated my hypothesis based on the correlation of environmental variables and genetic diversity, I stated that the colonies were in a productivity and temperature gradient, giving that the Gulf of California has been reported as with higher productivity in northern areas (Álvarez-Borrego 2002). Also, when I assessed the maps generated for the entire range of the studied colonies, the average values were behaving as a gradient of productivity and temperature, positioning to San Jorge as a colony with abundant resources and conditions for breeding (Castillo-Guerrero et al. 2016), whereas Marietas seemed to have the worst conditions for breeding. However, when I calculated the average values for the maximum foraging range reported for brown boobies (~40 km), I found out that a small area near to Marietas serve as hotspot with very high productivity (Figure S3.10). This could compensate for the fact that Marietas present very high temperatures, complicating some aspects of breeding like foraging and paternal care.

By performing an RDA, I quantified the relative contributions of environment using the "summer" variables and the "breeding" variables". The RDA showed that environmental variables explained 5.92% of the total variance composed by PP+SST ($F = 8.3124$; $df = 2$; p-value = .001), while isolated variables explained genetic diversity in different proportions depending on the time of the year as shown in Table 2.1. It seems that the individual variable that better explained the total variance was the primary productivity during summer with 4.33% ($F = 12.006$; $df = 1$; p-value = .001), whereas the SST during summer explained only 1.42% of the variation ($F = 3.8226$; $df = 1$; p-value = .001).

Individuals from San Jorge are highly positively correlated with high PP during the summer, Marietas SNP variability is correlated with low PP, and San Pedro showed no strong correlation for low or high productivity during summer. On the other hand, San Pedro showed a strong correlation with low SST during summer, whereas Marietas and showed no correlation (Figure 3.9 (A)). When comparing the environmental variables during the breeding season, Marietas showed a very strong correlation with high SST, whereas San Pedro and San Jorge showed a moderate correlation with lower temperatures. Finally, Marietas showed a very strong correlation with low primary productivity during the breeding season, whereas San Jorge and San Pedro showed a relatively high correlation with higher PP values (Figure 3.9 (B)). In summary, these results suggest that both SST and PP during summer and the breeding season have a significant effect in the genetic variability of the sampled colonies. Additionally, higher genetic diversity seems to be strongly associated with PP, especially in San Jorge, and negatively correlated in Marietas. However, SST seems to be strongly correlated with higher genetic diversity in Marietas but negatively correlated in the other two colonies. These results agree with my initial hypothesis that differences in environmental variables have a significant effect on the genetic diversity of brown booby colonies.

In a study by (Nunes & Bugoni, 2018) about the local adaptation to different environments in brown boobies by using microsatellites, they found that spatial autocorrelation among sampling locations is common, so that environmental distance can be confounded with geographical distance. They also discussed the contribution of foraging behaviour to local adaptation, arguing that different colonies have been reported that spend differences in the time spent when foraging based on food

availability. In a study carried by my collaborators in Marietas Island (Michael et al., 2018), a foraging range of 168.8 to 315.3 km (mean+-s.d. = 261.1+- 39.8) was reported by telemetry, finding a relationship between green ornaments and greater distances travelled. In contrast, brown boobies from San Jorge will travel shorter distances and prey on up to 27 species which reflects more food availability than other colonies outside the Gulf of California (Castillo-Guerrero et al., 2016). Such differences in foraging behaviour are very likely to be an additional factor for gene flow and differentiation in seabirds (Friesen, 2015).

Many studies have investigated the genomic footprints of divergent selection implicated in local adaptation in a wide range of species and systems (Savolainen, et al., 2013; Tigano & Friesen, 2016; Hohenlohe et al., 2010; Nielsen et al., 2009; Nunes, Beaumont, Butlin, & Paulo, 2010). Many of these species exhibit high natal philopatry (e.g., Huyvaert & Anderson 2004; Quinn, 1993), and such behaviour usually reduce further the gene flow amongst populations, therefore, it also promotes local adaptation and genetic differentiation even at fine spatial scales (Fraser et al., 2011). I acknowledge that my results about the influence of environmental variables explain a small, yet significant proportion of the distribution of genetic diversity in brown boobies. It is very likely that other factors like philopatry have a major effect in the high population structure in this species, however the real impact of site fidelity in differentiation of populations, remains to be measured and compared. In summary, both local adaptation to environmental variables and high philopatry should be considered in this kind of studies, given that both have the potential to be a major driver of population differentiation in wild populations (Sexton et al., 2014).

3.4.3 Distribution of genetic diversity and Demographic history

Watterson estimator and Tajima's D showed that San Pedro have higher genetic diversity and excess of rare alleles followed by Marietas and San Jorge respectively. However, Marietas presented a higher nucleotide diversity (pi) compared to the other islands (Figure 3.5). Additionally, Marietas showed significant higher heterozygosity and dissimilarity than San Pedro and San Jorge respectively (Figure 3.6). To try to explain better the distribution of the genetic diversity in these colonies, Watterson estimator and Tajima's D showed that San Pedro have higher genetic diversity and excess of rare alleles followed by Marietas and San Jorge respectively. This pattern could be explained by their geographic location and historical dispersion patterns, as San Pedro is in an intermediate point between San Jorge and Marietas, sharing some ancestry with both islands (see Figure 3.2). However, Marietas presented a higher nucleotide diversity (pi) compared to the other islands (Figure 3.5).

Additionally, Marietas showed significant higher heterozygosity and dissimilarity than San Pedro and San Jorge respectively (Figure 3.6). A possible explanation for these results can be that some kind of selection can be happening in Marietas because of the high temperatures and low productivity during breeding season like I observed in the RDA plots (Figure 3.9 (B)). This could cause selective pressures favouring more heterozygous individuals (e.g. good genes) and dissimilar individuals (e.g. compatible genes) simultaneously like I hypothesised in chapter 2 of this thesis. The gradient of diversity observed in the studied islands, where San Jorge present the lowest nucleotide and genetic diversity, also suggest the influence of additional evolutionary processes, like a bottleneck effect or a founder event.

The negative values of Tajima's D suggest the abundance of rare alleles or a recent selective sweep and population expansion after a recent bottleneck or founder event (Tajima 1989). Even though all the populations show such negative values of Tajima's D, San Pedro seems to be the one with the highest values and San Jorge with the lowest values (Figure 3.5). Although, I acknowledge that in other to confirm a founder hypothesis more research is needed like the use of other seabird genomes as outgroups for phylogeny analyses. However, the demographic reconstruction showed a stable effective population size (Ne) of 2-2.5 thousand individuals from 50 to 15 thousand of years ago (kya) for all colonies (Figure 3.7). This followed by a sudden rise in Ne that peaked at 4-5 thousand individuals, and then, an ongoing reduction that started 500 years ago (Figure S3.9). The abrupt change in population size in the reconstructions coincide with major climatic events like the Bølling-Allerød warming and the Younger Dryas (Zalloua et al. 2017). My demographic reconstruction suggest that other processes could be responsible of the abundance of rare alleles. Purifying selection on deleterious alleles thorough non-random mate choice could be one possible explanation for my results, increasing the proportion of rare variants in the populations (Jackson et al., 2015).

3.4.4 Final remarks

In summary, I found that brown boob populations in the Eastern Pacific seem to be highly differentiated and genetically isolated regardless of their relative proximity. To my knowledge, this is one of the first studies to use next generation sequencing to reveal population structure of brown boobies and the influence of environmental variables, highlighting the advantages of this approach for population genetic studies. I found a small but significant influence of environmental variables

on genomic variation, which could be subject to natural or sexual selection. In this context, genome wide scans for loci under selection would complement greatly my results and tell us more details about the actual regions of the genome subject to local adaptation.

CHAPTER 4: SIGNATURES OF SELECTION IN BROWN BOOBY COLONIES UNDER DIFFERING ENVIRONMENTS.

4.1 Introduction

Understanding the effects of selection on the genome has been a main goal in evolutionary biology (Nielsen 2005), and population genetics approaches attempt to discriminate loci under selection from neutral genetic variation (Ahrens et al., 2018; Beaumont, 2005; Oleksyk et al., 2010; Weigand & Lee, 2018). Additional challenges for evolutionary ecologist, are to investigate the relative contribution of demography and natural selection to spatial patterns of genetic variation (Collevatti et al., 2019), and to identify the genetic architecture underlying traits of ecological relevance (Ellegren & Sheldon, 2008; Slate et al., 2009). The use of next-generation sequencing is becoming a standard method in population genomics studies, which are now more feasible and allowing research in non-model organisms (Flanagan & Jones, 2017). Consequently, increasingly more powerful and accessible DNA sequencing technologies allow genome scans for the detection of candidate genomic regions presenting signals of adaptation (Collevatti et al., 2019). The application of such scans to large genomic data are particularly benefitted with such approach because adaptation usually shapes the patterns of genomic variation between and within loci (Kaplan et al. 1989).

Finding regions associated with variation in fitness-related traits is particularly interesting, as such traits and the loci underlying them are expected to be subject to strong selection (Miller et al., 2018). However, the interaction of natural selection with

other evolutionary forces, specific demographic events, and life history of the species makes the detection and interpretation of natural selection a very complex task. For instance, when there is low dispersal and local selection is strong enough, populations become differentiated genetically across the species distribution (Clausen et al. 1940). At the same time, Still, the opposite pattern could be true depending on the studied system , where the spatial variation in the pattern of natural selection could promote local adaptation and genetically distinct populations (Monnahan et al., 2015). Needless to say, differentiation of populations depends greatly on genetic drift, where small populations will have greater impact (Ouborg et al., 2010). Moreover, the gene flow of adaptive alleles into a population could also generate great variance in fitness, and the genomic consequences of such migration and selection depend on the basis and extent of the local adaptation (Monnahan et al., 2015). If few loci are promoting local adaptation, minimal gene flow is expected at such loci and at closely linked polymorphisms, but with effective homogenization elsewhere in the genome (Wu 2001; Nosil et al. 2009; Feder et al. 2012; Renaut et al. 2013). Therefore, selection imprints may be detected depending in factors like the number of generations since selection, the strength of such selection, and the extent of the recombination (Collevatti et al., 2019). However, caution is necessary when inferring selection as many demographic events like a founder event or the bottleneck effect can result in similar signatures of polymorphism (Hohenlohe et al. 2010; Nei et al. 2010). Additionally, gene flow might be reduced across the genome if many loci are under section with enough strength (Barton & Bengtsson 1986). The genetic variability underlying traits related to fitness should be fixed rapidly under strong directional

selection, however, what is observed in nature is that phenotypic variation in such traits is maintained (Kruuk, Slate & Wilson, 2008; Chenoweth & McGuigan, 2010).

Even when many loci contribute to local advantage, introgression of other alleles commonly occur unless the fitness of such alleles are very low (Monnahan et al., 2015). Studies regarding the balance between selection and migration have tried to infer such process from static genetic patterns, mainly from allele frequency divergence among populations (Bierne et al. 2013). Such approach is commonly used to study the effect of selection in wild populations by identifying regions in the genome that show an excess or a lack of divergence that cannot be explained only by genetic drift (Hohenlohe et al. 2010). Under demographic events like founder populations or bottleneck events, loci under selection are difficult to detect from neutral variation due to genetic drift, hiding the sometimes-weak signals of incomplete selection sweeps (Hermisson & Pennings, 2005), which can rise the false negative and positive rates from selection scans. Thus, studies between populations have their limitations because they perform well under only certain patterns of migration, samples from multiple populations are necessary, and they cannot differentiate the type of selection contributing to divergence without previous knowledge about specific traits under selection (Leinonen et al. 2006). Even though evidence for adaptation to novel environmental conditions on short, observable timescales has gathered in recent years (Carroll et al., 2007; Schoener, 2011), identifying different episodes of selection and associated genes in wild populations remain elusive.

Natural selection in the wild is composed by different types, such as viability selection, sexual selection, and gametic selection (Flanagan & Jones, 2017). Such

episodes of selection can provide insights into important biological aspects of a species like the mating system, ecology, and conservation (Emlen & Oring 1977; Loehle & Pechmann 1988; Stockwell et al. 2003). In this aspect, sexual selection is a form of natural selection where adaptations that attract mates or help outcompete other individuals, help to achieve increased reproductive success (Jaiswal et al., 2021). Some of the best examples of sexual selection have been documented in birds, where complex songs and ornamented plumage are commonly used to attract mates (Catchpole, 1980; Irestedt et al., 2009; Loyau et al., 2005; Hosken & House, 2011). The evolution of sexual traits and mating preferences has puzzled evolutionary biologist since the times of Charles Darwin (published in 1871), given that poses very interesting scenarios for traits related to fitness and many evolutionary contradictions that remain unsolved until this day. For instance, in mating systems with strong sexual selection in a particular trait, the variation on that trait should rapidly be reduced. However, in many species the genetic diversity is maintained, and to this effect it has been called the lek paradox (Borgia, 1979). Sexually selected traits are likely to evolve because non-random mating brings either direct or indirect benefits to the chooser (M. B. Andersson, 1994; M. Andersson & Simmons, 2006). Under sexual selection, mate choice is not random, is based on an ornament trait that may bring benefits to offspring (and the chooser), or members of one sex that compete for access to mates using morphological features that are secondary sexual characteristics (Miller et al., 2018). The preferences of females can evolve under natural selection for phenotypic benefits associated with male ornaments, like high quality territory, nutrition, parental care or protection (Price et al. 1993). Under such scenario, evolution should deplete the genetic variation underlying the selected trait over time and reduce offspring benefits,

however, in many systems choice for such traits remains unchanged (Miller et al., 2018). Therefore, numerous explanations to the maintenance of genetic variance and sexual selection have been proposed, like the association of condition dependant traits and its indirect genetic effect (Reid 2014; Tomkins et al. 2004; Miller 2007).

The brown booby, has been identified to experience sexual selection, where gular and feet coloration is carotenoid dependant and acts as an important condition-dependant signalling in sexual selection (Montoya et al., 2018).. Carotenoids are acquired through diet and might indicate a nutritional state and foraging efficiency (Hill et al. 2002; McGraw et al. 2003; Casagrande et al. 2006; García-Navas et al. 2012). In males, skin coloration displayed during courtship becomes greener after carotenoid ingestion, which suggests an interaction between the deposition of yellow carotenoids and the blue structural colour of the skin (Velando et al. 2006, Torres & Velando 2010). Presumably, this variation in skin colour has evolved as an honest sexual signal of condition (see chapter 2) and suggests that mate choice may be an important force favouring the evolution of sexual signals by reflecting overall quality and current condition (Montoya et al., 2018). Additionally, in chapter 2 I found a correlation between these condition dependant traits (gular and feet colour) and the levels of heterozygosity. However, these signals seemed to depend on the geographic location, which is very likely to be associated with environmental conditions (see chapter 3).

It is very important to highlight that, sexual selection can be displayed in several ways such as plumage and skin dimorphism, competitions for access to females (aggression), sperm competition (gametic selection), and preference to

specific mates or mate choice, which can affect the expression of associated traits (Wade & Arnold, 1980). Generally, most of the research about the genomic basis of sexual selection has been conducted on model species, under controlled conditions and using QTL (Erickson et al. 2004; Slate 2005; Andersson & Simmons 2006), while detecting signatures of sexual selection in wild populations has been difficult. In order to measure how selection affects the genome, it would be useful to use quantitative genetic theory, which focuses on individual episodes of selection (Arnold & Wade 1984b; Arnold & Wade 1984a), in combination with empirical work to identify signatures of selection in wild populations. Studies like the ones carried out by Flanagan & Jones (2015) and Monnahan et al. (2015) suggest that is possible to detect different aspects of sexual selection if the following characteristics are met: (1) the offspring and one or both parents are collected; and (2) one component of sexual selection or more must be strong in the studied system. The last point is critical for genomic scans to be able to detect signatures of selection and for the appropriate postulation of hypotheses (Flanagan & Jones, 2017). The present study meets both characteristics given that both parents and offspring were collected during the expeditions in three different breeding colonies of brown boobies where ornaments are known to be an important feature in sexual selection (Hill et al. 2002; McGraw et al. 2003; Casagrande et al. 2006; García-Navas et al. 2012; Velando et al. 2006, Torres & Velando 2010; Montoya et al., 2018). These colonies, present differences in environmental conditions like primary productivity and sea surface temperatures which are likely affecting the genetic diversity and mate choice of this species (see chapter 2 and chapter 3). I used a customised restriction-site associated DNA sequencing (3RAD sequencing) to produce genome-wide single nucleotide

polymorphism (SNP) data for three differentiated populations of brown boobies and used two genome-wide selection components scans based on population differentiation using an FST metric and using Fisher exact tests. I aim to (1) characterize the signatures of natural and sexual selection by comparing allele frequencies in males and females from different islands, (2) compare allele frequencies between males and females to detect if sex-specific viability selection is present between sexes, (3) compare allele frequencies between adults and offspring to detect gametic selection, and finally (4) use the detected loci under putative selection to investigate the biological function (Gene Ontology terms) of the genes near to such selected loci. I hypothesise that by comparing different groups of individuals with a particular sampling scheme depending on their sex, living stage, and geographic locations I can detect different episodes of selection affecting various biological functions in brown boobies.

4.2 Methods

For methods related to fieldwork, laboratory work, demultiplexing and alignment of sequencing please see the methods section in chapters 2 and chapter 3. I sampled complete families (both parents and offspring) from three different breeding colonies, thus I can compare groups of individuals at different life stages and between populations to detect different patterns of selection. Additionally, the SNP calling, and selection scan should be performed under specific sub-groups of individuals in order to detect different selection episodes (see below).

4.2.1 Data preparation and SNP calling

We performed two parallel bioinformatic pipelines for the same set of samples. The difference between pipelines consisted in separate groups of individuals

depending on the episodes of selection to detect. In one of the pipelines (hereafter "local" dataset), the individuals were separated by colonies to detect local natural selection and sexual selection in females and males. On the other hand, the second pipeline (hereafter "mating" dataset) separates the individuals by sex and offspring to detect sex-specific viability and gametic selection.

For both datasets I identified SNPs within the meta-population for each locus and then genotype each individual at each identified SNP by using the gstacks module from the STACKS version 2.6 pipeline (Catchen et al., 2013). Afterwards, SNPs were exported using the populations module, where the samples were grouped in their respective populations, and a locus was exported if it was present in 55% of the individuals in this population using the "r" parameter (-r 0.55) at a stack depth of at least 10 by using the "m" parameter (-m 10). I used the parameter "-write_single_snp" to avoid including SNPs in high linkage disequilibrium (LD), and I included a minor allele frequency of at least 0.025 to process a nucleotide site using the "–min_maf" parameter (--min-maf 0.025). Similarly, I used the same parameters to filter SNPs of sex-specific individuals and life-stage individuals (adults vs chicks). I used the output files generated by STACKS, to convert them to the appropriate formats for the following analyses. PGD-Spider (Lischer & Excoffier, 2012), plink (Purcell et al., 2007) and vcftools (Danecek et al., 2011) were used to convert the SNP data into PED and MAP format. In vcftools, the flags --depth and --site-depth were used to calculate read depth per individual and per SNP, and Binary files (BED, RAW and BIM) were generated from PED and MAP files using PLINK with the flags --make-bed, --recode A, --chr-set 95, and allow-extra-chr. SNP data management and analyses were performed in R-4.0.5 (R Core Team, 2019) using wrapper functions of the R package

SambaR (github page: https://github.com/mennodejong1986/SambaR). Once the data was imported into R, it was then stored in a genlight object using the function 'read.PLINK' of the R package adegenet-2.1.3 (Jombart, 2008; Jombart & Ahmed, 2011). Additional filtering was performed using the function 'filterdata' of the R package SambaR, with the parameters indmiss=0.5, snpmiss=0.5, min_mac=2, dohefilter=TRUE and min_spacing=500 to retain as many individuals as possible.

4.2.2 Genomic Selection Component Analysis framework

The classic selection component analysis (SCA) was proposed by Christiansen and Frydenberg (1973), and it combines the characteristics of the field observational study of selection (Lande & Arnold 1983) with a mating system estimation experiment (Ritland & Jain 1981). Recently, this approach was revisited and modified to include genomic data (Monnahan et al., 2015), by the collection of individuals through their lifetimes, scoring survival and measuring reproductive success. Here, fitness components are predicted from individual genotypes at single nucleotide polymorphisms (SNPs) across the genome, measuring life-stage specific episodes of natural selection. By designing specific sampling schemes, this method has the potential to detect different types of selection like viability, fecundity, gametic and sexual selection. For instance, viability selection can be detected by comparing the differential survival of zygotes and adults, sexual selection by comparing differences in mating success, and gametic selection by the segregation distortion when producing successful gametes (Table 4.1). Such data is collected randomly from several populations and sub-groups like adult males, females, and offspring. In the present study, I was able to meet all this sampling conditions, however, the number of unsuccessful individuals was very low and had to be excluded of the analysis.

Therefore, in order to detect sexual selection, I performed comparison of successful males (that were able to reproduce) from a specific island and compared with the total number of males sampled for this project. As I discussed in chapter 2 and chapter 3, its very likely that the mate preferences between colonies based in sexual ornaments are different and the distribution of genetic diversity are partially driven by differences on environmental conditions. Consequently, I expect to find signatures of sexual selection in males and females by comparing successful individuals of one colony with the rest of the sampled individuals. Additionally, loci under natural selection were scanned by comparing individuals from different islands, gametic selection scans were performed by comparing adults and offspring, and finally sex-specific selection was detected by the comparison of male vs female individuals.

4.2.3 Selection scans and Gene Ontology

In order to detect loci under positive selection, three selection scans were used: Genome Wide Differentiation Scan or GWDS (de Jong et al., 2021), OutFLANK (Whitlock & Lotterhos, 2014, 2015) and PCadapt (Duforet-Frebourg et al., 2014; Luu et al., 2017). GWDS is a newly developed approach based in population differentiation methods (Oleksyk et al., 2010; Weigand & Leese, 2018) and outlier analysis test (Ahrens et al., 2018), which searches for association between specific locus and allele frequencies by using the Fisher exact tests. OutFLANK outliers on the other hand, are detected using Holm-corrected p-values and Fst scores to quantify population differentiation. Lastly, the outliers detected by PCadapt use Bonferroni corrected p-values, a PCA based method. However, PCadapt cannot be forced to detect outliers for a prespecified population division, but instead outputs all outliers for all putative population divisions. For my analyses comparing different populations, PCadapt

showed to be unsuitable showing very abnormally high -log (test statistic) outlier loci and therefore was not considered for further downstream analyses. Even, though PCadapt did detected outlier loci for other types of selection when comparing within population individuals, I am only considering the detected loci by OutFLANK and GWDS for consistency. All the mentioned scans were implemented in the R package "SambaR" (https://github.com/menno dejong1986/SambaR; de Jong et al. 2021). I generated Fdist plots (Beaumont & Nichols, 1996) based in locus-specific estimates of H_E and F_{ST} (Cockerham & Weir 1987) to compare putative outlier loci. The Fdist method is a Fst-outlier based test that resort to computer simulations to model the behaviour of neutral loci under a symmetrical island model of population structure. The results of these selection scans were exported to a BED file containing a list with outlier SNPs and can be used to find nearby genes by BEDTOOLS2 version 2.26.0 (Quinlan & Hall, 2010). The genomic regions containing outlier loci were annotated by extracting the 100000 bp region surrounding the outlier SNPS (see Flanagan & Jones, 2015) from a reference genome of the masked booby (*Sula dactylatra*) published in Feng et al. (2020). I used such extracted regions in blastx to compare them to the non-redundant nucleotide database (Camacho et al. 2008). The choice for the 100000 bp window was made because it is difficult to predict the extent of sweeps under a reinforcement scenario without information about the strength of selection and the type of selection acting (hard vs soft sweep), but it is expected in most cases some regional patterns variability to be detectable within 100 to 200 thousand base pairs regions (Smadja et al., 2015). Lastly, blastx hits were used in the software Blast2Go (Conesa et al. 2005; Conesa and Gotz 2008; Gotz et al. 2008; Gotz et al. 2011) to find

the biological function associated with such region and plots of these biological functions were built for each type of selection.

4.3 Results

From the collected samples, 427 out of 441 individuals (43-114 per population) passed filters and were retained (Figure S4.6), of which, 27731 out of 102129 SNPs were retained after filtering and thinning for the “local dataset” (Figure S4.7). For the “mating dataset”, 399 out of 441 individuals (115-147 per population) were retained, of which 24442out of 160837 SNPs were retained. In general, I retained a similar number of females, males and offspring, however, the sample size from San Pedro is smaller because time limitations and dangerous weather conditions while collecting samples (Table S4.3) Moreover, the GC-content of this retained dataset equalled 0.59, and the 'transversion vs transition'-ratio equalled 0.66. These filtered SNPs and individuals were input in the genome-wide selection analyses following a specific sampling scheme where specific individuals are compared (e.g., adults vs offspring or males vs females) in order to detect different signatures of selection.

The amount and genomic location of outlier SNPs detected by the genomic scans varied widely depending on the comparison scheme (Table 4.1). The Manhattan plots clearly showed the significant outlier SNPs that where different from the rest of the putative neutral loci, however, only the outliers shared by GWDS and outFLANK analyses were considered (example of male sexual selection in Figure 4.1). For the complete set of Manhattan plots generated in this chapter, please check the Supplementary Figures S4.1, S4.2, S4.3, & S4.4. In summary, the detected loci for the different episodes of selection were as follows: for the natural selection scans 45-57 outlier loci were detected, for sexual selection 67-127 loci, for sex-specific viability selection 265 loci, and for gametic selection 294-580 loci were detected (Table 4.1). Additionally, Fdist plots were generated to visually assess and compare putative

outlier loci by comparing the locus-specific H_E and F_{ST} estimates of the different pairwise sub-groups (Figures 4.2, 4.3 & 4.4). In these plots is clear that most outlier loci detected by the GWDS and outFLANK analyses have higher Fst than the putative neutral loci, whereas the loci detected by PCadapt presented very low Fst and they are not differentiated from other loci. For most of the plots when comparing between colonies, it seems to be no clear pattern of loci segregation in specific colonies or opposing allele frequencies for either population.

However, in the male and female comparison it seems to be a clear segregation of loci specific to each sex (Figure 4.4).

Table 4.1. Total number of outlier loci. Comparisons that allow signatures of gametic, sexual, and viability selection to be inferred from genetic data.

Dataset	Type of selection	Compared groups	Group 1	Greoup 2	Number of Outlier SNPS
Local	Natural Selection	All individuals (Males, Females and Offspring) between colonies	Marietas	San Jorge	57
			Marietas	San Pedro	52
			San Jorge	San Pedro	45
	Sexual Slection	Only Males between colonies	Marietas	San Jorge	67
			Marietas	San Pedro	70
			San Jorge	San Pedro	68
		Only Females between colonies	Marietas	San Jorge	85
			Marietas	San Pedro	127
			San Jorge	San Pedro	83
Mating	Gametic-viability selection	Between Males and Offspring	Males	Offspring	580
		Between Females and Offspring	Females	Offspring	294
	Sex-specific viability Selection	Between Males and Females	Males	Females	265

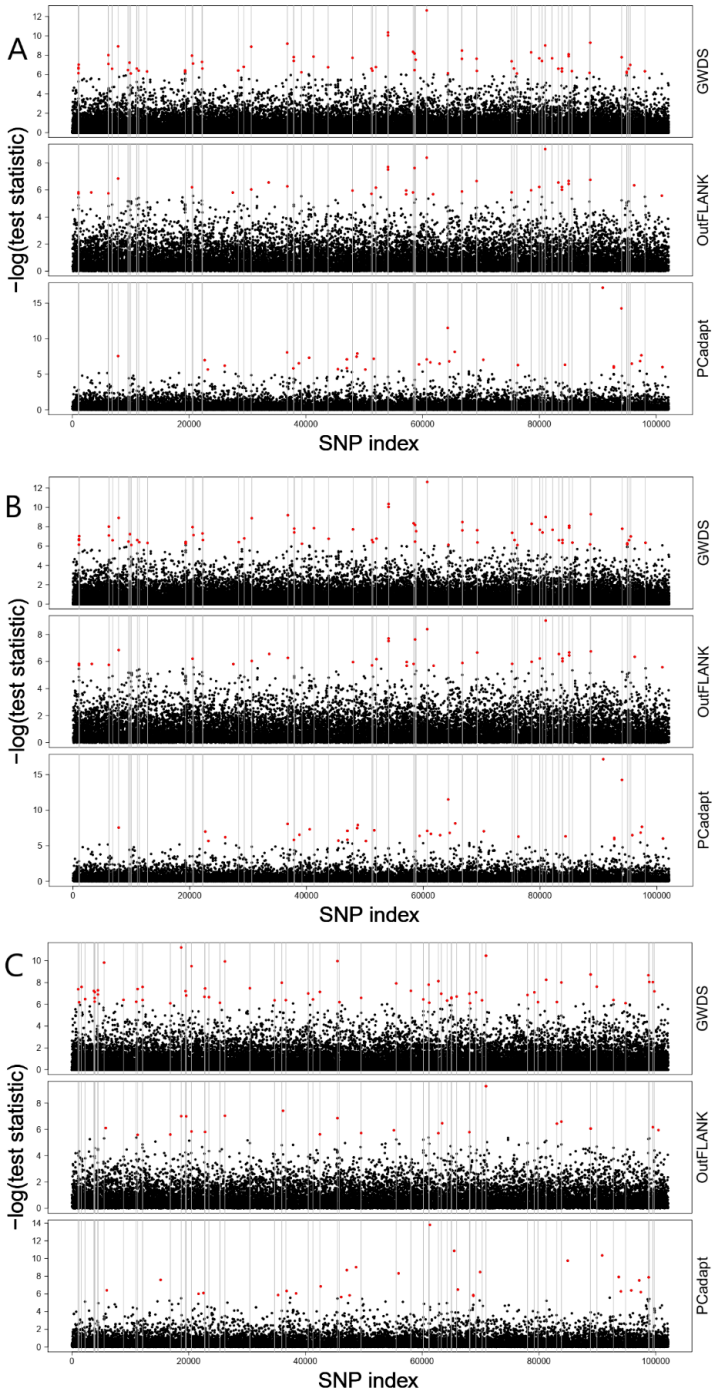


Fig 4.1. Genome Selection Scans to detect episodes of Sexual Selection in Males. Manhattan plot showing the outlier SNPs in red which are significantly different from zero. Vertical lines represent outlier loci shared between different scans A). Marietas vs San Jorge. B). Marietas vs San Pedro. C). San Jorge vs San Pedro.

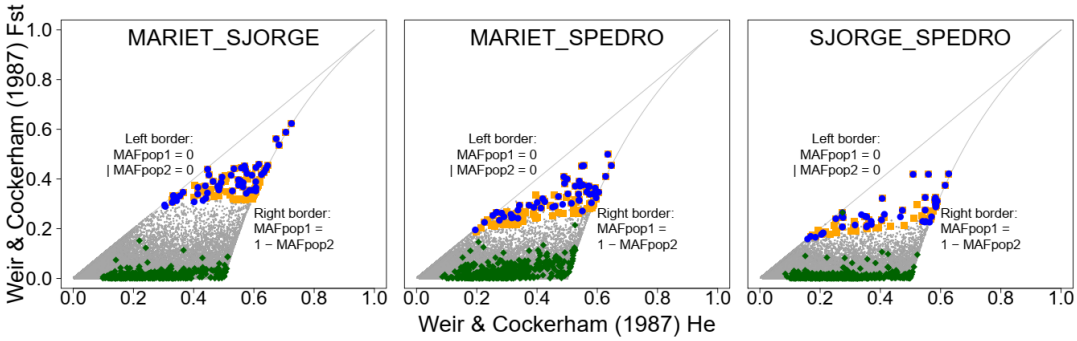


Fig 4.2. Fdist plots for the Natural Selection dataset. Pairwise comparisons for all individuals between populations by comparing putative outlier loci H_E and F_{ST} estimates. Green dots represent PCadapt, purple dots represent GWDS, and yellow represent OutFLANK analysis. Grey dots represent putative neutral loci.

The 10 0kb region surrounding these outlier SNPs showed recognizable similarity to known genes which have had level two (biological function) gene ontology annotations. I was able to detect 8-14 genes with biological function under natural selection, 14-33 genes under sexual selection, 43-55 under gametic selection, and 5 genes with biological function under sex-specific viability selection (Table S4.1 & Figure. S4.5). The number biological processes that have more Gene Ontology hits were cellular processes and biological regulations, metabolic processes, regulation of biological processes, response to stimulus and signalling. These processes were detected by all of the pairwise comparisons. On the other hand, there were some specific comparisons that detected fewer genes with biological functions that were associated with specific types of selection. For instance, immune system processes were detected by the natural selection comparison, one gene associated with rhythmic processes was detected by the sexual selection scan in males, and one gene associated with locomotion was detected by the sexual selection scan in females. Additionally, few genes were detected to be related with growth, interspecific interactions for those loci detected by the natural selection scan between San Pedro and the other two

islands. I generated bar plots for a visual assessment of these genes under each type of selection, which show the counts of genes involved in several biological functions (Figures 4.6, 4.7 & 4.8).

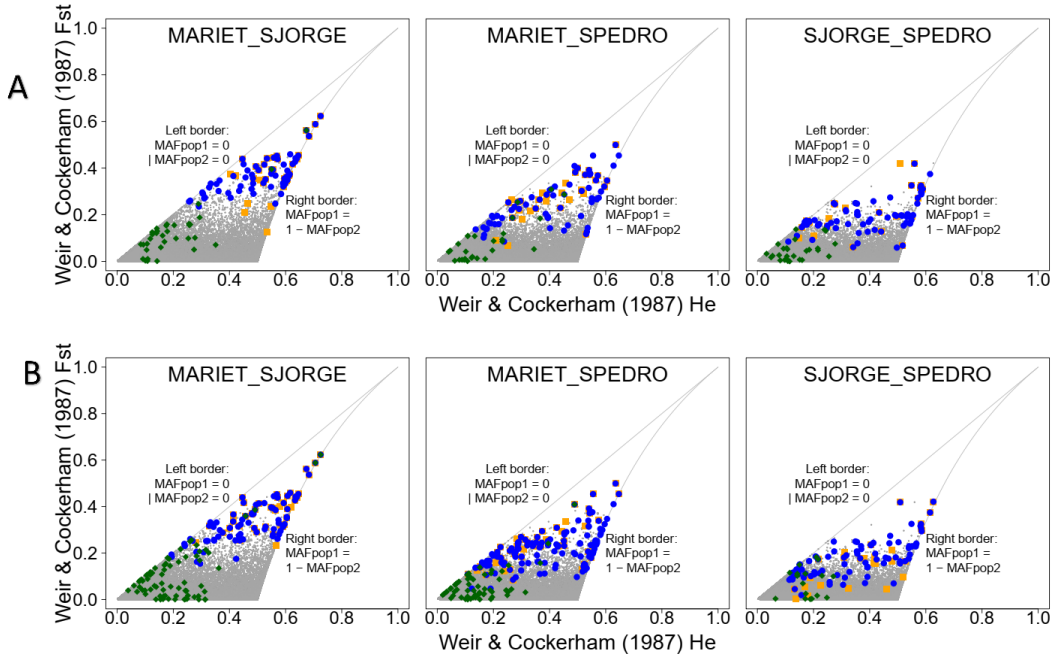


Fig 4.3. Fdist plots for the sexual selection dataset. Pairwise comparisons for all individuals between populations by comparing putative outlier loci H_E and F_{ST} estimates. Green dots represent PCadapt, purple dots represent GWDS, and yellow represent OutFLANK analysis. A). Only males considered. B). Only females considered. Grey dots represent putative neutral loci.

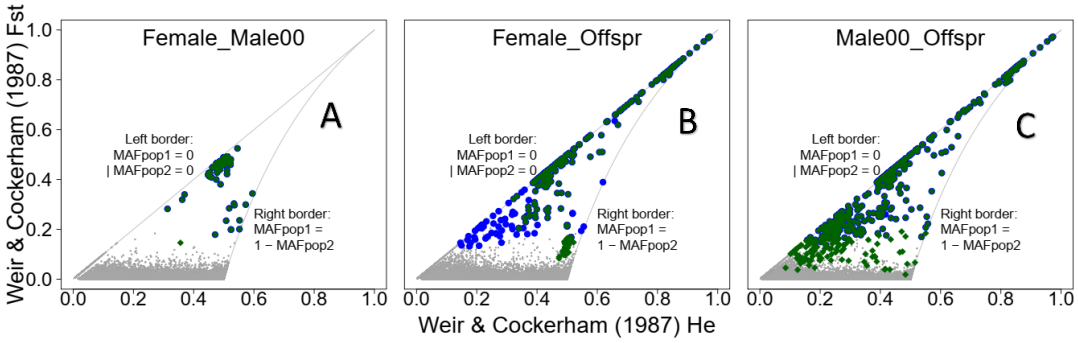


Fig 4.4. Fdist plots for the gametic and viability selection. Pairwise comparisons for all individuals between populations by comparing putative outlier loci H_E and F_{ST} estimates. Green dots represent OutFLANK analysis; purple dots represent GWDS. A). Viability Selection B). Gametic selection in females. C). Gametic selection in males. Grey dots represent putative neutral loci.

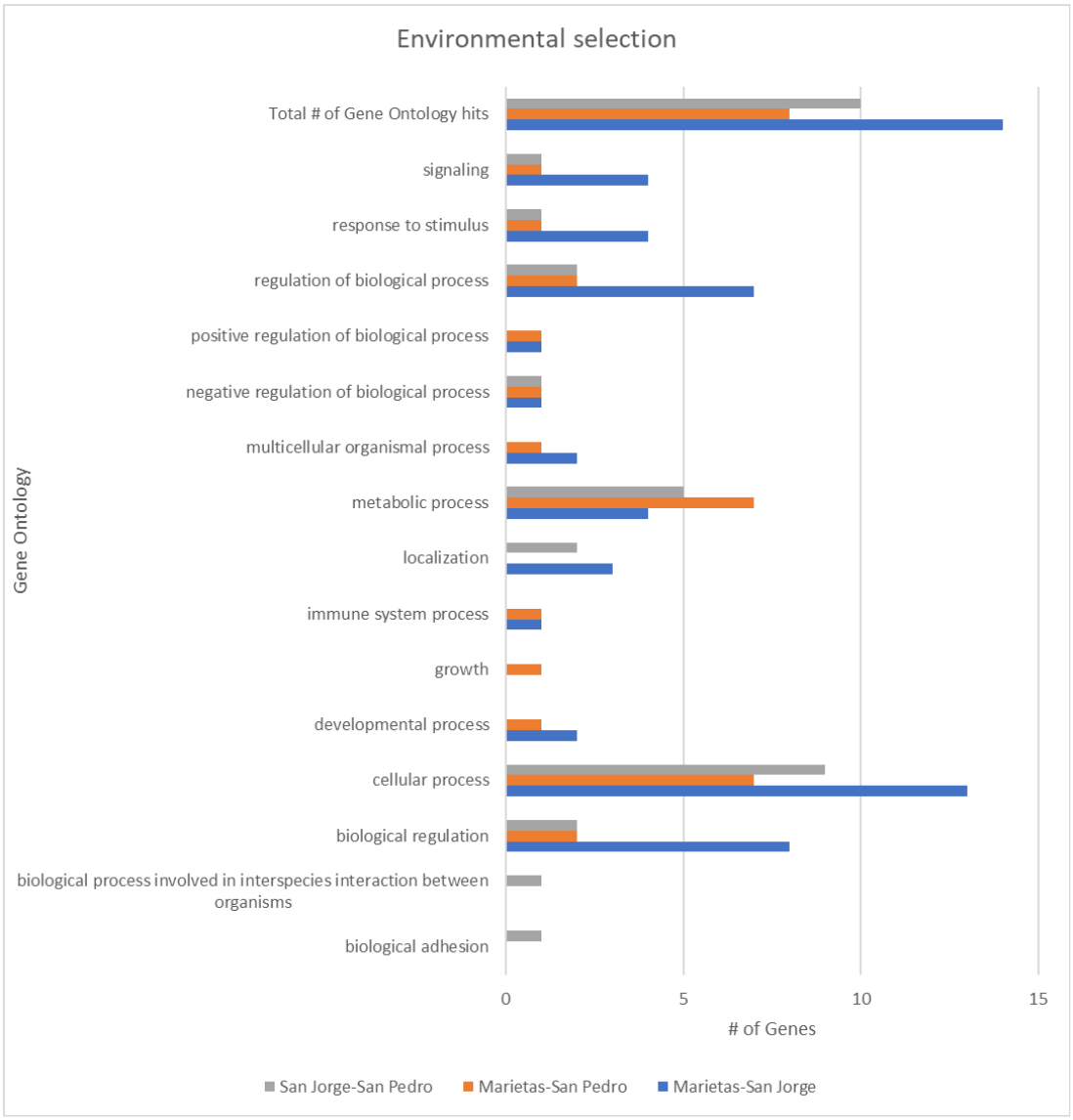


Fig 4.6. Gene ontologies describing the level 2 biological functions associated with environmental natural selection. Genes surrounding outlier loci comparisons for all individuals between different islands. The number of loci sequence with each gene ontology category are displayed in the bar chart and the total number of gene ontology hits for each comparison are also reported. Note that a single locus may belong to multiple gene ontology categories.

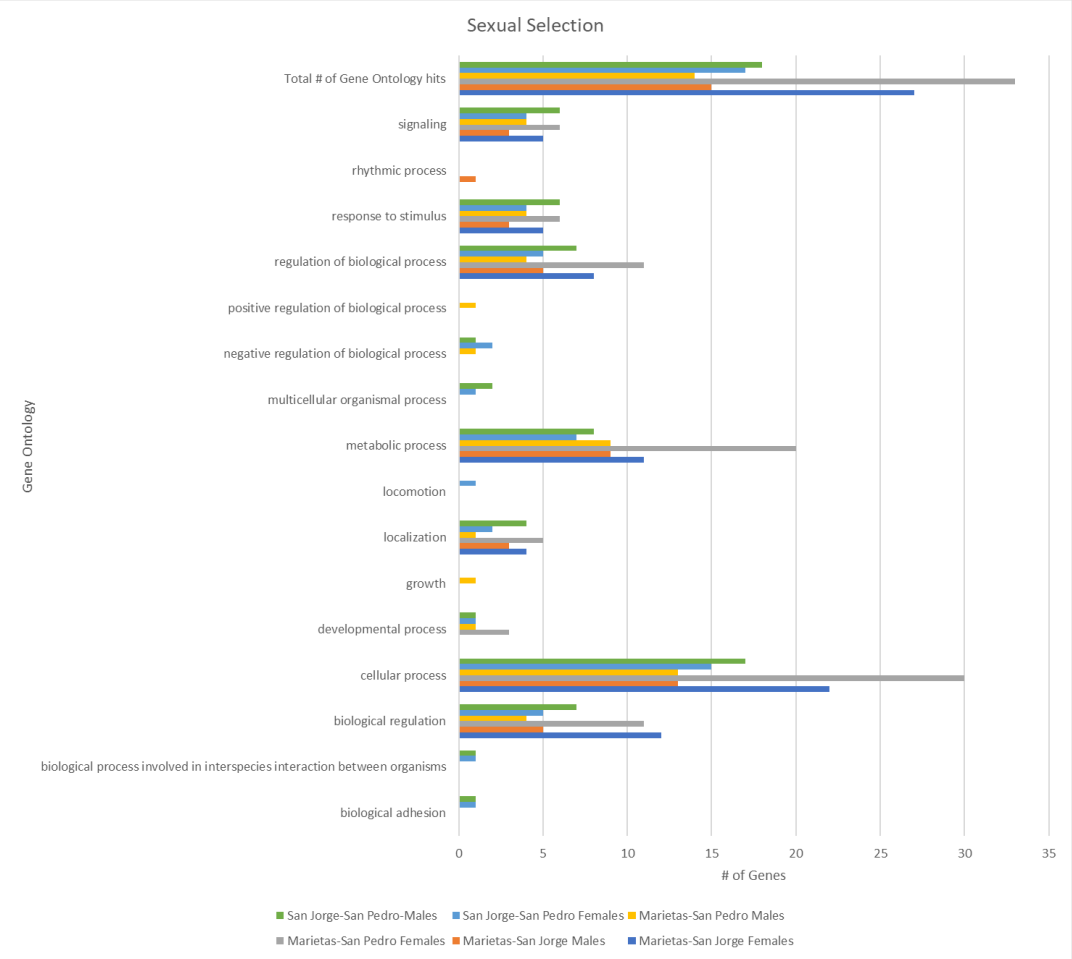


Fig 4.7. Gene ontologies describing the level 2 biological functions associated with sexual selection in Males and Females. Genes surrounding outlier loci comparisons for all individuals between different islands. The number of loci sequence with each gene ontology category are displayed in the bar chart and the total number of gene ontology hits for each comparison are also reported. Note that a single locus may belong to multiple gene ontology categories.

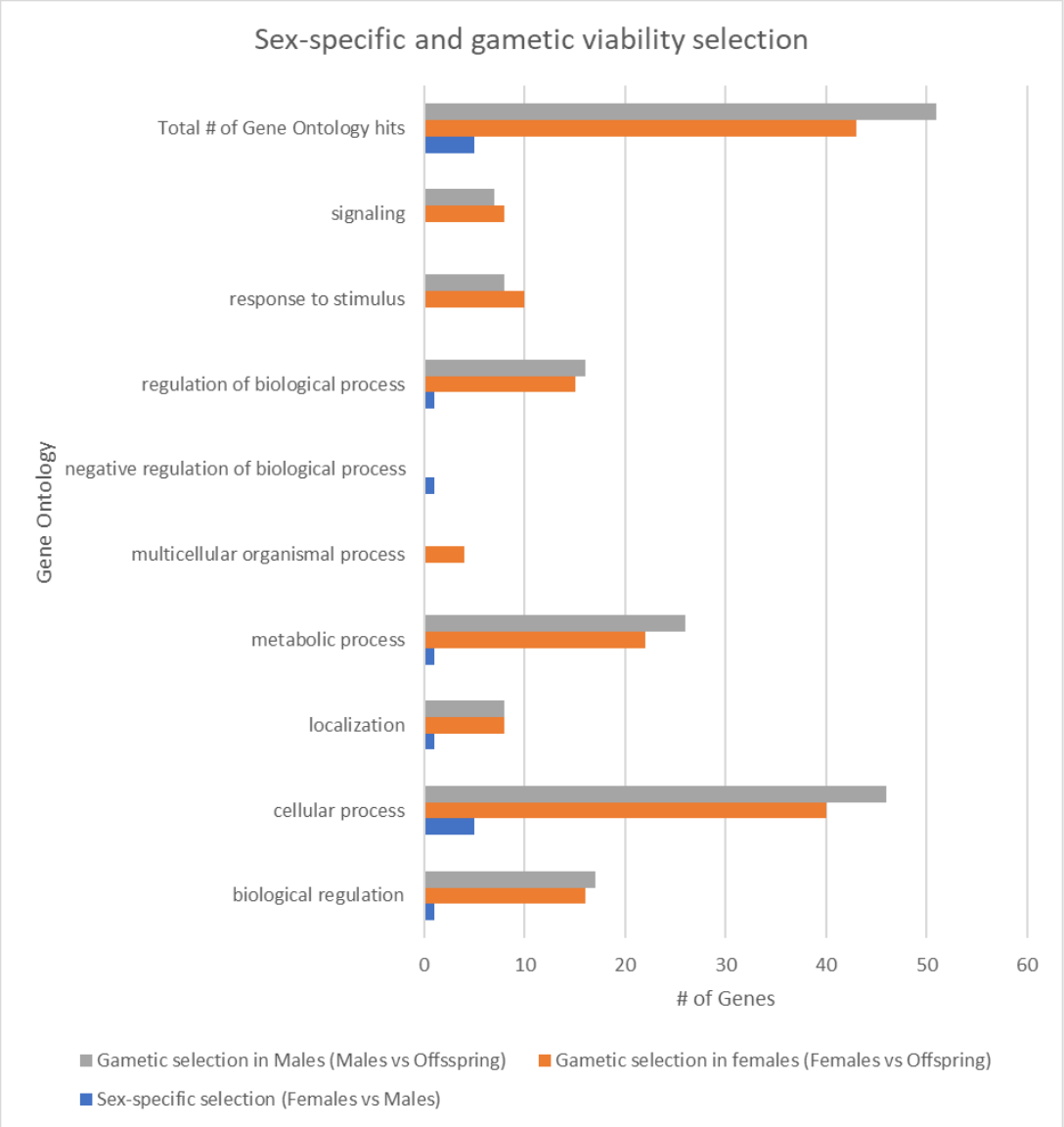


Fig 4.8. Gene ontologies describing the level 2 biological functions associated with sex-specific viability and gametic-viability selection. Genes surrounding outlier loci comparisons for all individuals between different islands. The number of loci sequence with each gene ontology category are displayed in the bar chart and the total number of gene ontology hits for each comparison are also reported. Note that a single locus may belong to multiple gene ontology categories.

4.4 Discussion

In the present chapter I explored the different forms of selection in Brown boobies, with special interest in sexual selection and how it changes between different environments. By the application of a selection component analysis framework to my genomic data, I performed GWDS and OutFLANK to identify loci putatively under different episodes of selection (Table 4.1). Additionally, the detected outlier loci were used to extract genomic regions surrounding such loci and were scanned against annotation databases to find Gene Ontology terms that would help to understand better the biological function of such regions under selection. Such results are discussed in terms of environmental heterogeneity and complemented by the conclusions from previous chapters when necessary. Overall, I have detected hundreds of genomic regions showing signals of differentiation compatible with natural selection, sexual selection, gametic-viability selection, and sex-specific viability selection. Therefore, my results show that genome-wide selection components analysis framework is a useful approach for differentiating signatures of selection in a wild population.

The selection component analysis framework is very flexible and can be adapted to a wide variety of study systems with different types of selection, making this approach suitable for a broad range of potential applications (Flanagan & Jones, 2017). My results propose that several episodes of selection that affect allele frequencies are present in studied brown booby colonies. In my original hypothesis I anticipated to find signatures of selection because brown booby males are known to be under strong sexual selection (Velando et al. 2006, Torres & Velando 2010;(Montoya et al., 2018), and I indeed found higher test statistic values in the

sexual selection comparisons related to females than those related to males (Figure S4.1-S4.4).

I also identified a considerably larger quantity of significant outlier SNPs in the male–female (sex-specific viability selection) comparison than in the comparison of sexual selection of males or females (Table 4.1). However, the highest numbers of outlier SNPs were found in male-offspring comparisons (580) and female-offspring comparisons (294). This could mean that more regions in the male genome are subject to gametic-viability selection than females, suggesting that differential selection on males and females may be having a persistent influence across the genome. Therefore, the male–female comparison could be detecting regions of the genome that are involved sex-biased survival, expression of sexually selected traits, or gamete competition. Additionally, strong sexual selection could be causing viability trade-offs between sexually selected traits and other vital functions. Since sexual selection in brown boobies seems to be strong (by female mate choice), an imbalance in the selection of traits could explain genome-wide allele frequency differences between the sexes. Only a few empirical studies that have implemented genome-wide selection components analysis examined several types of selection (Monnahan et al. 2015; Flanagan & Jones 2015; Robledo-Arnuncio & Unger, 2019). These studies reported similar results to ours in terms of proportion of detected loci for each type of selection.

4.4.1 Natural selection

We identified 45-57 outlier SNPs between colonies showing signature of local natural selection, with the highest number detected between San Jorge and Marietas while the lowest number was detected between San Pedro and San Jorge. I also identified several gene ontology categories only associated with regions near

significant loci in all the tests, with some biological functions that were unique for specific episodes of selection. For a full description of each biological process and the type of selection associated with it, please see Table S4.2. For instance, I detected signatures of natural local selection (Figure. 4.6) between San Jorge and San Pedro related to biological processes important for interspecies interactions between organisms. This biological function correspond to any process that results in a change of state or activity of a cell or organism in terms of movement, secretion, enzyme production, gene expression or similar process as a result of a stimulus from another living organism (Conesa & Götz, 2008). A possible explanation for this could be the contrasting environment and species composition between the two islands, given that San Jorge is a rocky island with no vegetation and doesn't share breeding grounds with any other seabird species. San Pedro on the other hand, is a larger island with a broad vegetation composition and a higher species richness, sharing breeding space with one of the largest populations of blue-footed boobies in the world (Tershy, 1997). Immune system processes were also subject to natural selection between Marietas and the other two colonies, which could suggest developmental functions calibrated for response to potential internal or invasive threats. During my expeditions I observed more stressors during incubating and rearing seasons in Marietas like higher temperatures and constant predators like crabs in the nesting areas. These threats were not present in the other islands, which could lead to immune system being a more critical function to be subject to selection in Marietas. Other factors such as high population density and human anthropogenic pollution could compromise the immune system and given that Marietas is considerably closer to human settlements this could

affect the health of the ecosystems. However more research would be necessary about population density and human impact in these birds before making any conclusions.

4.4.2 Sexual Selection

For the sexual selection comparisons, more loci under selection were detected in females than in males, especially between Marietas and San Pedro (127 outlier SNPS). For males, the sexual selection comparison detected a maximum of 70 outlier loci between Marietas and San Pedro (Table 4.1). This result is interesting given that the traditional view of sexual selection is only focused on males, however, mate choice occurs in both males and females in brown boobies (Andersson 1994). My results suggest that even the brown booby mating system is based in females evaluating and choosing high quality males, there might be equally important reciprocal sexual selection forces affecting more genomic regions in females than in males, even if these are not morphologically evident.

By scanning the outlier loci from males and females against the blasx databases, I found different biological functions depending on the islands being compared (see Table S4.1 for all comparisons), but here I am only mentioning the most relevant comparisons. Interestingly, male sexual selection (Figure. 4.7) seems to be associated with processes like growth between Marietas and San Pedro, and rhythmic processes between Marietas and San Jorge. As I explored in chapters 2 and 3 of this thesis, mate choice preferences for male traits seems to be different between colonies probably influenced for environmental conditions. Therefore, sexual selection related to growth and rhythmic processes is concordant with the differences found in body size and breeding timing between the different islands. Sexual selection in females seems to be affecting regions in the genome related to locomotion between

populations on San Jorge and San Pedro. In a recent study about the foraging habits of brown boobies and colour of sexual ornaments, it was reported great differences in foraging trips where San Pedro individuals tend to make shorter trips to get food, whereas the individuals in San Pedro Maritir travel the farthest (Michales et al. 2021 in prep).

4.4.3 Gametic viability selection

On the other hand, male gametic selection scans produced the highest number of outlier loci with 580 SNPs, whereas only 294 outlier SNPs were detected in females. This might suggest that more genomic regions associated with post-copulatory processes are being selected in this mating system, especially in males where sperm competition might be playing an important role in reproduction success. Moreover, sexual selection within the parental generation can influence a change within the population of successful gametes, as can gametic selection through either male or female function (Monnahan et al., 2015). Gametic-viability selection in males and females is the type of selection that was associated with more biological function (Figure. 4.8), which seems to be influencing mainly regions of the genome related to biological adhesion, cellular processes, metabolic processes, regulation, responses to stimulus, and signalling. However, most of these processes were also present in other types of selection, except biological adhesion which was exclusive of gametic viability selection.

4.4.4 Sex-specific viability selection

Lastly, 265 SNPs implicated in sex-specific (differential) viability selection, suggesting that sexual dimorphism resulting from sex-biased gene expression evolved in response to selection that acts differently on males and females in the current

generation (Cheng & Kirkpatrick, 2016). In previous analyses, more outliers were identified in the sex-specific viability comparisons than in the analysis of sexual selection (Flanagan & Jones, 2015, 2017; Monnahan et al., 2015) However, I found very few biological functions by blast2GO (only 5) for the outlier loci detected by the sex-specific selection scan (Figure. 4.7) even when many outliers were inputted (265). This suggest that the selected regions are very specific to brown boobies and current annotation databases are not able to match my sequences with similar GO terms, due to the lack if genomic information in this species. Additionally, I observed some consistent patterns in the shifting allele frequencies underlying the significant F_{st}-H_e results (Figure. 4.4). At the loci with values significantly different from zero in the comparison sex-specific viability selection, males tended to possess the minor allele and females had the major allele, suggesting opposing allele frequencies in males and females. This could be caused by both natural and sexual selection acting on a trait that is specific of each sex (Lucotte et al., 2016). Alternatively, the high number of outlier SNPs could be caused by differences in SNPs present on the Z chromosome, therefore, it would be recommendable to perform a new scan excluding the sexual chromosomes to avoid bias on the detection of SNPs under sex, specific viability selection. Unfortunately, the reference genome used in this study does not contain annotations to a chromosome level, so new alignments to a better annotated reference genome should be considered for future research.

4.4.5 Important considerations of the genome-wide selection components analysis framework

Genomic regions with biological functions that were significant in multiple analyses could suggest that those loci might be experiencing multiple forms of

selection (Figure. S4.5). If trade-offs between two or more types of selection (e.g., sexual and viability selection) occur for the same trait or genomic region, the same loci could show signatures of more than one types of selection, acting concordantly, at least within one sex (Andersson 1986; Kokko et al. 2002; Martin et al. 2014). However, in my study I found very few GO terms for the outlier loci related to sex-specific selection (only 5), regardless the high number of outlier SNPs found. A possible explanation for this could be false positives or that regions involved in sex-specific viability selection are not yet available in current annotation databases, thus are not able to match my sequences with similar GO terms. It is important to consider whether RAD-seq studies are prone to biases like intersecting outliers in shared regions with polymorphic restriction sites, or the proportion sequencing errors in specific regions that could affect F_{st} values (Flanagan & Jones, 2017).Therefore, cautious interpretation is necessary. Additionally, in the present study I was able to detect 27,000 SNPs which is a small dataset when compared with whole-genome sequencing, hence the chances of detecting polygenic traits are reduced greatly when using RAD-seq datasets. Regardless of the reduced numbers of SNPs, I was able to find hundreds of SNPs under putative selection under my specific selection scan framework, so the implementation of whole-genome sequences from this system seems very promising.

A commonly reported issue of using RAD-seq for selection scans are false homozygous genotypes caused by polymorphic restriction sites and PCR duplicates (Andrews et al. 2016; Monnahan et al. 2015). To reduce/remove issues with PCR duplicates, I implemented a custom ddRAD protocol (3RAD) where additional ambiguous indexed primers and the program clone_filter in STACKS 2.6, removed

PCR duplicates and reduced the undercalling of heterozygotes. Additionally, another disadvantage of studies implementing genome-wide selection component analysis is the inferring of paternal alleles through offspring and maternal genotypes (Monnahan et al. 2015; Flanagan & Jones 2015; Robledo-Arnuncio & Unger, 2019). In the present study I eliminated the need to infer mate genotypes because my sampling design allowed me to collect both parent and offspring, reducing the erroneous genotyping calls that could be amplified in the inferred allele frequencies.

4.4.5 Final remarks and recommendations

Regardless the mentioned constraints related to ddRAD and selection component analyses, the implementation of both approaches present great potential to identify regions involved in different episodes of selection. In the present study, I was able to identify unique genomic regions undergoing specific types of selection (e.g., natural and sexual selection), and also genomic regions influenced by several kinds of selection simultaneously (sex-specific viability selection and gametic viability selection). It is worth mentioning that I used a fragmented and unfinished version of the masked booby genome as a reference for my alignments. I used this genome as it was the closes relative available to the brown booby, however it lacks appropriate annotations to a chromosome level. Therefore, it is recommended to use a better annotated brown booby genome (or the closest good quality genome assembly), to perform again the analyses of this thesis for a more accurate interpretation of the genomic regions under selection. My findings suggest several factors that may be contributing to selection in brown boobies, which could not be easily inferred with a more traditional population genomic approach. As these kinds of studies become more common, it is very likely that the detection of false positives will improve like it is

already happening in many areas using next generation sequencing. One thing is clear, in order to better understand genomic evolution requires research from different disciplines and integral approaches, making this kind of studies more relevant to advance the knowledge of elusive evolutionary processes like sexual section. As a manner of final recommendations to improve or complement my study, it would benefit greatly from getting targeted individuals that failed to reproduce in the same season. This would provide greater insight about other regions in the genome involved in sexual selection pressures. Also, running additional sub-groups based in the genetic structures of the populations or running more tailored parent-offspring comparisons (by comparing adult males to only male offspring or adult females and offspring) might allow for a greater resolution when trying to discern more types of selection. Finally, investigate further the trade-offs of the implementation of less restrictive parameters in the blastx databases that could allow the detection of more genomic regions in non-model organisms. Therefore, as current databases continue to incorporate more annotated genes for biological functions in non-model organisms, these kinds of analyses will be able to better describe the biological functions of genomic regions under selection.

CHAPTER 5: GENERAL CONCLUSIONS

The data presented in this thesis has explored thoroughly some of the mechanisms involved in the genomic basis of sexual selection and the influence of environment on brown booby genetic diversity. Chapters 2, 3 & 4 described and tried to understand different aspects affecting the evolutionary processes of brown boobies

with special focus in sexual selection, explained in terms of environmental heterogeneity. The purpose of this last chapter is to try to integrate the findings of each data chapter and to discuss further the most important implications as a whole. Here I present three main themes to conclude this thesis: (1) Difference in mate choice and honesty of traits reflect genetic quality in different environments; (2) the search for genome-wide signatures of sexual selection and final remarks; (3) and the impact, recommendations and future work.

5.1 Difference in mate choice and honesty of traits reflect genetic quality in different environments

Mate choice is an important component of sexual selection and in brown boobies it is the female the one evaluating and choosing the quality of males in order to ensure the production and survival of the offspring. In the second chapter I examined traits involved in mate choice that could have an effect in shaping genetic composition of individuals and populations of brown boobies. Some of these factors are the coloration in bare parts like feet and gular, as well as other morphological traits like the length of beak, wings, legs, and total body weight. Additionally, I explored the proportion of extra pair paternity to assess the genetic quality between genetic fathers successfully raising one chick, extra-pair paternity and the rare cases where some genetic fathers were able to raise two chicks and how these proportions changed between breeding colonies. It is expected that monogamous species with biparental care usually have low levels of EPP, and both indirect and direct benefits are reflected by sexual ornaments (Kokko 1998). My findings in chapter 2 seems to support the previous statement, given that I reported only nine out of 174 presented EPP and ornament colours honestly reflected heterozygosity in the studied colonies.

Nevertheless, it seems that brown boobies in the Pacific Coast of Mexico excibit the lower percentages of EPP when compared with the most recent studies of fidelity on seabirds by using genome-wide SNPs to solve paternities. For instance, a recent study on Streaked Shearwaters revealed an average of 15% of EPP (Sakao et al., 2019), whereas other seabirds like the Magellanic Penguin and the Australasian Gannets precented average rates of 31% and 12% respectively (Hauber et al., 2018; Marasco et al., 2020).

Moreover, in a study carried by my collaborators in the Brown boobies from Marietas Island, it has been found that females paired with males with greener gulars may obtain indirect and direct benefits, given that, gular colour during courtship by rearing males is positive corelated to paternal care, chick body mass, and structural growth (Montoya & Torres, 2015). Initially, I predicted that if the social mate of female brown boobies has low genetic quality, then females try to gain any benefit from EPP by engaging in extra-pair copulation with males that have higher genetic quality or higher dissimilarity. However, I found few evidence of such behaviour as difference between those nests in most of the cases was not statistically significant. Therefore, it is likely that the occurrence of EPP and rare nests with two surviving chicks is more related with the nesting conditions of each island and the availability of food items for parental care, thus providing multiple mating opportunities across years.

Given that brown boobies are exposed to variable environmental conditions throughout their range (Nelson 1978, Schreiber & Norton 2002) with different sea surface temperatures (SST) and primary productivity (PP), which in return cause an

adjustment in foraging strategies defendant on prey sizes and abundances (Castillo-Guerrero et al. 2016). For instance, in the present study I did not find any nest with two chicks in Marietas while most of the EPP nests were found in San Jorge Island. The environmental measurements used as proxy in chapter 3 and my empirical observations while collecting samples showed a great variability in physical and ecological factors (e.g., habitat heterogeneity) that could affect nesting habitats and densities between the three colonies. All the mentioned factors also can potentially influence the honesty of the sexual ornaments or the patterns of extra-pair paternity (Emlen & Oring 1977). In this aspect, my results partially agree with my initial hypothesis where I stated that cuckolded males would have lower heterozygosity, however, the nests with two chicks showed also lower heterozygosity when compared with nests with only one chick, which is the opposite of what I expected. An alternative explanation could be that monogamy and the optimization of resources (raising only one chick) is associated with genetic quality, but further research with a bigger sampling size focusing on the genetic differences of EPP nests and within- pair nests should be done to explore such hypothesis.

Additionally, significant differences were found in body size and heterozygosity depending on the breeding colony. Marietas was the colony with lower body size and higher heterozygosity, whereas the opposite was true for San Jorge. This could suggest that the strong environmental pressures in Marietas might be shaping population for more heterozygous individuals. This makes sense if I consider the different levels of Primary Productivity and Sea Surface Temperature (Figure 1.2), suggesting that San Jorge have more availability of food year-round (Castillo-Guerrero et al. 2016), whereas Marietas have less food especially during the breeding

season (summer), making it energetically costly to maintain bigger bodies. San Pedro somehow, share conditions more similar to San Jorge and when I look at the distribution occurrence of nests with two chicks these two have the highest occurrence. Though, most of the nests that presented EPP were from San Jorge which contrasts with the initial hypothesis of chapter 2 that stating that in colonies with less resources will have a higher rate of EPP because females will try to obtain indirect benefits by higher quality EP offspring. In summary, these findings suggest that the occurrence of nests with two chicks and nests with extra-pair mates are mainly influenced by the habitat (Tables 2.1 & 2.2), and to some extent, to the low heterozygosity of the males (Figure 2.3). In the island of Johnston Atoll it has been suggested that nests with two chicks reflect a high availability of food in the area (Schreiber & Norton, 2002), so the same pattern could be true in San Jorge.

In previous studies, it has been reported that gular and feet coloration is carotenoid dependant and acts as an important condition-dependant signalling in sexual selection of brown boobies, because carotenoids are acquired through diet and might be indicating a nutritional state and foraging efficiency (Hill et al. 2002; McGraw et al. 2003; Casagrande et al. 2006; García-Navas et al. 2012). In this study, I found that traits such as green colour and body size was correlated with heterozygosity and inbreeding, however, some important differences were detected between males and females. In females I found that traits such as green colour in feet and gular were positive correlated with heterozygosity and negatively correlated with inbreeding in all the colonies (Figure 2.5). However, in males in each island showed different corelations between heterozygosity, colour, body size and genetic similarity. My results show some level of concordance with those reported in a cross-fostering

experiment carried in Marietas by (Montoya & Torres, 2015)**,** where they found that males with greener gulars provided better parental care and genetic quality to offspring; supporting the idea that in species with biparental care and low levels of extrapair copulations, sexual traits may signal direct and indirect benefits. My data suggest that female mate choice is shaping the honesty of ornament for genetic quality expressed in males in each colony. This poses more interesting questions about the local adaptations of mate choice in these colonies, where genetic structure might be playing a bigger role than I previously imagined. Finally, I found simultaneous correlations between heterozygosity and similarity acting in traits like body size and mass, suggesting that both the good genes-by-heterozygosity and the compatible gene models are present in this trait. Such findings are concordant with previous proposed hypotheses where both models could potentially provide indirect benefits, and where their contributions are not mutually exclusive because they contribute to different components of genetic variance (additive and nonadditive) (Colgrave et al. 2002; Neff & Pitcher 2005).

In chapter 3 I explored the genetic structure and the distribution of genetic variability associated with environmental heterogeneity, which complement greatly the results regarding to mate choice and honesty of ornament. In this thesis I found a great level of population structure between the sampled colonies with limited geneflow between them. Additional admixture analyses suggest that all three colonies are highly differentiated from each other, with San Pedro showing very marginal mixed ancestry coming from the other two colonies. Additionally, the phylogenetic reconstruction of the sampled individuals further supported this pattern of population structure (Figure. S3.7), suggesting that San Pedro and San Jorge were founded by

Marieta's descendants. However, I appreciate that possible to interpret ancestor and descendent relationships between these islands in the absence of outgroups, so these factors should be considered for future interpretations and research about the phylogenetic origin of theses colonies. The Fst and Nei's genetic distance also showed that Marietas and San Jorge were the most differentiated colonies, followed by San Pedro and Marietas, and finally San Jorge and San Pedro as the most closely related colonies (Figure 3.2). Even though, Brown boobies can travel long distances and no physical barriers are recognized between the sampled colonies, other factors like natal philopatry (Friesen et al. 2007), the different timings on breeding of each colony or habitat preferences are probably preventing gene flow and promoting their isolation. Moreover, male coloration is usually subject to sexual selection, and it has been cited numerous times as a reproductive isolation barrier in birds (Price 2008). For instance, environmental variations that influence selection pressures (either sexual or natural selection) can prevent gene flow if migrants are not adapted to new environments (Morris-Pocock et al., 2010). It is also known that morphological characteristics that are different among populations of brown boobies like the bare part, are important sexual ornaments that signal quality (Pierotti, 1987). In the present thesis I found that each colony present different coloration in bare parts and body size, which could cause negative selection against migrants which might present differences in sexual ornaments, exacerbating the differentiation between colonies even more.

Earlier studies about the genetic structure of Sulids have highlighted the role of local adaptation, behavioural and geographical isolation as main drivers of genetic differentiation in the absence of physical barriers Steeves et al. (2003). For instance, Steeves et al., (2005) found low levels of gene flow within Indo-Pacific and Atlantic

populations, concluding that limited natal dispersal combined with local adaptation and genetic drift were the main drivers of population differentiation, though the influence of these was not measured at that time. Regarding the genetic structure of brown boobies, Morris-Pocock et al., (2010) suggested that San Pedro diverged from colonies outside of the Gulf of California 130,000 years before the present in the absence of gene flow regardless of being inside a 500km range. They also suggested that factors such as salinity and sea surface temperature could act as cryptic barriers to dispersal. Even when all the mentioned studies have mentioned local adaptation and environment as potential drivers of population differentiation, to my knowledge, none of them have tried to quantify the influence of environmental variables with NGS data to explain such differentiation, thus highlighting the importance of my current study for the global knowledge of brown boobies and other seabird species.

One of the most important findings of the present thesis is the fact that an important portion of the genetic diversity can be explained by the influence of environmental variables like sea surface temperature and primary productivity. I found that the genomic variability of the individuals from San Jorge are highly correlated with high PP during the summer, Marietas SNP variability is correlated with low PP, and San Pedro showed no strong correlation for low or high productivity during summer. On the other hand, San Pedro showed a strong correlation with low SST during summer, whereas Marietas and showed no correlation. When comparing the environmental variables during the breeding season, Marietas showed a very strong correlation with high SST, whereas San Pedro and San Jorge showed a moderate correlation with lower temperatures. Finally, Marietas showed a very strong correlation with low primary productivity during the breeding season, whereas San

Jorge and San Pedro showed a relatively high correlation with higher PP values. These results showed that seasonality of variables play an important aspect in shaping the distribution of the genetic diversity in populations across environmental heterogeneity. This was especially true for Marietas and San Jorge, which presented contrasting values that explained the variability of their detected SNPs. I also found a strong correlation in the isolation by distance analysis as is more expected in species with broad distributions, suggesting that that environmental distance can be confounded with geographical distance. This allow me to suggest that the differences found in the mate choice of brown boobies between colonies and the correlation in the heterozygosity, similarity and phenotypic traits (as discussed in chapter 2), are in part driven for the environmental conditions. Finally, it is important to note that the small but significant influence of environmental conditions on genomic variation found in chapter 3, is very likely influencing at the same time some type of selection for specific regions in the genome. In order to detect natural selection or any other selective force (e.g., sexual section), genome wide scans for loci under selection are a great addition to find out more details about the actual regions of the genome subject to local adaptation like it has been shown in chapter 4.

5.2 The search for genome-wide signatures of sexual selection and the influence of environmental heterogeneity

The present dissertation has attempted to investigate several important evolutionary aspects of the brown booby populations in the East Pacific Ocean with the final goal of increase the knowledge of how sexual selection works at a genomic level. Even with all the new technological advancements, sexual selection in the wild remains elusive to researchers after more than 150 years from the publication of

Darwin's theory of natural selection. In chapter 4 I applied a new approach that combines recent developed tools to detect signatures of positive selection in the genome, and quantitative trait theory in the form of a sampling scheme in order to detect different episodes related to sexual selection. I was able to detect several hundred outlier loci putative under several types of selection like sexual, gametic, sex-specific viability, and local natural selection. Interestingly, I detected a higher number of outlier SNPs under sexual selection in females rather than males. This result is interesting given that the traditional view of sexual selection is only focused on males, however, mate choice occurs in both males and females (Andersson 1994). My results suggest that even the brown booby mating system is based in females evaluating and choosing high quality males, there might be equally important reciprocal sexual selection forces affecting more genomic regions in females than in males. Previous studies have investigated the genomic footprints of divergent selection implicated in local adaptation in a wide range of species and systems (Savolainen, et al., 2013; Tigano & Friesen, 2016; Hohenlohe et al., 2010; Nielsen et al., 2009; Nunes, Beaumont, Butlin, & Paulo, 2010). Additionally, most of these species exhibit high natal philopatry (e.g., Huyvaert & Anderson 2004; Quinn, 1993), and such behaviour usually reduce further the gene flow amongst populations (Fraser et al., 2011), therefore, extra caution should be taken in the interpretation of my results as certain population events can influence the detection of selection scans (Hermisson & Pennings, 2005).

In chapter 2 I found that females and males presented different degrees of correlation between traits like body size and colour in bare parts with genetic similarity and heterozygosity, whereas in chapter 3 I found that such genetic features are

explained by environmental variables. As such traits are known to be subject of sexual selection in brown boobies, the preference for certain variations in colour and body size might influence also certain genomic regions subject to such selection.

In my study I found several GO terms associated with sexual selection in males that affect biological functions like growth and rhythmic processes. This is most likely related to the differences found in body size and breeding timing between the studied islands as previously discussed in chapter 2 and chapter 3. On the other hand, sexual selection in females seems to be affecting regions in the genome related to locomotion, which could be explained with the differences in foraging habits recently found between colonies by My collaborators (Michael et al. in prep). Additionally, in the discussions of chapter 3 I hypothesized that the higher heterozygosity and dissimilarity found in Marietas could be the result a type of natural selection caused by the high temperatures and low productivity during the breeding season. This hypothesis seems to be further supported by my findings where immune system processes were also subject to natural selection between Marietas and the other two colonies which could suggest developmental functions calibrated for response to potential internal or invasive threats. Moreover, during my expeditions I observed more stressors during incubating and rearing seasons in Marietas like higher temperatures and constant predators like crabs. These threats were not present in the other islands, which could lead to immune system being a more critical function to be subject to selection in Marietas, where conditions are poor and weakened individuals could be more susceptible to pathogens. This could cause selective pressures favouring more heterozygous individuals (e.g., good genes) and dissimilar individuals (e.g., compatible genes) simultaneously like I hypothesised in the discussions of chapter 2.

These results partially agree with my hypothesis from chapter 4 which stated that more signatures of selection would be detected in contrasting environments.

Additionally, the gradient of diversity observed in my studied colonies, where San Jorge presented the lowest nucleotide and genetic diversity, also suggest the influence of additional evolutionary processes, like a bottleneck effect or a founder event. The negative values of Tajima's D suggest the abundance of rare alleles or a recent selective sweep and population expansion after a recent bottleneck or founder event (Tajima 1989). Even though all populations show such negative values of Tajima's D, San Pedro seems to be the one with the highest values and San Jorge with the lowest values. However, the demographic reconstruction showed a relative similar pattern in all islands which consist in a stable effective population size (Ne) of 2-2.5 thousand individuals from 50 to 15 thousand of years ago (kya) for all colonies. This followed by a sudden rise in Ne that peaked at 4-5 thousand individuals, and then, an ongoing reduction that started 500 years ago. An alternative explanation for the negative Tajima's D values in all islands could be due the overall reduction of brown booby populations in recent years detected by my demographic reconstruction, causing a generalized reduction in the overall genetic diversity of the species.

Finally, it is important to note that genomic regions with biological functions that were detected by multiple analyses could suggest that those loci might be experiencing multiple forms of selection, however the trade-off between two or more types of selection in single trait or genomic region remain to be investigated. Regardless the intrinsic constraints related to ddRAD as discussed in chapter 4, I was able to identify regions involved in different episodes of selection in the genome (like

natural and sexual selection) affecting very specific traits, and also genomic regions influenced by several kinds of selection acting simultaneously in various traits like the case of gametic, viability and sex-specific selection. These findings suggest that this approach has the potential identify several factors that may be contributing to the sexual selection in brown boobies, which could not be easily inferred with a more conventional population genomic approach. However, I humbly recognise that the information generated in this thesis is just scratching the surface of the actual evolutionary processes involved in the sexual selection in this system, but I think that at least it represents an example about the possible combination of approaches available to understand better how these evolutionary forces act in the nature.

5.3 Impact, recommendations, and future work

The results of this thesis can be complemented or serve as a foundation for future research aimed in several directions. The more I learned about the methodologies and theory behind each chapter, new questions arose that deserve to be investigated further. Additional research needs to be done on male phenotypic traits and the mechanisms of how they are detected as cues by females. This will help to understand better the mate choice, its evolutionary processes and how genetic diversity is gained in certain systems. For instance, as we detected regions of the genome that are being selected for biological functions related to immune system and growth, the use of candidate genes related to such functions should provide more precise information about the actual "good genes" being selected. Similarly, the Major Histocompability Complex (MHC) is a broad example of mate choice by dissimilarity and would provide information about compatibility at an individual level and on a gene-by gene basis.

In chapter 2, there were inconclusive results about the differences in quality from nests with extra-pair mates because of low sampling size of such individuals. A more extensive sampling effort would be ideal where a higher number of individuals are collected and if possible, both the social and the genetic father for a direct comparison about the genetic quality of such individuals, to generate more knowledge about the evolution of monogamy in birds. Similarly, the potential to raise two chicks per season poise interesting questions about the evolution of siblicide in these seabirds, as in some islands I was able to spot more nests with two chicks whereas in Marietas we found none. By increasing the sampling size on these rare cases, we can learn more about the reproductive strategies and the utilization of resources in seabirds.

In order to improve or complement my study in the future, it would benefit greatly from getting targeted individuals that failed to reproduce in the same season and also individuals that courted but did not form a pair. This would provide greater insight about other regions in the genome involved in sexual selection pressures. Similarly, as early breeders usually get better nesting areas, this behaviour can also be subject to sexual selection and a comparison against late breeders should also supplement the views on mate choice. Finally, running additional sub-groups comparisons considering the genetic structures of the populations or running more tailored parent-offspring comparisons might allow for a greater resolution when trying to detect more specific types of selection. For instance, by comparing surviving chicks to those chicks that did not survive or were killed by an older sibling could shed some like in the genomic adaptations of siblicide and how this also could shape the genetic diversity of these islands if different rates of these practices are found.

A couple of studies are being carried by my collaborators, which have direct relevance to my results and once they are published, it will allow us to revisit my conclusions under a new light. One of these studies is trying to investigate the foraging behaviour through the implementation of GPS trackers and stable isotopes to differentiate the assimilated prey between individuals and ecosystems on brown booby colonies to investigate whether ornament quality is related to dietary tendencies and at-sea behaviours. Another work is being done about the immune response of brown boobies in Marietas and the parental care behaviour related to coloration. Finally, a genetic analysis using metabarcoding on prey items to compare the brown booby diet in different islands is also being carried at the moment. The combination of all these different fields poses an exciting potential to comprehend in more integral way the evolutionary and ecologic factors influencing these seabirds.

To my knowledge, this is one of the first studies to use next generation sequencing to reveal signatures of sexual selection to a genomic level in monogamous species, the influence of mate choice by phenotypic cues, and the influence of environmental variables in genetic diversity. Which highlights the importance of this work to the overall knowledge of this species, and possible to other seabird species. Even though conservation is not the main focus of my thesis, as seabirds are being more threatened than ever by climate change, these kinds of studies are getting additional relevance to understand fragile ecosystems. The more we know about how species react to environmental variables, the better we can start constructing conservation plans for endangered systems. In this study I, briefly analysed the demographic history of my sampled populations, where I was able to detect a reduction in effective population size in the last five hundred years. Therefore, more

studies about evolutionary history and population differentiation should be considered by governmental agencies for the management of natural resources and conservation of vulnerable species to environmental alterations, such as seabird species.

APPENDICES

Appendix A: Supplementary material for chapter 2

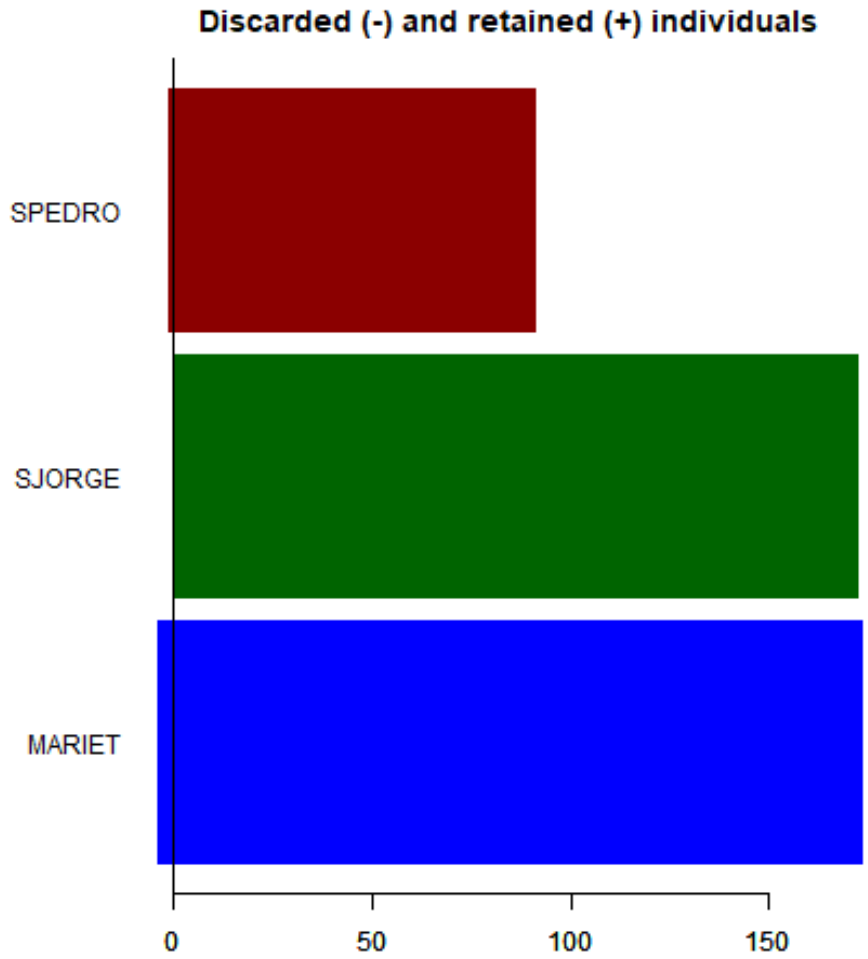


Fig S2.1. Proportion of retained individuals for the "parentage dataset". Bars before the 0 represent individuals that did not pass our quality filters.

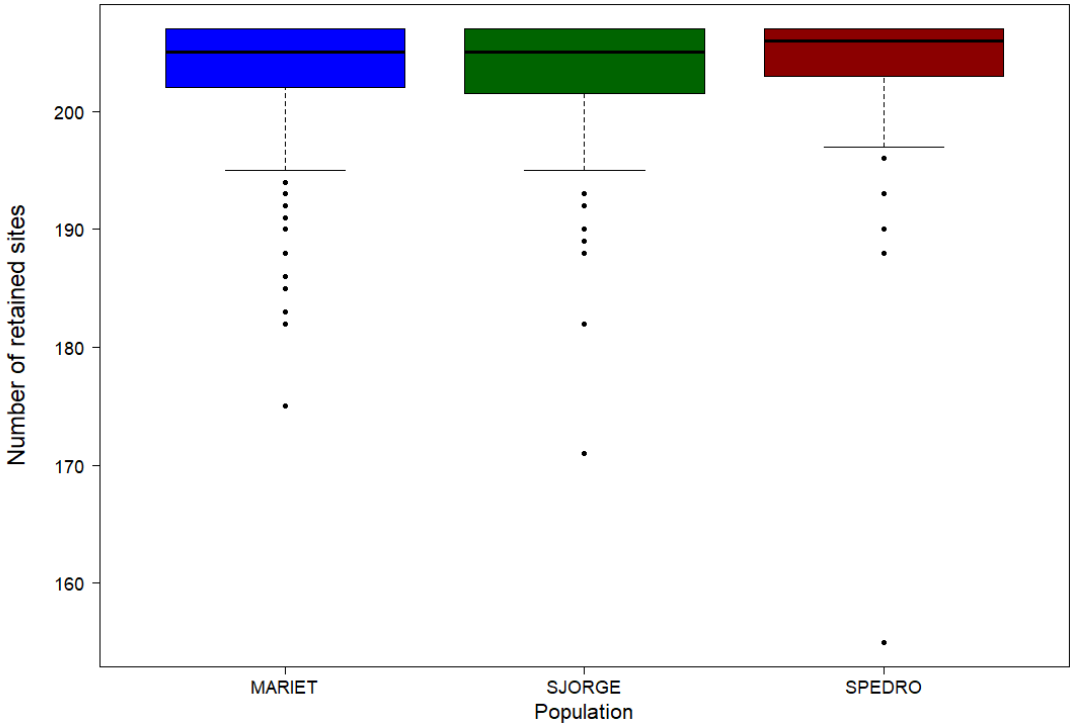


Fig S2.2. Number of retained SNPs per population in the "parentage dataset". Boxes represent the average of sites present in the sampled individuals.

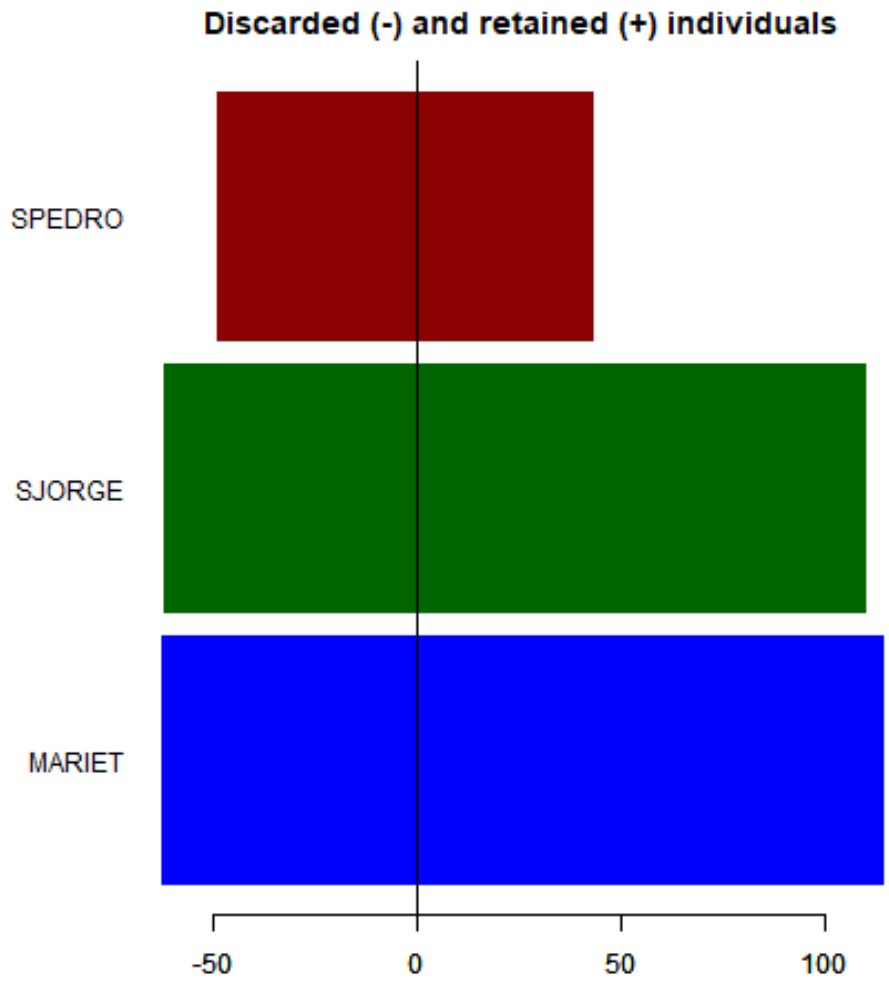


Fig S2.3. Proportion of retained individuals for the "genetic dataset". Bars before the 0 represent individuals that did not pass our quality filters.

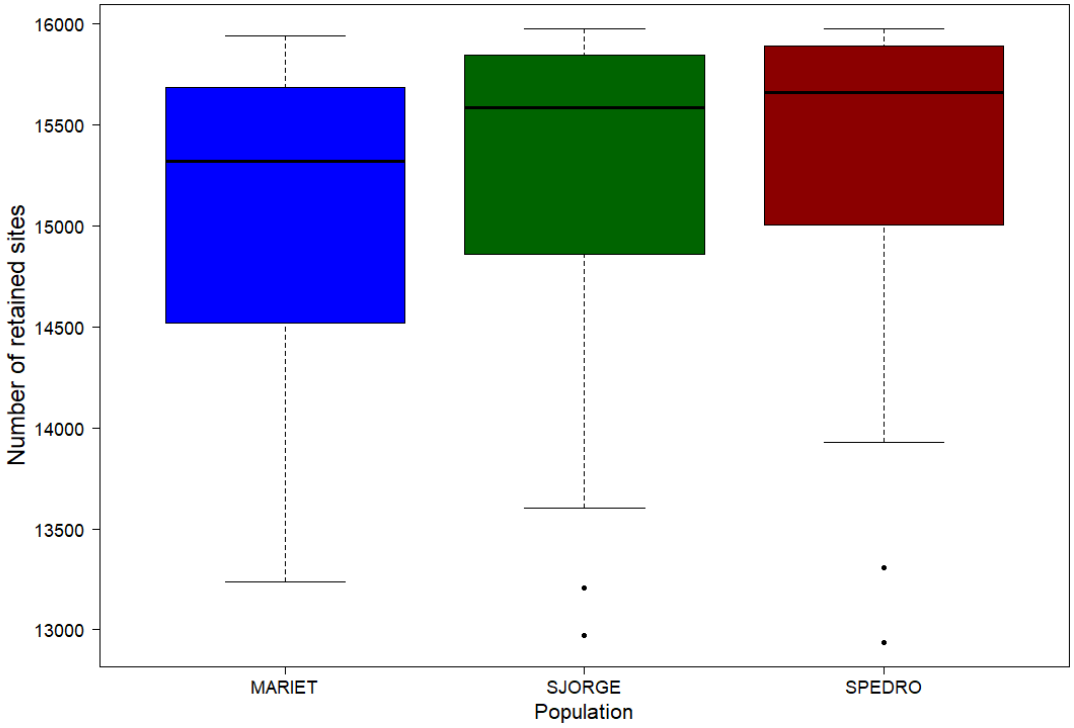


Fig S2.4. Number of retained SNPs per population in the "genetic dataset". Boxes represent the average of sites present in the sampled individuals.

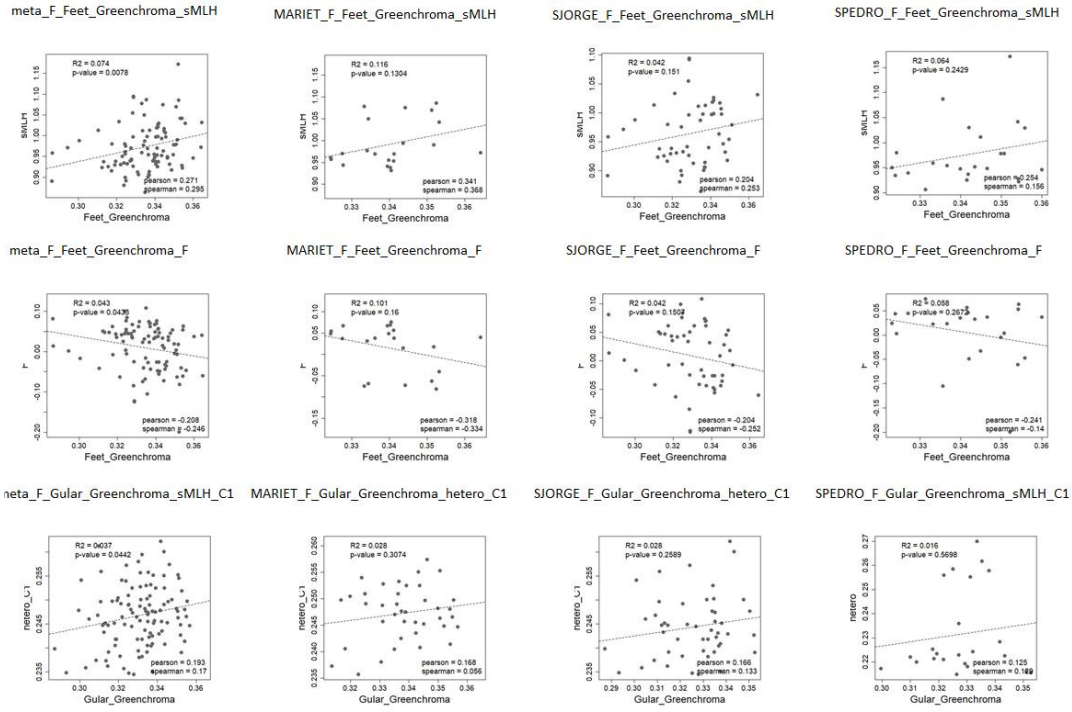


Fig S2.5. Correlation between genetic parameters and green colour in bare parts in females. Significant correlation of genetic parameters with green chroma when all females are grouped in a single metapopulation.

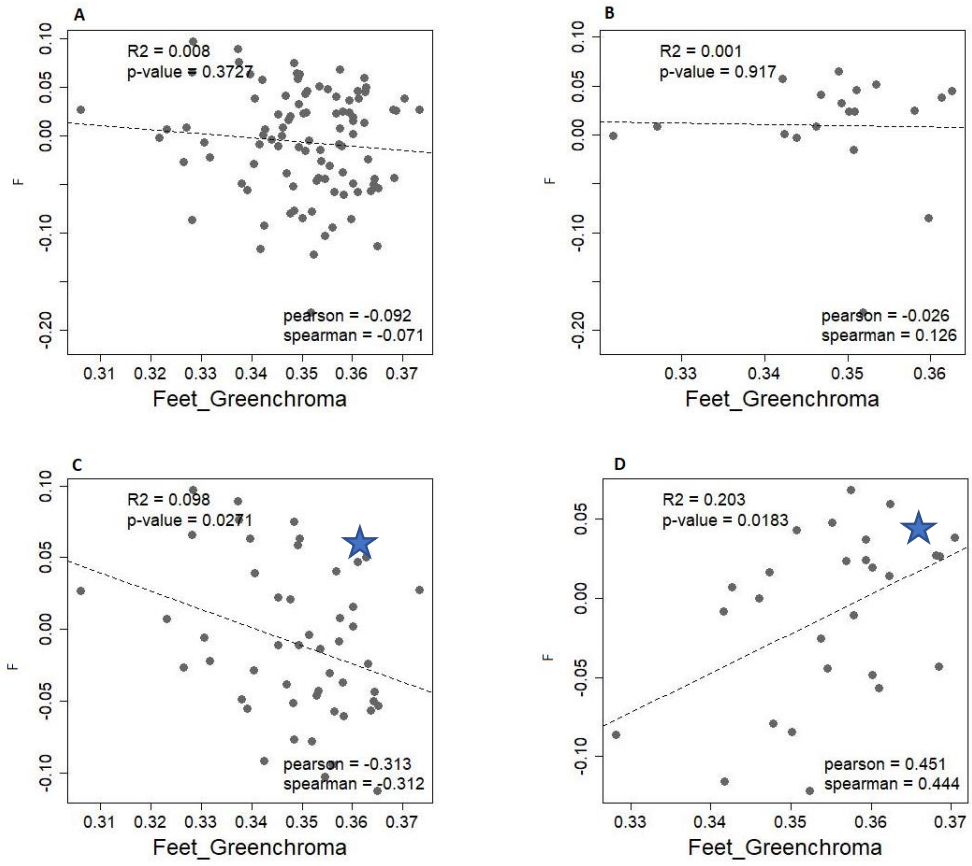


Fig S2.6. Correlation between inbreeding associated with green colour in feet (males). A). Metapopulation. B). Marietas. C). San Jorge. D). San Pedro. Star indicates plots with significant correlations.

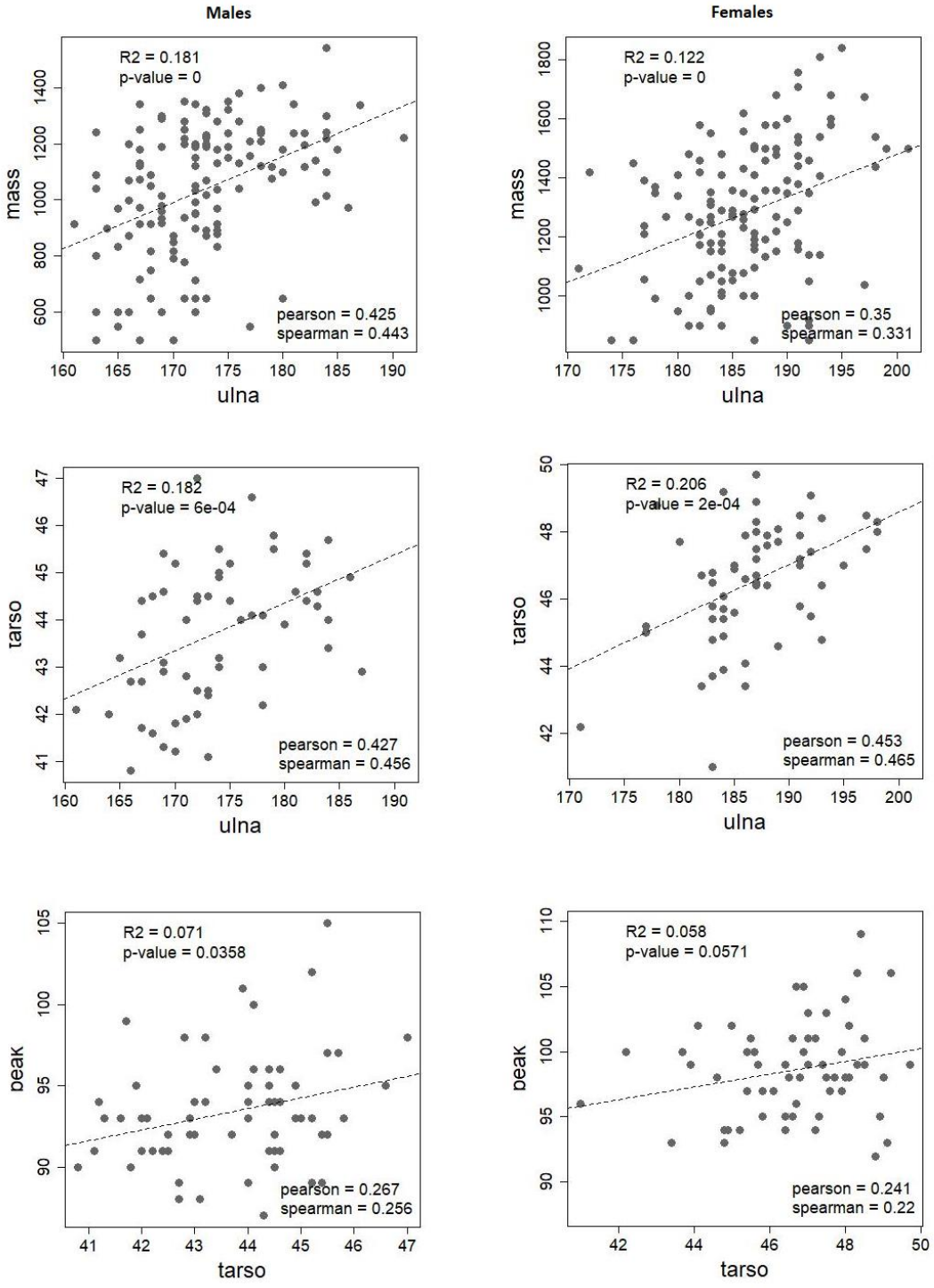


Fig S2.7. Significant correlation between traits related to body size. Phenotypes compared are showed in the Y and X axis of each plot. Sex of individuals are shown at the top of the figure.

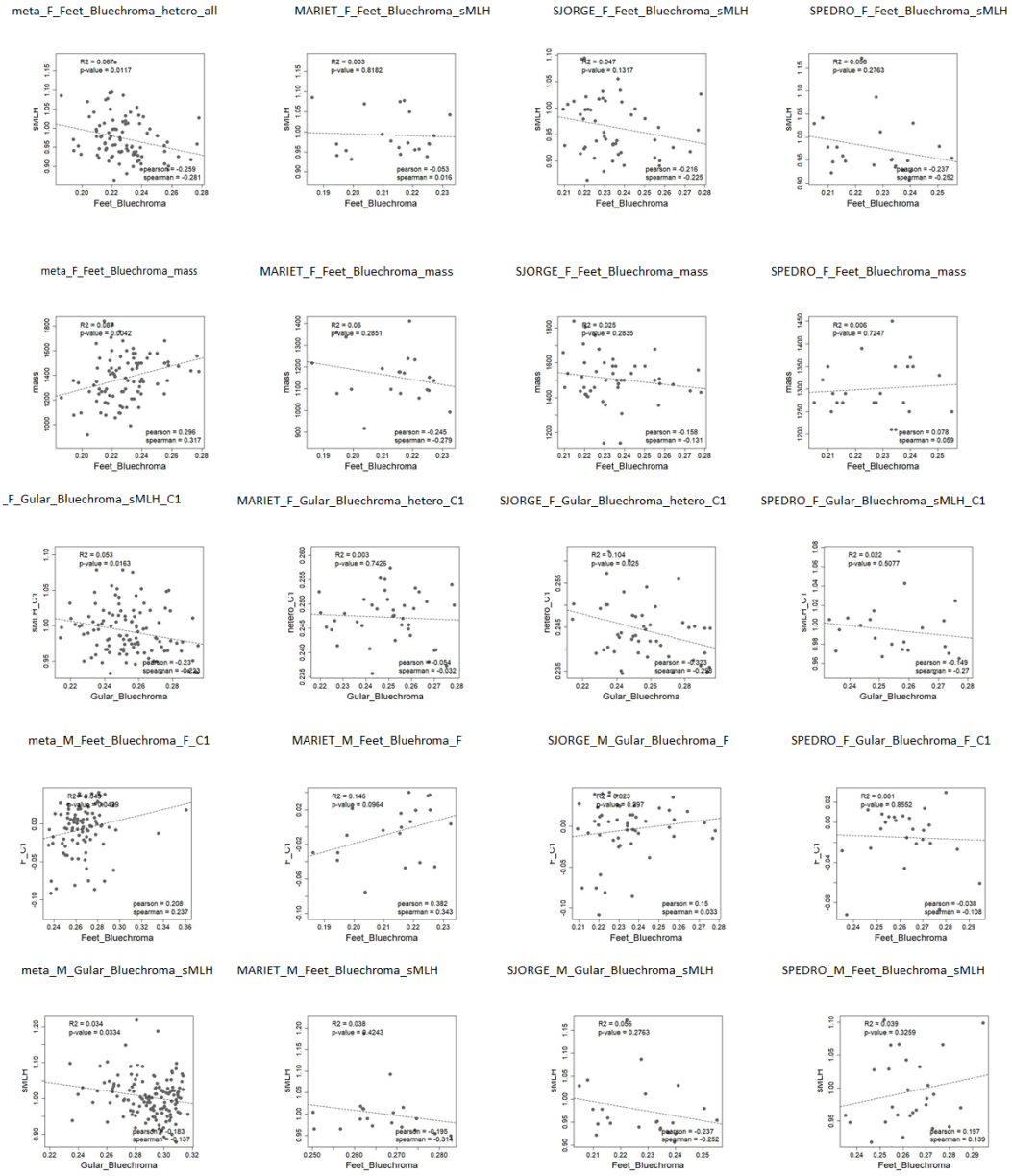


Fig S2.8. Other significant correlation between genetic parameters and blue colour in bare parts. Description is provided in the headers of each plot. Note that blue colour shows the opposite pattern than green colour when compared with the genetic parameters.

Appendix B: Supplementary material for chapter 3

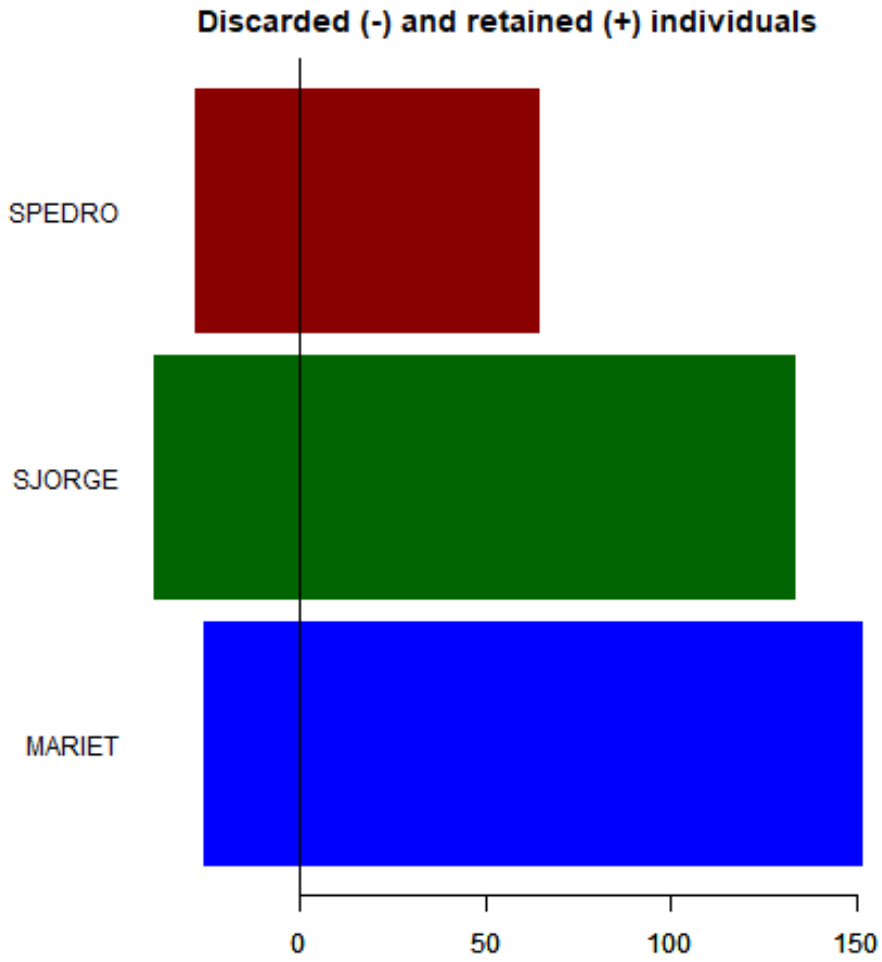


Fig S3.1. Proportion of retained individuals for the "structure dataset". Bars before the 0 represent individuals that did not pass our quality filters.

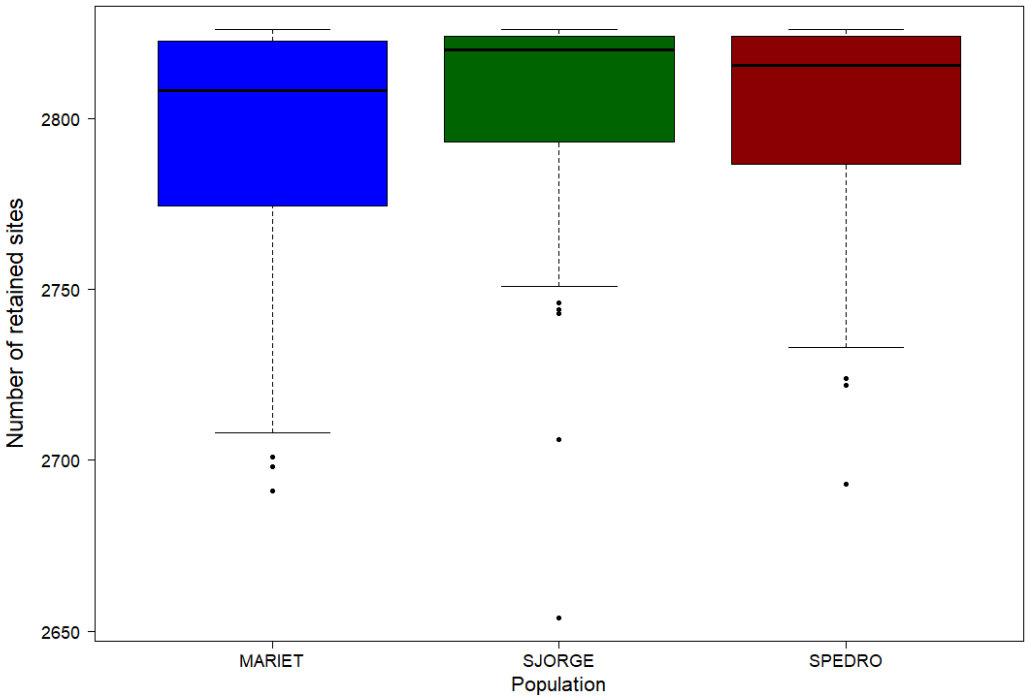


Fig S3.2 Number of retained SNPs per population in the "structure dataset". Boxes represent the average of sites present in the sampled individuals.

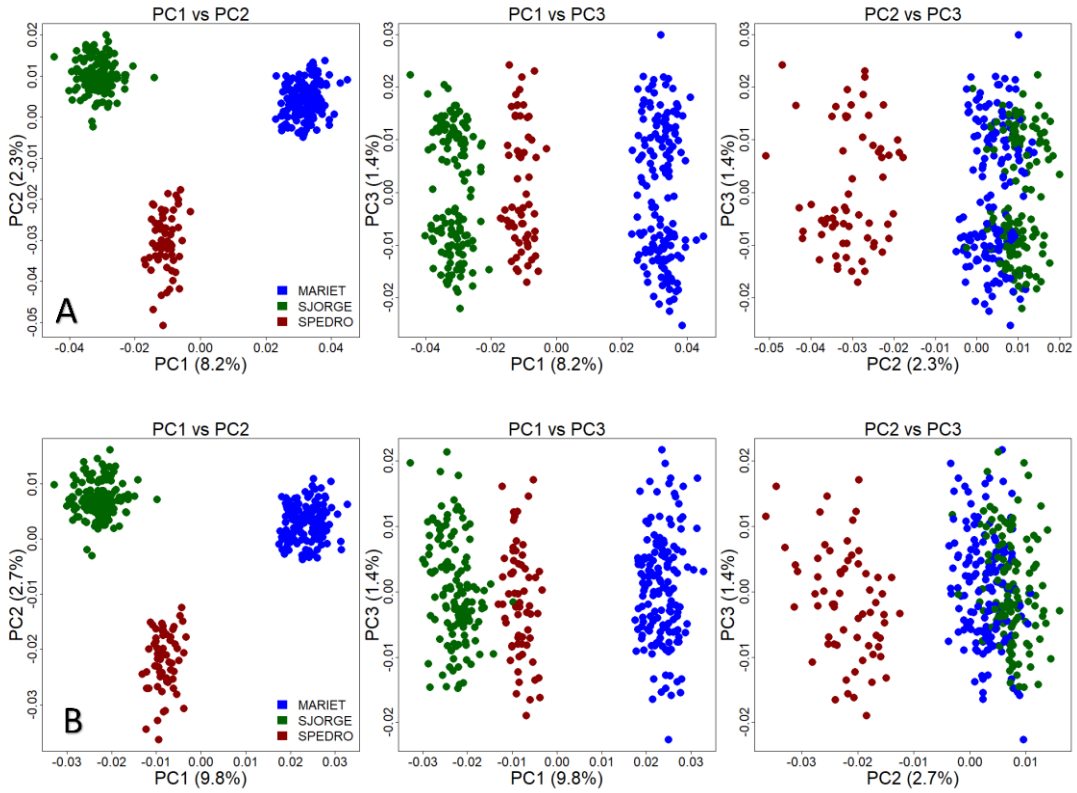


Fig S3.3. Principal Coordinate Analysis (PCoA) all different coordinates combinations. A. standardized data based on Hamming's genetic distance. B. standardized data based on Nei's genetic distance.

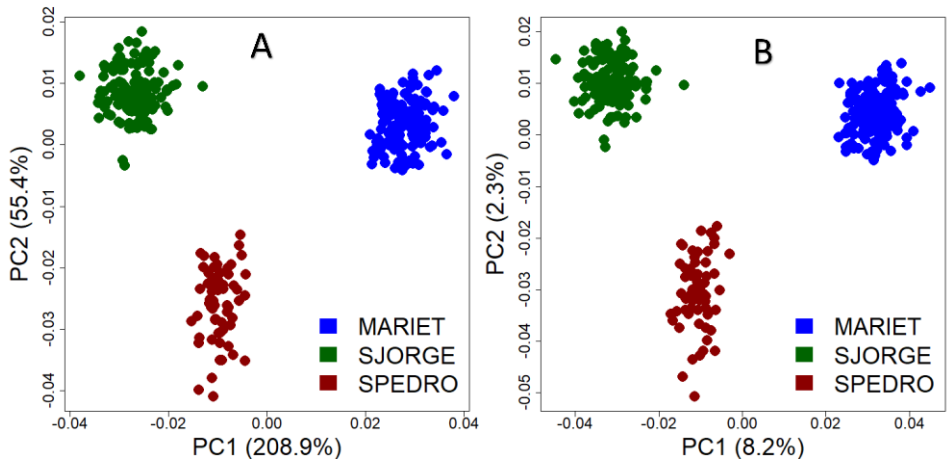


Fig S3.4. Principal Coordinate Analysis (PCoA) plot of the three sampled colonies. A. standardized data based on Pi (pairwise sequence dissimilarity). B. standardized data based on Hamming's genetic distance.

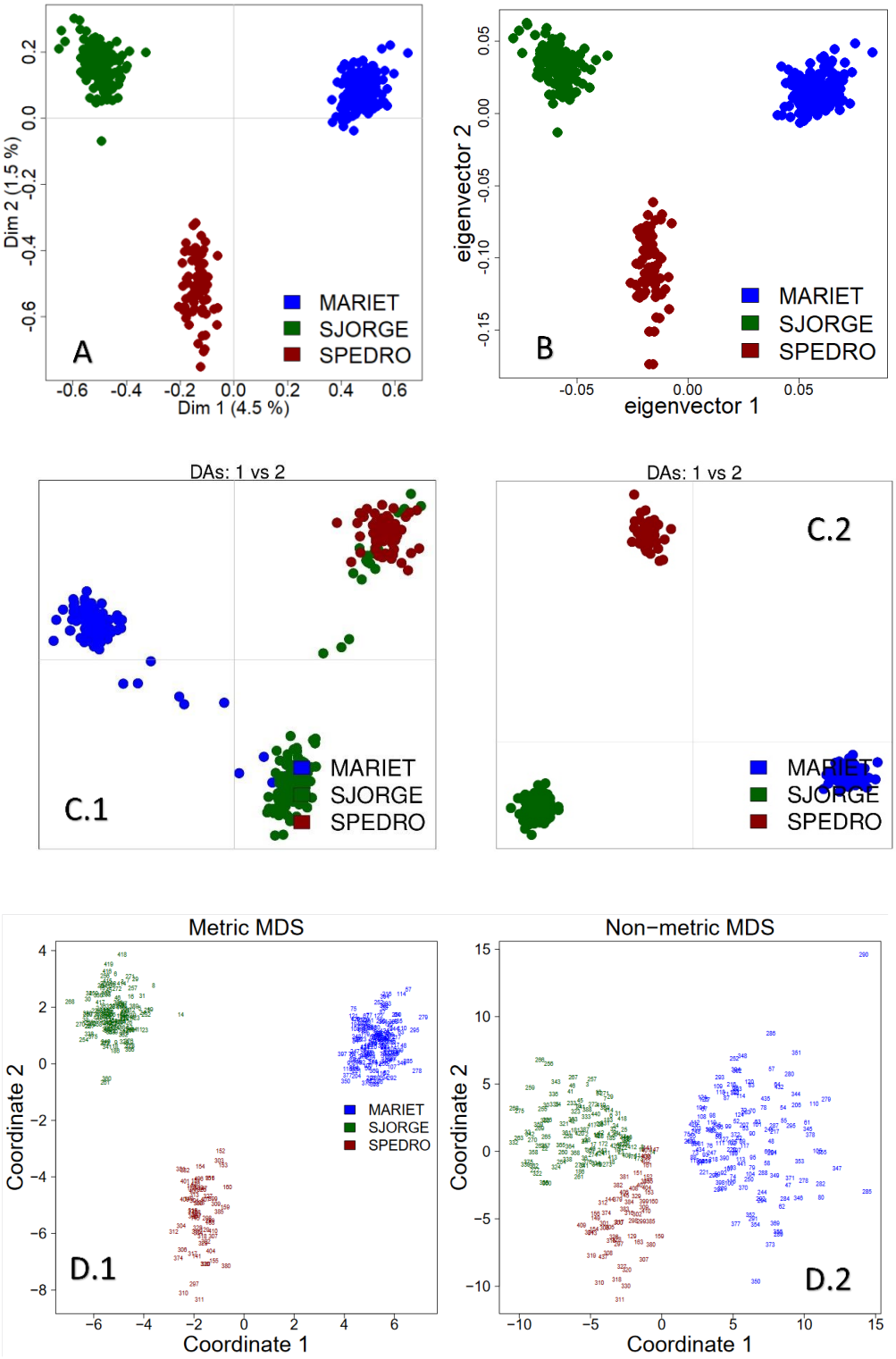


Fig S3.5. Various analyses showing similar patterns of population structure. A. Correspondence analyses (CA). B. Principal component analyses (PCA). C.1. DAPC analysis with prior population informationC.2. DAPC analysis without prior population information.D.1. & D.2. Multi-dimensional scaling (MDS)

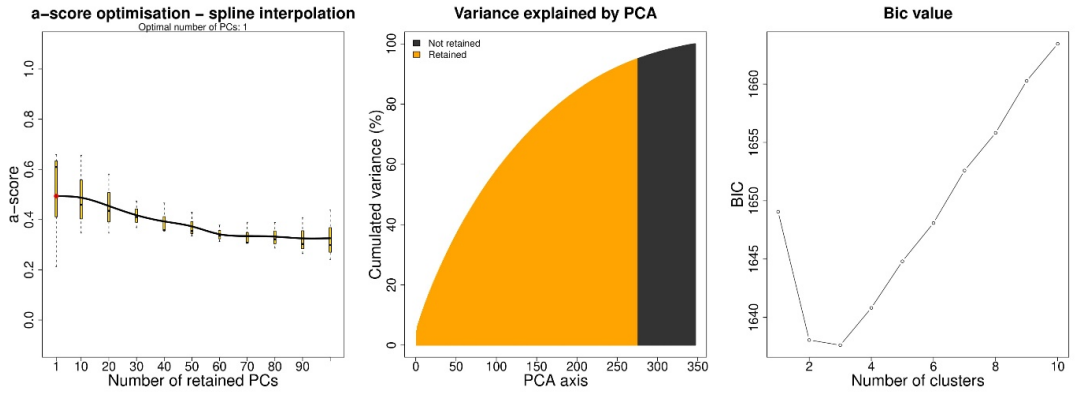


Fig S3.6. DAPC statistics. Support for optimal number of clusters for population structure.

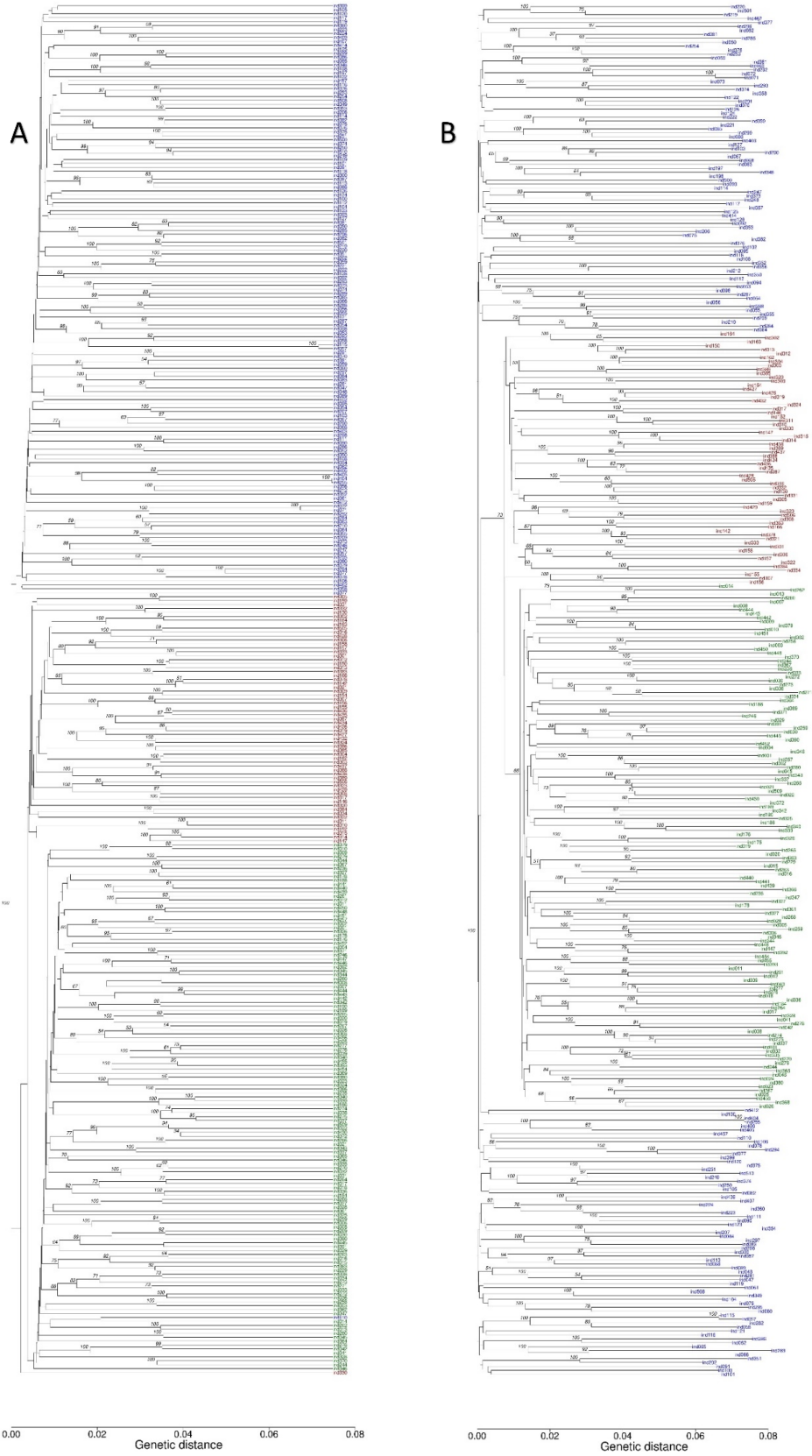


Fig S3.7. Phylogenetic tree reconstructions. A. Using the UPGMA method. B. Using the neighbour-joining method. Green=Marietas.

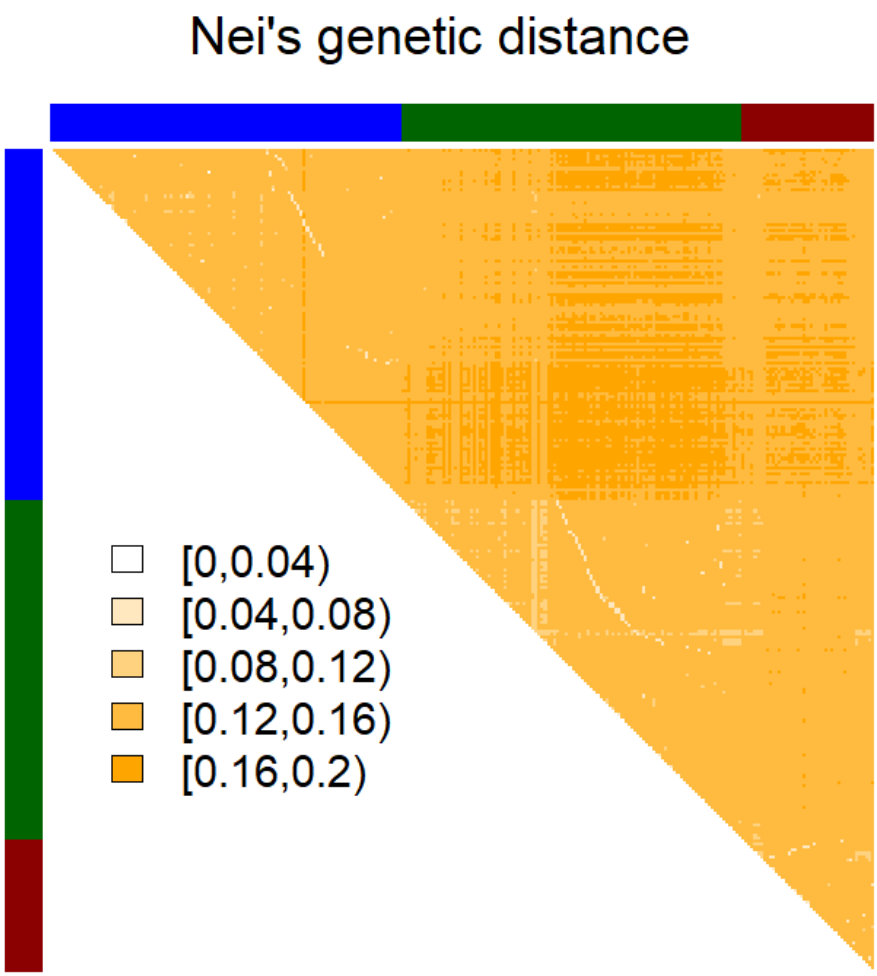


Fig S3.8. Nei's genetic distance between individuals. Genetic differentiation shown for individuals of Marietas (blue), San Jorge (green), and San Pedro (Brown).

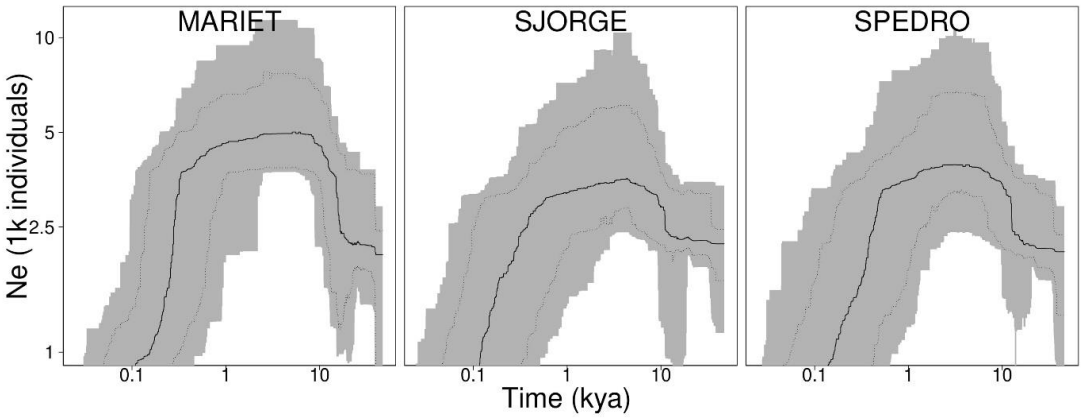


Fig S3.9. Scaled demographic reconstruction. Stairway plot showing the demographic history of the three colonies up to 15 kya. Time is shown in thousands of years ago.

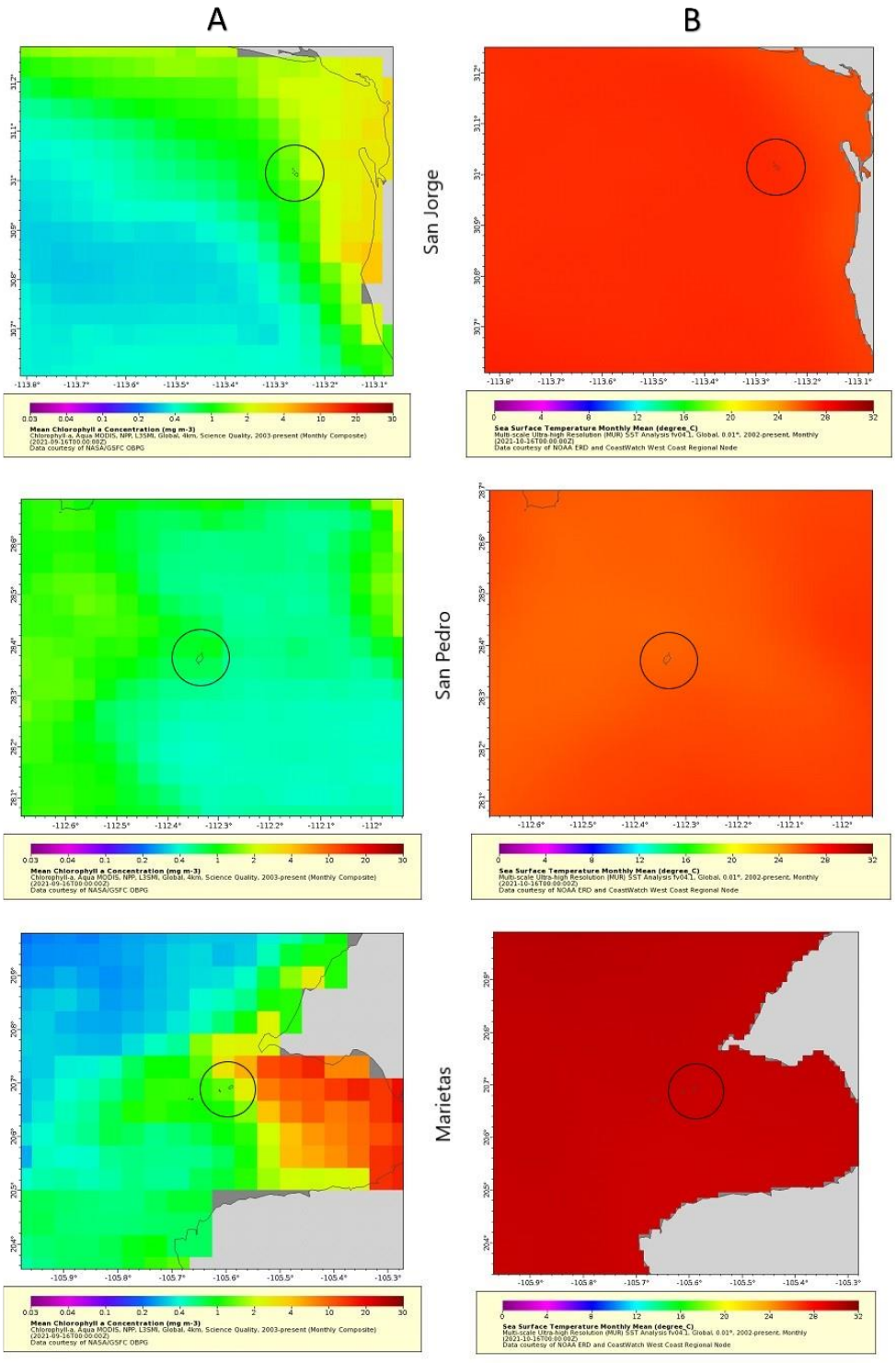


Fig S3.10. Maps of environmental variables in the three studied colonies. A. Primary Productivity. B. Sea Surface Temperature. 1. San Jorge. 2. San Pedro. 3. Marietas.

Appendix C: Supplementary material for chapter 4

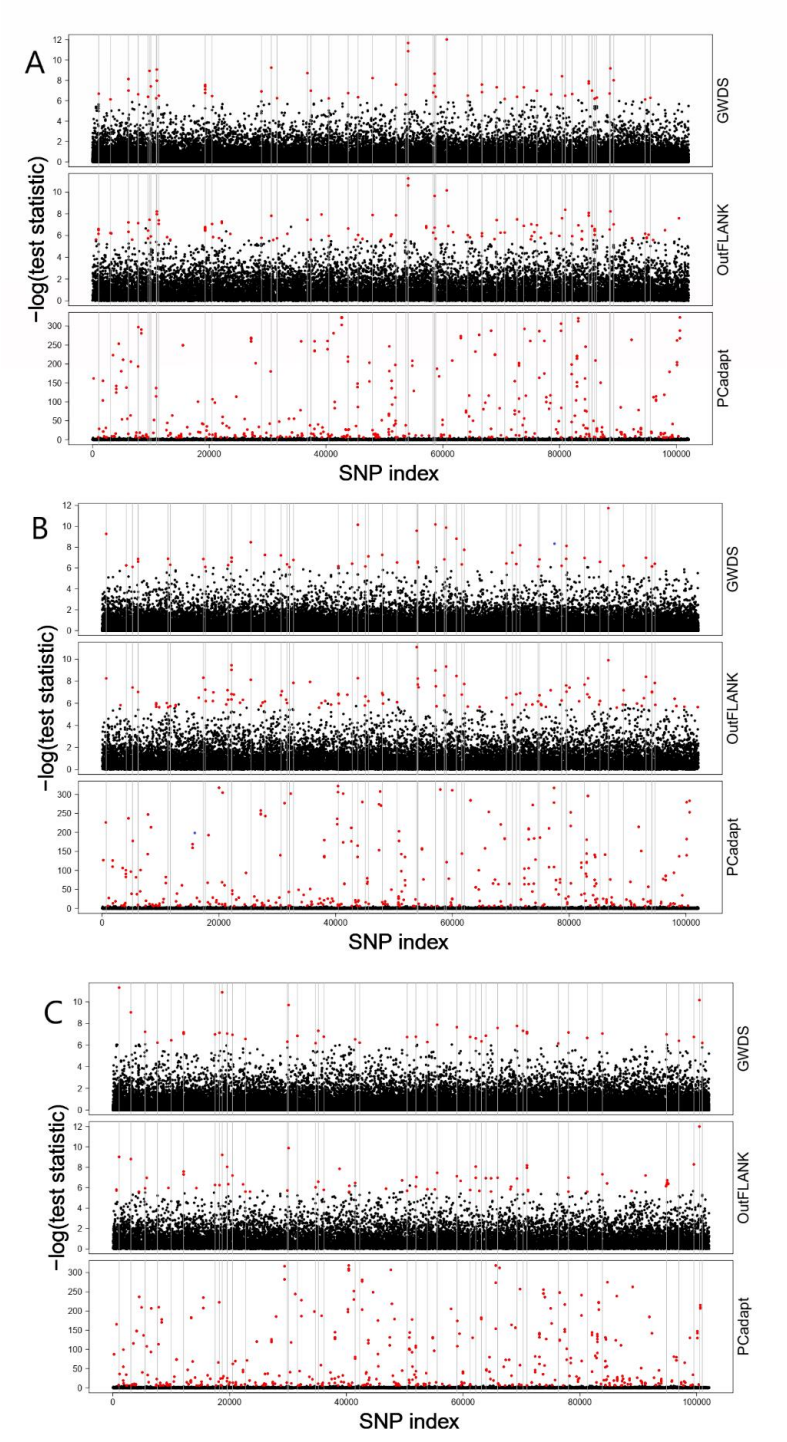


Fig S4.1. Genome Selection Scans to detect episodes of Local Selection. Manhattan plot showing the outlier SNPs in red which are significantly different from zero. Vertical lines represent outlier loci shared between different scans. A). Marietas vs San Jorge. B). Marietas vs San Pedro. C). San Jorge vs San Pedro.

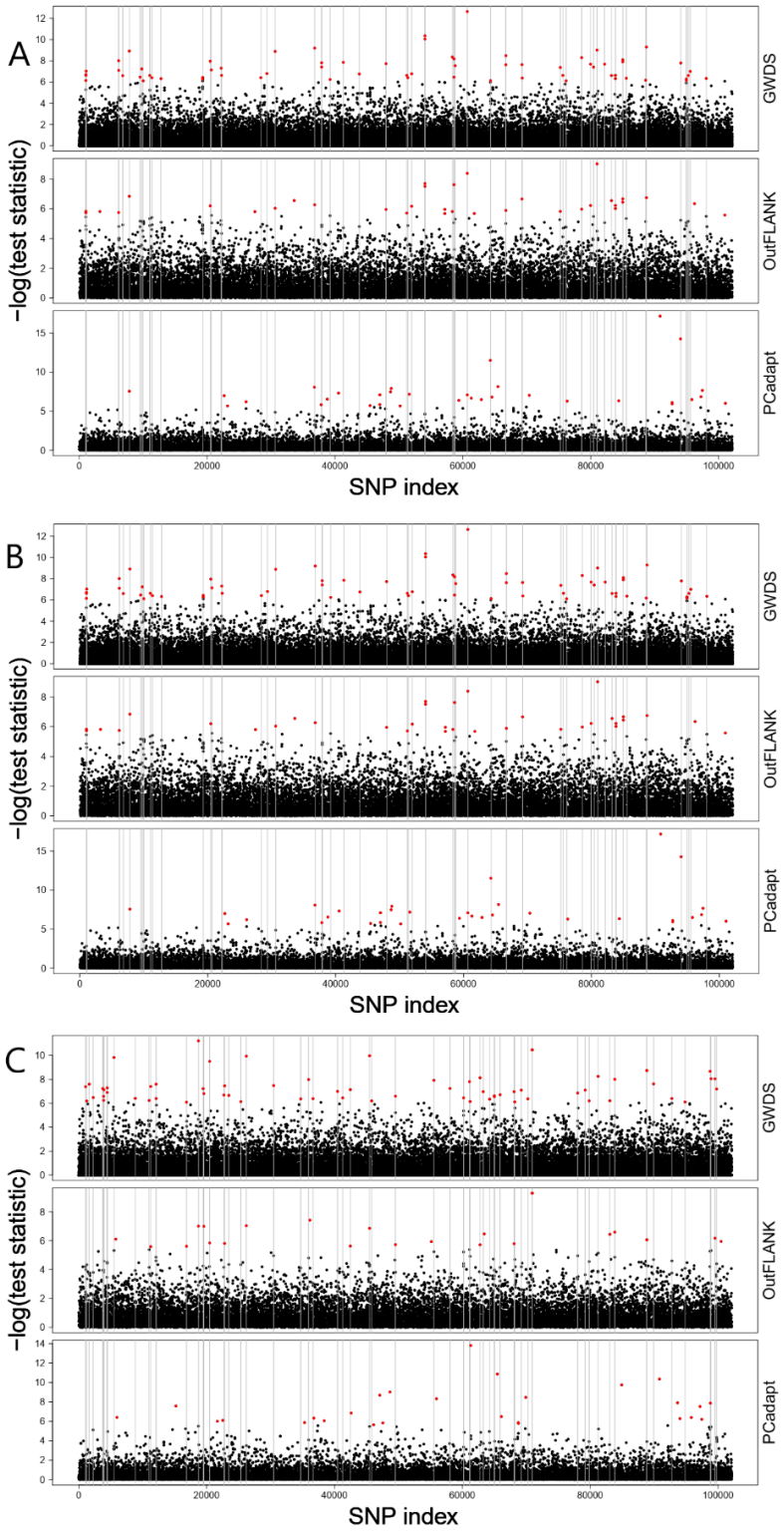


Fig S4.2. Genome Selection Scans to detect episodes of Sexual Selection in Males. Manhattan plot showing the outlier SNPs in red which are significantly different from zero. Vertical lines represent outlier loci shared between different scans A). Marietas vs San Jorge. B). Marietas vs San Pedro. C). San Jorge vs San Pedro.

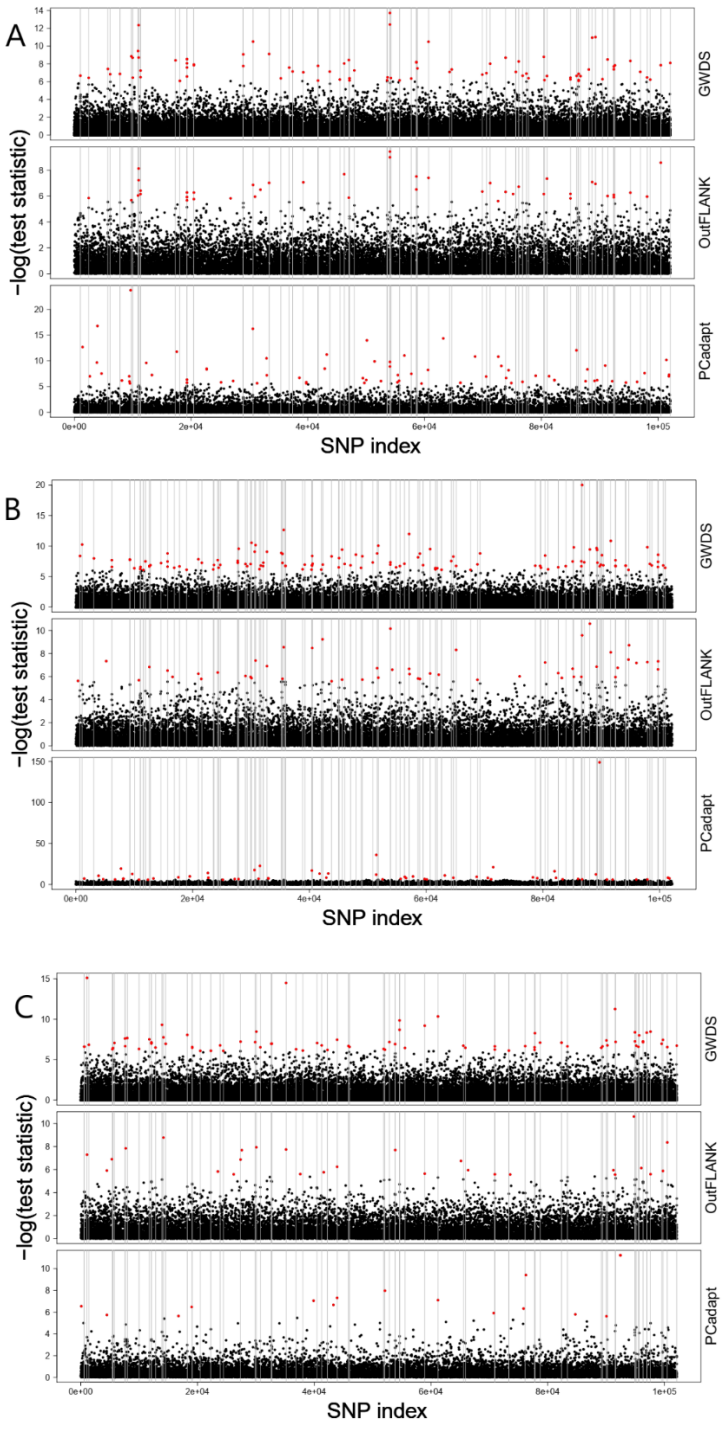


Fig S4.3. Genome Selection Scans to detect episodes of Sexual Selection in Females. Manhattan plot showing the outlier SNPs in red which are significantly different from zero. Vertical lines represent outlier loci shared between different scans A). Marietas vs San Jorge. B). Marietas vs San Pedro. C). San Jorge vs San Pedro.

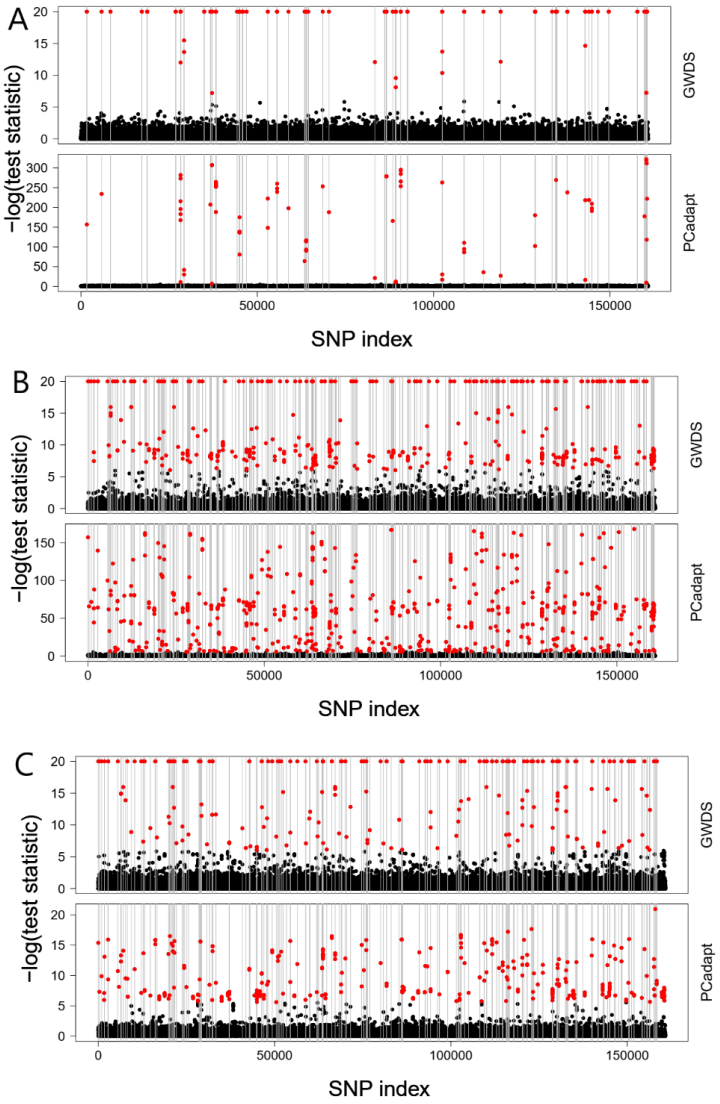


Fig S4.4. Genome Selection Scans to detect episodes of Gametic and viability Selection. Manhattan plot showing the outlier SNPs in red which are significantly different from zero. Vertical lines represent outlier loci shared between different scans A). Male vs Females (viability selection). B). Male vs Offspring (Male's gametic selection). C). Female vs Offspring (Female's gametic selection).

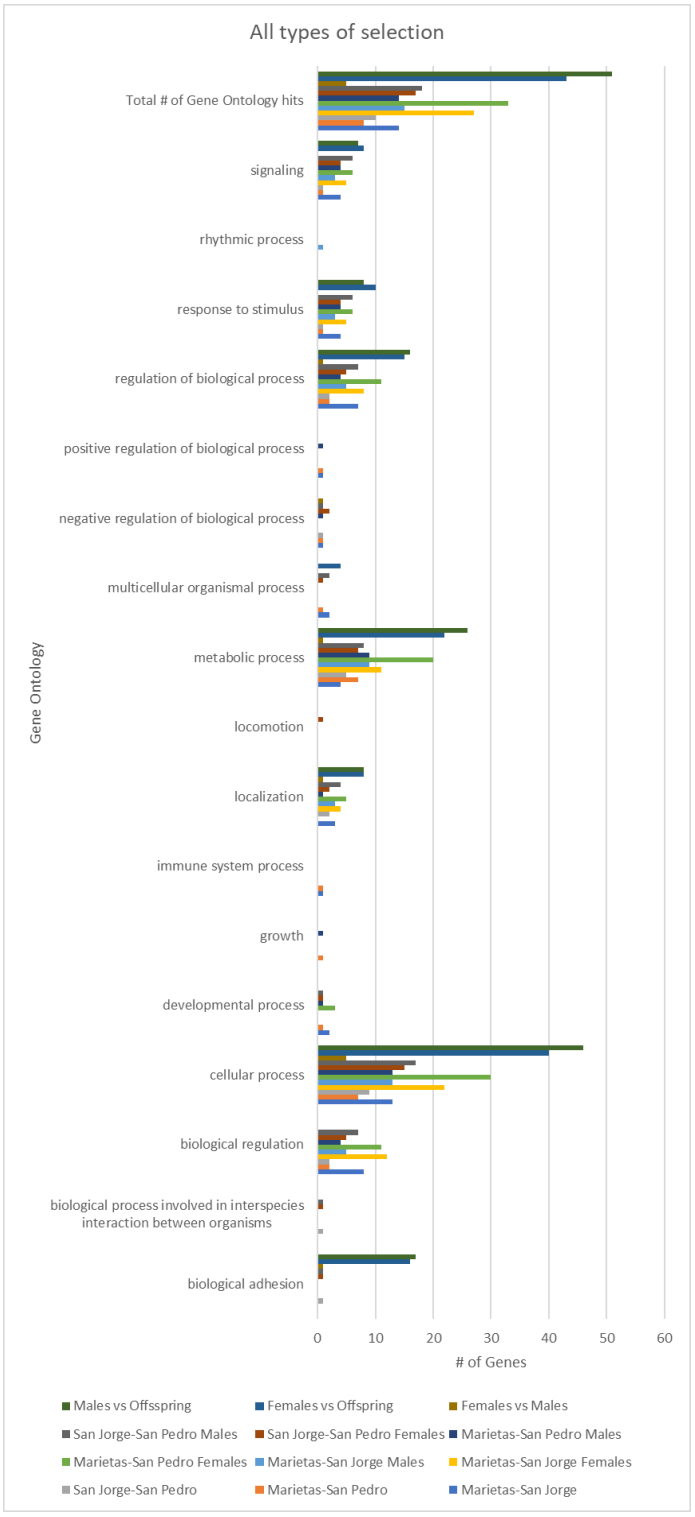


Fig S4.5. Gene ontologies describing the level 2 biological functions associated with all types of selection for comparison. Genes surrounding outlier loci comparisons for all individuals between different islands. The number of loci sequence with each gene ontology category are displayed in the bar chart and the total number of gene ontology hits for each comparison are also reported. Note that a single locus may belong to multiple gene ontology categories.

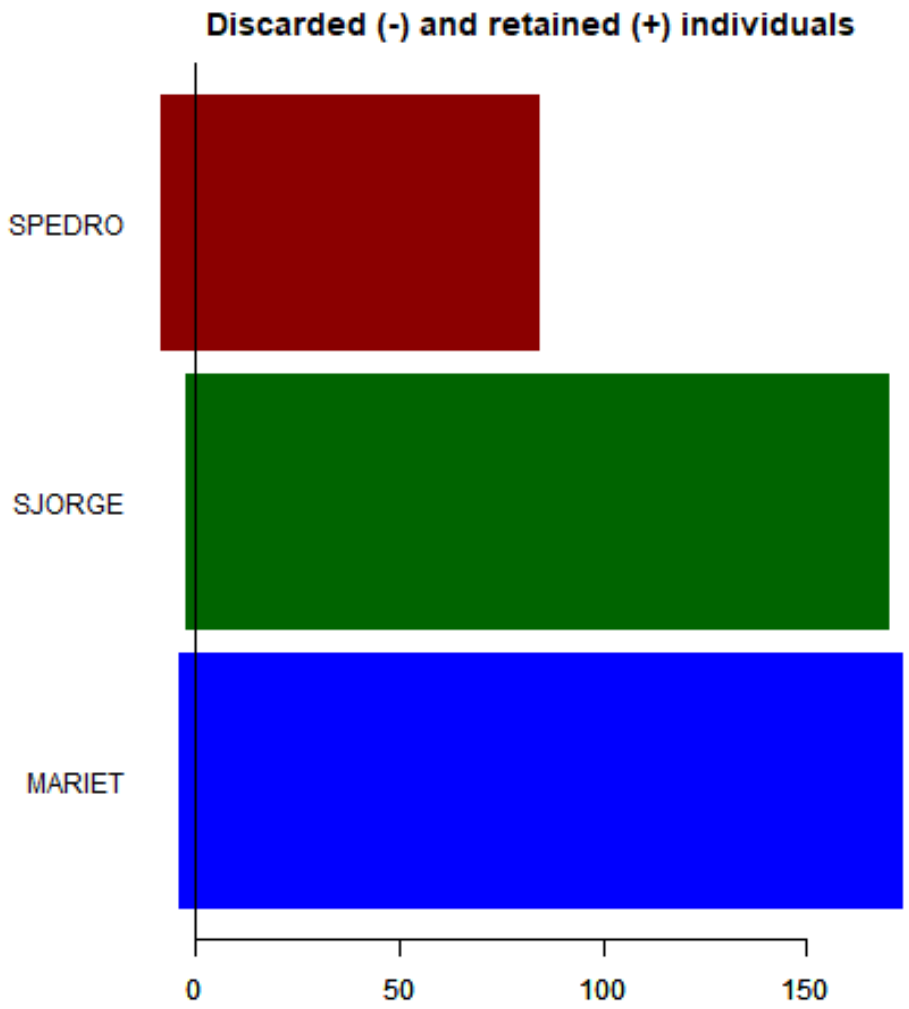


Fig S4.6. Proportion of retained individuals for the selection dataset. Bars before the 0 represent individuals that did not pass our quality filters.

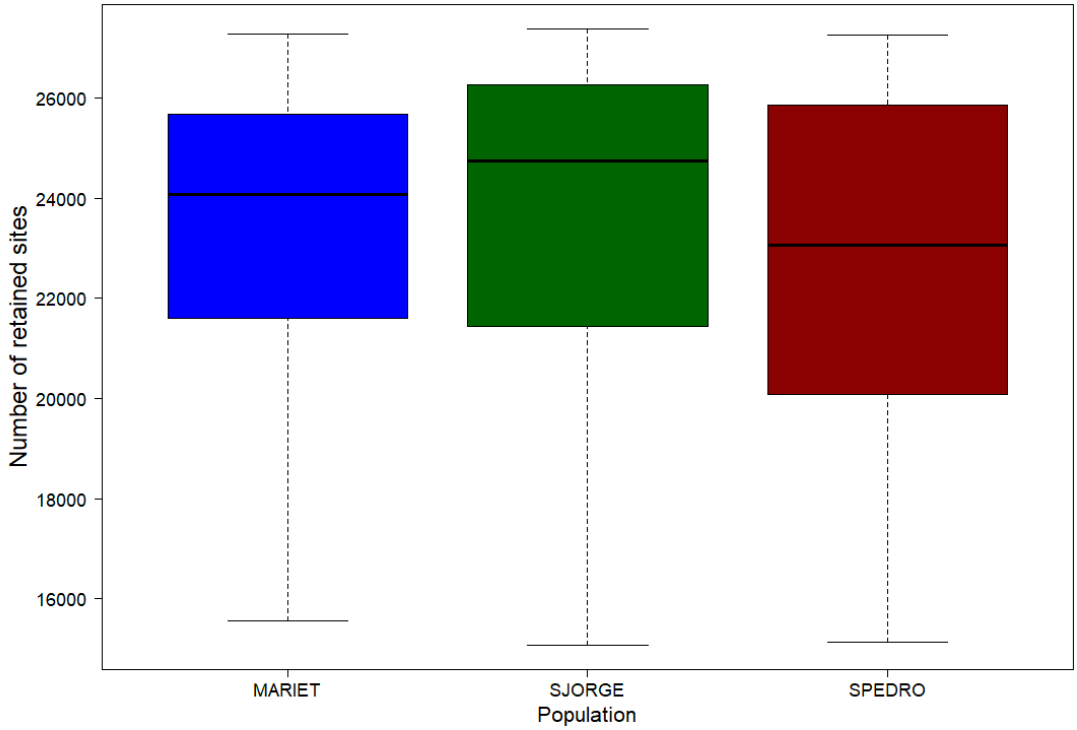


Fig S4.7. Number of retained SNPs per population in the selection dataset. Boxes represent the average of sites present in the sampled individuals.

Table S4.1. Total number of genes involved in biological functions in each episode of selection. Shaded numbers indicate underrepresented or absent biological functions in specific episodes of selection. Note that a single gene may belong to multiple gene ontology categories.

	Natural Selection			Sexual Selection (Males and Females)						Viability Selection	Gametic Selection	
GO Name	Marietas-San Jorge	Marietas-San Pedro	San Jorge-San Pedro	Marietas-San Jorge Females	Marietas-San Jorge Males	Marietas-San Pedro Females	Marietas-San Pedro Males	San Jorge-San Pedro Females	San Jorge-San Pedro Males	Females vs Males	Females vs Offspring	Males vs Offsspring
biological adhesion	0	0	1	0	0	0	0	1	1	1	16	17
biological process involved in interspecies interaction between organisms	0	0	1	0	0	0	0	1	1	0	0	0
biological regulation	8	2	2	12	5	11	4	5	7	0	0	0
cellular process	13	7	9	22	13	30	13	15	17	5	40	46
developmental process	2	1	0	0	0	3	1	1	1	0	0	0
growth	0	1	0	0	0	0	1	0	0	0	0	0
immune system process	1	1	0	0	0	0	0	0	0	0	0	0
localization	3	0	2	4	3	5	1	2	4	1	8	8
locomotion	0	0	0	0	0	0	0	1	0	0	0	0
metabolic process	4	7	5	11	9	20	9	7	8	1	22	26
multicellular organismal process	2	1	0	0	0	0	0	1	2	0	4	0
negative regulation of biological process	1	1	1	0	0	0	1	2	1	1	0	0
positive regulation of biological process	1	1	0	0	0	0	1	0	0	0	0	0
regulation of biological process	7	2	2	8	5	11	4	5	7	1	15	16
response to stimulus	4	1	1	5	3	6	4	4	6	0	10	8
rhythmic process	0	0	0	0	1	0	0	0	0	0	0	0
signaling	4	1	1	5	3	6	4	4	6	0	8	7
Total # of Gene Ontology hits	14	8	10	27	15	33	14	17	18	5	43	51

Table S4.2. Biological function associated with different types of selection. Description provided by Blast2Go (Gotz et al. 2008).

Biological function	Type of selection associated with function	Description of function
Biological adhesion:	Gametic-viability slection in males and females	The attachment of a cell or organisms to a substrate, another cell or other organism. Includes intracellular attachment between membrane regions.
Biological process involved in interspecies interaction between organisms:	Natural local selection in San Jorge-San Pedro	Any process evolved to enable an interaction with an organism of a different species.> Response to other organisms: Any process that results in a change in stat or activity of a cell or an organism (in terms of movement secretion, enzyme production, gene expression, etc.) as a result of a stimulus from another living organism.
Biological regulation:	Mostly sexual selection in females and also males. Absent in gametic-viability selection	Any process that modulates a measurable attribute of any biological process, quality or function. > Regulation of biological process: any process that modulates the frequency, rate or ectent of a biological process. Biological processes ate regulated by many means; examples include the control of gene expression, protein modification or interaction with a protein or substrate molecule .
Developmental process:	Natural local selection in Marietas-San Jorge, sexual selection between San Pedro and other islands, but absent in viability-gametic selection).	a biological process whose specific outcome is the progression of an integrated living unit: an anatomical structure (which may be a subcellular structure, cell, tissue or organ), or organism over time from an initial condition to a later condition.> Anatomical structure formation involved in morphogenesis: The developmental process pertaining to the initial formation of an anatomical structure from unspecified parts
Growth:	Sexual selection in males between Marietas-san pedro	The increase in size or mass of an entire organism or a cell.
Immune system process:	Natural selection between Marietas and the other colonies	Any process involved in the development or functioning of the immune system, an organismal system for calibrated response to potential internal or invasive threats.
Locomotion:	Sexual selection in females from San Jorge-San Pedro	Self-propelled movement of a cell or organism from one location to another. Response to an external stimulus.
Positive regulation of biological process:	Natural selection between Marietas and the other islands, and sexual selection in males from Marietas-San Pedro	Any process that activates or increases the frequency, rate or extent of a biological process. Biological processes ate regulated by many means; examples include the control of gene expression. Protein modification or interaction with a protein or substrate molecule.
Rhythmic process:	Sexual selection in males from Marietas-San Jorge	Any Process pertinent to the generation and maintenance of rhythms in the physiology of an organism.

Table S4.3. Retained individuals per islands after filtering

	Marietas	San Pedro	San Jorge	Total
Males	65	25	56	146
Females	62	22	50	134
Chicks	46	37	64	147
Total	173	84	170	427

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