SCALE-SPECIFIC PROCESSES UNDERLYING THE GENETIC POPULATION STRUCTURE OF SEABIRDS IN THE TROPICAL WESTERN INDIAN OCEAN.

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ABSTRACT

Global seabird populations have declined by 70%, since 1950, largely in response to human mediated threats. Identifying the pressures that the remaining populations face has therefore become a top priority. Many breeding colonies are now monitored annually, though others have been almost completely neglected. Considerable bias also exists towards higher latitude species, while fewer studies have been conducted on tropical groups. Beyond tracking population sizes, numerous knowledge gaps also exist that severely restrict conservation efforts. This includes the understanding of seabird meta-population structure and the processes underlying population divergence. The importance of these studies lies in the fact that the preservation of biodiversity requires the conservation of diversification processes. Generating this knowledge is therefore an important first step towards recognising responses to episodic disturbance and long-term environmental change, as well as recovery potential. In this context, the present study employed microsatellite analysis and ringing information to investigate the processes underlying the metapopulation structure of seabirds in the tropical western Indian Ocean. Three species were selected as proxies to cover a range of population sizes, distributional ranges, and intrinsic behavioural (e.g. migratory behaviour) and morphological (e.g. polymorphism) characteristics. These were the Sooty Tern (*Onychoprion fuscatus*), Red-footed Booby (*Sula sula*), and Barau's Petrel (*Pterodorma baraui*). The overall objective was to provide insight into the mechanisms underlying divergence across a range of scales.

Microsatellite information highlighted that genetic populations of the Red-footed Booby and Barau's Petrel were weakly, though significantly, structured. For the Barau's Petrel, this was supported by ringing information that indicated extreme colony fidelity. Some gene flow appears to occur among the breeding colonies of the Red-footed Booby, though the scale and frequency of this remains uncertain as banding information is insufficient at this stage. Nevertheless, though populations of both species were genetically structured, the processes underlying divergence were different. Extreme natal philopatry appears to have driven divergence between the two colonies of the Barau's Petrel, while local selective forces (e.g. kleptoparasitism risk and/or selection against immigrants) appear to have isolated the three studied breeding colonies of the Red-footed Booby. Conversely, microsatellite information identified a total lack of genetic structure among breeding colonies of the Sooty Tern in the western Indian Ocean, and between colonies in the western Indian and Eastern Pacific Oceans. This accords with banding recoveries, which illustrate considerable inter-colony exchange of individuals among most islands of the Seychelles and between breeding colonies in the western Indian and West Pacific Oceans. The processes underlying the genetic population structure (or, in this case, lack thereof) in the Sooty Tern therefore appear to operate at extremely large scales. The species' low natal philopatry and high dispersal capabilities, combined with an importance of social stimulation and a reliance on seasonally favourable marine conditions, appears to influence the decisions of where and when individual Sooty Terns choose to breed. Anthropogenic disturbance at breeding sites, particularly that related to egg harvesting activities, also appears to drive dispersal in the Sooty Tern.

These results improve our understanding of the mechanisms underlying the genetic population structure in seabirds at low latitudes. However, numerous questions remain unanswered and warrant further study. Clear conservation implications were also identified for the three studied species. Nevertheless, caution should still be applied when extrapolating this information across other species.

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Seabird vulnerability, conservation status, and management priorities

Around 350 bird species, broadly including those from the Orders Sphenisciformes (i.e. penguins), Procellariiformes (i.e. petrels, albatrosses, and allies), Pelecaniformes (i.e. pelicans), Suliformes (i.e. boobies, frigatebirds, and allies), Phaethontiformes (i.e. tropicbirds), and Charadriiformes (i.e. terns, gulls, and allies), are mostly or entirely dependent on marine habitats for foraging (Brooke 2002; Schreiber & Burger 2002; Croxall *et al.* 2012). Referred to as seabirds – and collectively amounting to about 3.5% of all extant bird species (Croxall *et al*. 2012) – this group is one of the most biologically diverse of all avian assemblages. It includes a spectrum of species ranging from those that endure the Antarctic winter while breeding (e.g. the Emperor Penguin [*Aptenodytes forsteri*]; Le Maho 1977), to those that nest on deserted and exposed tropical islands (e.g. the Black-naped Tern [*Sterna sumatrana*]; Hulsman & Smith 1988). Feeding methods of seabirds are just as varied, including such unusual examples as species that strain plankton from the surface of the water using specialised bill lamellae (e.g. the Salvin's Prion [*Pachyptila salvini*]; Gartshore *et al.* 1988), groups that dive to around 500 metres (m) below in search of pelagic fish and krill (e.g. the Emperor Penguin; Le Maho 1977; Kooyman & Kooyman 1995), and those that plunge dive into water from great heights after mesopelagic fish (e.g. the Masked Booby [*Sula dactylatra*]; Machovsky-Capuska *et al.* 2016). Despite this diversity, all seabirds share one common trait. With the exception of the Sphenisciformes, seabirds are among the most aerial of all birds and can spend weeks, months, or (in some cases) even years, flying out at sea (e.g. the Wandering Albatross [*Diomedea exulans*]; Weimerskirch *et al.* 2006). Many penguins are also seasonal dispersers that spend months or years foraging at sea before sexual maturity is reached (e.g. the King Penguin [*Aptenodytes patagonicus*]; Pütz *et al.* 2014), or just subsequent to each breeding season (e.g. the Macaroni Penguin [*Eudyptes chrysolophus*]; Bost *et al.* 2009). Yet all seabirds must return to a few isolated breeding colonies to reproduce, at which point adults are constrained by the energy demands of their chicks (Weimerskirch *et al.* 1993; Schreiber & Burger 2002). This 'central place foraging,' within the expansive marine environment, demands unique behavioural, demographical, physiological, and morphological adaptations (Weimerskirch *et al.* 1993; Schreiber & Burger 2002). Notably, seabirds exhibit dramatically different life-history and demographic characteristics from most land birds (Schreiber & Burger 2002). Some authors have even gone so far as to describe seabirds as extreme, in that most breed synchronously in vast colonies (often amounting to millions of individuals; Feare 1976a, b), have extended longevity (20 to 60 years; Clapp & Sibley 1966), delayed sexual maturity (breeding age sometimes delayed until 10 years of age; Weimerskirch 1992), low reproductive output (in many cases one egg per year or every second year; Weimerskirch 1992), and lengthy chick-rearing periods (up to a year in some species; Weimerskirch *et al.* 1993). Passerine birds, by contrast, typically have much shorter lives (three to five years), larger annual reproductive outputs (eight to 10 eggs per clutch in some species), and greatly reduced chick-rearing periods (usually less than 30 days; Tinbergen 1987; Hamer *et al.* 2002; Schreiber & Burger 2002). Together these traits render seabirds especially vulnerable to drastic temporal changes in marine and terrestrial ecosystem state (Hamer *et al.* 2002). For example, over 95% of seabirds are highly synchronous colonial breeders though coloniality renders these birds susceptible to complete breeding failure in the event of episodic prey shortages, extreme environmental conditions (e.g. El Niño-Southern Oscillation [ENSO] events; Surman & Nicholson 2009), and thermal stress (Baird 1990; Iglesias-Vasquez *et al.* 2017). That said – the life-history attributes of seabirds are such that their populations are robust in terms of inter-annual variations in breeding success, but highly sensitive to slight changes in adult mortality. Poor reproductive output must be long term and extensive to decrease populations, yet even slight increases in adult mortality have lasting effects (Montevecchi 2002).

Investigations into the fossil record indicate that seabird extinction has been relatively rare in comparison with other avian groups (Steadman & Olson 1985; Steadman 1995; Steadman 2006). However, some 28% of the 346 extant seabird species are now considered threatened with extinction with 5% in the highest category of critically endangered and a further 10% considered near threatened (Croxall *et al.* 2012). This exceeds the vulnerability of all other comparable groups of birds: 26% of Psittacidae (i.e. parrots; 374 species), 19% of Columbidae (i.e. pigeons/doves; 318 species), and 18% of Accipitridae (i.e. raptors; 238 species) are listed in one or another threat category on the International Union for Conservation of Nature (IUCN) Red List, whilst 12% or less of all other bird families are considered threatened (Croxall *et al.* 2012). These statistics mirror global patterns of biodiversity loss with between 10% and 50% of well-studied species currently identified as being at risk of extinction (Paleczny *et al*. 2015). Unsurprisingly then, one of the most important current conservation challenges is to maintain or improve the status of the many globally threatened species, including that of seabirds. This demands knowledge of their overall biology and the identification of population-specific management priorities. Salafsky *et al.* (2008) broadly classified management priorities for global biodiversity as follows: the need for on-going monitoring, the control of invasive species, breeding site discovery and enhanced site protection, improved legislation and best-practice standards, education for increased public awareness and support, stringent harvest management protocols, reintroductions and habitat control to promote species recovery, assessment of the potential impacts of global warming, and generating knowledge of the genetic diversity within as well as an understanding of the nature of gene flow among populations. Considering the broad spectrum of threats that seabird species face (reviewed in Chapter 1.5), and their overall conservation status (Croxall *et al.* 2012), these general actions are especially applicable for seabirds.

Seabirds have become exceptionally well-studied within the last decade and knowledge of their overall conservation status is now considered more comprehensive than for any other group of marine organisms (Croxall *et al.* 2012). However, Paleczny *et al.* (2015) assessed population trends for the world's seabirds and noted that as little as 19% of the global seabird population had been monitored more than five times between 1950 and 2010. Most colonies in the northwest Atlantic, southwest Pacific, Indian Ocean, and polar regions have been almost completely neglected. In recognising a similar issue, Lewison *et al*. (2012) identified six research themes that require additional attention to optimise management strategies for global seabird populations. These topics included basic aspects of at-sea spatial ecology, trophic dynamics, community roles of seabirds, direct and indirect impacts of fisheries, population responses to environmental variability, and management of anthropogenic impacts. Particular emphasis was also placed on the subpopulation dynamics of seabird species, including aspects of colony connectivity. Of these topics, the need to understand seabird population dynamics was recognised as one of the most important in that it has such profound implications for conservation and management.

1.2. Importance of genetics to conservation

Effective decision making is crucial in this era when humans govern the likelihood of a species' survival. The importance of conservation genetics, a relatively new field of molecular ecology, is thus being increasingly recognised within the scientific community since it employs sensitive genetic markers to explore the effects of contemporary changes in genetic structure on the long-term survival of a species (Frankham *et al.* 2002; Wan *et al.* 2004). The field aims to preserve species as dynamic entities, capable of coping with environmental and anthropogenic change and disturbances (Frankham *et al.* 2002; Wan *et al.* 2004). This demands the recognition of basic speciation concepts since the preservation of biodiversity ultimately requires the conservation of these diversification processes (Frankham *et al.* 2002; Wan *et al.* 2004). Conservation genetics broadly encompasses the management of genetic diversity in small populations, the resolution of taxonomic uncertainties, the definition of management units within species, and the use of molecular genetic analyses in forensics and the understanding of species biology (Frankham *et al.* 2002). In addition, because endangered species also often have small or declining populations and since small populations are more susceptible to inbreeding and the associated loss of genetic diversity resulting in elevated extinction risks, a major concern in conservation genetics is the effect of small population size (Frankham *et al.* 2002). It is in this context that the field of population genetics, often considered one of the most important components of conservation genetics, focusses on the mechanisms through which evolutionary changes occur by means of population connectivity and isolation (Wan *et al.* 2004). In this context, populations are defined as units with genetic panmixia (Stow & Magnusson 2012). The broad aim of this is to provide a greater understanding of the effects of various forces, which result in evolutionary changes, in species over time (Wan *et al.* 2004). Thus, whilst conservation genetics typically emphasizes the importance of genetic structuring in preserving species as separate entities, population genetics emphasizes the roles that different forces have played towards contemporary genetic structure (Frankham *et al.* 2002; Wan *et al.* 2004). Both are vitally important and have implications for conservation. Since genetic diversity is profoundly linked to resilience, knowledge of population connectivity and meta-population structure are critical first steps towards recognising population/species responses to episodic disturbance and/or environmental unpredictability, as well as their recovery potential (Morris-Pocock *et al.* 2016). Assuming local populations differ genetically, the loss of even a single population may result in the loss of important genetic variation, including local adaptations (e.g. timing of breeding), that might ultimately affect the species' ability to adapt to changing conditions and possibly also to speciate (Frankham *et al.* 2002; Wan *et al.* 2004).

Speciation is a fundamental process responsible for the generation of biodiversity on Earth (Fitzpatrick *et al.* 2009). Accepting a Biological Species Concept, speciation is considered complete upon the evolution of reproductive isolation among populations (Fitzpatrick *et al.* 2009). As a function of population connectivity, speciation operates at two levels. The ecological connectivity of populations involves the movement of individuals in sufficient numbers to allow colony interchange including colonisation, while evolutionary connectivity serves only to maintain gene flow among populations and therefore requires the exchange of far fewer individuals (as little as one migrant per generation is sometimes required to prevent divergence among populations due to genetic drift; Frankham *et al.* 2002; Noreen 2010). Both are influenced by a suite of intrinsic (pre- or post-zygotic) and extrinsic barriers to dispersal (reviewed in Friesen *et al.* 2007). Differing selective forces within a species' range often lead to significant adaptive variances. Understanding these features of connectivity therefore provides insight into a species' ability to recolonise abandoned breeding sites, through ecological connectivity, and to adapt genetically to changing conditions, through evolutionary connectivity. Unsurprisingly this topic, the importance of gene flow during population divergence, has become a major focus of modern speciation research (Via 2009).

It is also on this basis that conservation and population genetics have been recognised for their ability to define the appropriate scale of monitoring or managing populations/species, providing means for the identification of individuals in non-natal breeding colonies, and to test for dramatic changes in effective population size, connectedness, and divergence over time (reviewed in Wan *et al.* 2004). Indeed, the study of intraspecific population genetic differentiation is an effective method with which to study the early phases of population divergence and speciation. This type of research, notably, allows for the identification of Evolutionary Significant Units (ESUs) and Management or Functional Units (MUs; Moritz 1994; Wan *et al.* 2004; Palsbøll *et al.* 2006; Robertson *et al.* 2014). This approach to conservation was developed to provide an objective strategy to prioritising units for conservation, at both the species and sub-species levels, since losses to local genetic diversity may outweigh the significance of losses to population size (Moritz 1994; Palsbøll *et al.* 2006). ESUs, which sometimes encompass multiple MUs, are recognised to partition genetic diversity and assess conservation value. They are identified as populations characterized by significant phylogenetic divergence (Moritz 1994; Wan *et al.* 2004; Robertson *et al.* 2014), and are consequently considered synonymous with the term 'subspecies' since ESUs demonstrate highly restricted gene flow with other lineages within the higher organizational species level (Moritz 1994; Wan *et al.* 2004). MUs, by contrast, are demographically independent populations that have distinct allele frequencies, regardless of the level of genetic divergence (Moritz 1994; Wan *et al.* 2004; Palsbøll *et al.* 2006). They represent contemporary structuring, rather than historical divergences, and need not demand detailed genetic management but indicate that populations should be treated separately (Moritz 1994; Palsbøll *et al.* 2006). According to such genetic information, best practice management strategies can be implemented at the appropriate scales to slow or halt the decline of threatened species (Moritz 1994; Wan *et al.* 2004; Palsbøll *et al.* 2006).

1.3. Using molecular methods to study population differentiation in seabirds

Contemporary research has revealed a profound influence of the frequency and scale of dispersal, here defined as movement of individuals among breeding sites, on the demographic and evolutionary trajectories of animal populations (Kisel & Barraclough 2010; Morales *et al.* 2010; Yamamichi & Innan 2012). Dispersal represents the fundamental basis of gene flow and influences individual fitness, with resultant significant consequences for population differentiation, resilience, and viability (Kisel & Barraclough 2010; Morales *et al.* 2010; Yamamichi & Innan 2012). Sparsely distributed populations, such as those on islands, are expected to converge towards panmixia given regular dispersal among them. In such instances, kin competition is reduced within populations thereby increasing the overall population fitness (Morales *et al.* 2010). At intermediate levels, dispersal influences subpopulation dynamics through the effects of natural selection, mutation, and genetic drift (Morales *et al.* 2010). Genetic drift, or the random change in allele frequencies between generations (Wright 1937), is one of the main mechanisms driving divergence among animal populations and has its most profound impact when dispersal is low and population sizes are limited (Friesen *et al.* 2007). The random frequency change of alleles associated with genetic drift can lead to the extinction of alleles, thus lowering the amount of genetic variation found in populations with genetic drift compared to those without genetic drift (Star & Spencer 2013). In this sense, an animal's dispersal ability (or lack thereof) is thought to reflect some form of adaptive evolution and hence a proper understanding of the demographic and evolutionary processes within a population requires knowledge of the extent to which it interacts with others. Seabirds, in particular, have an ability to forage over extraordinarily long distances at sea where they encounter few physical barriers to dispersal. Their flight potential also implies that the probability of individual birds encountering, and therefore remaining to reproduce in, non-natal populations is greatly elevated (Friesen *et al.* 2007). Yet seabirds represent something of a paradox to genetic theory. Their extreme vagility implies that individuals can disperse freely among breeding colonies, maintaining high levels of gene flow, yet strong breeding and natal philopatry (combined with other extreme reproductive characteristics) frequently result in pronounced genetic differentiation among their populations (Friesen *et al.* 2007; Friesen 2015; Coulson 2016).

The field of population studies has developed dramatically in recent years, owing to technological advances. Dispersal in seabirds was traditionally estimated using capture-mark-recapture studies (Weimerskirch *et al.* 1985; Lebreton *et al*. 2003). However, for most species, this approach was impractical in that unrealistic effort usually only offers recovery information of very limited resolutions (Fiedler 2009). Access to isolated breeding sites of seabirds is also often restrictive. To illustrate these points, Weimerskirch *et al.* (1985) noted a recovery rate of only 0.31% among nearly 62 000 banded seabirds in the French Austral and Antarctic territories. Though recovery rates do vary among taxonomic groups, and in relation to the distribution of effort, Weimerskirch *et al.* (1985) recorded a maximum recovery rate of only 1.06% in the Brown Skua (*Stercorarius antarcticus*; n = 1 040). Nevertheless, any resightings of banded birds in non-natal breeding colonies provides direct evidence for immigration, but this is dependant on knowing whether or not these individuals contribute to the new population in a reproductive manner. Animal-borne tracking devices (e.g. Argos satellite global positioning system [GPS] trackers) have, more recently, been used to study the long-distance movements of seabirds (Fiedler 2009). However, this work almost never offers insight into gene flow among breeding colonies in that these studies often focus on birds that are already engaging in, or are just completing, a breeding attempt (Fiedler 2009). Such studies on non-breeding or pre-mature birds are also limited in that the recovery of tracking devices is often necessary to secure any information collected (Fiedler 2009). In the last two decades, molecular techniques have been increasingly employed to monitor seabird dispersal and to assess the demographic and evolutionary trajectories of their populations (Friesen 2007; Taylor & Friesen 2012). Genetic approaches such as mitochondrial, and mini- and microsatellite deoxyribonucleic acid (DNA) markers have provided important insights that have critically affected management decisions for threatened species or populations (Friesen 2007; Abdul-Muneer 2014).

DNA is found within both the nucleus (nuDNA) and mitochondria (mtDNA) of animal cells. The mtDNA genome, which is maternally inherited and does not undergo recombination, comprises a circular 'chromosome' that typically consists of 36 or 37 genes in verterates. Two of these are ribonucleic acids (RNAs), 22 are transfer RNA (tRNAs), and 12 or 13 are subunits of multimeric proteins of the inner mitochondrial membrane (Wan *et al.* 2004; Figure 1.1). There is an additional noncoding sequence termed the mitochondrial control region (mtDNA CR), which plays a vital role in replication and transcription. It is subdivided into three domains that differ in base composition and their rate and mode of evolution (Wan *et al.* 2004; Figure 1.1). Exons within the mtDNA genome are tightly packed, with no spacing between the introns. mtDNA is also histone-free, has a limited repair ability, and therefore has a relatively high inter-generational mutation fixation rate though the rates of evolution are variable among different regions of the genome (Wan *et al.* 2004). The 12s RNA region of mtDNA is highly conserved and has been used to illustrate phylogeny of higher categorical levels such as in phyla and subphyla, while the 16s RNA has a higher mutation rate and is used in phylogenetic studies at mid-categorical levels such as among families or rare genera (Hwang & Kim 1999; Wan *et al.* 2004). The protein-coding regions of mtDNA (i.e. Cytochrome *b*, NADH Dehydrogenase, Cytochrome Oxidase, and ATP Synthase subunits) have quicker evolution rates than the RNA regions and are considered powerful markers for inferring evolution history in lower categorical levels such as among families, genera, and species (Hwang & Kim 1999). The mtDNA CR is often the most useful mtDNA marker and is the only region that can be used for intraspecific studies of population differentiation, in that the two domains flanking the central sequence region (termed domains I and II) are hypervariable in base substitutions and indels (Hwang & Kim 1999; Wan *et al.* 2004). Nevertheless, these sites have limited base substitution variation among individuals of the same species implying that contemporary changes to genetic variation are frequently left with little trace (Wan *et al.* 2004). The use of mtDNA is further limited by its uniparental inheritance, and in that it consists of a single nonrecombinating 'super-gene' (Wan *et al.* 2004). As such, the most common applications of mtDNA markers in seabird studies are in taxonomic clarification among species and understanding divergence among deeply rooted populations where it is most commonly used in combination with nuDNA markers (e.g. Avise *et al.* 2000; Burg & Croxall 2001; Welch *et al.* 2011; Genovart *et al.* 2013; Iglesias-Vasquez *et al.* 2017). The focus of conservation and populations genetics is on contemporary genetic structuring, depending on both maternal and paternal variation, therefore demanding DNA markers that have higher evolutionary rates and wider application than mtDNA (Frankham *et al.* 2002; Wan *et al.* 2004).

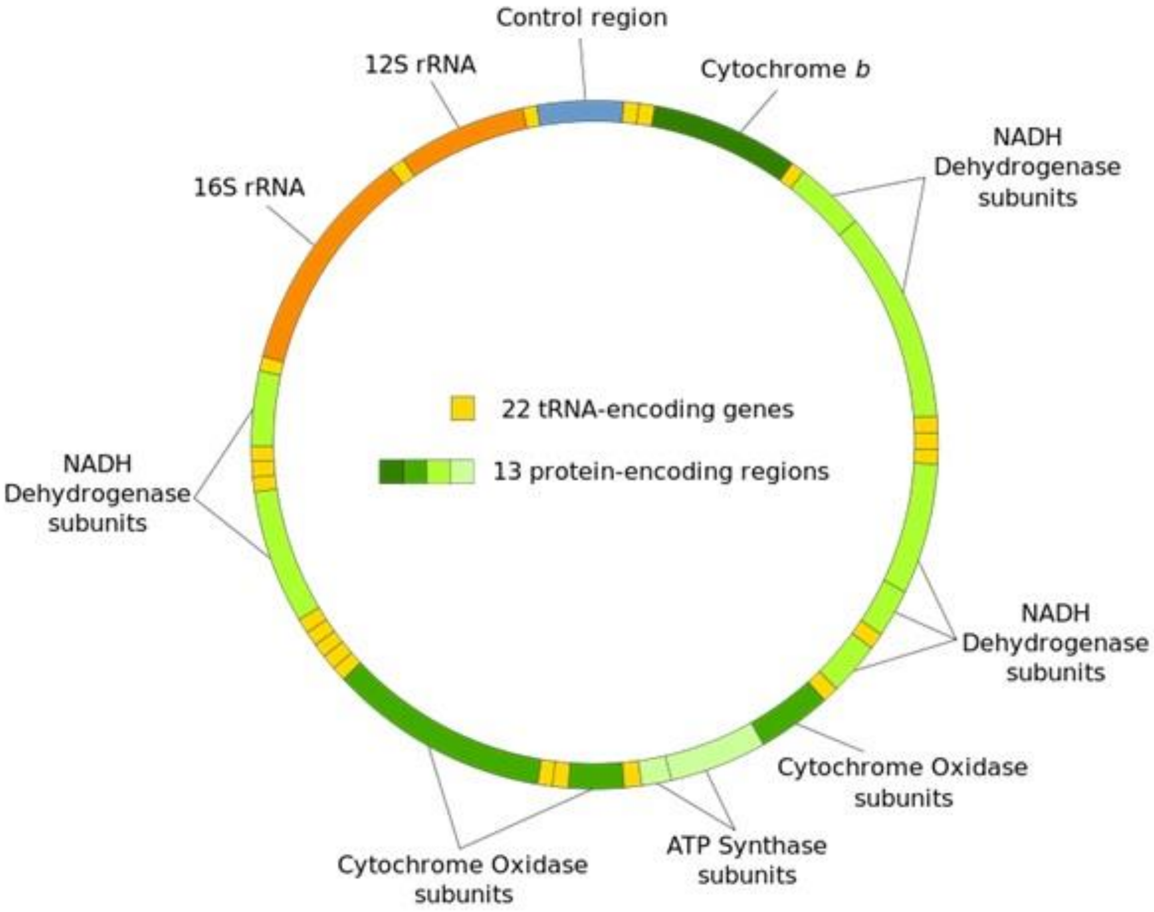

Figure 1.1: Structure of the vertebrate mitochondrial DNA genome, highlighting major regions employed in studies of mtDNA (Source: https://en.wikipedia.org/wiki/Mitochondrial_DNA).

Variable number of tandem repeats (VNTR) analysis techniques include nuclear DNA micro- and minisatellites, both of which are characterized by a high degree of length polymorphism (Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015; Figure 1.2). The VNTR loci in both mini- and microsatellites exhibit mutation rates several orders of magnitude higher than those of other DNA sequences (including mtDNA). They are therefore suited to analysing recent changes to genetic structure (Wan *et al.* 2004; Abdul-Muneer 2014; Thashi Bharadwaj & Grace 2015). Minisatellites are tandemly repeated motifs, interspersed in telomeric regions of the nuclear genome, of eight to 100 base pairs (bp) that repeat between two to several hundred times at each locus. Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are randomly scattered throughout the nuclear genome but frequently appear in transcription units (Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015; Figure 1.2). They are common repeated sequences of one to six bp repeat motif units (Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015; Figure 1.2). The differences both in length of repeat unit, and in number of tandem repeats, determines the different applications and limitations for the two kinds of VNTR markers. Their applications are broadly similar, however (Wan *et al.* 2004). The flanking regions of VNTR repeat sequences are conservative, while the repeat motifs are highly variable among individuals of the same species (Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015). Locus specific oligonucleotide primers, based on the conserved sequences flanking the mini- or microsatellite, are therefore used to amplify the core repeat sequences by way of polymerase chain reaction (PCR; Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015; Figure 1.2). Genetic polymorphisms among individuals/populations can then be detected via electrophoresis or can be size-fractioned using specialized DNA analysers (Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015). The advantages of using VNTR markers in population and conservation genetic research are diverse and include a requirement of low quantities of DNA (10 – 100 nanograms [ng]), their co-dominant nature which allows for the identification of homozygote and heterozygote individuals, the high accuracy and reproducibility, and the fact that different loci can rapidly be multiplexed in PCR (Wan *et al.* 2004; Abdul-Muneer 2014; Thashi Bharadwaj & Grace 2015). They often show such high allelic diversity that, even with a small number of loci and a large sample size, all individuals will have a unique multilocus genotype (Wan *et al.* 2004). However, microsatellites only sample a very small portion of the genome and suffer from a number of other limitations that relate to their complex pattern of evolution. Oligonucleotide primers for VNTR analysis are often genus or family specific, for example, and heterozygotes can be misclassified when null-alleles occur because of mutations in the primer annealing sites (Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015). Stutter bands may also complicate accurate scoring of polymorphisms, in that mutations occur in a stepwise fashion (Hardy *et al.* 2003; Wan *et al.* 2004; Thashi Bharadwaj & Grace 2015). Despite these disadvantages, mini- and microsatellite markers are still considered the most useful tools for the investigation of genetic variation within species (Wan *et al.* 2003; Thashi Bharadwaj & Grace 2015). Microsatellites, in particular, are the preferred marker for assessing the relationships among populations of seabird species (e.g. Burg & Croxall 2001; Friesen *et al.* 2006; Quillfeldt *et al.* 2017).

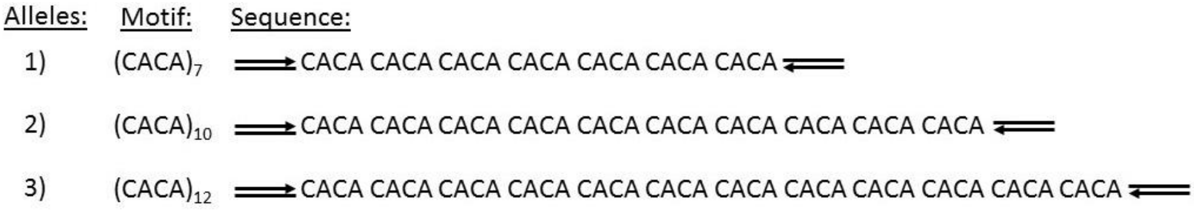

Figure 1.2: Structure of VNTR markers, in this case microsatellites, used in ecological and genetic studies. Arrows represent the conserved forward and reverse flanking sequences used for oligonucleotide primer development (adapted from Thashi Bharadwaj & Grace 2015). Nucleotides are abbreviated as follows: C = cytosine, A = adenine.

1.4. Processes underlying seabird population genetic structure

The barriers to gene flow and mechanisms underlying genetic population differentiation, or speciation, in birds are now relatively well understood. That said, these processes are far better known from terrestrial bird assemblages than for seabirds and the barriers to gene flow in marine systems (including seabirds) mostly differ from those in terrestrial ecosystems (Friesen *et al.* 2007; Friesen 2015). Several recent reviews identified a number of factors/processes that influence population differentiation in seabirds (Friesen *et al*. 2007; Friesen 2015; Munro & Burg 2017). The most important mechanism through which seabird populations diverge seems to be the presence of physical barriers to gene flow (or allopatric speciation) including major land or ice barriers (Friesen *et al.* 2007; Friesen 2015; Thanou *et al.* 2017). Friesen *et al.* (2007) noted that, among 11 mtDNA studies on seabirds the breeding distributions of which are (or were) fully or partially fragmented by land or ice barriers, all exhibited significant genetic differentiation including six species that were also phylogenetically structured. However, not all seabirds show genetic population structure in the presence of land or ice suggesting that some have an ability to cross physical barriers (Morris-Pocock *et al.* 2016; Quillfeldt *et al.* 2017). One such species is the Magnificent Frigatebird (*Fregata magnificens*), which appears to exchange genes across the Isthmus of Panama even though this physical feature limits gene flow in several other species (Steeves *et al.* 2003, 2005a; Hailer *et al*. 2010; Morris-Pocock *et al.* 2010, 2016; Nuss *et al.* 2016). Not all barriers to dispersal are obvious, and comparative phylogenetic studies have sometimes also revealed the presence of cryptic physical barriers to gene flow (Friesen *et al.* 2007; Morris-Pococok *et al.* 2010, 2016). This implies that dispersal among populations can be limited by the presence of salinity and temperature barriers, often linked to ocean currents, that are not always immediately obvious. The rationale behind this is that the distribution of habitat suitable for foraging and nesting is patchy, with large areas of ocean (e.g. eastern Pacific basin; Morris-Pocock *et al.* 2010, 2016) being unsuitable for most species. Some species therefore show divergence on either side of such unsuitable foraging habitats (i.e. warm ocean currents) or nesting areas. As an example – connectivity between Atlantic and Mediterranean populations of several marine species, including seabirds (e.g. *Calonectris* shearwaters; Gómez-Díaz *et al.* 2009; Genovart *et al.* 2013; Thanou *et al.* 2017), are limited by the Strait of Gibraltar and the Almeria-Oran oceanographic temperature front. However, these simple models fail to explain the many examples of genetic population differentiation in seabirds that exist in the absence of tangible or cryptic physical barriers to dispersal (Friesen *et al.* 2007; Quillfeldt *et al.* 2017).

Several seabird species show genetic population differentiation among islands of the same archipelago (e.g. Galapagos Petrel [*Pterodroma phaeopygia*]; Friesen *et al.* 2006) or among populations on the same islands (e.g. Band-rumped Storm-Petrel [*Oceanodroma castro*]; Monteiro 1998; Friesen *et al.* 2007; Smith & Friesen 2007). It was therefore suggested that gene flow in seabirds may act as a function of colony dispersion, with a greater influence of genetic drift expected under a one-dimensional stepping-stone model in which individuals disperse to neighbouring colonies rather than at random (*n*-island model). This hypothesis has received limited support (Friesen *et al.* 2007), though patterns of isolation by distance among breeding colonies have been observed in several species including the Hawaiian Petrel (*Pterodroma sandwichensis;* Welch *et al.* 2012). Isolation by distance implies that more distant colonies show greater levels of divergence from each other than those in close proximity (Welch *et al.* 2012). Another suggestion was that gene flow among breeding colonies may relate to a species' non-breeding distribution. This implies that the probability of an individual bird encountering (and therefore potentially breeding at) non-natal colonies elevates with increased distance travelled (Friesen *et al.* 2007; Quillfeldt *et al.* 2017). Genetic population structure is also theorised to be higher in species that have multiple population-specific breeding and non-breeding distributions (Friesen *et al.* 2007; Quillfeldt *et al.* 2017). To illustrate, Burg and Croxall (2001) found genetic differences among populations of two albatross species with different non-breeding areas. However, Quillfeldt *et al.* (2017)

observed no evidence for a relationship between non-breeding distribution and genetic structure among populations of each of the Antarctic (*Pachyptila desolata*) and Thin-billed (*P. belcheri*) Prions, and Blue Petrel (*Halobaena caerulea*). Other drivers of population divergence in seabirds may include natal or breeding philopatry (reviewed in Chapter 3), population bottlenecks and associated genetic drift in founder populations, differences in the timing of breeding (allochrony; reviewed in Chapter 4), and retained ancestral variation following range expansions or contractions (Friesen *et al.* 2007; Rawlence *et al.* 2014; Friesen 2015).

Though our understanding of seabird population dynamics, and the mechanisms through which divergence occurs, has increased within the last decade or so, most population genetic studies on seabirds have focussed on temperate and polar species. Comparatively few studies have investigated population structure and patterns of gene flow in tropical systems (e.g. Avise *et al.* 2000; Hailer *et al*. 2010; Morris-Pocock *et al.* 2016; Nuss *et al.* 2016). It is widely believed that the mechanisms of population differentiation in seabirds differ by latitude. To illustrate – Friesen *et al.* (2007) noted that, in species with tropical to temperate distributions, the greatest genetic differences involved the tropical populations. Studies of population genetic differentiation in temperate and polar latitudes are often also confounded by historical associations among breeding colonies. This implies that population genetic differentiation in higher latitude species may reflect past differentiation in multiple glacial refugia rather than contemporary restriction of gene flow (Friesen *et al.* 2007). Conversely, a lack of differentiation among higher latitude breeding colonies of seabirds may reflect past association in a single refugium rather that contemporary panmixia (Moum *et al*. 2002; Morris-Pocock *et al*. 2008). At lower latitudes, Pleistocene refugia probably had less of an effect on population differentiation in seabirds and fewer features exist that could act as tangible or cryptic physical barriers to gene flow (Morris-Pocock *et al.* 2010). This implies that the process of population differentiation in tropical systems are more likely to be governed by non-physical barriers (e.g. natal or breeding philopatry, differing breeding schedules) than at higher latitudes (e.g. Steeves *et al*. 2005a, b; Hailer *et al*. 2011; Welch *et al*. 2011).

1.5. Seabird populations of tropical western Indian Ocean: status, trends, and threats

The islands of the western Indian Ocean, accounting for as little as 1.9% of the total land area of Africa and its associated islands, have been recognised as one of the 34 'Hotspots of Global Biodiversity' (Myers *et al.* 2000). The region further includes United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Listing for six insular nature sites and three cultural sites. These landmasses are floristically and zoologically exceptional amongst island ecosystems (Le Corre & Safford 2001; Vences *et al.* 2009; Cheke & Hume 2010; Hume 2013). Collectively Madagascar, Mauritius, Réunion Island, the Comoros, the French îles Eparses, and the Seychelles support over 13 000 species of plant, 380 reptiles, 310 birds, 240 mammals, and countless invertebrates of which there are many endemic lineages. Madagascar alone hosts well over 8 000 unique species rendering it the nation with the highest overall endemism in the Afrotropical ecozone and one of the most biodiverse countries on the planet. Madagascar's main 'associated islands,' including Mauritius, Réunion Island, the Comoros, and the Seychelles, once also supported one of the richest faunas found on any oceanic archipelago including the second most phylogenetically distinct assemblage of vertebrates (Holt *et al.* 2013). Most of the region's granitic islands originate as "geological castaways" from the Indian subcontinent as it separated from Gondwanaland between about 160 and 115 million years ago (MYA; Rabinovitz *et al.* 1983; Briggs 2003; Harmon *et al.* 2008; Kunter & Agnarsson 2011). Added to these are several coralline and volcanic islands of varying ages, sizes, and origins. The volcanic development of the Comoros is thought to have occurred some 15 to 10 MYA (Montaggioni & Nougier 1981; Emerick & Duncan 1982; Nougier *et al.* 1986), while the emergence of Rodrigues, Mauritius, and Réunion Island has been dated at between 9.9 and 1.5 MYA (Montaggioni & Nativel 1988; Moore *et al.* 2011). These estimates are based on recent exposed lavas, while other evidence suggests that the subaerial origins of these masses may have occurred much earlier (Bonneville *et al.* 1988; Gillot *et al.* 1994). Sea level fluctuations within the last five million years are then thought to have had a profound effect on the presence, size, and shape of the many coralline islands in the region (Haq *et al.* 1987; Miller *et al.* 2005). The time during which many of these islands have been isolated, combined with their diverse geological histories, underlies these unique evolutionary dynamics. It also explains such unusual phenomena as gigantism in Elephantbirds (*Aepyornis maximus*) and Aldabra Giant Tortoises (*Aldabrachelys gigantea*), and flightlessness in Dodos (*Raphus cucullatus*) and Réunion Solitaires (*Threskiornis solitaries*; Cheke & Hume 2010; Kunter & Agnarsson 2011). Though marine productivity is generally quite low in the Indian Ocean, numerous large oceanographic (e.g. seasonal Somalia upwelling; Schott *et al.* 2009) and climatic features (e.g. Indian Ocean Dipole [IOD; also called the Indian Ocean Zonal Mode]; Schott *et al.* 2009) also exist that support significant concentrations of emblematic or economically important marine species. This includes more than 40 species of cetacean (Balance & Pitman 1998), five species of marine turtle (Frazier 1975), massive populations of sharks, tuna, and billfish (Worm *et al*. 2005), and a great diversity of resident and migratory seabirds (Le Corre & Jaquemet 2005). Nonetheless, though the population status of many of these organisms is not well known, most appear to have been reduced to a fraction of their historical numbers.

The degree of exploitation that natural systems of the western Indian Ocean bioregion endure is a function of human population size and level of socio-economic development

(UNEP-Nairobi Convention & WIOMSA 2015; Obura *et al.* 2017). As of July 2016, an estimated 182 million people were known to reside in the 10 nations that bound the western Indian Ocean. Human densities vary among these countries, between islands and the mainland, and in relation to distance from the coast (Obura *et al.* 2017; Table 1.1). Small islands generally host the highest population densities with virtually minimal distinction between coastal and inland populations (reviewed in UNEP-Nairobi Convention & WIOMSA 2015). Human densities currently exceed 190 individuals per square kilometre (/Km^2) in the Seychelles, 390 individuals/Km^2 in the Comoros, and 630 individuals/Km^2 on Mauritius (Obura *et al.* 2017). Though Madagascar has a greater internal extent than the aforementioned island states (total island size approximatey 587 000Km^2), around 35% of its population also resides within 100 kilometres (Km) of the coast (Obura *et al.* 2017). Concerning mainland countries – a third of Mozambicans and South Africans, and a quarter of Tanzanians, live in close proximity to the Indian Ocean. It is further expected that at-least 50% of these mainland populations will be living within littoral areas by 2020 (Francis & Torell 2004; UNEP-Nairobi Convention & WIOMSA 2015). The countries that bound the western Indian Ocean are also characterised by extremely high population growth (UNEP-Nairobi Convention & WIOMSA 2015; Obura *et al*. 2017), with auto-recruitment in most nations exceeding 2% of the total population per year (CIA World Factbook 2016). Coupled with these statistics are some of the lowest overall Gross Domestic Products (GDP; rarely surpassing US$500 per head) and Human Development Indexes (HDI) among all listed countries (per the World Bank [IBRD-IDA]; CIA World Factbook 2016; Table 1.1). Most of these communities are therefore heavily reliant on natural habitats (particularly littoral ecosystems) for their economic, social, and cultural security (UNEP-Nairobi Convention & WIOMSA 2015; Obura *et al.* 2017). The economies of many of these nations also often depend on the goods and services provided by marine systems, with the annual “Gross Marine Product” (equivalent to GDP) of the western Indian Ocean region exceeding US$20.8 billion (Obura *et al.* 2017). These statistics altogether highlight the major impact of human activities, and emphasize that the considerable and growing economic and social benefits drawn from the western Indian Ocean bioregion are becoming increasingly undermined by the intensifying pressures imposed on it.

Table 1.1: Demographic and economic statistics of the 10 nations that bound the western Indian Ocean.

Country	Land area (Km^2)	Population in millions[1]	Proportion (%) of population living near the coast[2]	Gross Domestic Product rank[3]	Human Development Index rank[4]
Comoros	2 170	0.79	100	186	160
Kenya	582 650	48.46	8	68	146
Madagascar	587 040	24.89	34	133	158
Mauritius	2 040	1.26	100	122	64
Mozambique	801 590	28.83	70	127	181
Réunion	2 517	0.87	100	-	-
Seychelles	455	0.94	100	174	63
Somalia	637 657	14.32	55	149	-
South Africa	1 219 912	56.01	40	38	119
Tanzania	946 087	55.57	25	81	151

[1]Estimate as of 1 July 2016; [2]CIA World Factbook (2016); [3]Based on 195 countries listed by the World Bank [IBRD-IDA] in 2016 (CIA World Factbook 2016); [4]Based on 188 countries listed by the World Bank [IBRD-IDA] in 2016 (CIA World Factbook 2016).

The breeding seabird population of the western Indian Ocean, amounting to roughly 19 million individuals of 30 species (Danckwerts *et al.* 2014; Table 1.2), appears to have declined to a great extent (Feare 1978; Le Corre 1996; Cheke 2001; Bowler *et al.* 2002; Feare & Lesperance 2002; Le Corre *et al.* 2015). Following human settlement on the main oceanic islands, the distribution and abundance of many species was significantly altered. Anthropogenic presence on the many coralline islands, including the complete transformation of some systems, also deleteriously affected the region’s seabird populations.

Fifteen of the 46 known Sooty Tern (*Onychoprion fuscatus*) colonies were driven to extinction within the last century, whilst eight additional colonies are known to have been greatly reduced in size (approximatey 27% reduction in some cases; Feare & Lesperance 2002). This is by far the dominant species in the region, accounting for more than 70% of the contemporary seabird biomass (Danckwerts *et al.* 2014). In addition, at-least half of the Red-footed (*Sula sula*), Masked (*S. dactylatra*), and Brown Booby (*S. leucogaster*) colonies have been extirpated while, the now critically endangered, Abbot's Booby (*Papasula abbotti*) is considered regionally extinct. This latter species previously bred on Rodrigues, Mauritius, and Assomption Island in the Seychelles (Feare 1978; Cheke 2001). A number of other terns and tropicbirds have also declined in recent timescales, as a consequence of a broad range of threats (e.g. human disturbance, increased chick mortality), with White-tailed Tropicbird (*Phaethon lepturus*) numbers on Aride (Seychelles) having decreased by as much as 60% between 1988 and 2000 (Bowler *et al.* 2002; Catry *et al.* 2009). Population count data for other groups (e.g. frigatebirds, shearwaters) are mostly insufficient to establish trends at this stage, yet most species appear to have declined with at-least some colonies of some species having been extirpated altogether (e.g. Le Corre 1996; Bowler *et al.* 2002). That all said, appropriate conservation action has proven effective in reversing some of these negative trends. The complete eradication of Norway Rats (*Rattus norvegicus*) from Tromelin Island has, for example, prompted a rapid recovery of the island's seabird community (Le Corre *et al.* 2015). The breeding populations of the Red-footed and Masked Boobies increased by about 23% per year following the successful eradication of the rats, and the Sooty and White (*Gygis alba*) Terns and Brown Noddies (*Anous stolidus*) are now breeding annually for the first time since about 1856 (Le Corre 1996; Le Corre *et al.* 2015; Le Corre, M. unpub. data). For the first time ever, the Brown Booby was also recorded breeding in 2014 (Le Corre *et al.* 2015), and permanent activity of Red-tailed (*Phaethon rubricauda*) and White-tailed Tropicbirds suggests that these two species may re-attempt breeding on Tromelin Island in the near future (Le Corre, M. pers. comm.).

Table 1.2: Breakdown of the breeding seabird population of the western Indian Ocean, amounting to roughly 19 million individuals of 30 species (from Danckwerts *et al*. 2014).

Order:	Common name:	Scientific name:	Approximated regional population (individuals):
Procellariiformes	Round Island Petrel complex	*Pterodroma sp.*	500
	Barau's Petrel	*Pterodroma baraui*	25 000
	Mascarene Petrel	*Psudobulweria aterrima*	250
	Wedge-tailed Shearwater	*Ardenna pacificus*	475 000
	Persian Shearwater	*Puffinus persicus*	25 000
	Tropical Shearwater	*Puffinus bailloni*	174 000
	Bulwer's Petrel	*Bulweria bulwerii*	5
	Jouanin's Petrel	*Bulweria fallax*	7 500
Phaethontiformes	Red-billed Tropicbird	*Phaethon aethereus*	250
	Red-tailed Tropicbird	*Phaethon rubricauda*	21 000
	White-tailed Tropicbird	*Phaethon lepturus*	28 000
Suliformes	Great Frigatebird	*Fregata minor*	13 000
	Lesser Frigatebird	*Fregata ariel*	21 000
	Abbott's Booby	*Papasula abbotti*	0[1]
	Masked Booby	*Sula dactylatra*	33 000
	Brown Booby	*Sula leucogaster*	50 000
	Red-footed Booby	*Sula sula*	93 000
	Socotra Cormorant	*Phalacrocorax nigrogularis*	38 000
Charadriiformes	Brown Noddy	*Anous stolidus*	400 000
	Lesser Noddy	*Anous tenuirostris*	1 200 000
	Sooty Gull	*Ichthyaetus hemprichii*	500
	White-eyed Gull	*Ichthyaetus leucophthalmus*	2 500
	Caspian Tern	*Sterna caspia*	400
	Great Crested Tern	*Thalasseus bergii*	30 000
	Saunder's Tern	*Sternula saundersi*	250
	Bridled Tern	*Onychoprion anaethetus*	14 000
	Sooty Tern	*Onychoprion fuscatus*	16 150 000
	Roseate Tern	*Sterna dougallii*	46 000
	White-cheeked Tern	*Sterna repressa*	750
	Black-naped Tern	*Sterna sumatrana*	600
	White Tern	*Gygis alba*	88 000
		Total:	18 937 505

[1] last bred in the region in about 1920

Threats to seabird populations are diverse, frequently colony specific, and often act synergistically and so are difficult to mitigate. Though most islands are now formally listed as or included into nature reserves, legislation to protect remote oceanic islands is virtually impossible to enforce (Feare 1978). In the western Indian Ocean, the most widespread cause of seabird population declines has been the destruction of nesting colonies resulting in increased adult mortality, lower breeding success, or colony abandonment (Feare *et al.* 2007). In a recent review of the causes of extinctions in marine ecosystems, Dulvy *et al.* (2003) noted that 37% (n = 133) of all extinctions were a result of habitat alteration and destruction. Notably, guano collection and coconut planting on islands during the late 19th century had catastrophic effects where entire seabird communities were often eliminated (e.g. booby populations on the Providence group, Seychelles; Feare 1978). The introduction of non-native species, particularly land mammals, has additionally had a profound impact on the region's seabird communities (Trevino *et al.* 2007; Falquier *et al.* 2009; Russel & Le Corre 2009; Dumont *et al.* 2010). Though all islands of the western Indian Ocean have now been invaded, the diversity of introduced mammals and their introduction histories varies greatly (Russel & Le Corre 2009; Russel *et al.* 2016). Feral cats (*Felis catus*), Norway and Black (*R. rattus*) Rats, and House Mice (*Mus musculus*) are almost ubiquitous among all island groups, though cultural contingency has also led to regional invasions by dogs, goats, lemurs, civets, and tenrecs (Cheke 2010; Russel *et al.* 2016). The Mascarene Archipelago (Mauritius, Rodrigues, and Réunion Island) alone has lost more than 74 endemic vertebrate species, including several seabirds, as a direct consequence of the impacts imposed by invasive mammals (Russel *et al.* 2016). Réunion Island's two endemic petrels, the Mascarene (*Pseudobulweria aterrima*) and the Barau's (*Pterodroma baraui*) Petrels, are principally under threat through predation by cats, rats, and mice (Falquier *et al.* 2009; Pinet *et al.* 2009; Dumont *et al.* 2010). Falquier *et al.*

(2009) noted that more than 54% of feline scats collected around the breeding colonies of Barau's Petrel contained feathers, and suggested that 0.57 ± 0.55 Barau's Petrels are killed daily by a single cat with some 22 juveniles and 70 adults taken per individual cat per petrel breeding season. In addition, though mostly limited to the Seychelles and Madagascar, most seabird species seem to be exploited at-least occasionally. Exploitation is the leading cause of population declines and extinction in marine systems in general, responsible for more than 55% of all documented species extinctions (Dulvy *et al.* 2003). Commercial, though apparently regulated (see Chapter 4.1), exploitation of eggs of the Sooty Tern and Brown Noddy occurs on the Seychelles and Amirantes Islands (Ridley & Percy 1958; Feare 1976a, b; Feare *et al.* 2007; Croxall *et al.* 2012). This is coupled with unknown magnitudes of illegal and unregulated exploitation. Chicks of the Wedge-tailed Shearwater (*Ardenna pacifica*) are also commercially exploited for food off Madagascar and in the Seychelles (Ridley & Percy 1958), and Feare (1978) observed the remains of exploited frigatebirds and tropicbirds on the outer Seychelles islands. Feare (1978) additionally speculated that the eggs of Bridled (*Onychoprion anaethetus*) and Greater Crested Terns (*Thalasseus bergii*) are harvested annually along with those of the Sooty Tern and Brown Noddy. Harvesting has a profound impact on seabird populations, especially when adults are taken. About 50 years of unregulated exploitation (combined with threats imposed by invasive mammals) on Tromelin Island led to the local extinction of the Great (*Fregata minor*) and Lesser (*F. ariel*) Frigatebirds, Red-tailed Tropicbird, Sooty Tern, Lesser (*Anous tenuirostris*) and Brown Noddy, and White Tern. Other forms of disturbance, such as episodic geomorphological changes to islands (e.g. Africa Banks 1976; Feare 1979), increased tourism activity (Pinet *et al.* 2009), and urbanization have also affected some breeding species on some islands. Finally, our impacts on seabird foraging habitats in the Indian Ocean are not well understood but are largely attributed to commercial fisheries (reviewed in Montevecchi 2002; Karpouzi *et al.* 2007), the removal of subsurface predators (i.e. tunas and billfish) with which many of these birds associate while foraging (Danckwerts *et al.* 2014), and chronic plastic and heavy-metal pollution (e.g. Yamashita *et al.* 2011; Tanaka *et al.* 2013; Lavers *et al.* 2014; Wilcox *et al.* 2015). The impacts of global climate change on seabird populations are also of increasing concern. To illustrate, Legarnd *et al.* (2016) noted the potential for an 11% reduction in suitable habitat for wintering Barau's Petrels by 2100 based on several Intergovernmental Panel on Climate Change (IPCC) climate change prediction models.

1.6. Thesis overview: principal aims and objectives

As we are the primary drivers of extinction in so many species, not only seabirds, it is incumbent on us to understand and mitigate population declines as far as possible whilst also preserving genetic diversity for the future. From a more selfish human perspective, the well-being of seabirds and other marine top predators is intricately linked to the proper functioning of marine ecosystems on which our livelihoods depend (Vader *et al*. 1990; Surman & Nicholson 2009). Losses to these populations will likely have powerful effects on the functioning of marine and terrestrial ecosystems worldwide, with resultant impacts on social and economic spheres. The loss of such top predators frequently has unforeseen negative consequences including trophic cascades and the burgeoning of mesopredator populations (Heithaus *et al.* 2007). Therefore, with the primary objectives of identifying the mechanisms underlying tropical seabird colony connectivity and understanding how the loss of individual populations will affect genetic diversity and population resilience, this study aims to assess population structure for three species in the western Indian Ocean using polymorphic microsatellite analysis. Key questions include: (1) what common physical barriers (e.g.

Madagascan landmass, ocean currents) prevent gene flow among colonies? (2) does population genetic structure exist in the absence of physical barriers to dispersal? and (3) what non-physical barriers (e.g. natal or breeding philopatry) may be restricting gene flow? By covering a range of life-history strategies, these species will act as proxies for a wider range of taxa as they are representative of the diversity of ecologies and life history strategies found in tropical seabirds. This information will aid in the identification of the populations or species that are most at risk of extinction, and should offer insight into how such risk can be mitigated. These data can then be projected into conservation initiatives where ESUs and MUs can be defined to strengthen management practices.

If the seabird community of the western Indian Ocean (limits 50°E and 75°E, and 30°S and 20°N) is classified on the basis of where each of the different species breed, the region can be divided into five distinct sub-bioregions including: (1) the southern Mozambique Channel, (2) the northern Mozambique Channel, (3) the Seychelles, (4) the Mascarenes, and (5) Somalia and the Red Sea (refer to Danckwerts *et al*. 2014). These biogeographic divisions are important because, along with physical data, they suggest that various biotic and abiotic factors have influenced the distributions of organisms at the intra-specific population level. Population structure is considered in the context of four of the Indian Ocean sub-bioregions. The Somalia and the Red Sea region was excluded for the time being as it is dominated by a suite of species (e.g. Socotra Cormorant [*Phalacrocorax nigrogularis*]) not found elsewhere in the western Indian Ocean (Danckwerts *et al*. 2014). On the basis of life-history characteristics, a sixth ‘biogeographic’ category (island endemic species) is also recognised to illustrate another set of processes which lead to quite different evolutionary patterns.

Three species were selected for microsatellite analysis, including the Sooty Tern, Red-footed Booby, and Barau’s Petrel. These species exhibit a range of population sizes, distributional ranges, and intrinsic behavioural (e.g. migratory behaviour) and morphological (e.g. polymorphism) characteristics that render them advantageous for study. The Sooty Tern is an exceptionally abundant species that dominates the seabird community of the western Indian Ocean (Feare *et al.* 2007; Danckwerts *et al.* 2014). Colonies of several thousand breeding pairs occur in the Mozambique Channel, Mascarenes, and the Seychelles. The largest of these colonies (each over 500 000 pairs) are on Bird Island (Seychelles), Juan de Nova (northern Mozambique Channel), and Europa Island (southern Mozambique Channel; Danckwerts *et al.* 2014). Geographic variations exist in the species' breeding phenology, according to spatial variations in oceanographic conditions (mainly sea-surface temperature [SST] and chlorophyll *a* concentration; Jaquemet *et al.* 2007). Outside of the breeding period, Sooty Terns disperse widely across the Indian Ocean to a broad area from Seychelles to East India and the Bay of Bengal (Jaeger *et al.* 2017). In contrast, the Barau's Petrel is an island endemic with a relatively small population (approximatey 14 000 pairs) and a highly restricted and yet naturally fragmented breeding range on the two main mountain masses of Réunion Island (Pinet *et al.* 2009). Its breeding is highly synchronous among the different breeding colonies and, rather unlike the movement of Sooty Terns, adults have regular foraging patterns in the breeding season and make predictable post-breeding migrations (Pinet *et al.* 2009, 2011a, 2011b, 2012). Finally, the Red-footed Booby breeds on six large and (apparently; Le Corre 1999) discreet populations across the western Indian Ocean (Danckwerts *et al.* 2014). It is highly polymorphic in its plumage, with five distinct morphs occurring at different morph ratios on the different islands (Le Corre 1999). Breeding is mostly aseasonal in this species (Le Corre 1996, 2001) and, unlike either the Barau's Petrel or the Sooty Tern, adults are thought to remain around their breeding colonies year-round (Diamond 1974). These three species also exhibit a range of philopatric tendencies and of nest site fidelity. Petrels are highly philopatric, typically returning to the same colony in which they hatched or even to within metres of the natal nest (cf natal philopatry; Warham 1996). In contrast, an appreciable proportion of terns move among populations and become recruited into non-natal colonies (e.g. Roseate Tern [*Sterna dougallii*]; Tree, A. unpub. data). Philopatric tendencies of terns do vary among species, however (Coulson 2016), though individuals are typically more faithful to colonies where they have previously bred successfully than to colonies in which they were born (cf breeding philopatry; Lebreton *et al.* 2003; Møller *et al.* 2006; Draheim *et al.* 2010). Less is known about colony fidelity in boobies, though it would seem that at-least some exchange of individuals occurs among breeding colonies of most species (e.g. Kim *et al.* 2007; Levin & Parker 2012; Morris-Pocock *et al.* 2010, 2016). Populations of the Nazca Booby (*Sula granti*) on different islands of the Galapagos Archipelago also display contrasting levels of natal and breeding philopatry (Levin & Parker 2012), suggesting that this group may be intermediate in its behaviour between the terns and petrels.

As the number of studies on seabird population genetics has increased, so has our understanding of mechanisms underlying population differentiation. In a recent review, Friesen *et al.* (2007) identified seven general factors that influenced population genetic structure at various scales. These were broadly categorised as follows: the presence of physical land or ice barriers, geographic distance between colonies, colony dispersion patterns, nonbreeding distribution, foraging range, population bottlenecks, and retained ancestral variation. However, the factors that promote population differentiation, especially the barriers that disrupt gene flow in different organisms, are multifaceted and many additional questions remain unanswered. Considerable bias also exists towards higher latitude species, while tropical groups have largely been neglected, and the mechanisms of population differentiation in seabirds are thought to differ by latitude (Friesen *et al.* 2007).

Our ability to predict population structure for conservation purposes, especially considering the diversity of behaviour and life-history strategies of seabirds, therefore remains a challenge. With that in mind, the extent of population genetic structure is hypothesized to be highly variable among the three study species and may not necessarily correlate to scale. However, where genetic structure exists, the subpopulation dynamics of the three study species will most likely be governed by non-physical barriers to dispersal (e.g. natal or breeding philopatry, differing breeding schedules), as has already been documented for several other species (e.g. Steeves *et al*. 2005a, b; Hailer *et al*. 2011; Welch *et al*. 2011), and the conditions at (e.g. disturbance) and around (e.g. food availabitiy) the breeding colonies will likely contribute to the scale and frequency of immigration.

1.7. References

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CHAPTER 2: EVIDENCE OF BIOGEOGRAPHIC ISOLATION AND PHENOTYPIC ASSORTATIVE MATING IN THE RED-FOOTED BOOBY (*SULA SULA*).

2.1. Introduction

The term polymorphism describes the coexistence of two or more distinct and genetically determined forms (or morphs) within a population, the least plentiful of which exists in too great an abundance to be due solely to some recurrent mutation (Ford 1940, 1945; Huxley 1995). Polymorphism can involve a wide range of traits that are either directly advantageous to the organism, or which act as non-adaptive correlates to physiological or fitness characters of selective value (Galeotti *et al.* 2003). Examples include traits involving morphology (e.g. bill size and shape in birds; reviewed in Durell 2000), physiology (e.g. salinity and temperature tolerances in molluscs; Sokolova & Berger 2000), and behaviour (e.g. differential migration strategies in birds; Ray Chandler & Mulvihill 1990). It is traditionally thought to be an evolutionary adaptation to altering extremes in conditions, or to a wide range of niches present within a species' breeding area (Galeotti *et al.* 2003). The existence of permanent polymorphism implies a selective balance among morphs, each benefitting from some selective advantage in certain instances but suffering disadvantages elsewhere (Fisher 1930). Spatial variations in the relative frequencies of morphs often exist as a result (e.g. MacColl & Stevenson 2003; Nebel 2006; Lee & Jablonski 2012), with alternative morphs found dominating at the extremities of such so-called 'ratio-clines' (Galeotti *et al.* 2003). Studying ratio-clines may therefore provide clues to the differing selective forces, or genetic barriers, operating across a polymorphic system including the patterns of gene flow and a species' metapopulation structure (Galeotti *et al.* 2003). Theory also suggests that the processes that maintain differences within a polymorphic system can promote and accelerate speciation events (Hugall & Stuart-Fox 2012).

Polymorphism in colour (hereafter polymorphism) is a taxonomically widespread rare phenomenon predominantly in vertebrates (Galeotti *et al.* 2003). It has independently appeared multiple times in the class Aves and is now seen in about 3.5% of all extant bird species including some owls and diurnal raptors (Galeotti & Rubolini 2004), waders (e.g. the Ruff [*Philomachus pugnax*]; Küpper *et al.* 2016), herons (e.g. the Eastern Reef Heron [*Egretta sacra*]; Itoh 1991), cuckoos (Trnka & Grim 2013), swallows (e.g. the Brown-throated Martin [*Raparia paludicola*]; Earlé & Tree 2005), and shrikes (e.g. African *Malaconotus* shrikes; Hall *et al.* 1966*)*. Concerning seabirds, plumage polymorphism is found in four families including the Procellariidae (i.e. petrels and allies; seven species of 70 total; e.g. Graff *et al.* 2015), Hydrobatidae (i.e. northern storm-petrels; two species of 20 total; e.g. Robertson *et al.* 2016), Stercorariidae (i.e. skuas and allies; four species of seven; e.g. Bengtson & Owen 1973), and Sulidae (i.e. boobies and gannets; two species of 10; Nelson 1978; Le Corre 1999). The family Sulidae includes ten species, most of which show some degree of sexual dimorphism in size and/or intensity of colouration on the bare regions of the face (Nelson 1978). Only two species, the Red-footed (*Sula sula*) and Brown (*S. leucogaster*) Boobies, display plumage polymorphism, however. The Brown Booby exists in only white and brown-headed forms (Vanderwerf *et al.* 2008), while the Red-footed Booby is considered one of the most polymorphic of all bird species. The number of recognised adult plumage types in the Red-footed Booby varies between three (white-tailed white, white-tailed brown, and all brown; Nelson 1978) and five (white, light, mixed, brown, and dark; Schreiber *et al.* 1996), though several intermediate forms also exist (Nelson 1978; Del Hoyo *et al.* 1992). Many Red-footed Booby morphs overlap in their distributions and can freely interbreed, though some are highly restricted in their ranges (Nelson 1978; Baião & Parker 2008). But, whilst allelic variation at the melanocortin-1 receptor (Mc1r) locus has been identified as the genetic basis for polymorphism in the Red-footed Booby (Baião *et al.* 2007), the processes underlying the complex distributions of the various morphs are not yet fully understood (Le Corre 1999; Baião & Parker 2008).

To date, there have been no conclusive studies of the possible adaptive significance of plumage polymorphism in Red-footed Boobies or of the processes underlying the distribution of the various morphs. Nelson (1978) first proposed that plumage polymorphism in the Red-footed Booby may constitute intra-specific adaptive radiation, in the form of disruptive colouration, helping birds exploit food more efficiently. In this scenario, extreme individuals, within an otherwise normally distributed population, would have an advantage since they are less familiar to potential prey (Wilson *et al.* 1988; Galeotti *et al.* 2003). Despite this, no apparent fitness differences have been detected among the different morphs (Baião & Parker 2008). Similarly, though in a converse situation, selection might act through crypsis with less conspicuous forms being favoured in populations that are sympatric with frigatebirds (Nelson 1978; Le Corre 1999; Galeotti *et al.* 2003). For example, white-tailed brown morph birds may be less susceptible to kleptoparasitism in habitats based on darker substrata than white-tailed white forms (Le Corre 1999). However, no evidence exists to support differential vulnerability of the various morphs to kleptoparasitism in colonies where different morphs occur sympatrically. Another hypothesis, that of sexual selection, has been proposed to explain plumage polymorphism in bird species in general (Galeotti *et al.* 2003). This theory states that individuals may select partners based on common (cf assortative mating) or unfamiliar (cf dissassortative mating) traits ultimately leading to deviations in genotype (and phenotype) frequencies (Galeotti *et al.* 2003; Jiang *et al.* 2013). Assortative and dissassortative mating have been observed in several avian groups, including seabirds (e.g. Boland *et al.* 2004; Cohen & Dearborn 2004; Eda *et al.* 2016), but Le Corre (1999) and Baião & Parker (2008) found no deviations from random mate choice in three breeding colonies of the Red-footed Booby.

Red-footed Boobies are abundant and widely distributed in the western Indian Ocean, occupying several offshore islets in the outer Seychelles, southern Mozambique Channel, Mascarenes, and central Indian Ocean (Diamond 1974; Feare 1978; Cheke 2001; Le Corre, M. unpub. data; Figure 2.1). An apparent ratio-cline exists throughout the species' range (reviewed in Le Corre 1999 and Nelson 1978). By far the dominant morph in this region, amounting to some 33 000 breeding pairs and dominating four colonies in the outer Seychelles and northern Mozambique Channel (Le Corre, M. unpub. data), is the white-tailed white morph (Le Corre 1999; Figure 2.1). Several extinct populations are also known to have been dominated by this morph including colonies on the Amirantes, Assomption, Providence, Astove, Agalega, Mauritius, and Rodrigues (reviewed in Feare 1978; Le Corre 1999; Cheke 2001; Figure 2.1). This is, in fact, the main morph globally (Nelson 1978). Only two colonies are (or were) dominated by the rarer white-tailed brown morph (Le Corre 1999; Figure 2.1). Europa Island (hereafter Europa), in the Southern Mozambique Channel, remains a stronghold and supports approximately 6 000 breeding pairs (Le Corre, M. unpub. data), more than 90% of which are of the white-tailed brown morph (Le Corre 1999; Figure 2.1). The breeding colony on Glorieuses is additionally believed to have supported mostly white-tailed brown birds, but was extirpated by the early 20th century (Ridgway 1895; Benson *et al.* 1975; Feare 1978; Cheke 2001; Figure 2.1). Finally, Tromelin Island (hereafter Tromelin) hosts a medium-sized breeding colony (approximatey 6 000 pairs; Ringler *et al.* pers. comm.) and is unique in that it supports an unusual mix of white-tailed white and white-tailed brown morph

Red-footed Boobies, including three discreet intermediate forms, at a ratio that has been stable for at-least 50 years (Le Corre 1996; Le Corre *et al.* 2015; Figure 2.1). The favoured hypothesis for this observed variation in morph ratios among breeding colonies of the Red-footed Booby, in the western Indian Ocean, proposes that there are strong isolating mechanisms in place which limit the exchange of birds among colonies (Le Corre 1999; Steeves *et al.* 2003). Birds living on different island groups could also be subjected to different selective forces giving rise to the different morph ratios (Le Corre 1999).

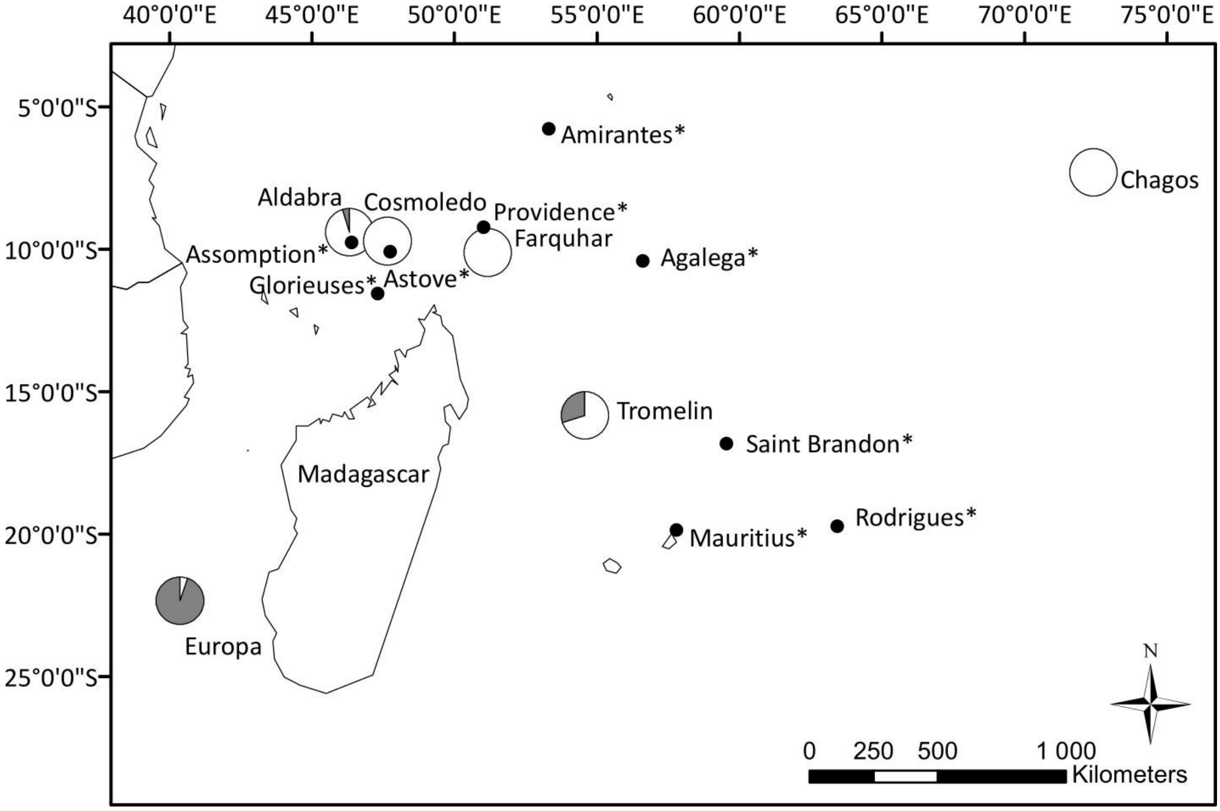

Figure 2.1: Distribution of all extant and extinct (denoted with asterisks) Red-footed Booby breeding colonies in the western Indian Ocean. Approximate morph ratios for extant colonies are indicated with pie charts where grey and white represent total contribution of white-tailed brown and white-tailed white morphs in each population, respectively. Data taken from Le Corre (1999).

The aim of this chapter is to assess the genetic population structure of the Red-footed Booby in the western Indian Ocean, in the context of the species' ratio-cline in plumage polymorphism. White-tailed brown (i.e. Europa), white-tailed white (i.e. Aldabra Atoll [hereafter Aldabra]), and mixed (i.e. Tromelin) breeding colonies are compared with one another, using polymorphic nuclear microsatellite analysis, to understand the scale and frequency of gene flow among colonies allowing the identification of barriers to gene flow and possible isolating mechanisms. It is predicted that spatial variations within the species' polymorphic system are the result of complex patterns of isolation and connectivity among colonies, and that these are mirrored in the species' population genetic structure. The breeding populations on Aldabra and Europa are expected to differ genetically since these colonies are each at opposite ends of the ratio-cline within the species' polymorphic system. The colony at Tromelin should, however, show relations to each of the other two breeding colonies since it supports both white-tailed white and white-tailed brown morph Red-footed Boobies. A degree of inbreeding between these two morphs on Tromelin is expected to have eroded any genetic differentiation among the different morphs breeding on this colony, however. To quantify this genetic mixing, and to understand the processes underlying Tromelin's unique pattern, the morph ratio and mating structure of the Tromelin population is additionally readdressed at a much larger scale than previously attempted. Regardless of Le Corre (1999) and Baião & Parker (2008), it is still suspected that a significant level of non-random mating, with respect to colour morph, is responsible for the maintenance of this colony's unusual colour morph ratio.

2.2. Materials and methods

2.2.1. Plumage polymorphism and mating pattern at Tromelin

Field work, specifically to quantify plumage polymorphism and assess the mating pattern of Red-footed Boobies on Tromelin, was conducted between the 16th of April 2013 and 22nd of May 2013. This time-frame coincides with a peak egg-laying period on the island (Le Corre 1996). The Red-footed Booby is monogamous, with no evidence of extra-pair fertilisation (Baião & Parker 2009), and both parents share incubation and chick-guarding duties (Nelson 1978).

A survey of the number of breeding pairs was conducted using coordinated counts of active nests across the whole island (approximatey 100 hectares [ha]). Nests were found in stands of *Tournefortia argentea,* a flowering shrub of littoral zones and small islets, sometimes at high density. All nests were numbered, using labelled plastic ribbons, and the colour morphs of both adults recorded. More often than not, only a single adult was present at each nest. In such cases, to enable partner identification, lone adults were marked on the upper breast or tail with waterproof orange paint as in Le Corre (1999). Thereafter, nests were visited at regular intervals until the colour morph of the mate was observed. Two main colour morphs are represented at the colony, alongside at-least three intermediate morphs. These were broadly categorised as follows: white-tailed brown morph birds, white-tailed white morph birds, white morph birds with brown scapulars, brown morph birds with yellow heads and white scapulars, and brown morph birds with a yellow head and brown scapulars (Figure 2.2). These morphs are discreet and are found only in adults older than three years of age (Nelson 1978). Immature birds, largely chocolate brown in colour, gradullay attain adult plumage (beginning in their second year) but are differentiated from adults based on the variable brown streaking on their bodies, the duller bill, and blackish brown flight feathers

(Nelson 1978). Adults, in attendance at nests, were treated as partners when parental reproductive (i.e. brooding, feeding chick, or mating) or nest guarding behaviours (i.e. defensive postures or attacks) were observed. All inactive nests, indicated by the absence of adults over the entire duration of the study, were disregarded. Effort was equally distributed across the entire island, including all habitat types, for the duration of the study period.

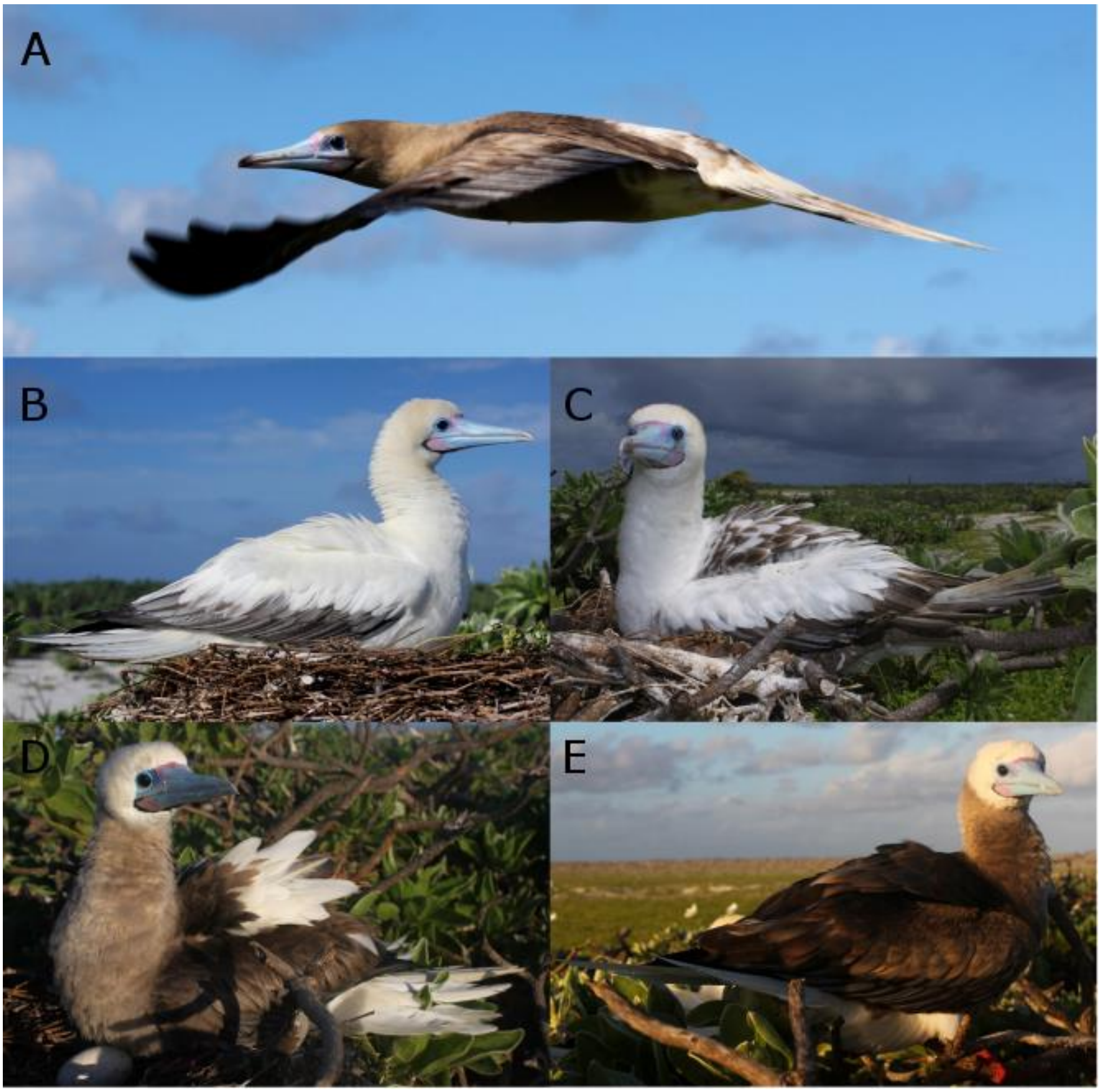

Figure 2.2: The Red-footed Booby is a strongly polymorphic species and five distinct colour morphs are represented on Tromelin Island in the South-western Indian Ocean. These include a (a) white-tailed brown morph, (b) a white-tailed white morph, (b) a white morph with brown

scapulars, (c) a brown morph with a yellow head and white scapulars, and (d) a brown morph with a yellow head and brown scapulars. Photo credit: Daniel Keith Danckwerts.

This technique was performed with prior support from the *Centre de Recherches sur la Biologie des Populations d'Oiseaux* (CRBPO; Muséum Paris), *Direction de l'Environnement, de l'Aménagement et du Logement de la Réunion* (DEAL Réunion), and *Terres Australes and Antarctiques Françaises* (TAAF).

2.2.2. Blood collection

Eighty-six adult Red-footed Boobies were captured, using a neck-noose attached to a 3 metre (m) telescopic pole, at three breeding colonies in the western Indian Ocean (Table 2.1). All were banded, facilitating individual identification, and assigned to one of the five broad morphotypes outlined in Chapter 2.2.1 (Figure 2.2). A small sample of whole blood (approximatey 0.2 millilitre [mL]; maximum of 1.0% of body weight; Fair *et al.* 2010) was collected through medial metatarsal or basilic venipuncture using sodium-heparin lined BD Micro-Fine^{TM} 1.5mL 27-gauge syringes. Blood samples were placed in 2mL Eppendorf tubes, in an insulated box containing ice packs, and centrifuged within four hours for serum collection and serological analyses (Lebarbenchon *et al*. 2015; Jaeger *et al.* 2016). Blood cells were preserved in 70% ethanol in the field, were frozen at -20 degrees Celsius (°C) within five hours of collection, and later held at -80°C at the *Centre de Recherche et de Veille sure les Maladies Emergentes dans l'Océan Indien* (CRVOI; Saint Denis, Réunion) upon return to Réunion Island.

All sampled individuals at Europa and Aldabra were of the white-tailed brown and white-tailed white morphs, respectively. At Tromelin, 10 individuals were of the white-tailed white morph, seven were of the white-tailed brown morph, two were white-tailed brown morph birds with yellow heads and white scapulars, and one was a white-tailed brown morph bird with a yellow head and brown scapulars. No white morph birds with brown scapulars were sampled at Tromelin. Finally, for two individuals collected during an independent sampling season at Tromelin, no colour morph data was attained. Though these two individuals were retained for comparisons among colonies, they were excluded in all tests pertaining to morphs.

The techniques employed to capture, and sample, Red-footed Boobies were approved by the Ethics Committee of Rhodes University (reference Number ZOOL-01-2013). Bird capture, handling and the collection of biological material were performed under research programs approved by the CRBPO (permit 1392). Sample collection on Tromelin and Europa was conducted with additional support by DEAL Réunion, and TAAF. Bird capture and collection of biological material at Aldabra, including sample export to Réunion Island, was performed with the approval of the Seychelles Bureau of Standards and Ministry of Environment, Energy, and Climate Change.

Table 2.1: Eighty-six (86) adult Red-footed Boobies were blood-sampled at three breeding colonies in the western Indian Ocean.

Breeding colony	Latitude/Longitude	Approximate colony size (pairs)	Approximate morph ratio (white : brown : intermediate)	Sample size
Tromelin Island	15° 53’S 54° 31’E	6 919[a]	69.4 : 30.6[d, e]	22
Aldabra Atoll	09° 25’S 46° 20’E	8 250[b]	95 : 5 : 0[f]	28
Europa Island	22° 22’S 40° 21’E	3 000[c]	91.3 : 1.5 : 7.2[g]	36

[a]Ringler *et al.* pers. comm.; [b]Le Corre, M. unpub. Data; [c]Le Corre & Jouventin 1997; [d]Brygoo 1955, Staub 1971, Le Corre, 1996; [e]includes 7.2% intermediates; [f]Diamond 1974; [g]Le Corre & Jouventin 1997

2.2.3. DNA extraction

Deoxyribonucleic acid (DNA) was extracted from blood cell subsamples using the DNeasy® Blood & Tissue kit (QIAGEN), following the procedure developed for animal tissue with minor adjustments. This technique, outlined below, was used since it yielded higher DNA concentrations when tested against the methods developed for both nucleated and nonnucleated blood.

Approximately 50 to 100 microlitres (µL) of each blood cell sample was added to 180µL of Buffer ATL in labelled ultra-violet (UV) light sterilised microcentrifuge tubes. Subsequently 20µL of the enzyme proteinase K was added to each, before thorough mixing on a vortex for approximately five seconds. Following optimisation experiments aimed at maximising protein yield, the samples were incubated overnight at 56°C on a Heidolph Titramax 1 000 thermomixer, set to mix at 450 revolutions per minute (rpm). The proteinase K digests all cellular constituents and releases the DNA into the stable lysis buffer. This buffer contains a high concentration of chaotropic salts (e.g. guanidine thiocyanate, lithium perchlorate) that destabilise the hydrogen bonds of proteins, including those of nucleases, ultimately disrupting their association with water. In doing so, the buffer provides optimal conditions for the transfer of DNA to silica. Next, 200µL of both Buffer AL (QIAGEN) and 96% ethanol were added into each microcentrifuge tube to further enhance the binding potential of nucleic acids to silica. After mixing once more, the solutions were transferred into individual DNeasy Mini Spin-columns housed within 2mL collection tubes. All were centrifuged at 8 000 rpm (6 000g) for one minute. The lysate containing proteins and polysaccharides, which descended during centrifuging, was discarded and the spin-columns were each given new collection tubes. During centrifuging, most of the DNA remained bound to the silica membrane within each spin-column. Next, 500µL of the Buffer AW1 was added into each spin-column and all were centrifuged at 8 000 rpm (6 000g) for another minute. This buffer, containing 96% ethanol and a low concentration of chaotropic salts, washed the silica membrane of all unwanted proteins. The removal of these residues was crucial to high DNA yields and purity. A second wash was also performed using 500µL of the Buffer AW2, in which all were centrifuged at 14 000 rpm (20 000g) for three minutes. This buffer contains a higher ethanol concentration and a lower concentration of chaotrophic salts than Buffer AW1. At both wash steps, the spin-columns were transferred into new collection tubes and the flow-through liquid discarded. The higher rate of centrifuging in the second step ensured complete flow through and removal of all salt and protein residues, acting also to dry the silica membrane ready for elution. DNA was finally eluted from the spin-columns, into labelled UV light sterilised 2mL microcentrifuge tubes. In this step, 150µL of Buffer AE was added atop the silica membrane. Prior to centrifuging, at 8 000 rpm (6 000g) for one minute, all were incubated at room temperature for five minutes. This step was repeated using 50µL of Buffer AE, ensuring maximum DNA yield. The spin-columns were then discarded. At each step, centrifuging was repeated if necessary to ensure complete flow-through.

Quantification of DNA in 2µL aliquots of each sample was then performed using a Thermo Scientific NanoDrop 2 000c UV-Vis Spectrophotometer. Measured nucleotide concentration varied greatly, between 1.8 and 57.0 nanograms per microliter (ng/µL). Samples with DNA concentrations higher than 40ng/µL were diluted with Buffer AE. Extracted DNA was stored at -20°C pending further analysis. This, and subsequent sample manipulation, for the Red-footed Booby was performed at the *Université de La Réunion*.

2.2.4. Microsatellite selection and polymerase chain reaction (PCR) technique

A 3-primer polymerase chain reaction (PCR) approach, using an M13 tail (5’-CACGACGTTGTAAAACGAC-3’) for the forward primer, was used for microsatellite loci amplification following Schuelke (2000). Fifteen previously developed dinucleotide microsatellite markers were used (Morris-Pocock *et al.* 2010a; Table 2.2), alongside four fluorescently-labelled dyes (6-FAM, PET, VIC, and NED) for the universal M13 forward primer enabling fragment analysis multiplexing (Schuelke 2000). Simplex PCR amplifications, targeting one locus at a time, were performed using a GeneAmp PCR System 9 700 (Applied Biosystems) in a 10µL reaction volume containing 5µL of GoTaq® G2 Hot Start Colorless Master Mix applied 2x (Promega), 0.25µL of the forward primer with M13 5’-tail [1 micromoles {µM}], 0.25µL of the reverse primer [10µM], 0.25µL of the dye (6-FAM, NED, PET or VIC; [10µM]), and 2µL of genomic DNA [approximatey 40ng/µL]. Reaction volumes were completed with sterile deionised water. PCR amplifications were carried out under the following conditions: an initial denaturing step at 95°C for five minutes, followed by 43 primer annealing cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. A final elongation stage was then employed where temperature was held at 72°C for 20 minutes. PCR products were stored at -20°C prior to product size determination.

Up to four different simplex PCR plates, each with a different dye (Table 2.2), were mixed and PCR product sizes determined using a 3 730XL DNA analyser (Applied Biosystems), by Gentyane platform (Clermont-Ferrand, France). Product sizes were estimated with the LIZ(500) standard using GeneMapper version 4.0 (Applied Biosystems). Values of null allele sizes, not exceeding 5% of the total genotyping dataset, were accepted.

2.2.5. Data analysis

2.2.5.1 Mating pattern at Tromelin Island

Fifteen possible mating combinations exist between the different morphs of the Red-footed Booby on Tromelin and a Chi-Square (χ^2) test was used to determine whether the observed mating pattern differed from a random mating pattern. Expected values were calculated based on the probability of a single bird selecting another at random, using the morph ratio in the same sample of breeding adults as the observed values. Intermediate plumage combinations were lumped with one another, fulfilling the χ^2 assumption that more than 80% of all expected counts must contain at-least five observations (Yates *et al.* 1999). Thus, for the purposes of these statistical calculations, a distinction was made between white-tailed white, white-tailed brown, and intermediate morphs.

2.2.5.2 Primer screening and estimates of genetic diversity

Mistyped allele sizes, deviations from repeat motifs, and evidence of null alleles, large-allele dropout, and stutter peaks were first examined among all loci, for each colony/morph, using MicroChecker version 2.2.3 (van Oosterhout *et al.* 2004). Each sample-locus combination was then tested for linkage disequilibrium (LD) using GenePop version 4.0.10 (Rousset 2008), with the exact probability test (Markov chain parameters: 10 000 dememorizations, 100 batches, 1 000 iterations per batch). All resulting p-values were corrected using sequential Bonferroni correction for multiple comparisons (Rice 1989). Presence/absence of LD was confirmed with the method of Index of Association (Agapow & Burt 2001), in the package poppr (Kamvar *et al.* 2014, 2015), developed for R (R Development Core Team 2017). Additionally, to assess the overall power of all subsequent analyses, a multi-locus genotype accumulation curve was constructed using the package poppr (Kamvar *et al.* 2014, 2015), in R version 3.4.0 (R Development Core Team 2017).

Table 2.2: Characteristics of 15 dinucleotide repeat microsatellite markers employed for the Red-footed Booby (adapted from Morris-Pocock *et al.* 2010a). Nucleotides are abbreviated as follows: A = adenine, G = guanine, C = cytosine, T = thymine.

Name	Primer Sequences (5'-3')	Repeat motif	Fragment size (base pairs)	GenBank Accession No.	Mix no.	Dye
Ss2b-138	F: CAGTTCTCAGCCCTTGTTTT R: GTCAAGACTTTTCAGGAACCA	$(TG)_{13}$	354 – 360	GU175417	1	6-FAM
Ss1b-142	F: TTCCTGATTGACACCTTTGA R: AGGGACTGTGGTGTTTCTGT	$(AC)_{11}$	344 – 346	GU175422	1	NED
Ss2b-153	F: TCAAGACCTGTTTGATGGTG R: AGAAATGTGCCATGCTTCTC	$(TG)_{12}$	322 – 326	GU175424	1	PET
Ss2b-88	F: CTGGGAAGCAAGAAGTCTGT R: TCTTCCTCCTGCCTCTTATG	$(AC)_{11}$	307 – 315	GU175427	1	VIC
Ss2b-71	F: CCACAATCCATGACAGAAGA R: TTTCTCCAGCTATCCTGCTT	$(CT)_{14}$	193 – 215	GU175416	2	6-FAM
Ss2b-48	F: TTTTCAGCCTTGTTATTCAGC R: GTAGTCATTAACAGGATCAGGA	$(CA)_{13}$	157 – 171	GU175420	2	NED
Ss2b35	F: ACCTCCTGATCCTCTTGC R: TAGTGCAGCTTTCACCTCCT	$(TC)_{10}$	131 – 143	GU175428	2	PET
Ss2b-2	F: ATGGAGGCGTATGTTTTGAT R: GTAAAATTCAGCTCCCCTGA	$(TG)_{12}$	250 – 264	GU175419	2	VIC
Ss2b-110	F: CCAGAGAGAATTTCCATTGC R: CCATCTGTGTTGAAGGGGTA	$(TG)_{11}$	169 – 183	GU175418	3	6-FAM
Ss2b-92	F: CAGTGGCATTACACCTCAAT R: TCTCAAAACAGCTGGAAACA	$(AG)_9$	154 – 156	GU175430	3	NED
Ss1b-57	F: CTGGGAAGCAAGAAGTCTGT R: TCTGACCACTTGTTTGGTGT	$(AC)_9$	232 – 240	GU175425	3	PET
Ss1b-16	F: TGCACCTAACAGTAGCTCACA R: ACTGAGTTCTCACGCCTACC	$(TG)_{10}$	259 – 267	GU175426	3	VIC
Ss1b-51	F: CAGGTGGTGAGCAATTGAA R: TTTAACCCCAGTCACCAAAT	$(CA)_{12}$	233 – 245	GU175421	4	6-FAM
Ss1b-98	F: TTATCCCTTTGCTTTGCTTT R: TGGGAATGACGAAGGTAAAT	$(GT)_{10}$	247 – 253	GU175423	4	NED
Ss1b-106	F: GTGCACATGGGTCAACTTTA R: CCCAACTTCCCAGTTACATC	$(AG)_{10}$	158 – 162	GU175429	4	PET

Standard estimates of genetic diversity, including the mean number of alleles per locus (N_a), private allele richness (P_A) in each population, the effective number of alleles (A_E), observed heterozygosity over all loci (H_o), and unbiased expected heterozygosity over all loci (uH_e; Nei 1978), were calculated for all breeding colonies and morphs using GenAlEx version 6.6 (advance notice based off Peakall & Smouse 2012). The statistical significance of differences in means of both N_a and A_E, between white-tailed white and white-tailed brown morph birds, were estimated using a non-parametric Wilcoxon Signed Rank test as implemented in PAST version 3.1.5 (Hammer *et al.* 2001). Intermediate morphs were excluded from this test because of the low sample size. A Kruskal-Wallis test of equal medians was similarly used to test the significance of differences in N_a and A_E among breeding colonies of the Red-footed Booby, using PAST version 3.1.5 (Hammer *et al.* 2001). Where p-values were lower than 0.05, pairwise Mann-Whitney tests were used post hoc. Allelic richness (AR; El Mousadik & Petit 1996) was additionally calculated for each colony/morph using FSTAT version 2.9.3 (Goudet 1995, 2001), and compared among colonies/morphs using 10 000 permutations of the alleles. This latter index accounts for differences in the number of sampled individuals per population (Foulley & Ollivier 2006), allowing for comparisons of genetic diversity among breeding colonies or morphs. Intermediate morphs were excluded from calculations of AR as too few samples were collected. Deviations from Hardy-Weinberg proportions were assessed at each locus, for each breeding colony and morph, with the pegas package (Paradis 2010) in R version 3.4.0, using the exact test based on 1 000 Monte Carlo permutations of the alleles. Finally, Wright's inbreeding coefficient, within individuals relative to the subpopulation, (F_{IS}; Wright 1922) was also computed for each breeding colony and morph using GenAlEx version 6.6 (advance notice based off Peakall & Smouse 2012). This index quantifies the degree to which heterozygosity is reduced among individuals in a subpopulation, relative to what would be expected if mating was panmictic within each subpopulation (Wright 1922). Values of F_{IS} range between -1 and +1. Those close to zero are expected under random mating, while substantial positive values indicate inbreeding possibly due to assortative mating or undetected null alleles and population sub-structuration (Wright 1922, 1965). Negative values of F_{IS} indicate excess heterozygosity, due either to disassortative mating or selection for heterozygotes (Wright 1922).

2.2.5.3 Genetic differentiation among populations

Wright's overall multi-locus fixation index ($F_{ST;}$ Wright 1922), and its associated p-value, was computed over all loci and populations following Weir and Cockerham (1984) using the package adegenet (Jombart 2008; Jombart & Ahmed 2011), in R version 3.4.0. F_{ST}, when calculated over all loci and populations, quantifies levels of population subdivision by describing the mean reduction in observed heterozygosity, in pre-defined subpopulations, relative to the expected heterozygosity of the total population. It assumes an Infinite Allele Model (IAM; Kimura & Crow 1964) and rests on the underlying assumption that higher F_{ST} values are expected in situations where fewer migrants are shared among populations. Theoretically, the division of a population into smaller subpopulations decreases genetic diversity (measured as heterozygosity) due both to a reduction in effective population size (relative to the parent or total population) and an increased effect of genetic drift (Nei 1978). F_{ST} thus provides an indirect measure of gene-flow (or lack thereof) among populations. Values of F_{ST} can change depending on how the subpopulations are defined within the analyses, however. The statistical significance of differences in these indices, among pairs of populations/morphs, were thus tested using 10 000 random permutations of the raw genotypes using the package adegenet, in R version 3.4.0.

2.2.5.4 Migration rates and effective population size

Wright's F_{ST} has widely been used to estimate migration rates among genetic populations, of a given species, based on a generalised formula (Wright 1943). This is:

$$F_{ST} = \frac{1}{(1 + 4Nm)}$$

Unfortunately, the underlying mathematical model makes many biologically unrealistic assumptions (Whitlock & McCauley 1999), including equal effective sizes of subpopulations (N), no mutation, a migration/drift equilibrium, and uniform migration rates among all paris of populations (m; Yamamichi & Innan 2011). Almost all natural populations violate these expectations, implying that there is often limited information to be gained about dispersal from multi-locus allele frequency data (Whitlock & McCauley 1999). The extent of the bias caused by such violations has not yet been fully explored, however.

An alternative approach, based on Faubet *et al.* (2007), was therefore adopted to calculate directional migration rates among populations per generation following a Markov Chain Monte Carlo (MCMC) method. This assumes only that loci are unlinked and that individuals were sampled at random from their source population. Directional migration rates (m) among all pairs of populations were calculated with the software Bayesian Inference of imMigration rates (BIMr), using 10 MCMC iterations of the raw genotype data and default values for burnin and thinning. The mean value of m, for each pair of populations in each direction (from, into), was subsequently multiplied by an estimate of the effective population size (N_e) for each colony to determine the number of migrants (Nm) per generation.

N_e, or the size of a hypothetical population that would lose heterozygosity at a rate equal to that of the observed population (Wright 1931, 1938; Crow & Kimura 1970; Wang 2005), is widely regarded as one of the most important parameters in both evolutionary

(Charlesworth 2009), and conservation biology (Nunney & Elam 1994; Frankham 2005). It is estimated based on the time-period encompassed by the sampling effort, and can either be a single sample or two sample (temporal) estimate (Wang 2005; Waples & Do 2010). Contemporary estimates of N_e were calculated for the three Red-footed Booby breeding colonies using a single-sample estimator based on the linkage disequilibrium (LD) model implemented in both LDNE (Waples & Do 2008), and NeEstimator version 2 (Do *et al.* 2014). Though temporal methods of estimating N_e have been more widely applied, the LD method generally provides better precision when samples are temporally spaced nearer to one another as is the case in the present study (Waples & Do 2008). The non-random association (or LD) between alleles is produced by several factors, including migration, direct or indirect selection, and genetic drift within finite populations. For neutral loci in isolated populations, with random mating, LD would come exclusively from genetic drift and can be used to estimate N_e (Hill 1981). N_e can also be calculated, based on the LD model, for monogamous animals as is more typical for seabirds (Schreiber & Burger 2002). A mean value was calculated across the two software platforms (LNDE and NeEstimator version 2), assuming monogamous mating within the three breeding populations of the Red-footed Booby. Two allele frequencies were adopted as cut-offs: 0.02, which is recommended for samples of less than 25 individuals per population (Waples & Do 2010), and 0.05. Estimates of 95% confidence intervals (CI) were calculated by jackknifing over loci for each estimate.

2.2.5.5 Population structure

Genetic diversity within, and genetic differentiation among, populations was principally assessed using Principal Coordinate Analyses (PCoA) based on scaled overall multi-locus genotypes, using a Euclidian distance matrix between individuals among the three populations. PCoA is often used to investigate genetic structuring among individuals, offering a simplified and preliminary (yet meaningful) view of genetic variability among populations within a multi-dimensional space (Jombart *et al.* 2009). The benefit of this multivariate test lies in its ability to extract information from genetic markers, without requiring rigid assumptions about genetic models (e.g. Hardy-Weinberg equilibrium or LD). These analyses were implemented using the package adegenet (Jombart 2008; Jombart & Ahmed 2011), in R version 3.4.0.

Assignment tests, based on the multi-locus genotypes, were additionally used to assess differentiation and clustering among populations and morphs of the Red-footed Booby. These were evaluated using a model-based Markov Chain Monte Carlo (MCMC) clustering procedure, implemented with a Bayesian algorithm, in STRUCTURE version 2.3.3 (Pritchard *et al.* 2000). The model assumes several genetic clusters, denoted as K, each of which is characterised by a unique pattern of allele frequencies at each sampled locus (Pritchard *et al.* 2000). Individuals in the sample are assigned (probabilistically) to the genetic clusters, or jointly to two or more clusters if their genotypes indicate that they are admixed (Pritchard *et al.* 2000). Membership coefficients (termed *Q*) are assigned to all individuals for each value of K (Pritchard *et al.* 2000).

Multiple independent simulations using a burn-in period of 5 000 simulations, followed by 10 000 repetitions, were first run in STRUCTURE version 2.3.3 using different settings (e.g. admixture versus no-admixture model, LOCPRIOR verses no-LOCPRIOR) to assess convergence and determine the importance of model choice. An admixture model (Dirchelet parameter; $\alpha = 1.25 \pm 0.25$), assuming sampling location (LOCPRIOR; $r = 0.95 \pm 0.22$; Hubisz *et al*. 2009), was finally used. This model, when compared to all other STRUCTURE algorithms, is best suited to populations with weak genetic structure or datasets employing less than 20 microsatellite loci (Hubisz *et al*. 2009). Correlated allele frequencies were assumed to account for the association between linked loci that arise in admixed populations (Pritchard *et al*. 2000; Falush *et al.* 2003). To estimate K, 10 independent runs of 10 000 iterations each (after a burn-in of 5 000 steps) were performed for K = 1 to K = 5. The optimal value of K was subsequently determined using the mean overall log-likelihood given the number of clusters (lnP(X|K); Pritchard *et al*. 2000) and the second-order rate of change of lnP(X|K) (ΔK; Evanno *et al*. 2005). Ten independent simulations, at the optimal value of K, were then run using 1 000 000 iterations each after a burn-in period of 500 000 steps. The simulation with the highest value of lnP(X|K), at the optimal value of K, was finally used to plot the estimated membership of all individuals to the different clusters. To assess the population structure of the Red-footed Booby in the western Indian Ocean, irrespective of sampling location, a second admixture model (Dirchelet parameter; $\alpha = 1.49 \pm 0.23$) was employed using morph as the LOCPRIOR factor ($r = 0.99 \pm 0.15$) and three morph categories (white-tailed white, white-tailed brown, intermediate) as prior information.

2.3. Results

2.3.1. Plumage polymorphism at Tromelin Island

Eight hundred and fifty-one active nests were found on Tromelin, suggesting that there were over 1 700 breeding adults in attendance during the study period. The colour morphs of 1 335 (78% of breeding population) breeding adults were identified. The white-tailed white morph dominated the colony, accounting for 62% of all breeding birds, yet white-tailed brown birds were also particularly numerous (26%; Figure 2.3). White birds with brown scapulars (3%), brown birds with yellow heads and white scapulars (6%), and brown birds with yellow heads and brown scapulars (3%) all occurred, though in low abundances (Figure 2.3).

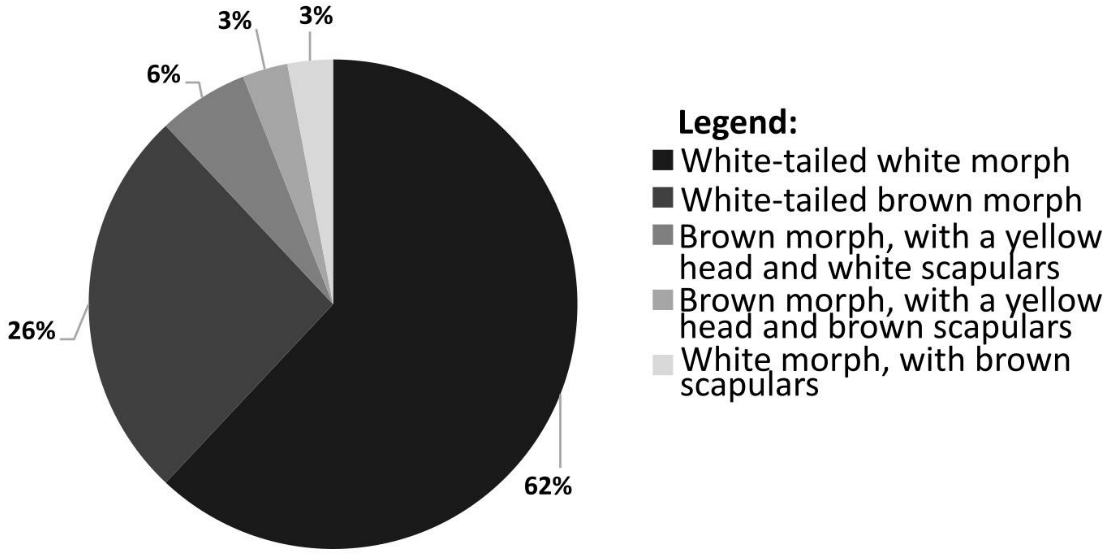

Figure 2.3: Frequency of abundance of five morphs among 1 335 breeding adult Red-footed Boobies on Tromelin island between 16 April 2013 and 22 May 2013.

2.3.2. Mating pattern at Tromelin Island

The colour morphs of both adults were recorded at 520 nests on Tromelin, representing approximately 60% of all breeding pairs on the island at the time (Figure 2.4). All possible mating combinations were found, except the paired combination of white morph birds with brown scapulars and brown morph birds with yellow heads and brown scapulars (Table 2.3). Fifty-four percent of all couples were of the same colour morph, whilst an additional 24% of the couples were of a white-tailed white and white-tailed brown morph combination. Additionally, intermediate forms more frequently mated with white-tailed brown birds compared with white-tailed white individuals. The overall mate selection differed from the expected pattern ($\chi^2 = 29.68$; df= 5; p-value < 0.05; Table 2.3), suggesting that mate preference was assortative with respect to plumage polymorphism.

Table 2.3: Observed and expected mate combinations, by colour morph, among Red-footed Boobies at Tromelin Island. Shaded mating combinations were lumped for the purposes of χ^2 analysis giving a total of 6 possible mate combinations. Colour morphs are abbreviated as follows: W = white-tailed white, B = white-tailed brown, WBS = white morph, with brown scapulars, BYB = brown morph, with yellow head and brown scapulars, BYW = brown morph, with yellow head and white scapulars, I = lumped intermediate morphs.

	Expected frequency of pair combinations (n= 1 038)	Expected mating pattern (n=519)	Observed mating pattern (n= 519)
W/W	0.384	199.157	227
W/B	0.326	169.113	127
B/B	0.069	35.900	49
W/BYW	0.075	39.026	34
B/BYW	0.032	16.569	25
W/WBS	0.044	22.920	19
B/WBS	0.019	9.731	13
B/BYB	0.011	5.786	10
W/BYB	0.026	13.628	9
WBS/WBS	0.001	0.659	2
WBS/BYW	0.004	2.246	1
BYB/BYB	4.0×10^{-4}	0.233	1
BYB/BYW	0.003	1.335	1
BYW/BYW	0.004	1.912	1
WBS/BYB	0.002	0.784	0
I/I	0.014	7.170	6
B/I	0.062	32.087	48
W/I	0.145	75.574	62
Total:	1	519	519

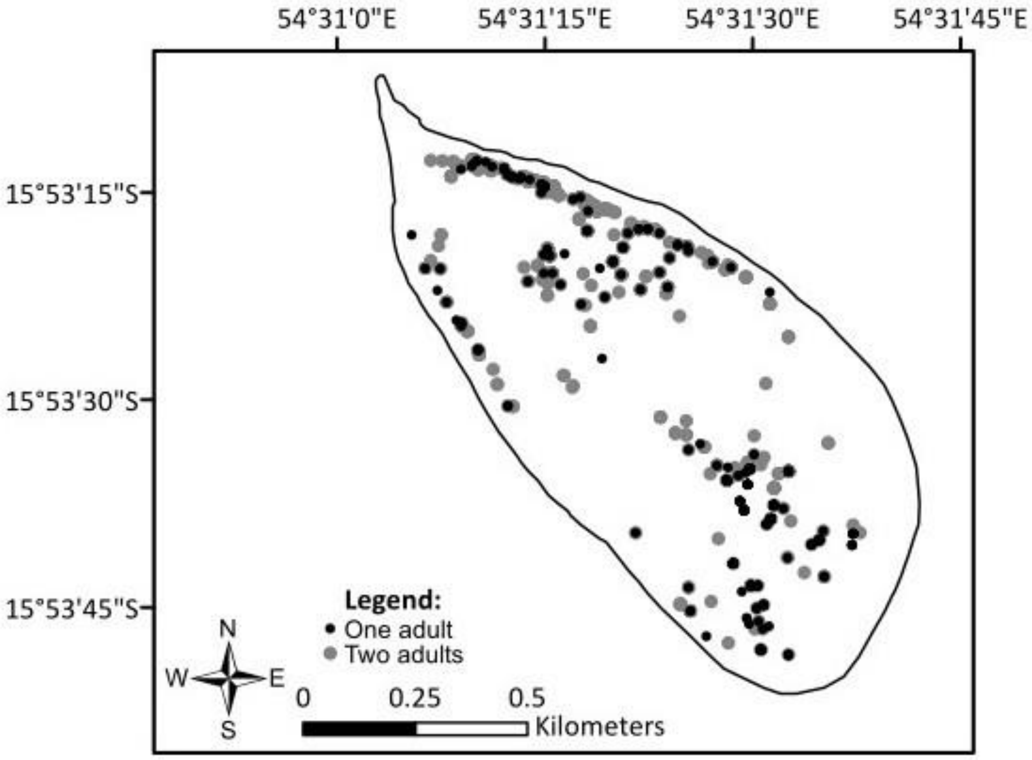

Figure 2.4: The distribution of Red-footed Booby nests on Tromelin Island, between 16 April 2013 and 22 May 2013, indicating those for which the colour morph of one and both adults were attained.

2.3.3. Description of data, tests of assumptions, and genetic diversity

All fifteen nuclear microsatellite loci successfully amplified in more than 97% of all samples from the three Red-footed Booby breeding colonies. As little as 0.62% of the global dataset consisted of null values, with a maximum of 4.55% missing data at any one locus in each population.

No significant linkage disequilibrium was observed among any loci (corrected exact Markov chain probability test, all p-values > 0.05; Index of Association method, p-value > 0.05). Null allele frequency was low, ranging from -0.21 to 0.28 among all colonies, though presence was detected at loci Ss2b-88, Ss2b-35, and Ss2b-92 at Aldabra, Ss1b-142, Ss2b-35, and Ss2v-92 at Europa, and Ss1b-142 at Tromelin. All loci for which null alleles were detected were associated with homozygote excesses. This overall pattern was not consistent among the separate colonies, however. No large-allele dropout or stutter peaks were observed among any loci. N_a ranged between 4.67 ± 0.49 at Europa and 5.27 ± 0.67 at Aldabra (grand mean across populations 4.89 ± 0.32), while A_E varied between 2.78 ± 0.28 at Europa and 3.06 ± 0.37 at Aldabra (grand mean across populations 2.90 ± 0.18; Table 2.4a). Differences in both N_a and A_E among the three breeding colonies of the Red-footed Booby were not statistically significant (Kruskal-Wallis test: both p-values > 0.09). Discrepancy between estimates of A_E and N_a, along with allelic frequency distributions (Figure 2.5), indicated a high number of rare alleles (frequency < 0.20) within each of the three breeding colonies. AR, based on a minimum sample size of 21 diploid individuals, ranged between 4.36 ± 1.70 at Europa and 4.98 ± 2.23 at Aldabra (grand mean across populations 4.90 ± 1.79; Table 2.4a) with no significant differences among colonies (p > 0.10). The three populations all contained P_A with Aldabra and Tromelin showing the highest and lowest counts, respectively (Table 2.4a). Only two P_A had a frequency greater than 0.05, one each for Aldabra and Europa, but all accounted for more than 0.01 of the allelic variation at each locus in their respective populations. Locus specific deviations from Hardy-Weinberg proportions at the 99% confidence interval were observed at loci Ss2b-88, Ss2b-35, and Ss2b-92 at Aldabra, Ss1b-142, Ss2b-35, and Ss2b-91 at Europa, and Ss1b-142 at Tromelin. This corresponds to those loci, in each population, for which significant presence of null alleles was detected. However, the global data showed no significant deviations from Hardy-Weinberg proportions (p-value = 0.14). All microsatellite loci were therefore retained in all analyses. F_{IS} was generally low, never exceeding 0.11 ± 0.07 in all three breeding colonies of the Red-footed Booby, suggesting random mating (Table 2.4a). H_o was lowest at Europa (0.53 ± 0.06) and highest at Tromelin (0.58 ± 0.06; grand mean across populations 0.56 ± 0.04; Table 2.4a). uH_e was also lowest at Europa (0.58 ± 0.05) and highest at Tromelin (0.62 ± 0.04; grand mean across populations 0.60 ± 0.03; Table 2.4a). The number of multi-locus genotypes recorded across all populations remained stable after 9 loci, suggesting that this use of all 15 polymorphic microsatellite loci was sufficient to describe the genetic diversity and population structure of Red-footed Boobies in the western Indian Ocean (Figure 2.6).

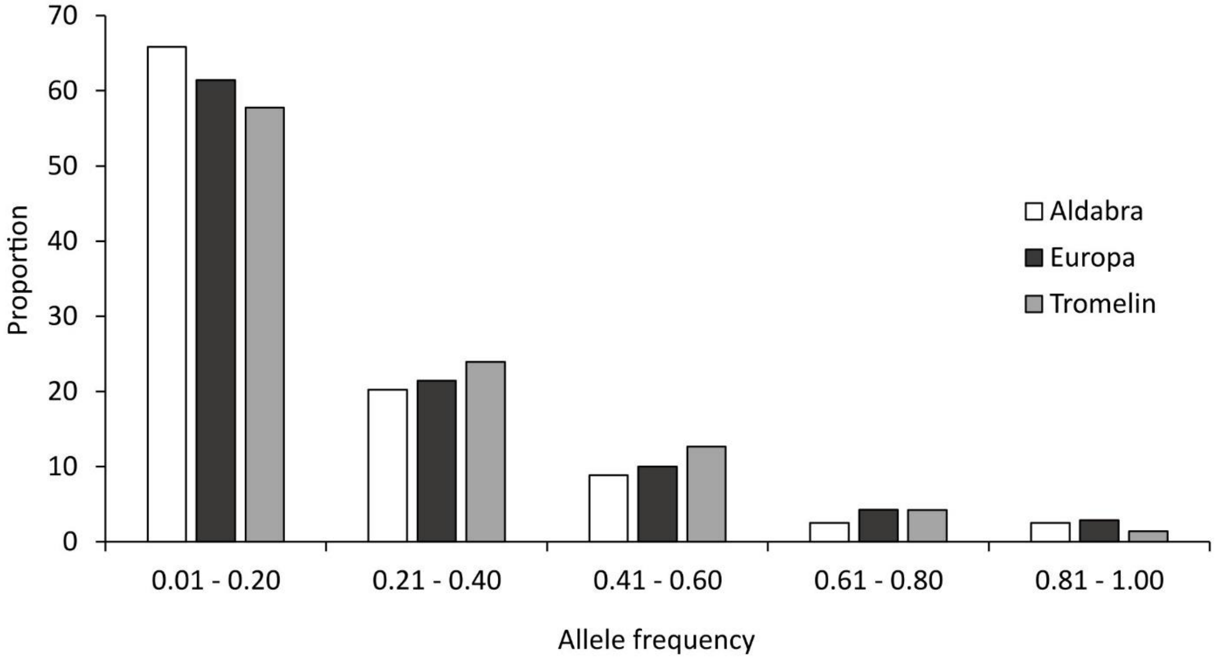

Figure 2.5: Allele frequency distributions, standardised across 15 microsatellite loci, of Red-footed Boobies sampled at three breeding colonies in the western Indian Ocean.

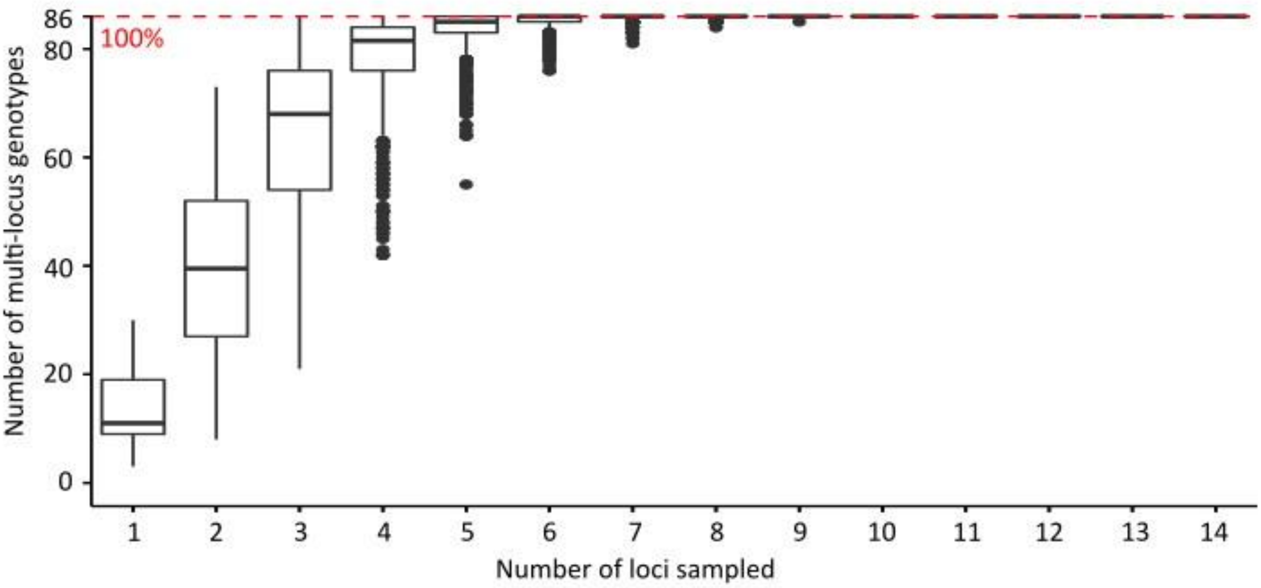

Figure 2.6: Multi-locus genotype accumulation curve based on 15 dinucleotide repeat microsatellite loci, employing 86 individual Red-footed Boobies sampled at three breeding colonies.

Concerning genetic diversity by colour morph. N_a ranged between 5.13 ± 0.47 in white-tailed brown morph birds and 5.40 ± 0.70 in white-tailed white morph individuals (grand mean across morphs 4.33 ± 0.35; Table 2.4b). The difference in N_a between these two morphs was not statistically significant, however (Wilcoxon test: W = 60; p-value = 1). Intermediate forms had a lower N_a (2.47 ± 0.31; Table 2.4b), but this was almost certainly an artefact of the low sample sizes. A_E was also similar (Wilcoxon test: W = 47; p-value = 0.26), between the two dominant morphs: 2.99 ± 0.36 and 2.96 ± 0.31 in white-tailed white and white-tailed brown morphs, respectively. Differences between estimates of N_a and A_E, combined with the allele frequency distribution (Figure 2.7), indicated many rare alleles within the two dominant morphs. AR, based on a minimum sample size of 37 diploid individuals, ranged between 4.73 ± 0.51 and 5.64 ± 0.64 in white-tailed brown and white-tailed white morph individuals, respectively (grand mean 5.44 ± 0.50; Table 2.4b), with no significant differences among morphs (p-value > 0.30). P_A were found in both dominant morphs (Table 2.4b), though only two in white-tailed brown birds had a frequency greater than 0.05. Allelic frequency of all other P_A exceeded 0.01 of the total variation at each locus within each morph, however. F_{IS} was generally low, never exceeding 0.11 ± 0.07, in all three morph classes of the Red-footed Booby (Table 2.4b). H_o and uH_e were similar among the three morphs, ranging from 0.55 ± 0.06 to 0.56 ± 0.11 in intermediate morph birds and 0.56 ± 0.07 to 0.61 ± 0.05 in white-tailed brown morph birds (Table 2.4b).

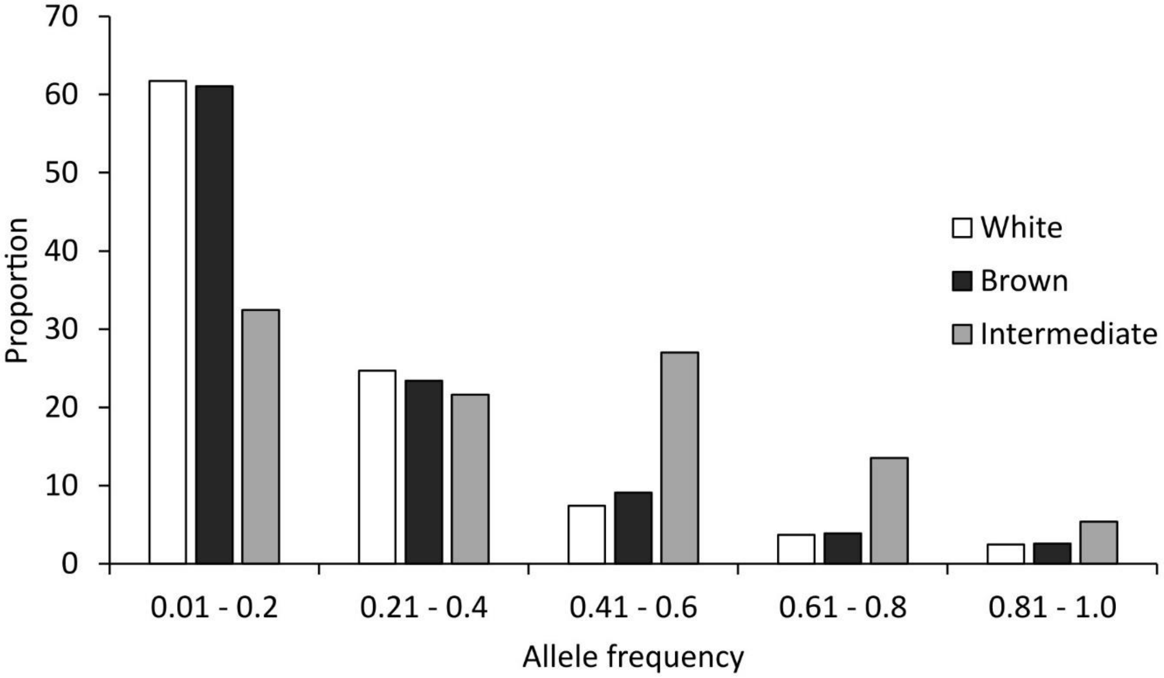

Figure 2.7: Allele frequency distributions, standardised across 15 microsatellite loci, of three Red-footed Booby morph classes in the western Indian Ocean.

Table 2.4: Estimates of allelic diversity at a) three breeding colonies and b) in three colour morph classes of Red-footed Booby in the western Indian Ocean, based on 15 polymorphic dinucleotide repeat microsatellite loci.

a)

Breeding colony	**n**	**N_a**	**AR**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
Aldabra	27.73 ± 0.11	5.27 ± 0.67	4.98 ± 2.23^a	14 (18%)	3.06 ± 0.37	0.56 ± 0.06	0.61 ± 0.05	0.10 ± 0.06
Europa	35.80 ± 0.12	4.67 ± 0.49	4.36 ± 1.70^a	5 (7%)	2.78 ± 0.28	0.53 ± 0.06	0.58 ± 0.05	0.11 ± 0.07
Tromelin	21.93 ± 0.07	4.73 ± 0.05	4.76 ± 1.88^a	3 (4%)	2.86 ± 0.26	0.58 ± 0.06	0.62 ± 0.04	0.07 ± 0.07
Total	28.49 ± 0.86	4.89 ± 0.32	4.90 ± 1.79	N/A	2.90 ± 0.18	0.56 ± 0.04	0.60 ± 0.03	0.09 ± 0.04

b)

Colour morph	**n**	**N_a**	**AR**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
White-tailed White	37.67 ± 0.16	5.40 ± 0.70	5.64 ± 0.64^b	16 (20%)	2.99 ± 0.36	0.56 ± 0.07	0.60 ± 0.05	0.11 ± 0.07
White-tailed Brown	42.80 ± 0.15	5.13 ± 0.47	4.73 ± 0.51^b	13 (18%)	2.96 ± 0.31	0.55 ± 0.06	0.61 ± 0.05	0.10 ± 0.07
Intermediate	3.00 ± 0.00	2.47 ± 0.31	N/A	0 (0%)	2.21 ± 0.24	0.56 ± 0.11	0.56 ± 0.07	N/A
Total	27.82 ± 2.66	4.33 ± 0.35	5.44 ± 0.50	N/A	2.72 ± 0.18	0.55 ± 0.05	0.59 ± 0.03	0.02 ± 0.07

n = mean number of individuals per locus ± s.e.; N_a = mean number of alleles per locus ± s.e.; AR = mean allelic richness per locus ± s.e. based on a minimum sample size of 21 diploid individuals (superscripts indicate statistically homogenous groups); P_A = private allele richness (proportion of private alleles from total allelic richness in parenthesis); A_E = mean number of effective alleles ± s.e.; H_o = observed heterozygosity over all loci ± s.e; uH_e = unbiased expected heterozygosity ± s.e.; F_{IS} = fixation index calculated based on Weir and Cockerham (1984)

2.3.4. Effective population size and migration rates among breeding colonies

Europa had the lowest overall estimates of $\mathrm{N_e}$; between 116.35 and 142 at the cut-offs of 0.05 and 0.02, respectively. The highest overall estimates of N_e were calculated as between 510.65 and 1636.5 for Aldabra, at the cut-offs of 0.05 and 0.02, with Tromelin showing intermediate N_e estimates of between 103.75 and 478.5 (Table 2.5a and 2.5b).

Using these estimates of N_e and calculated m from BIMr, it appears that there is considerable exchange of individuals among the three sampled breeding colonies of the Red-footed Booby in the western Indian Ocean (Figure 2.8). Europa, Tromelin, and Aldabra were all connected to one another with movement in both directions among all pairs of populations (Figure 2.8). The greatest movement occurred into and out of Aldabra, with more connectivity to Tromelin than to Europa; the highest estimates of migration occurred in the direction of Aldabra to Tromelin (Figure 2.8).

Table 2.5: Estimates of effective population size ($\mathrm{N_e}$) and their associated 95% confidence intervals (CI), calculated using two approaches, at three Red-footed Booby breeding colonies based on allele frequency cut-offs of a) 0.05 and b) 0.02.

		Ne estimator method		**LNDE method**		
a)	**Population**	**Ne**	**95% CI**	**Ne**	**95% CI**	**Mean Ne**
	Aldabra	505.1	127 – ∞	516.2	128 - ∞	510.65
	Europa	116.2	72 – 245	116.5	72 - 246	116.35
	Tromelin	103.4	54 – 490	104.1	54 - 505	103.75

b)	**Population**	**Ne**	**95% CI**	**Ne**	**95% CI**	
	Aldabra	1584	172 – ∞	1689	173 – ∞	1636.5
	Europa	142	87 – 319	142	87 – 321	142
	Tromelin	472	111 – ∞	485	111 – ∞	478.5

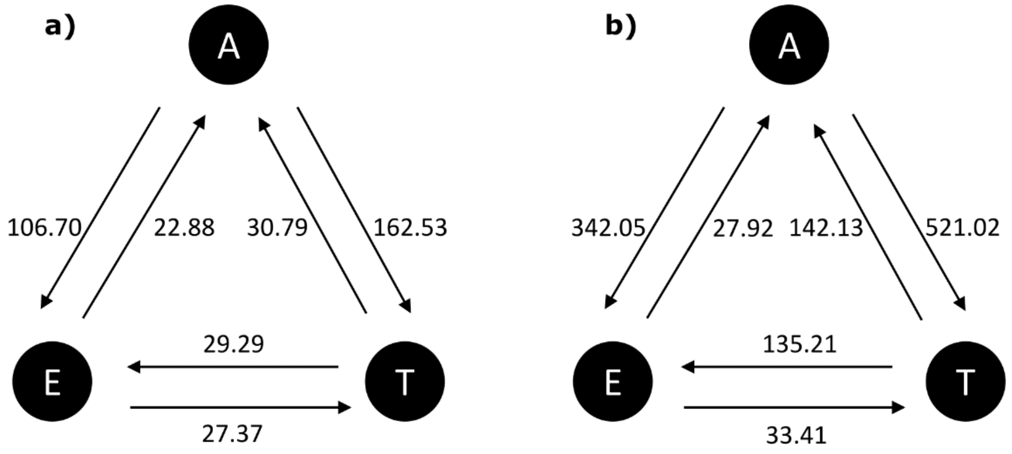

Figure 2.8: Estimates of the number of migrants per generation (Nm) among breeding colonies of Red-footed Booby in the western Indian Ocean, based on mean estimates of effective population size (N_e) at the allele frequency cut-offs of a) 0.05 and b) 0.02. Breeding colonies are abbreviated as follow: A = Aldabra, E = Europa, T = Tromelin. Arrows denote direction of movement.

2.3.5. Genetic differentiation among populations/morphs

Among the colonies, pairwise F_{ST} values were all 0.02 with a global F_{ST} of 0.02 (p-value< 0.05; Table 2.6). All pairwise F_{ST} values were significant, indicating three distinct genetic groups of birds (Table 2.6). The Aldabra and Europa populations were highly significant from each other (p-value < 0.01; Table 2.6), while Tromelin was significantly different from each of the other two colonies (p-value < 0.05; Table 2.6). These F_{ST} values indicated weak, though statistically significant, genetic structure across the sampled populations of the Red-footed Booby.

Among the different colour morphs, pairwise F_{ST} values ranged between 0.01 and 0.02 with a global F_{ST} of 0.01 (p-value < 0.01; Table 2.7). As was expected, white-tailed white and white-tailed brown morph birds were significantly different from one another (Table 2.7).

Intermediate morphs were, similarly, significantly different from both the white-tailed white and white-tailed brown morph birds (Table 2.7). These F_{ST} values indicated weak, although statistically significant, genetic structure across the different morphs.

Table 2.6: Pairwise F_{ST} estimates (below diagonal), and levels of statistical significance (above diagonal), among three breeding colonies of Red-footed Booby from the western Indian Ocean. Significant differences are indicated by asterisks.

	Aldabra	**Europa**	**Tromelin**
Aldabra	N/A	2.0 x10^{-3}*	0.02*
Europa	0.02	N/A	0.02*
Tromelin	0.02	0.02	N/A

Table 2.7: Pairwise F_{ST} estimates (below diagonal), and levels of statistical significance (above diagonal), among colour morphs of Red-footed Booby from three breeding colonies in the western Indian Ocean. Significant differences are indicated by asterisks.

	White-tailed White	**White-tailed Brown**	**Intermediate**
White-tailed White	N/A	0.01*	0.03*
White-tailed Brown	0.01	N/A	0.02*
Intermediate	0.02	0.02	N/A

The first two axes of the Euclidean distance-standardised PCoA, representing both breeding colonies and morphs, accounted for less than of 10% of the total genetic variation in the sampled Red-footed Boobies (Figure 2.9). This, at the very most, indicates weak differentiation among populations/morphs within the western Indian Ocean.

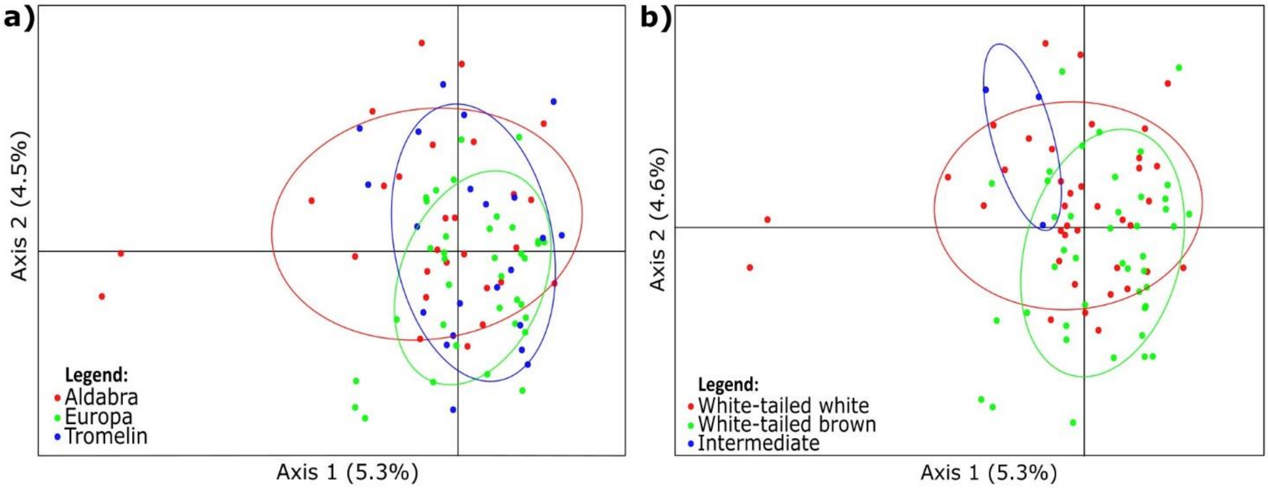

Figure 2.9: First and second axes of the Euclidian-distance standardised PCoA, including standard inertia ellipses, visualising the genetic diversity within, and genetic differentiation among, a) individuals from three breeding colonies and b) individuals of three colour morph classes of Red-footed Booby in the western Indian Ocean.

The best-supported model for the Bayesian clustering analysis, performed in STRUCTURE version 2.3.3, representing both breeding colonies and morphs (based on Evanno *et al.* 2005) was that of two genetic clusters (maximum value of ΔK at K = 2; Figure 2.10; results not presented for morphs). This was supported by the method of Pritchard *et al.* (2000; lowest standard error [s.e.] for lnP(X|K) at K = 2; Figure 2.10). These corresponded to the predicted scenario; Europa (overall $Q_{cluster1}$ = 7%, overall $Q_{cluster2}$ = 93%) and Aldabra (overall $Q_{cluster1}$ = 98%, overall $Q_{cluster2}$ = 2%) were each assigned to different genetic populations (Figure 2.11a). Individuals sampled at Tromelin showed varying levels of admixture to the two genetic clusters (overall $Q_{cluster1}$ = 65%, overall $Q_{cluster2}$ = 35%), however (Figure 2.11a). Upon closer inspection, the individual values of *Q* were unrelated to plumage polymorphism (mean assignment values for white-tailed white individuals: $Q_{cluster1}$ = 65%, overall $Q_{cluster2}$ = 35%; mean assignment values for white-tailed brown individuals $Q_{cluster1}$ = 60%, overall $Q_{cluster2}$ =

40%). Though when modelled using colour morph, disregarding sampling location, white-tailed white morph birds were all assigned to a single major genetic cluster (overall $Q_{cluster1}$ = 99%, overall $Q_{cluster2}$ = 1%), while white-tailed brown morph individuals constituted another cluster (overall $Q_{cluster1}$ = 4%, overall $Q_{cluster2}$ = 96%; Figure 2.11b). The three brown-based intermediate forms were all assigned closer to white-tailed brown morph birds (overall $Q_{cluster1}$ = 53%, overall $Q_{cluster2}$ = 47%; Figure 2.11b).

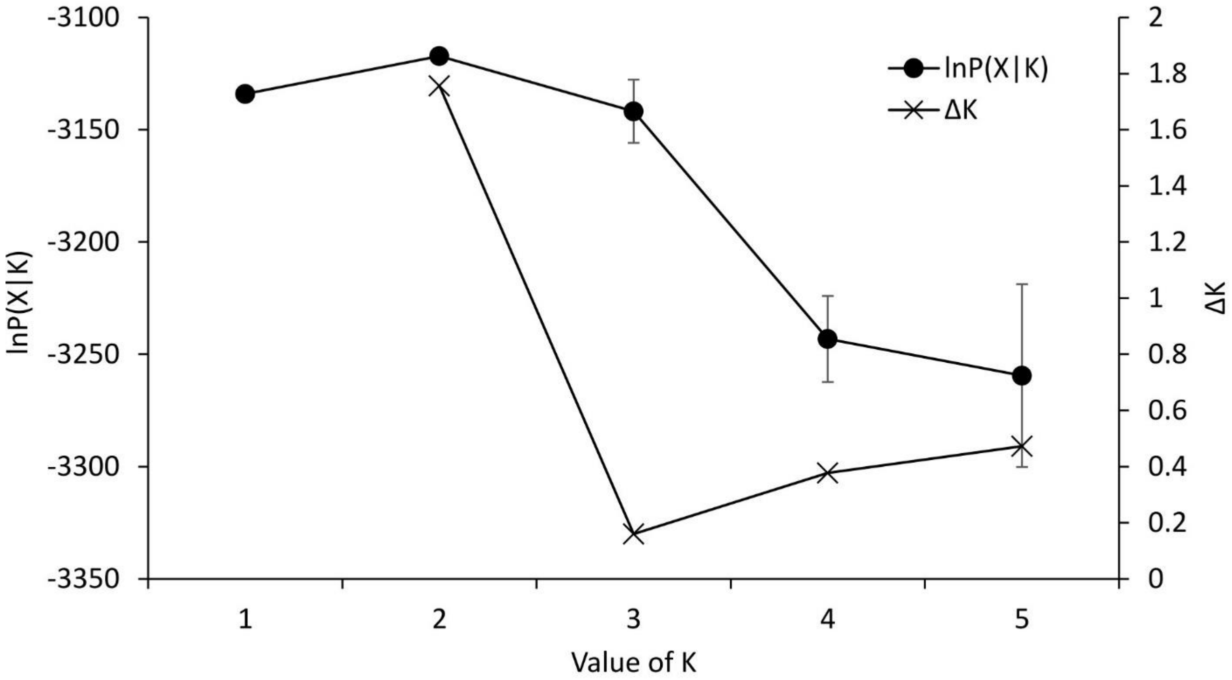

Figure 2.10: lnP(X|K) and ΔK (Evanno *et al*. 2005), as obtained in STRUCTURE version 2.3.3 representing breeding colonies of the Red-footed Booby, with Kmax ranging between 1 and 5. Each value was obtained by averaging the posterior probabilities of 10 independent runs.

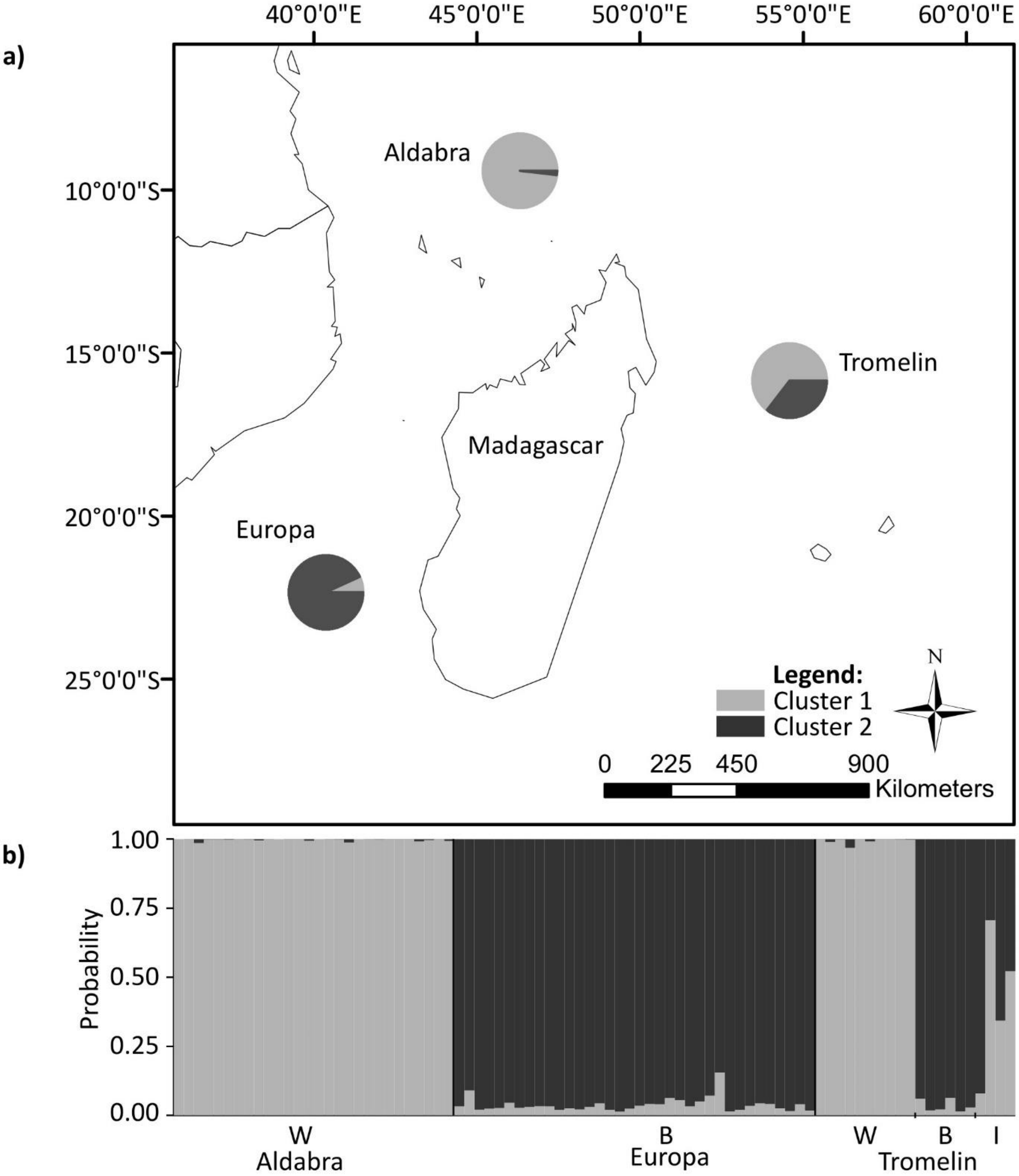

Figure 2.11: Results from two independent MCMC clustering procedures, performed in STRUCTURE version 2.3.3, visualising a) the overall membership of three Red-footed Booby breeding colonies, assuming sampling location, to two genetic clusters and b) proportional membership of different Red-footed Booby colour morphs, regardless of sampling location, to the two genetic clusters. Vertical bars represent different individuals and colour morphs

are abbreviated as follows: W = white-tailed white morph, B = white-tailed brown morph, I = intermediate morphs.

2.4. Discussion

2.4.1. Overall population structure of Red-footed Boobies

The population structure of Red-footed Boobies in the western Indian Ocean, with reference to plumage polymorphism, was first addressed by Le Corre (1996, 1999). Based on the observed distribution of the various morphs, Le Corre classified breeding colonies into three broad groups: the white-tailed white morph dominated colonies, the white-tailed brown morph dominated colonies, and the mixed colony on Tromelin. From this, he inferred limited admixture among the three populations and proposed at-least two genetic clusters of birds. The rationale behind this was that spatial differences in morph ratios were thought to be attributed to local selective forces, operating at extremely fine scales, or limited degrees of genetic isolation or connectivity among islands (Nelson 1978). Le Corre (1996, 1999) additionally predicted that the white-tailed brown morph dominated population on Europa, in the southern Mozambique Channel, was strongly isolated from all other extant breeding colonies in the western Indian Ocean. This was due to both the geographic isolation of, and the local marine environment surrounding, the island (Le Corre 1999). Polymorphism was since identified to have a genetic basis in the Red-footed Booby (Baião *et al.* 2007), providing indirect support for Le Corre, though its exact relation to the species' overall population structure has, until now, not been investigated. In addressing the relationship between genetic population structure and plumage polymorphism in the Red-footed Booby, the present study offers definitive support for Le Corre (1996, 1999). Wright's overall multi-locus fixation index (F_{ST}; Wright 1922, 1965) highlighted weak, though highly significant, genetic structure between the Aldabra and Europa populations of the Red-footed Booby. This was supported by the Bayesian assignment tests, performed in STRUCTURE version 2.3.3, which identified two weakly differentiated genetic clusters of birds. These correspond to the white-tailed white (i.e. Aldabra) and white-tailed brown (i.e. Europa) morph dominated breeding populations. These populations were characterised by distinct allele frequencies and a moderate proportion of private alleles. F_{ST} additionally indicated that both the Aldabra and Europa populations of the Red-footed Booby were weakly, though significantly, different from the mixed colony on Tromelin. However, the Bayesian assignment tests indicated that this colony is characterised by an intermediate overall genetic structure with individually varying degrees of relation to each of the other two populations. Generally, white-tailed white and white-tailed brown morph birds from Tromelin clustered alongside those sampled at Aldabra and Europa, respectively. These results were unsurprising and, to confirm the predictions of Le Corre (1996, 1999), can be explained by two non-mutually exclusive hypotheses.

First, the ratio-cline in plumage polymorphism may be related to varying degrees of genetic connectivity among islands. BIMr indicated that Europa and Aldabra populations were mostly isolated from one another on either side of the Mozambique Channel, suggesting that limited gene flow occurs between them, whereas birds breeding on Tromelin show relation (and possible ancestry) to white-tailed brown and white-tailed white morph dominated populations elsewhere. However, the estimation of migration rates from multi-locus genotype data is notoriously difficult and is unlikely to be accurate given the level of genetic differentiation among the three populations. Importantly though, the STRUCTURE analysis indicated that no first-generation migrants were sampled suggesting that gene flow into Tromelin may either have been historical, or that it is irregular within contemporary time-scales (see Chapter 2.4.2). The STRUCTURE analysis could also imply that the colony on

Tromelin exists as a result of at-least two independent colonisation events, from separate populations elsewhere (Mayr 1942). Whether or not this includes Europa and Aldabra, as the current results suggest, remains unclear since many other important breeding colonies (e.g. Cosmoledo) were not included in the present study. Initial colonisation of Tromelin by seabirds also occurred long prior to the first visits by man (Le Corre *et al*. 2015), at a time when sulid populations in the region far exceeded their present numbers (Feare 1978). Birds on Tromelin could thus have originated from other breeding colonies that are not active today (e.g. Glorieuses). Though necessarily speculative, this hypothesis is consistent with the microsatellite results of the present study and the observed ratio-cline in the region.

Selective forces could also result in fitness differences among the different morphs, thus contributing to the divergence of the Aldabra and Europa populations on either side of the Mozambique Channel. This hypothesis would assume that these two breeding colonies are genetically isolated from one another, enabling divergent evolution, or that gene flow between them is extremely rare. Variables of selective significance to plumage polymorphism may include intrinsic characteristics of the birds themselves, including their behaviour, and/or characteristics of the islands on which they breed. Intrinsic differences among individuals may, as examples, include their ability to regulate temperature, differences in breeding phenologies or foraging behaviour, differential vulnerability to kleptoparasitism, or some undescribed form of social interaction or cue among birds (Le Corre 1999). Selective forces at, and surrounding, breeding colonies may include ambient temperature, sea-surface temperature (SST), surface chlorophyll *a* concentration, substrata colour, and the presence or absence of frigatebirds. A third scenario is that the high overall SST of the Mozambique Channel may constitute a cryptic physical barrier to gene flow and may thus limit movement of birds between Aldabra and Europa. To expand this theory, the mixed population on

Tromelin may be subjected to intermediate (or more variable) selective forces benefitting both dark and pale plumaged birds. Indeed, Pocklington (1979) noted that, while islands to the north of Madagascar are permanently surrounded by warm water of intermediate salinity and Europa is situated in a zone where warm summer water is routinely replaced by cooler water in the winter, Tromelin is positioned in an area of mixed and highly variable oceanographic conditions.

The theory that selective forces could result in fitness differences among the different morphs, thus driving divergent evolution among colonies of the western Indian Ocean, may be supported by the occurrence of the last remaining white-tailed brown morph dominated population at Europa. The Mozambique Channel is characterised by high overall SST, and Le Corre and Jouventin (1999) and Cheke (2001) both argued that this has contributed towards the isolation of Europa's seabird community. A similar biogeographic pattern has been observed in the White-tailed Tropicbird (Le Corre & Jouventin 1999; Humeau *et al.* in prep); tropicbirds on Europa are regarded as an independent subspecies (*Phaethon lepturus europae*), characterized by a smaller size than other races and with a distinctive golden wash over the plumage similar to that of the 'Christmas Island' Tropicbird (*Phaethon lepturus fulvus*; Le Corre & Jouventin 1999). Similarly, marine conditions in the southern and central Mozambique Channel are believed to have maintained the genetic isolation of local populations of the Green Turtle (*Chelonia mydas*; Bourjea *et al.* 2007). However, the formerly large white-tailed brown morph dominated breeding colony on Glorieuses complicates this interpretation (Diamond 1974; Benson *et al*. 1975; Le Corre 1999). While Diamond (1974) doubted the existence of white-tailed brown morph birds on the Glorieuses Archipelago, due to its proximity (approximatey 200 kilometres [Km]) to Aldabra and the recent scarcity of dark morph birds in that region, the descriptions and collections by Abbott (in Ridgway 1896) and

Nicoll (1906) clearly show that white-tailed brown morph birds were largely dominant there (90% of the population according to Nicoll 1906). Glorieuses and Aldabra are similar in geological structure and experience comparable oceanographic conditions with weakly defined seasonal shifts in SST and surface chlorophyll *a* concentration (Pocklington 1979). It is therefore unlikely that local selective forces could have driven divergence between these two proximate populations. Le Corre (1999), considering all extant and extinct breeding colonies, additionally found no latitudinal gradient in the ratio-cline of Red-footed Boobies in the western Indian Ocean. Aldabra and Europa both also host significant populations of Great (*Fregata minor*) and Lesser (*F. ariel*) Frigatebirds. Moreover, no fitness differences have been observed among the different morphs at breeding colonies where they co-exist (Le Corre 1999; Baião & Parker 2008). Thus, while local selective forces may have contributed to the observed distribution of the different morphs, it seems unlikely that they are solely responsible for the overall patterns of plumage polymorphism in the western Indian Ocean. This highlights the potential importance of dispersal to divergence among populations of the Red-footed Booby.

2.4.2. Gene flow among breeding colonies of the western Indian Ocean

The migration rate among populations (*m*), and approximated effective population size (N_e) on which estimates of the number of migrants (*Nm*) per generation are based, are both notoriously difficult to calculate. These Bayesian estimates of *m* are almost certainly overestimated, due to the levels of pairwise genetic differentiation among breeding colonies (global $F_{ST} < 0.05$; Faubet *et al.* 2007), while estimates of N_e are far lower than surveyed approximations of the three breeding colonies (refer to Chapter 2.2.2). With these important caveats in mind, present estimates of *Nm* indicated contemporary exchange of individuals among the three sampled breeding colonies of the Red-footed Booby. Whilst substantial inter-island movements of both adult and immature Red-footed Boobies are known to occur in the central Pacific Ocean (Harrington 1977; Woodward in Nelson 1978), consistent with the ratio-cline among those breeding colonies (Nelson 1978), it is curious to note that no direct evidence exists in support of individual exchanges among colonies in the western Indian Ocean. On the contrary, though effort has not been great, there has been no recovery of banded birds at non-natal breeding colonies (Le Corre *et al.* 2015; Le Corre pers. comm.). The ratio-cline within the region, particularly the stability of the morph ratio at Tromelin, additionally suggests that very few (if any) inter-island exchanges have occurred within contemporary time-scales (Le Corre *et al.* 2015). The population on Tromelin has, however, increased disproportionately to the species' reproductive capabilities following Norway Rat (*Rattus norvegicus*) eradication in 2005/2006. While Le Corre *et al.* (2015) concluded that the increase was a result of augmented breeding success and an associated increase in the countability of adults, they also noted an obvious potential for individual-exchanges among breeding colonies. Despite the high flight potential and pelagic foraging habits of Red-footed Boobies (Weimerskirch *et al.* 2005; Mendez *et al.* 2016), Diamond (1974) found that adults are largely sedentary at their breeding colonies with non-breeding and pre-mature birds typically gathering in large 'gangs.' The number of birds counted in these aggregations, over multiple field seasons in various months, are insufficient to have solely resulted in such dramatic increases in population size at Tromelin (Le Corre. pers. comm.). This would seem to support the notion that individual exchanges occur among islands of the western Indian Ocean.

Morris-Pocock *et al.* (2010b, 2016) have recently also studied the ecological and biogeographical factors that influence the global population structure of Red-footed Boobies.

Four distinct genetic clusters of birds were identified across the species' entire pan-tropical distribution, broadly corresponding to the major oceanographic basins, including populations of the Atlantic, Indian, Western and Central Pacific, and Eastern Pacific Oceans. Substructure analyses within each of these four clusters revealed no significant differences and the authors proposed that, although the Red-footed Booby breeding colonies appeared to have evolved in isolation from one another, rare gene flow has occurred between all major genetic groups. Concerning Indian Ocean breeding colonies only, Morris-Pocock *et al.* (2016) noted genetic admixture among the two weakly-differentiated genetic clusters of birds: the Aldabra and North-Keeling, and the Christmas Island populations (Morris-Pocock *et al.* 2016). The observation of a white-tailed brown morph Red-footed Booby on Christmas Island (Hennicke 2009), and of individual brown-tailed white (Le Corre, M. pers. comm.) and brown-tailed brown (pers. obs.; Figure 2.12) morph birds on Tromelin, could provide support for these large-scale inter-colony exchanges of Red-footed Boobies. Specifically, the two brown-tailed forms are the rarest of all the morphs of the Red-footed Booby and are limited to only a few colonies on the Galapagos, Cocos, and Clipperton Islands in the eastern Pacific Ocean (Nelson 1978). No colonies are known to support this colour morph in the western Indian Ocean. Though no genetic support for any of these records was ever attained, plumage polymorphism is not caused by single spontaneous mutations in the Red-footed Booby (Baião *et al*. 2007). This would imply that these individuals must have originated elsewhere, and highlights that movement of Red-footed Boobies may occur through the Indo-Pacific basin.

Figure 2.12: On 1 May 2013, a brown-tailed brown morph Red-footed Booby was discovered breeding on Tromelin Island. This record, along with the observation of a brown-tailed white morph Red-footed Booby on Tromelin, suggests that large-scale movements occur among breeding island. These two morphs are atypical of colonies in the western Indian Ocean. Photo courtesy of Clara Morey-Rubio, with permission.

2.4.3. The case of Tromelin Island

Tromelin was first sighted by European explorers in 1722 (Rébillon 1935), though it remained undisturbed until the l'Utile shipwreck in 1761 when around 183 people were marooned on the island (Gardiner & Cooper 1907). These people lived on the island for 15 years, eating seabirds, turtles, and fish. It was not until November 1776 that the eight remaining survivors were rescued. The island subsequently went undescribed until Layard visited in December 1856 (Brooke 1981). Layard noted eight seabird species, among which six were breeding at the time. Additionally, Layard noted the presence of both Norway Rats and

House Mice (*Mus musculus*) on the island for the first time (Brygoo 1955). A century later, in 1954, French meteorologists set up a station on the island with permanent residents. Only four seabird species were recorded as breeding, including around 200 pairs of the Red-footed Booby. Two thirds of this population were described as the "white form", the rest being "brown and white". Staub visited the colony in August of 1968 and noted some 300 pairs of the Red-footed Booby. The morph ratio was exactly as described by Layard (Staub 1970). Later, in 1993/1994, only two seabird species remained including around 150 pairs of the Red-footed Booby. Le Corre (1996) noted that 69.4% of them were of the "white morph", while 30.6% were of the "brown morph." Around 130 pairs of Red-footed Booby bred on the island in December 2005, 66% of which were the white-tailed white morph and 34% were of the white-tailed brown morph. This decline in Tromelin's seabird community has been attributed to anthropogenic disturbance and threats posed by invasive mammals (Le Corre 1996; Le Corre *et al.* 2015). Less than eight years later, subsequent to the eradication of rats, the population of Red-footed Boobies had grown by around 23% per year to 855 pairs (Le Corre *et al.* 2015). In August 2012, among the 625 breeding adults for which the colour morph was noted, 65.4% were of the white-tailed white morph and 33.6% were of the white-tailed brown morph (Le Corre *et al.* 2015). All three intermediate morphs were also noted as being present. Although the morphs have been described differently over the years, the results from the present study confirm that the morph ratio has remained stable in the colony of Red-footed Boobies on Tromelin. This is in spite of massive increases in population size; the most recent estimates suggest that some 6 000 pairs of Red-footed Booby breed on the island each year and yet the morph ratio still remains unchanged (Ringler *et al*. pers. comm.).

The fact that Tromelin's morph ratio has remained stable, despite fluctuations in population size, is compelling. The processes which maintain plumage polymorphism in birds have been highly debated though, in this context, sexual selection for colour morph seems responsible. Le Corre (1999) and Baião and Parker (2008) previously assessed the mating pattern of Red-footed Boobies with respect to plumage polymorphism and found no evidence for non-random mating in three breeding populations. Interestingly, Le Corre (1999) found no evidence for non-random mating at this very same breeding colony. This distinction could be the result of a higher sampling effort in the present study, and a different methodological approach by which intermediate forms were separated from the two dominant morphs. Adults on Tromelin appear to select mates based on common phenotypes, while assortment concerning intermediate forms is based on body colour rather than the colour of the plumage extremities (i.e. scapulars, tail, and head colouration). White-tailed brown morph birds frequently mated with brown morph birds with yellow heads and white scapulars and brown morph birds with yellow heads and brown scapulars, whereas white-tailed white morph birds more typically paired with white morph birds with brown scapulars. In this species, polymorphism has been related to allelic variation at the Mc1r locus (Baião *et al*. 2007). Alleles Val85 and His207 are homozygous in white morphs, whereas brown and intermediate birds are homozygous and heterozygous at alleles Met85 and Arg207, respectively (Baião *et al*. 2007). Assortative mating should therefore maintain homozygosity and is thus expected to have maintained the morph ratio at Tromelin.

Assortative mating has several important consequences for population genetics (Lynch & Walsh 1998). One mechanism through which it can be achieved is imprinting. If chicks imprint on the colour morphs of their parents, later seeking family-coloured mates of their own, the morph ratio will be maintained through biological segregation among the morphs (O'Donald 1983; Lank 2002). Imprinting, if complete, then has the potential to lead to genetic isolation (i.e. speciation) of the different morphs after only a few generations (Cooke & Cooch 1968).

It may even result in selection against immigrant genotypes, if phenotypes differ between breeding colonies (e.g. Aldabra versus Europa). This has been experimentally shown in the Lesser Snow Goose (*Chen caerulescens caerulescens*; Cooke & Mirsky 1972), but has never been empirically tested in the Red-footed Booby. Nevertheless, the speciation potential is eroded in situations in which assortative mating is incomplete among co-existing morphs (Lack 2002). This seems to be the case at Tromelin, where around 45% of couples consist of mixed pair combinations. However, the level of assortative mating in this colony seems sufficient enough to have maintained genetic differences among white-tailed white and white-tailed brown morph individuals. An alternative scenario suggests that inter-individual variation in mate choice decisions may be genetically pre-determined, promoting biological segregation of the morphs, though this remains a little-known topic (reviewed in Bakker & Pomiankowski 1995).

2.4.4. Further work

Though the present study has offered insight into the population structure underlying the ratio-cline in plumage polymorphism of Red-footed Boobies in the western Indian Ocean, there is an obvious need for an increased sampling effort. First and foremost, it is necessary to include other important breeding colonies, such as Cosmoledo and Chagos, to allow a proper assessment of genetic diversity within the region and to understand the true nature of gene flow among geographically proximate and distant islands. Samples of rare, atypical, morph birds from each of Aldabra and Europa may also provide insight into the relationships between these apparently isolated breeding colonies. It is likely these individuals may include first generation migrants from other breeding colonies. Further, greater sampling effort is necessary at Tromelin to understand the existence of intermediate morphs, and to assess the long-term implications of assortative mating on this colony's genetic diversity and population structure. A study investigating the patterns of morph inheritance among different pair combinations of Red-footed Boobies would be especially informative. One could theorise that intermediate forms are the result of cross-morph pair combinations and that their scarcity could be linked to a reduction in fitness or mateability. The inclusion of historical samples, taken from museum specimens, might also shed light on the relationships among extant and extinct breeding colonies particularly concerning the white-tailed brown morph dominated population that once existed on the Glorieuses Archipelago.

To separate the two non-mutually exclusive hypotheses, explaining the population structure of Red-footed Boobies (selection vs. connectivity among islands), it is also necessary to study variables of presumed adaptive significance. Firstly, the risk of kleptoparasitism to the various morphs of the Red-footed Booby should be quantified, especially at breeding colonies where the different morphs co-occur. Frigatebirds and skuas are both prolific kleptoparasites of boobies, and this has implications for chick-feeding rates, breeding success, and ultimately population trajectories of boobies. Such has been observed in the population of the Masked Booby at Tromelin, which increased subsequent to the local extinction of the Great Frigatebird (Le Corre 1999). Next, though a latitudinal gradient is not present in the ratio-cline of plumage polymorphism in Red-footed Boobies from the western Indian Ocean, differing ambient temperatures at breeding islands could result in fitness differences among different morphs. Physiological studies, assessing the temperature regulation abilities of the various morphs, could be informative. Studies evaluating the importance of communicative ritualized behaviour could also shed insight into the adaptive significance of plumage polymorphism in the Red-footed Booby. Flank-touching in Abbott's Booby (*Papasula abbotti*), bill clapping in Pink-backed Pelican (*Pelecanus rufescens*), areal display flights in Red-tailed

Tropicbirds, parading in Blue-footed Boobies (*Sula nebouxii*), and forward-head waving in Brown Boobies are all recognised as forms of ritualized behaviour (reviewed in Nelson & Baird 2002). It is likely that plumage polymorphism may constitute a similar adaptive or communicative function in the Red-footed Booby, especially since it appears important to mate choice. Finally, tracking studies employing modern bird-borne video cameras, time-depth (immersion) loggers, Argos global positioning system (GPS) transmitters and global location sensors (GLS) would offer insight into foraging relationships between individual Red-footed Boobies and their marine environment during both the breeding and non-breeding periods (e.g. Weimerskirch *et al.* 2005; Pinet *et al.* 2011, 2012; Mendez *et al.* 2016). The information these devices would provide could also shed light on juvenile and adult dispersal patterns, as well as cues used while foraging.

2.4.5. Management implications, in relation to genetic diversity

Red-footed Booby populations in the western Indian Ocean have undergone significant declines within the last century. Only six of the 15 known breeding sites remain occupied today, while all lasting populations are believed to be greatly reduced in size. This decline is attributable to direct human exploitation of adults and their eggs, predation of eggs and chicks by introduced predators, the destruction and transformation of breeding areas including the historical collection of guano, and increasing tourism and human encroachment at their breeding islands (Carr 2011). Though similar threats affect most other seabirds in the region, Red-footed Boobies seem to have suffered more extensively due to a combined effect of an intolerance of disturbance at breeding sites and their specific nesting requirements (demanding tall woody shrubs or trees). Red-footed Boobies are protected through multi-national legislation, but this is virtually impossible to enforce on oceanic islands. Enhanced protection at breeding colonies, and at sea, is necessary to ensure the persistence and recovery of this species in the western Indian Ocean (Carr 2011).

Results from the present study indicate that the conservation of Red-footed Boobies, in the western Indian Ocean, is mostly dependent on scale-specific management of their populations. Though the three sampled populations appear connected through rare gene flow, they also seem to have evolved in isolation from one another. They should thus be categorised as separate management units (MUs). In light of this, the persistence of the species in the region is dependent on the protection of the three main breeding populations including those on Aldabra, Chagos, and Cosmoledo. Despite Aldabra being the smallest of these main colonies, it hosts high overall genetic diversity. This includes many rare and private alleles not found in either of the other two, albeit smaller, sampled populations. Protection of the three medium-sized breeding populations, including Tromelin, Europa, and Farquhar, is also crucial to the maintenance of high overall genetic diversity since at-least two of these populations also contain unique alleles. The population on Europa is of particular conservation importance, due to its apparent isolation from all other breeding colonies except, according to these results, Tromelin. The maintenance of the species' genetic diversity is of paramount importance to its resilience and ability to recolonise the many abandoned breeding sites.

The recovery of the Red-footed Booby populations in the western Indian Ocean, including the recolonization of abandoned breeding sites, is also dependent on the restoration of 'environmentally traumatised' islands (Carr 2011). Human influence at former breeding colonies of the Red-footed Booby, such as at Glorieuses, has been catastrophic (Ridgway 1895; Benson *et al.* 1975). Invasive mammals have supressed breeding numbers and annual reproductive success, while the conversion of native forest to coconut plantations has deleteriously altered breeding opportunities for Red-footed Boobies (Carr 2011). Returning these islands to a predator free, coconut managed, state would rapidly assist passive recovery and recolonization as is already exemplified in at-least two cases from the western Indian Ocean (Carr 2011; Le Corre *et al.* 2015).

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CHAPTER 3: EXTREME PHILOPATRY DRIVES UNPRECEDENTED FINE-SCALE POPULATION STRUCTURING IN THE ENDANGERED ENDEMIC BARAU'S PETREL (*PTERODROMA BARAUI*) FROM RÉUNION ISLAND.

3.1. Introduction

As the human influence on natural ecosystems has increased, conservation biologists have grown more and more concerned that wildlife refugia are too small, and conservation efforts too slight, to avert extinctions of the now numerous threatened species worldwide (Diamond *et al.* 1976; Rodrigues *et al.* 2004). Island ecosystems pose special challenges for conservation (Myers *et al.* 2000). Their unique evolutionary environments and opportunities culminate in high overall concentrations of endemic species, coupled with moderate levels of species richness (Myers *et al.* 2000; Tye *et al.* 2002; Whittaker & Fernández-Palacios 2007; Kier *et al.* 2009). These endemics often diverge rapidly from sister taxa, driven by isolation, often small population sizes, and the influence of very specific selective forces, implying that their populations are typically more unstable than those of widespread species of continental origin (Frankham 1997; Whittaker & Fernández-Palacios 2007). Island restricted species additionally tend to be highly specialised and lack defences against a broad range of predators, competitors, or diseases from which they have often evolved in isolation. This is exemplified by the extinction of indigenous forest birds, fruit bats, and reptiles on Guam following an accidental introduction of the Brown Tree Snake (*Bioga irregularis*) in about 1944 (Savidge 1987; Rodda *et al.* 1997; Wiles *et al.* 2003). Due to a complete lack of competition, the introduced population of Brown Tree Snakes exploded to over 2 million individuals with densities exceeding 5 000 individuals per square kilometre (/Km^2; Rogers *et al.* 2017). This resulted in extremely high predation rates on indigenous fauna, as many lacked defences (e.g.

flightlessness) and most had a sense of fearlessness against the novel predators. Island populations of animals are also often genetically isolated and small in size. They therefore experience increased levels of inbreeding and a greater impact of genetic drift. As a result, these populations generally exhibit lower levels of genetic diversity and fitness compared to counterparts found in mainland populations (Frankham 1997). To illustrate – Furlan *et al.* (2012) observed perilously low genetic diversity in two small isolated island populations of the Platypus (*Ornithorhynchus anatinus*), noting severe implications for population resilience. Threats to island ecosystems are diverse but are mostly of anthropogenic origin or influence (Trevino *et al.*2006). Common underlying causes of decline in island restricted species include the introduction of invasive species, unsustainable development and tourism, increasing climate variability, natural disasters including extreme weather events, the overexploitation of natural resources, and land degradation and marine pollution (reviewed in Whittaker & Fernández-Palacios 2007). The result is frequent conflict between socio-economic development, within already limited spaces on islands, and national obligations to conserve sensitive biodiversity.

Seabirds often dominate the biota and are central to the function of islands. In fact, most marine bird species are entirely dependent on high-density nesting colonies, often amounting to thousands or millions of breeding pairs, on scattered coastal or oceanic islands (Schreiber & Burger 2002). Generally high rates of breeding site fidelity, along with other extreme life-history and demographic characteristics, have contributed to the isolation and speciation of various seabird lineages among island groups (e.g. Levin & Parker 2012; Rawlence *et al.* 2014; Thanou *et al.* 2017). One of the most diverse families is the Procellariidae, including the fulmars (i.e. *Fulmarus, Daption*), petrels (i.e. *Pterodroma, Bulweria, Procellaria,* and *Pseudobulweria*), diving-petrels (i.e. *Pelecanoides*), prions (i.e.

Pachyptila), shearwaters (i.e. *Ardenna, Calonectris,* and *Puffinus*), and the miscellaneous petrels (i.e. *Macronectes, Aphrodroma, Halobaena,* and *Pagodroma*). These diverse birds are distributed throughout the world, with the greatest abundance and diversity of species occurring between 37°S and 59°S in all ocean basins (Chown *et al.* 1998). The most specious clade among the procellarids are the gadfly petrels (i.e. *Pterodroma*), comprising 37 highly oceanic tropical and subtropical seabirds of variable plumage, morphology, and complex taxonomy (Imber 1985; Jesus *et al.* 2009). Breeding for a significant proportion of these birds is confined to one or a few nearby, though often extremely remote, islands (Imber 1985; Chown *et al.* 1998; Brooke 2002). Given their limited breeding range and the difficulty often associated in researching them – the gadfly petrels have recently been recognised as one of the least known and most threatened of all avian groups (Croxall *et al.* 2012). They are thus highlighted for special conservation status and action under a range of international, regional, and national agreements. This includes the Agreement on the Conservation of Albatrosses and Petrels, the European Union Birds Directive, the Convention on the Conservation of Migratory Species of Wild Animals, and the Convention on the Conservation of European Wildlife and Natural Habitats (Croxall *et al.* 2012). Various national and international working groups, including the Gadfly Petrel Conservation Group, have also been structured to study their overall distribution, population status, behaviour, and vulnerabilities. This information is urgently required to inform effective management regimes for their populations, providing a foundation for the assessment of the status of and threats to their existing populations, in the planning of future management actions, and to enable directed monitoring of population status and recovery (Salafsky *et al.* 2008).

The islands of the South-western Indian Ocean host a surprising diversity of petrels (Pinet *et al*. 2009; Brown *et al*. 2011; Shirihai *et al*. 2014). Réunion is the only island in the world that supports two endemic species; the Mascarene (*Pseudobulweria aterrima*) and the Barau's (*Pterodroma baraui*) Petrels. Both have unfavourable conservation status, suffering from threats imposed during human colonisation and expansion including light-induced mortality of fledglings and predation by invasive mammals (Le Corre *et al*. 2002; Le Corre 2008; Falquier *et al.* 2009; Pinet *et al*. 2009; Dumont *et al.* 2010). Though both species remained little known until about 1995, considerable scientific attention within the last decade has furthered our understanding of Reunion's two endemic petrels. In fact, these birds have become the primary focus of the LIFE+ Pétrels project (2014 – 2020) of the European Union Life programme. This multi-institutional initiative has the primary aim of halting declines in populations of the Mascarene and Barau's Petrels, bearing in mind Reunion's urban context and the importance of stakeholder involvement in conservation. The biology of the endangered Barau's Petrel is now fairly well-known (e.g. Pinet *et al.* 2011a, 2011b, 2012; Danckwerts *et al.* 2016a), while recent developments, including the discovery of three active breeding colonies, have shed insight into the virtually unknown and critically endangered Mascarene Petrel (Pinet, P. pers. comm.). Conservation awareness is also vastly improved and resulted in the establishment of a large national park in 2008, which encompasses all known breeding sites of the two species (Pinet *et al.* 2009). Rescue programs for grounded birds have also been operating effectively since 1995, dramatically reducing light-induced mortality of fledglings (Le Corre 2002), while the control of cats is undertaken year-round within the limits of French legislation (Pinet *et al.* 2009). Nevertheless, numerous knowledge gaps still exist that severely limit efforts to conserve Réunion's two endemic petrels (Pinet *et al.* 2009). This includes an understanding of population structure including their genetic diversity and the connectivity that exists among breeding colonies. This information is vital towards defining effective management regimes for their populations.

The aim of this chapter is to assess genetic diversity and effective population size of the Barau's Petrel, using polymorphic nuclear microsatellite analysis, to inform scale-dependant management of its populations. Its two main breeding colonies, situated on Piton-des-Neiges (*Bras-des-Etangs*) and Grand Benare (*Vallee des Deux Miches*), are additionally compared with one another to investigate the small-scale geographic relationships between them including estimated rates of inter-colony exchange. To augment these data, an analysis of band recoveries is performed based on the long-term annual monitoring programs at the two breeding colonies of the Barau's Petrel. It is predicted that the species' life-history and reproductive characteristics (e.g. high presumed natal philopatry, nest site, and mate fidelity), combined with Réunion Island's unique geography, will have caused fine-scale divergence between the two breeding colonies of the Barau's Petrel. Patterns of genetic diversity should be reflective of this, with each colony containing distinct multilocus genotype signatures and private alleles (P_A). Banding information should provide additional support for limited inter-colony exchanges of individuals, considering the species' high assumed natal philopatry. In addition to offering insight into the population structure of the Barau's Petrel, this information should enable the identification of possible isolation processes that underlie species diversity and endemism in gadfly petrels and which are important for their overall conservation.

3.2. Materials and methods

3.2.1. Sample collection

Fieldwork was performed over three austral summers, between 2008/2009 and 2012/2013, at two large breeding colonies of the Barau's Petrel on the central massif of Réunion Island including those at the *Vallee des Deux Miches* (Grand Benare massif) and *Bras-*

des-Etangs (Piton-des-Neiges massif; Table 3.1, Figure 3.1). Adult birds were captured at their nests and banded, facilitating individual identification. A small whole blood sample (approximatey 0.2 millilitres [mL]; maximum of 1.0% of body weight; Fair *et al.* 2010) was additionally collected from all individuals through medial metatarsal or basilic venipuncture using sodium-heparin lined BD Micro-Fine^{TM} 1.5mL 27-gauge syringes. Blood samples were stored cold (approximatey 5 degrees Celsius [°C]) in 70% ethanol in 2mL Eppendorf microcentrifuge tubes for up to 48 hours. All were then archived at -20°C at the *Université de La Réunion* pending further analysis.

Table 3.1: Two-hundred and fifty-nine adult Barau's Petrels were sampled at two breeding colonies on Réunion Island between the austral summers of 2008/2009 and 2012/2013.

Massif	Colony name	Latitude Longitude	Number of monitored nests	Sample size
Grand Benare	*Vallee des Deux miches*	21° 06' 52.4"S 55° 25' 20.9"E	100	117
Piton-des-Neiges	*Bras-des-Etangs*	21° 06' 06.5"S 55° 28' 31.6"E	250	142

The techniques employed to capture, and sample, Barau's Petrels were approved by the Ethics Committee at Rhodes University (reference Number ZOOL-01-2013). A research and blood collection permit was obtained from the *Centre de Recherches sur la Biologie des Populations d'Oiseaux* (CRBPO; Muséum Paris; permit 1392), with additional support from *Parc National de La Réunion*.

3.2.2. DNA extraction technique

Total deoxyribonucleic acids (DNA) were extracted from whole blood subsamples using the QIAmp Blood & Tissue kit (QIAGEN), following the procedure developed for animal tissue with minor adjustments (refer to Chapter 2.2.3). Quantification of DNA in two microlitre (µL) aliquots of each sample was subsequently performed using a Thermo Scientific NanoDrop 2 000c UV-Vis Spectrophotometer. Measured nucleotide concentration varied between 1.8 and 594.4 nanograms per microlitre (ng/µL). Samples with DNA concentrations higher than 40ng/µL were diluted with Buffer AE (QIAGEN). Extracted DNA was stored at -20°C pending further analysis. This, and all subsequent sample manipulation for the Barau's Petrel, was performed at the *Université de La Réunion*.

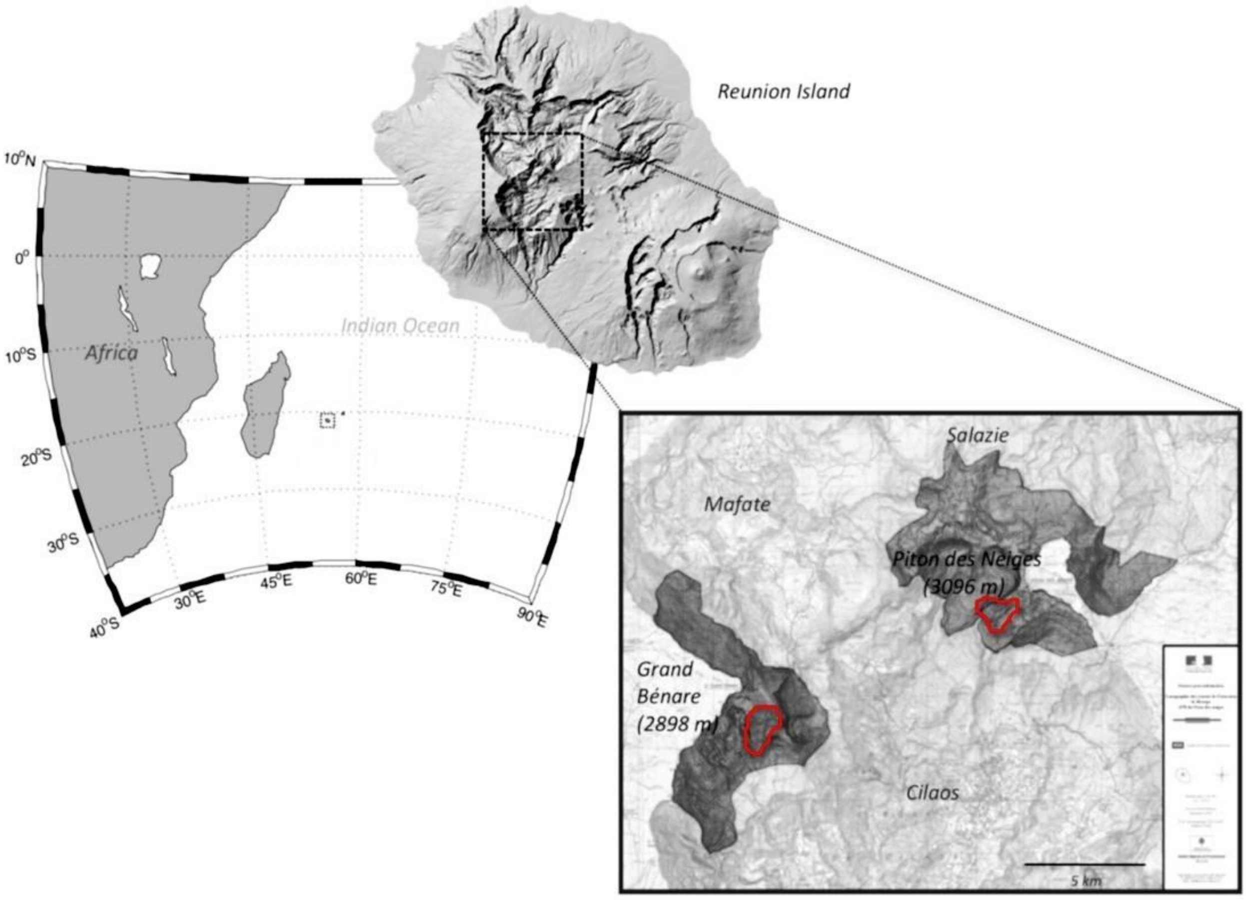

Figure 3.1: Barau's Petrel breed only on Réunion Island, in the western Indian Ocean. Breeding colonies are located in high altitude elfin forest (shaded in dark grey) on the two main mountain masses, Grand Benare and Piton-des-Neiges. Samples employed in the present study were taken from within the *Vallee des Deux Miches* (Grand Benare) and *Bras-des-Etangs*

(Piton-des-Neiges) breeding colonies; circled in red. Figure adapted and reproduced with permission from the authors of Pinet *et al.* (2009).

3.2.3. Oligonucleotide primer development

Until the present study, microsatellite loci had not previously been isolated for the Barau's Petrel (results from this study published in Danckwerts *et al.* 2016b). A genomic library was therefore constructed using DNA of a single individual collected at the *Vallee des Deux Miches* breeding colony. Microsatellite sequences were then isolated by Ecogenics GmbH (Zurich, Switzerland) based on this library. Size-selected fragments from genomic DNA were enriched for simple sequence repeat (SSR) content using magnetic streptavidin beads and biotin-labelled GATA and GTAT repeat oligonucleotides. The SSR-enriched library was analysed on an Illumina MiSeq platform using the Nano 2x250 version 2 format.

After assembly, 417 contigs or singlets contained a microsatellite insert with a tetra- or a trinucleotide repeat sequence of at-least six units, or a dinucleotide repeat sequence of at-least 10 units. Suitable primer design was possible in 300 microsatellite candidates, of which 36 were tested for functionality and polymorphism. Polymorphism was tested using an additional eight individuals from the *Vallee des Deux Miches* breeding colony. Among these 36 candidate loci and associated primers for polymerase chain reaction (PCR) – four were monomorphic and 17 showed a high number of stutters, comprising more than two alleles in some samples, or were difficult to interpret. Fifteen primer pairs gave satisfactory amplification patterns, for which oligonucleotide primers were developed (Table 3.2). These microsatellite loci all exhibited tetranucleotide repeat motifs with at-least seven repeat sequences (Table 3.2).

Table 3.2: Characteristics of 15 tetranucleotide repeat microsatellite loci developed for Barau's Petrel (adapted from Danckwerts *et al.* 2016b). Nucleotides are abbreviated as follows: A = adenine, G = guanine, C = cytosine, T = thymine.

Name	Primer Sequences (5'-3')	Repeat motif	Fragment size (bp)	GenBank Accession No.	Mix no.	Dye
PB_2859	F: TGCCTTGCGTCCTATAAATGC R: TAGTCAGCTATCCGTCCAGC	(TATG)$_7$	152 – 164	KX138620	1	6-FAM
PB_2001	F: GGTTTAAGCCAGCTAACACTTCC R: TCTTGTTCAGGGCATCTGGG	(TGTT)$_8$	252 – 270	KX138615	1	NED
PB_0316	F: GAAAACTGGGAACCAAGGGG R: CCCTAACATGGTGGTGCTTC	(TTTG)$_8$	110 – 126	KX138609	1	PET
PB_1201	F: TCTGTGCCAACTACTCCCAG R: TTGTACCTGCGTCTGCTTTC	(GATA)$_7$	207 – 247	KX138613	1	VIC
PB_2742	F: TCTTGCCCACTTTTTCCAGC R: TCTTTGCCTTTCCCAGGTTG	(AAGA)$_8$	159 – 185	KX138619	2	6-FAM
PB_3916	F: CGGAGACCATCACTCCTCTG R: ATGCTTGCAAAGCTGCCG	(TCTT)$_7$	238 – 252	KX138621	2	NED
PB_1890	F: AACCAAGCTGTTGTGTGAGC R: TCCAGCTTTCCTGACTTTCC	(AAGG)$_{14}$	126 – 174	KX138614	2	PET
PB_0760	F: AGCAGGCTTTCGAGAATAGG R: AGGATCCAGTCCTACATCCAC	(TTCC)$_7$	199 – 235	KX138610	2	VIC
PB_0931	F: CACCAGGACATGCAGAAACC R: CCCAAGGCAGGCTAAAAACC	(TAGA)$_{10}$	194 – 214	KX138611	3	6-FAM
PB_4708	F: AATTACCCAGTTATGCTTCTGC R: AGGTGGAGCTTGTTCTGTCC	(TATG)$_{10}$	225 – 253	KX138622	3	NED
PB_5759	F: AGTCGAGGAGGTGAATGTAGC R: GATACCAGTCAGGCAGAGGC	(AGAT)$_{11}$	142 – 170	KX138623	3	PET
PB_2206	F: TGATTGCCCAGTAAACTGCC R: TGTTTTGGCACTCAGGTTCG	(ACAA)$_8$	213 – 231	KX138617	3	VIC
PB_2653	F: GCCTCCTCTCAGTTCCTACC R: ACAGCACCAGTGTTCATTTG	(AGAT)$_8$	208 – 236	KX138618	4	6-FAM
PB_1030	F: AGCCCTCTGAAGGTTCTGG R: CAGCTGACTCAAGACAAAAGATATAG	(ATGT)$_9$	170 – 186	KX138612	4	PET
PB_2138	F: AATACTTCTTCCGAGTTTTGGG R: TGGGTCAGAAACTGTGAATGC	(TTTC)$_9$	222 – 254	KX138616	4	VIC

3.2.4. Polymerase chain reaction (PCR) technique

A 3-primer PCR approach using the universal M13 tail (5'-CACGACGTTGTAAAACGAC-3') for the forward primer was used for microsatellite loci amplification (Schuelke 2000). Four different fluorescently-labelled dyes (6-FAM, PET, VIC, and NED) were used alongside the universal M13-tail forward primer to enable fragment analysis multiplexing (Table 3.2). Simplex PCR amplifications, targeting one locus at a time, were performed using a GeneAmp

PCR 9 700 System (Applied Biosystems) in 10µL reaction volumes containing 5µL of MasterMix Applied 2x (Applied Biosystems), 0.25µL of the forward primer with the M13 5'-tail [1 micromole {µM}], 0.25µL of the reverse primer [10µM], 0.25µL of the dye (6-FAM, NED, PET or VIC; [10µM]), and 2µL of genomic DNA [approximatey 40ng/µL]. Reaction volumes were completed with sterile deionised water. PCR amplifications were carried out under the following conditions: an initial denaturing step at 95°C for five minutes, followed by 43 primer annealing cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. The central annealing temperature was adjusted to 55°C for locus PB_1030, based on its lower melting temperature (Danckwerts *et al.* 2016b). A final elongation stage was then employed where samples were held at 72°C for 20 minutes. PCR products were stored at -20°C prior to product size determination.

Up to four different simplex PCR plates, each with a different dye (Table 4.2), were mixed and PCR product sizes determined using a 3 730XL DNA analyser (Applied Biosystems), by Gentyane platform (Clermont-Ferrand, France). Product sizes were estimated with the LIZ(500) standard using GeneMapper version 4.0 (Applied Biosystems). Values of null allele sizes, not exceeding 5% of the total genotyping dataset, were accepted. To achieve this, locus-specific amplifications for certain samples were repeated.

3.2.5. Data analysis

3.2.5.1 Genetic diversity

Mistyped allele sizes, deviations from the tetranucleotide repeat motifs, and evidence of null alleles, large-allele dropout, and stutter peaks were first examined among all loci, for each breeding colony, using MicroChecker version 2.2.3 (van Oosterhout *et al.* 2004). Each sample-locus combination was then tested for linkage disequilibrium (LD) using GenePop version 4.0.10 (Rousset 2008), with the exact probability test (Markov chain parameters: 10 000 dememorizations, 100 batches, 1 000 iterations per batch). The resulting p-values were corrected using sequential Bonferroni correction for multiple comparisons (Rice 1989). Presence/absence of LD among loci was confirmed with the method of Index of Association (Agapow & Burt 2001), in the package poppr (Kamvar *et al.* 2014, 2015), in R version 3.4.0 (R Development Core Team 2017). Additionally, to assess the overall power of all subsequent analyses, a multi-locus genotype accumulation curve was constructed using poppr (Kamvar *et al.* 2014, 2015), in R version 3.4.0.

Standard estimates of genetic diversity, including the mean number of alleles per locus (N_a), P_A richness in each population, the effective number of alleles (A_E), observed heterozygosity over all loci (H_o), and unbiased expected heterozygosity over all loci (uH_e; Nei 1978), were calculated for both breeding colonies of the Barau's Petrel using GenAlEx version 6.6 (advance notice based off Peakall & Smouse 2012). The statistical significance of differences in means of N_a and A_E, between the two breeding colonies of the Barau's Petrel, were assessed using non-parametric Wilcoxon Signed Rank tests as implemented in PAST version 3.1.5 (Hammer *et al.* 2001). AR (El Mousadik & Petit 1996) was calculated for each breeding colony using FSTAT version 2.9.3 (Goudet 1995, 2001), and compared between populations using 10 000 permutations of the alleles. Deviations from the Hardy-Weinberg proportions were assessed at each locus, for each breeding colony, with the package pegas (Paradis 2010), in R version 3.4.0, using the exact test based on 1 000 Monte Carlo permutations of the alleles. Finally, Wright's inbreeding coefficient, within individuals relative to the subpopulation (F_{IS}; Wright 1922, 1965) was also computed for each breeding colony using GenAlEx version 6.6 (advance notice based off Peakall & Smouse 2012).

3.2.5.2 Genetic differentiation among populations

Wright's overall multi-locus fixation index (F_{ST}; Wright 1922), and its associated p-value, were computed over all loci and both breeding colonies following Weir and Cockerham (1984) using the package adegenet (Jombart 2008; Jombart & Ahmed 2011), in R version 3.4.0 (R Development Core Team 2017). The statistical significance of this index, between the pair of breeding populations, was then tested using 10 000 random permutations of the raw genotypes, again with the package adegenet, in R version 3.4.0.

Mutation processes occur at relatively high rates and with stepwise changes in allele sizes at microsatellite loci. This introduces bias into classical measures of population differentiation such as F_{ST} (Hardy *et al.* 2003). Therefore, to corroborate the results of F_{ST} at this very fine scale where stepwise mutations may have biased statistical outcomes for some loci (refer to Chapter 3.3.1), two additional measures (namely R_{ST} and G_{ST}; Nei 1973; Slatkin 1995) of population differentiation were calculated using SPAGeDi (Hardy & Vekemans 2002). R_{ST} was defined by Slatkin (1995), and uses a stepwise mutation model to assess variances in allele sizes rather than allele frequencies (as in F_{ST}). This measure better reflects population differentiation among microsatellite loci in instances when stuttering is observed (refer to Chapter 3.3.1), but suffers high sampling variance (Hardy *et al.* 2003). The simple test of permutated R_{ST} (denoted as pR_{ST}) developed by Hardy *et al.* (2003), based on a randomization procedure of allele sizes, was used to determine whether stepwise-like mutations contributed to genetic differentiation (i.e. do the results of F_{ST} equal those of R_{ST}). When R_{ST} is greater than pR_{ST}, any observed genetic differentiation could be the result of random mutations and not genetic drift (Hardy *et al.* 2003). Finally, G_{ST} was used as an extension of Nei's (1972) estimate of genetic distance between pairs of breeding populations.

3.2.5.3 Migration rates and effective population size

An alternative approach, based on Faubet *et al.* (2007), was therefore adopted to calculate directional migration rates among populations per generation following a Markov Chain Monte Carlo (MCMC) method. This assumes only that loci are unlinked and that individuals were sampled at random from their source population. Directional migration rates (*m*) among all pairs of populations were calculated with the software Bayesian Inference of imMigration rates (BIMr), using 10 MCMC iterations of the raw genotype data and default values for burnin and thinning. The mean value of *m*, for each pair of populations in each direction (from, into), was subsequently multiplied by an estimate of the effective population size (N_e) for each colony to determine the number of migrants (*Nm*) per generation.

Contemporary estimates of N_e were calculated for the two Barau's Petrel breeding colonies using a single-sample estimator based on the LD model implemented in both LDNE (Wright 1931, 1938; Waples & Do 2008), and NeEstimator version 2.0 (Do *et al.* 2014). N_e was calculated assuming a monogamous mating pattern, as is typical for seabirds (Schreiber & Burger 2002). A mean value was calculated across the two software platforms (LNDE and NeEstimator). Two allele frequencies were adopted as cut-offs: 0.02 and 0.05. Estimates of 95% confidence interval (CI) were calculated by jackknifing over loci for each estimate.

3.2.5.4 Population structure

Genetic diversity within, and genetic differentiation between, breeding colonies of the Barau's Petrel were initially addressed using Principal Coordinate Analyses (PCoA) based on scaled overall multi-locus genotypes. These analyses were implemented by means of a Euclidean distance matrix among individuals in each breeding colony, using adegenet (Jombart 2008; Jombart & Ahmed 2011), in R version 3.4.0.

Assignment tests, based on the multi-locus genotypes, were additionally used to assess differentiation between and clustering among individuals from the two colonies of the Barau's Petrel. These were evaluated using a model-based Markov Chain Monte Carlo (MCMC) clustering procedure, implemented with a Bayesian algorithm, in STRUCTURE version 2.3.3 (Pritchard *et al.* 2000). Multiple independent simulations, using a burn-in period of 5 000 simulations followed by 10 000 repetitions, were first run using different settings (e.g. admixture versus no-admixture model, LOCPRIOR verses no-LOCPRIOR) to assess convergence and determine the importance of model choice. An admixture model (Dirchelet parameter; $\alpha = 1.88 \pm 0.53$), assuming sampling location (LOCPRIOR; $r = 1.17 \pm 0.18$; Hubisz *et al*. 2009), was finally used. Correlated allele frequencies were assumed (Pritchard *et al.* 2000; Falush *et al.* 2003). To estimate the number of genetic clusters (K), 10 independent runs of 10 000 iterations each (after a burn-in of 5 000 steps) were performed for K = 1 to K = 5. The optimal value of K was subsequently determined using the mean overall log-likelihood given the number of clusters ($\ln P(X|K)$; Pritchard *et al*. 2000), and the second-order rate of change of $\ln P(X|K)$ (ΔK; Evanno *et al*. 2005). Ten independent simulations, at the optimal value of K, were then run using 1 000 000 iterations each (after a burn-in of 500 000 steps). The simulation with the highest value of $\ln P(X|K)$, at the optimal value of K, was finally used to plot the estimates of individual membership for all genetic clusters. Membership coefficients (termed *Q*) were assigned to all individuals for each genetic cluster at the optimal value of K.

3.2.5.5 Banding/ringing data

Annual monitoring at the *Vallee des Deux Miches* and *Bras-des-Etangs* breeding colonies was commenced over the summers of 2008/2009 and 2002/2003, respectively. All re-sightings at nests, up until the present day (9 and 15 years of data for the *Vallee des Deux*

Miches and *Bras-des-Etangs* colobies, respectively), were collated for the two colonies to quantify natal philopatry and levels of individual exchange. Re-sightings of birds banded at two age groups (adults, pre-fledglings), within and between each breeding colony, were determined using sub-setting functions in R version 3.4.0 (R Development Core Team 2017).

3.3. Results

3.3.1. Description of data, tests of assumptions, and genetic diversity

All fifteen nuclear microsatellite loci successfully amplified in more than 55% of all samples from the two breeding colonies of the Barau's Petrel. Around 3.09% of the global dataset consisted of null values, with a maximum of 47.18% missing data at any one locus. Locus PB_1030 contained this extremely high proportion of missing data, with 31.62% and 47.18% missing data within the *Vallee des Deux Miches* and *Bras-des-Etangs* breeding populations respectively. Removal of this locus increased the percentage of samples for which successful amplification was achieved at all loci to more than 94%. As little as 0.44% of the reduced dataset consisted of null values, with a maximum of 2.56% of missing data at any one locus in each breeding colony.

No significant LD was observed among any loci in either the global or the reduced dataset (corrected exact Markov chain probability test, all p-values > 0.05; Index of Association method, p-value > 0.05). Null allele frequency was low, ranging from -0.07 to 0.19 in the two colonies, though presence was detected at loci PB_1890, PB_2742, PB_4708, and PB_1030 in both colonies, and PB_3916 in the *Vallee des Deux Miches* breeding colony only. All loci for which null alleles were detected were associated with homozygote excesses. No large-allele dropout was detected at any loci. The only examples of stuttering were at loci PB_2742 and PB_3916 in both breeding populations, as was indicated by the highly significant shortage of heterozygote genotypes with alleles of one repeat unit difference. Estimates of N_a per locus were similar in the global and the reduced datasets, but were always higher in the *Bras-des-Etangs* colony (grand mean across populations 5.93 ± 0.33 and 6.00 ± 0.35 for the global and the reduced dataset respectively; Table 3.2a, b). Locus specific differences in N_a were not significantly different between the two breeding colonies, however (Wilcoxon test: W = 47; p-value = 0.26). A_E was also similar between the two breeding colonies (grand mean across populations 2.89 ± 0.20 and 2.97 ± 0.21 for the global and the reduced dataset, respectively; Table 3.2a, b), but was always slightly higher in the *Vallee des Deux Miches* colony (Wilcoxon test: W = 59; p-value = 0.71). Differences in A_E and N_a, along with patterns of allele frequency distributions in the two breeding populations, indicated a high proportion of rare (allele frequency < 0.20) alleles. Estimates of AR were not significantly different between the two colonies in both the global and the reduced dataset, but were always lower in the *Vallee des Deux Miches* colony (grand mean across populations 5.81 ± 0.41 and 6.23 ± 0.44 for the global and the reduced dataset, respectively; Table 3.2a, b). Both breeding colonies of the Barau's Petrel contained P_A, with the *Bras-des-Etangs* colony showing the highest count (Table 3.2a, b). Five and one of these alleles accounted for more than 0.01 of the total allelic variation at each locus in the *Bras-des-Etangs* and *Vallee des Deux Miches* colony, respectively. No P_A accounted for more than 0.05 of the total allelic variation at each locus in either of the two breeding colonies. Locus-specific deviations from Hardy-Weinberg proportions at the 99% confidence interval were observed at loci PB_1890, PB_2742, PB_4708, PB_1030, and PB_3916 across both breeding populations. These deviations from the Hardy-Weinberg proportions were not consistent between the two breeding colonies and were mostly associated with significant presence of null alleles. The global dataset showed significant deviations from the Hardy-Weinberg proportions (p-value = 0.004), however.

Removal of locus PB_1030, which contained the highest proportion of null allele values, dramatically improved this result in the reduced dataset to within acceptable limits (p-value = 0.01). F_{IS} was generally low, never exceeding 0.07 ± 0.04 in either dataset, respectively (grand mean across populations 0.06 ± 0.02 and 0.05 ± 0.02 for the global and the reduced dataset, respectively; Table 3.2a, b). This suggested random mating and highlighted the adverse influence of locus PB_1030 on the global dataset. H_o and uH_e were similar between the pair of breeding populations (grand means across populations for H_o and uH_e were 0.55 ± 0.03 and 0.59 ± 0.03, respectively in the global dataset; grand means across populations for H_o and uH_e were 0.57 ± 0.04 and 0.61 ± 0.04, in the reduced dataset; Table 3.2a, b). The genetic variability of multi-locus genotypes recorded across all populations remained stable after eight loci, suggesting that the use of 14 polymorphic microsatellite loci was sufficient to describe the genetic diversity and population structure of the Barau's Petrel (Figure 3.3).

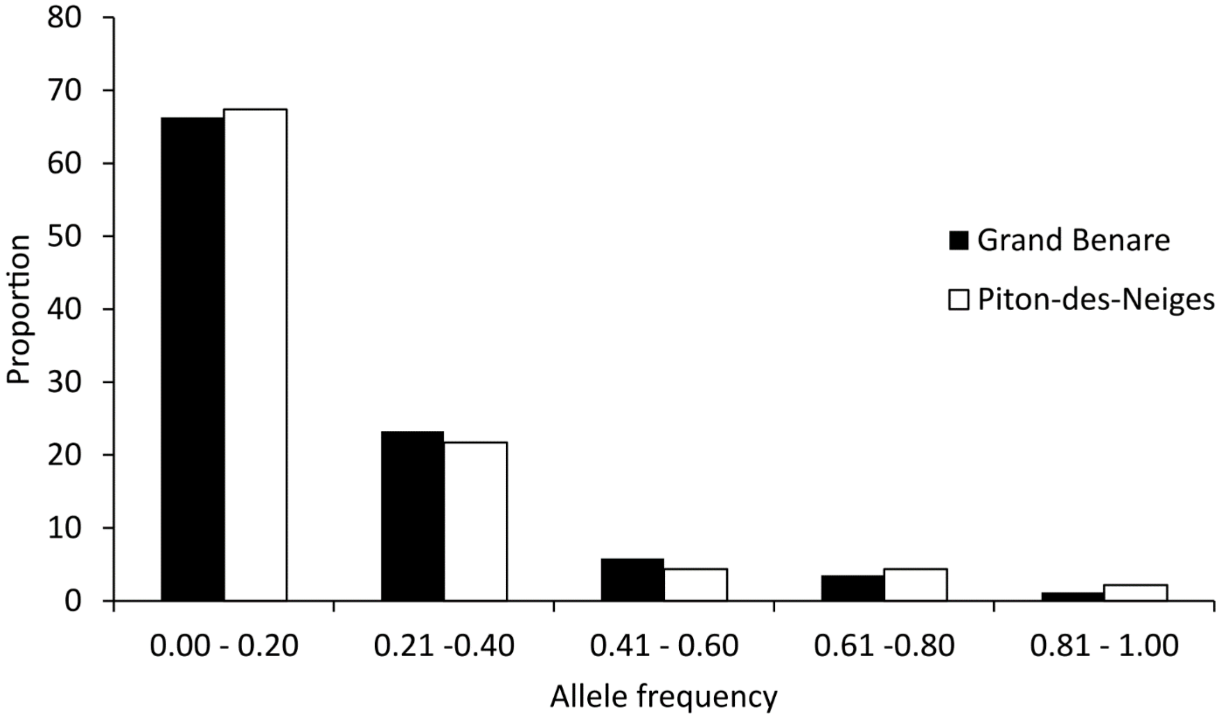

Figure 3.2: Allele frequency distributions, standardised across 14 microsatellite loci, in Barau's Petrels sampled at two breeding colonies on Reunion Island.

3.3.2. Effective population size and migration rates among breeding colonies

Approximated N_e for the *Bras-des-Etangs* genetic population (1622.7 and 1755.8 individuals at the cut-offs of 0.05 and 0.02, respectively) was more than double that of the *Vallee des Deux Miches* genetic population (576.5 and 850.9 at the cut-offs of 0.05 and 0.02, respectively; Table 3.4 a and b). Based on these estimates of N_e and calculate *m* from BIMr, it appears that considerable exchange of individuals occurs between the two breeding colonies of the Barau's Petrel (Figure 3.4). The greatest movement was estimated in the direction of *Bras-des-Etangs* to *Vallee des Deux Miches.*

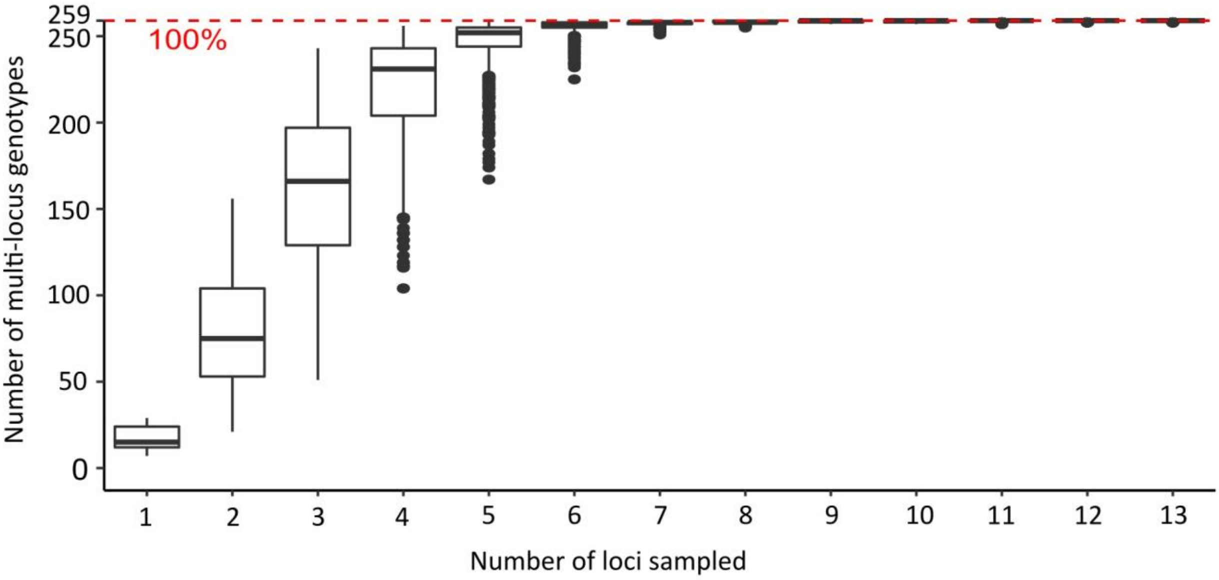

Figure 3.3: Multi-locus genotype accumulation curve based on 14 tetranucleotide repeat microsatellite loci, employing 259 individual Barau's Petrels sampled at two breeding colonies.

Table 3.3 Estimates of genetic diversity at two breeding colonies of the Barau's Petrel, based on a) 15 and b) 14 tetranucleotide microsatellite loci.

a)

Breeding colony	**n**	**N_a**	**AR^a**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
Vallee des Deux Miches	113.93 ± 2.45	5.73 ± 0.43	5.49 ± 0.39^c	4 (5%)	2.91 ± 0.27	0.57 ± 0.04	0.61 ± 0.04	0.07 ± 0.04
Bras-des-Etangs	137.07 ± 4.44	6.13 ± 0.52	5.74 ± 0.47^c	10 (10%)	2.87 ± 0.30	0.54 ± 0.06	0.58 ± 0.06	0.06 ± 0.02
Total	125.50 ± 3.29	5.93 ± 0.33	5.81 ± 0.41	N/A	2.89 ± 0.20	0.55 ± 0.03	0.59 ± 0.03	0.06 ± 0.02

b)

Breeding colony	**n**	**N_a**	**AR^b**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
Vallee des Deux Miches	116.36 ± 0.34	5.79 ± 0.46	5.82 ± 0.43^d	4 (5%)	2.99 ± 0.28	0.58 ± 0.04	0.62 ± 0.04	0.06 ± 0.04
Bras-des-Etangs	141.50 ± 0.23	6.21 ± 0.55	6.20 ± 0.51^d	10 (11%)	2.96 ± 0.31	0.55 ± 0.06	0.59 ± 0.06	0.05 ± 0.02
Total	128.93 ± 2.43	6.00 ± 0.35	6.23 ± 0.44	N/A	2.97 ± 0.21	0.57 ± 0.04	0.61 ± 0.04	0.05 ± 0.02

n = mean number of individuals per locus ± s.e.; N_a = mean number of alleles per locus ± s.e.; AR = mean allelic richness per locus ± s.e. (superscripts indicate statistically homogenous groups); P_A = private allele richness (proportion of private alleles from total allelic richness in parenthesis); A_E = mean number of effective alleles ± s.e.; H_o = observed heterozygosity over all loci ± s.e; uH_e = unbiased expected heterozygosity ± s.e.; F_{IS} = fixation index calculated based on Weir and Cockerham (1984)

[a]=estimate based on a minimum sample size of 75 diploid individuals. [b]= estimate based on a minimum sample size of 114 diploid individuals.

Table 3.4: Estimates of effective population size (N_e) and their associated 95% confidence intervals (CI), calculated using two approaches at two Barau's Petrel breeding colonies based on allele frequency cut-offs of a) 0.05 and b) 0.02.

a)		**Ne estimator method**		**LNDE method**		
	Breeding colony	**Ne**	**95% CI**	**Ne**	**95% CI**	**Mean Ne**
	Vallee des Deux Miches	576.1	288.7 - 3664.7	576.9	288.8 - 3714.2	576.5
	Bras-des-Etangs	1621.2	481.5 - ∞	1624.2	481.8 - ∞	1622.7

b)		**Ne estimator method**		**LNDE method**		
	Breeding colony	**Ne**	**95% CI**	**Ne**	**95% CI**	**Mean Ne**
	Vallee des Deux Miches	849.5	393.8 - ∞	852.3	394.4 - ∞	850.9
	Bras-des-Etangs	1754	577.4 - ∞	1757.6	577.8 - ∞	1755.8

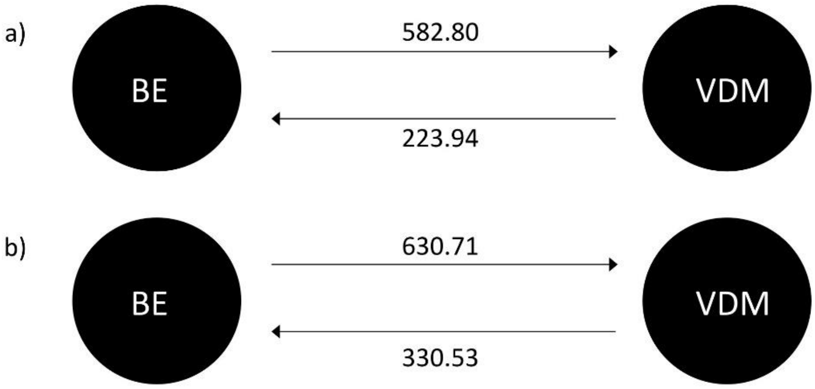

Figure 3.4: Estimates of the number of migrants per generation (*Nm*) between breeding colonies of the Barau's Petrel, based on mean estimates of effective population size (N_e) at the allele frequency cut-offs of a) 0.05 and b) 0.02 and estimates of migration rate (*m*) as calculated using BIMr. Breeding colonies are abbreviated as follow: BE = *Bras-des-Etangs*, VDM = *Vallee des Deux Miches*. Arrows denote direction of movement.

3.3.3. Genetic differentiation, and overall population structure

The pairwise F_{ST} value, between the two breeding colonies of the Barau's Petrel, was 0.01 (p-value = 1.0×10^{-4}). These values indicated weak, though highly statistically significant, genetic structure across the two genetic populations of the Barau's Petrel. Estimates of G_{ST} (0.01; pairwise p-value < 0.001) and R_{ST} (0.01; pairwise p-value < 0.001) both also indicated weak, though highly statistically significant, genetic differentiation between the two breeding colonies of the Barau's Petrel. The comparison between F_{ST} and R_{ST} showed no difference ($pR_{ST} = 0.008$, p-value = 0.1487) indicating no major contribution of stepwise mutations to the observed genetic differentiation (Hardy *et al.* 2003). Consequently, stepwise mutations can be considered negligible (in relation to the influence of genetic drift), in explaining the differentiation between the two breeding colonies of the Barau's Petrel.

The first two axes of the Euclidean distance-standardised PCoA, representing individuals from both breeding colonies, accounted for around 6% of the total genetic variation in the sampled Barau's Petrels (Figure 3.5). This, at the very most, indicates weak differentiation between the two sampled breeding populations of the Barau's Petrel.

The best-supported model for the Bayesian clustering analysis performed in STRUCTURE version 2.3.3 (based on Evanno *et al.* 2005), was that of two genetic clusters (maximum value of ΔK at $K = 2$; Figure 3.6). This was supported by the method of Pritchard *et al.* (2000; lowest standard error [s.e.] for $\ln P(X|K)$ at $K = 2$; Figure 3.6). These genetic clusters corresponded to the two breeding colonies of Barau's Petrel and are indicative of low admixture (Figure 3.7). $Q_{cluster1}$ and $Q_{cluster2}$ were 0.17 ± 0.002 and 0.83 ± 0.002 in the *Vallee des Deux Miches* breeding colony, and 0.91 ± 0.001 and 0.09 ± 0.001 in the *Bras-des-Etangs* colony.

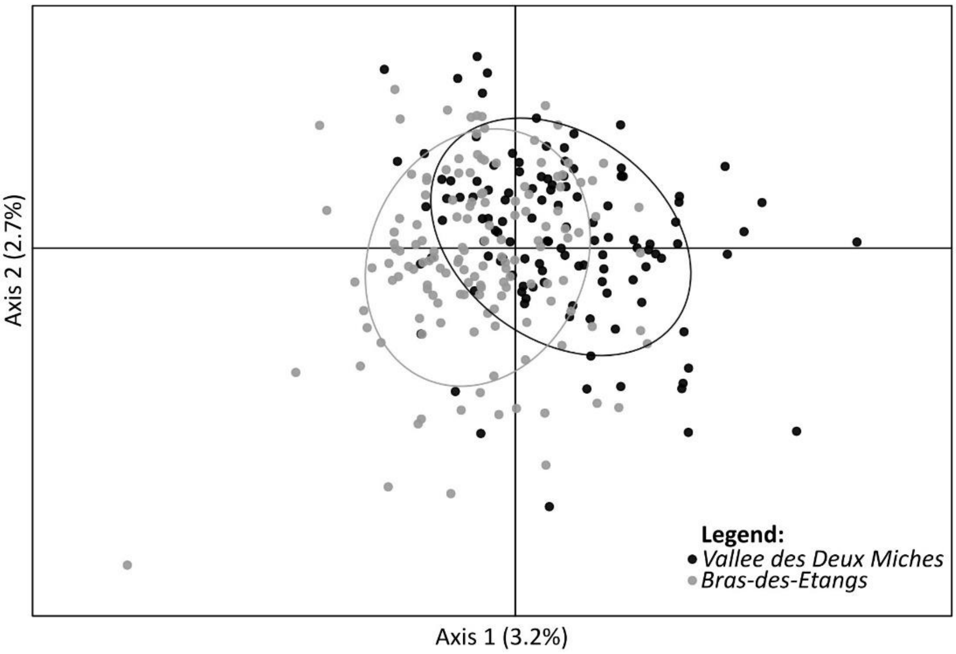

Figure 3.5: First and second axes of the Euclidian-distance standardised PCoA, including standard inertia ellipses, visualising the genetic diversity within, and genetic differentiation between, two breeding colonies of Barau’s Petrels.

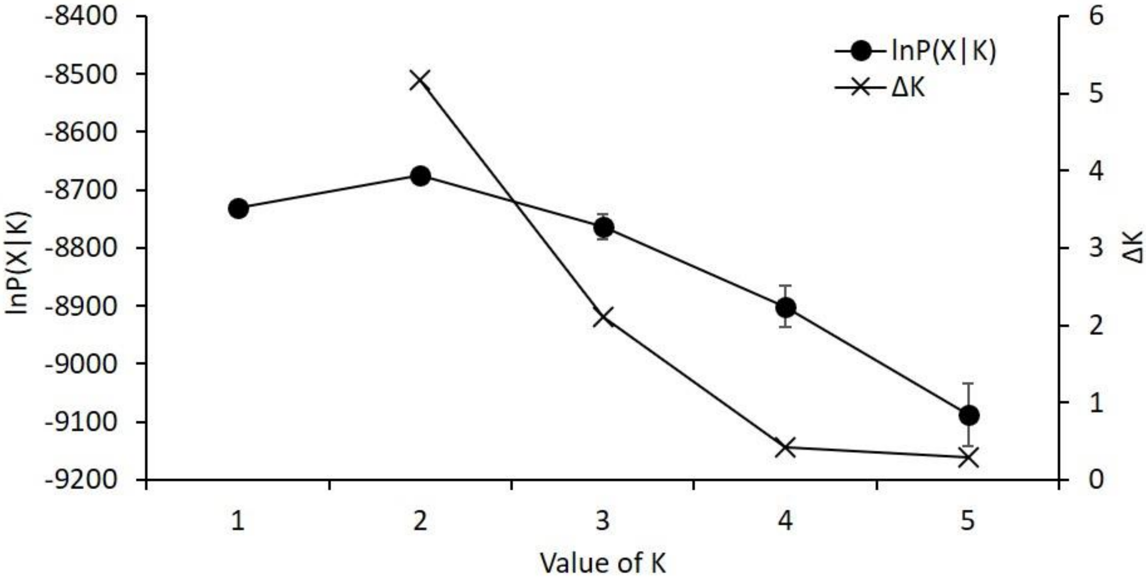

Figure 3.6: lnP(X|K; ± s.e.; Pritchard *et al.* 2000) and ΔK (Evanno *et al*. 2005), as obtained in STRUCTURE version 2.3.3, with Kmax ranging between 1 and 5. Each value was obtained by averaging the posterior probabilities of 10 independent runs.

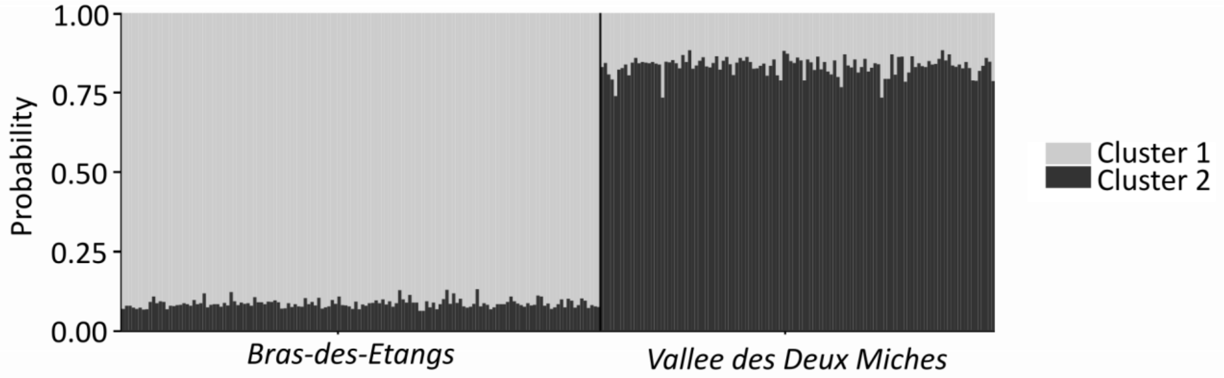

Figure 3.7: Results of a MCMC clustering procedure, performed in STRUCTURE version 2.3.3, visualising the membership of individual Barau's Petrels sampled at breeding colonies, assuming sampling location, to two genetic clusters. Vertical bars represent individuals.

3.3.4. Ringing/banding information

Since the inception of the ringing programme, until the present day, 1 105 and 392 individual Barau's Petrels have been banded at the *Bras-des-Etangs* and *Vallee des Deux Miches* breeding colonies respectively (Table 3.5). Of these, between 66% and 68% of adults have been re-sighted at nests in the colonies in which they were first banded. Only 4% of pre-fledglings have been recovered in the colonies in which they had previously been banded. Pre-fledglings typically returned to the colonies between three and six seasons subsequent to when first left. However, these ringing data provide no evidence of individual exchange between the two breeding colonies (Table 3.5).

3.4. Discussion

3.4.1. Overall population structure and potential isolating mechanisms

In addressing the population structure of the threatened endemic Barau's Petrel for the first time, this study has identified a scale of inter-colony divergence that is finer than in any other seabird species that has been studied to date. Wright's overall multi-locus fixation index

(F_{ST}; Wright 1922) highlighted weak, though statistically significant, genetic differentiation between the two sampled breeding colonies of the Barau's Petrel. Although somewhat surprising, this result was maintained by two similar estimates of genetic differentiation between populations, namely R_{ST} and G_{ST}. These results were also supported by the assignment tests, performed in STRUCTURE version 2.3.3, which indicated two distinct genetic clusters of birds corresponding to the two sampled breeding colonies. PCoA lacked the resolution to detect differences between the two genetic populations of the Barau's Petrel, however. Bayesian estimates of *Nm* were high, suggesting an inter-colony migration rate of a few hundred individuals in each direction per generation. Yet these estimates of *Nm* were almost certainly unrealistic due to violated assumptions of the Bayesian model through which they were calculated, including the low observed genetic differentiation between the breeding colonies (global $F_{ST} < 0.05$). Importantly, Faubet *et al.* (2007) indicated that F_{ST} values greater than 0.05 were necessary to obtain reliable estimates of the *m* among populations. This inference, that the Bayesian estimates of migration rates are unrealistic, is corroborated by the long-term banding information where fidelity of breeding birds to breeding colonies was estimated as 100%. Though a similar pattern of genetic differentiation was expected between the two breeding colonies of the Barau's Petrel – the scale at which this species' populations are structured is surprising given its small global population size (approximatey 14 000 pairs; Le Corre, M. pers. comm), restricted breeding range on the central massif of Réunion Island (< 10 identified breeding colonies; Pinet *et al.* 2009), and the proximity of the two sampled breeding colonies (approximatey 6 kilometres [Km]).

Table 3.5: Summary of banding information derived from the long-term monitoring programmes at the two main colonies of the Barau's Petrel.

	Individuals banded		Individuals re-sighted at colony		Number of breeding immigrants	
Breeding colony:	Adults	Pre-fledglings	Adults	Pre-fledglings	Banded as adults	Banded as pre-fledglings
Bras-des-Etangs (Piton-des-Neiges)	447	658	303 (68%)	28 (4%)	0 (0%)	0 (0%)
Vallee des Deux Miches (Grand Benare)	250	142	164 (66%)	5 (4%)	0 (0%)	0 (0%)

Similar genetic differentiation has, however, been documented in the four other gadfly petrels for which published population genetic studies currently exist. Friesen *et al.* (2006) identified three distinct island populations of the Galapagos Petrel (*Pterodroma phaeopygia*), based on structuring in microsatellite genotypes and mitochondrial DNA (mtDNA) ATPase 6/8 gene sequences. An insignificant isolation-by-distance relationship among island populations was additionally noted, although some of the highest pairwise F_{ST} values for the microsatellite dataset were reported between the most proximate of the breeding colonies (Floreana/Isabela [F_{ST} = 0.35; ± 86Km], Santa Cruz/Floreana [F_{ST} = 0.18; ± 72Km], Santiago/Santa Cruz [F_{ST} = 0.12; ± 67Km]; Friesen *et al.* 2006). Though Browne *et al*. (1997) and Nunn and Anderson (1999) noted low variability in allozymes and mtDNA cytochrome *b* gene sequences among colonies of the Galapagos Petrel, the result of Friesen *et al.* is consistent with inter-island differences in breeding phenology, morphology, egg size, and vocalizations (Cruz & Cruz 1990; Tomkins & Milne 1991). Restricted inter-colony exchanges were also reported to occur among most of the five breeding islands of the Galapagos Petrel (Friesen *et al.* 2006), as is supported by banding studies (Simons 1985; Cruz & Cruz 1990). Rayner *et al.* (2010) more recently described significant population genetic structure, using mtDNA Cytochrome Oxidase subunit one gene sequences, between the two remnant populations of the Cook's Petrel (*Pterodroma cookii*) on New Zealand. The colonies on Little Barrier and Codfish Islands, which are separated by approximately 1 300Km, both contained unique genetic diversity. They were thus recognised as two distinct management units (MUs) for which conservation of genetic as well as behavioural and morphological differences were recognised as a priority (Rayner *et al.* 2007a, b; Rayner *et al.* 2010). Variation in microsatellite genotypes, and mtDNA Cytochrome *b* and nuclear DNA (nuDNA) intron sequences, have similarly revealed structure among the four contemporary breeding colonies of the Hawaiian Petrel (*Pterodroma sandwichensis*; Welch *et al.* 2011, 2012). As in the Galapagos Petrel – the most proximate of these genetic populations, located some 75Km apart on the islands of Maui and Lanai, expressed some of the highest pairwise F_{ST} values for both the nuDNA intron (F_{ST} = 0.543) and microsatellite (F_{ST} = 0.095) datasets (Welch *et al.* 2012). Wiley *et al.* (2011) estimated a strikingly low inter-colony dispersal rate of fewer than one migrant per 1 000 generations in the Hawaiian Petrel, based on the mtDNA and nuDNA intron gene sequences. These results were initially surprising, since (like in the Barau's Petrel, and unlike the Galapagos Petrel) Hawaiian Petrels are morphologically indistinguishable among breeding colonies (Wiley *et al.* 2011). The observed patterns are in accordance with inter-island differences in foraging behaviour, however (Wiley *et al.* 2011). Finally, Iglesias-Vasquez *et al.* (2017) assessed the extent of genetic differentiation between New Caledonian (ssp. *caledonica*) and Australian (ssp. *leucoptera*) populations of the Gould's Petrel (*P. leucoptera*) using two mtDNA genes and five nuDNA introns. Their results highlighted weak, though significant, differentiation among these populations in accordance with strong ecological distinctiveness (foraging distribution, winter distribution, and breeding phenology). They were thus categorised as separate genetic MUs.

The degree to which the genetic populations of the Galapagos and Hawaiian Petrels are structured is higher than for any other seabird that has been studied to date (Friesen *et al.* 2006; Wiley *et al.* 2011), bar the present study on the Barau's Petrel. In combination with the other published studies on gadfly petrels (i.e. Rayner *et al.* 2010; Iglesias-Vasquez *et al.* 2017), this information altogether suggests that the isolation processes that drive population divergence in petrels operate at extremely fine scales. In seabirds, population genetic structure is usually explained by the interaction of natal and breeding philopatry (e.g. Rawlence *et al.* 2004; Faria *et al.* 2010; Levin & Parker 2012), mate fidelity (Friesen *et al.* 2006), mate choice (e.g. Taylor *et al.* 2012), differences in breeding and non-breeding distributions (e.g. Burg & Croxall 2001; Wiley *et al.* 2011), and divergent breeding timetables (allochrony; e.g. Monteiro 1998; Smith & Friesen 2007). High mate and breeding philopatry are believed to have driven divergence in the Galapagos Petrel (Friesen *et al.* 2006), while Rayner *et al.* (2010), Welch *et al.* (2012), and Iglesias-Vasquez *et al.* (2017) concluded that population specific non-breeding distributions and strong natal philopatry have reduced gene flow and promoted population divergence in the Cook's, Hawaiian, and the Gould's Petrels. Tracking studies on the Barau's Petrel have indicated that no such differences in foraging behaviour occur between breeding colonies (Pinet, P. pers. comm.). Birds from both the *Bras-des-Etangs* and *Vallee des Deux Miches* breeding colonies routinely occupy areas to the South and West of Madagascar while breeding, targeting two specific areas including subtropical waters between the Agulhas Current and the southern East Madagascar Current, and the recently discovered subtropical South Indian Ocean Counter Current (Pinet *et al.* 2012). They then migrate to the central Indian Ocean for the non-breeding period, where they occupy an area centered between 74°E and 97°E around the Ninety East Ridge (Pinet *et al*. 2011b). Barau's Petrel are additionally known to be highly synchronous summer (September – April) breeders

(Pinet *et al.* 2009, 2011a, b, 2012). They typically arrive back at the breeding colonies from their non-breeding migration on the 10th of September ± 7 days (Pinet *et al.* 2011b, 2012), always synchronised with the lunar cycle (Pinet *et al.* 2011a). The birds spend 11 ± 6 days at the colonies for pair bonding, nest construction, and mating, after which they depart on an extended pre-laying exodus. During this period, they spend 46 ± 9 days foraging in areas to the South and West of Madagascar (Pinet *et al.* 2012). Both sexes then return to the breeding colonies, where a single egg is then laid. Incubation lasts 52 ± 7 days, with alternate incubation shifts of 12.1 ± 2.3 days in males and 15 ± 3.2 days in females (Pinet *et al.* 2012). The chick-rearing period then lasts until mid-April, with peak fledging occurring in a narrow window between about the 13th and 27th of April each year (Pinet *et al.* 2009). In the absence of any other obvious physical barriers to gene flow, it would appear that (as is the case with the Galapagos Petrel; Friesen *et al.* 2006) strong natal philopatry, and to a lesser degree mate fidelity, have driven genetic population divergence in the Barau's Petrel.

Philopatry is believed to have evolved from the many benefits of coloniality including defence against predation, social stimulation, conspecific facilitation of location and capture of prey, and possibly mate choice (Coulson 2016). Seabirds in general are well known for their philopatric tendencies and, though strong natal philopatry is not universal among all lineages (e.g. comparatively low in the family Sternidae; Feare & Lesperance 2002; Coulson 2016), Friesen (2015) noted that the tendency of birds to return to their natal colony is probably the second most obvious potential barrier to gene flow after the physical isolation of breeding colonies (i.e. allopatric speciation). In its original definition (cf natal philopatry), a high degree of philopatry implies very limited gene flow among breeding sites of a given species (Coulson 2016). More recent treatment of the term encompasses breeding site fidelity in adults, not including natal breeding sites, where gene flow is possible (refer to Chapter 4; Coulson 2016).

In theory though, natal philopatry has the potential to lead to reproductive isolation and speciation among populations given sufficient time (Coulson 2002, 2016). Banding studies on the Procellariidae indicate high natal philopatry in most species (Weimerskirch *et al.* 1985). As with the present results where no inter-colony exchanges were detected despite a significant number (≥ 8%) of the breeding population of Barau's Petrel being banded, Weimerskirch *et al.* (1985) noted that natal fidelity was nearly 100% (39 recoveries outside of natal colonies in total) among more than 15 000 petrels banded in the French Austral and Antarctic Territories between 1951 and 1982. In most instances, natal philopatry drives population divergence in combination with mate fidelity (Friesen *et al.* 2007; Friesen 2015; Coulson 2016). Mate fidelity is known to be extremely high in all Procellariiformes, including the Barau's Petrel (Boucher, S. unpub. data), with 'divorce' rates occurring in under 3% of all monitored pairs of some species (e.g. Northern Fulmar [*Fulmarus glacialis*]; Macdonald 1977). Some strongly philopatric species, including members of the order Procellariiformes, have shown no evidence of restricted gene flow among breeding sites, however (Burg & Croxall 2001; Friesen 2015). To illustrate, no evidence of genetic structure has been detected among breeding colonies of the Grey-headed Albatross (*Thalassarche chrysostoma*) using both mtDNA and microsatellite data. This is in spite of the species' high apparent natal philopatry (Burg & Croxall 2001). Grey-headed Albatrosses form one globally panmictic genetic population, and it was suggested that mixing of birds may take place at sea or that female-mediated gene flow may occur among colonies but at very low frequencies (Burg & Croxall 2001). In addition to this, Laurence *et al.* (2014) observed panmixia among 13 breeding colonies of the Grey-faced Petrel (*Pterodroma gouldi*) on New Zealand. Though this species was believed to be highly philopatric, regular gene flow appears to occur among all of the surveyed populations.

3.4.2. Effective population size and genetic diversity

The most recent population estimates suggest that around 14 000 pairs of Barau's Petrel breed annually on Réunion Island (Le Corre, M. pers. comm.), though radar surveys indicate that nearly 40 000 birds fly over the coastline towards the sea each morning during the breeding season (Gineste, B. unpub. data). These birds are highly selective in their breeding habitats, occupying upland elfin forests (broadly 2 200 – 2 800 metres above sea level [m a.s.l.]) dominated by *Philippia montana* (Ericacae)*, Hypericum lanceolatum* (Hypericaceae)*, Phylica nitida* (Rhamnacae)*, Sophora denudate* (Fabaceae)*, and Erigeron karwinskianus* (Asteraceae; Probst *et al.* 2000). A thick, undisturbed, loam or humus layer appears to be a prerequisite for burrow establishment though total vegetation cover, slope, and altitude also appear to be important variables (Probst *et al.* 2000; Pinet *et al.* 2009). Barau's Petrel will, however, nest in habitats that are less favourable where they more typically occupy natural crevices under rocks or on steep cliffs (Probst *et al.* 2000; Pinet *et al.* 2009). The density of burrows varies in relation to habitat type, with an observed nest density of around 0.03 nests per metre squared (/m^2) in suboptimal and 0.35 nests /m^2 in optimal areas (Probst *et al.* 2000). Some 100 and 250 active burrows are monitored annually in each of the *Vallee des Deux Miches* and *Bras-des-Etangs* breeding colonies, respectively, but these populations are known to extend well outside the boundaries of the two study areas (Le Corre, M. pers. comm.). The colony at *Bras-des-Etangs* is the largest known colony of the Barau's Petrel and is situated in an area of optimal habitat conditions at around 2 400m a.s.l. Nests occur at extremely high density and, as is characteristic of this species, are typically located in cinder soils in thick elfin forest. The colony at the *Vallee des Deux Miches*, by contrast, is at the upper limit of the altitudinal range of the species (2 600 – 2 800m a.s.l) where conditions are less suitable. Nests in this colony occur at low density and are typically placed in natural crevices under rocks, though a portion of the colony (named MM) is in thicker elfin forest at slightly lower altitude (approximatey 2 400m a.s.l.). The present estimates of N_e for the two breeding colonies are therefore well within realistic limits when compared to surveyed estimates of population sizes (800 – 850 pairs at *Bras-des-Etangs,* and 300 – 400 pairs at *Vallee des Deux Miches*), and indicate that both colonies contain a significant proportion (> 5%) of the species' total breeding population. It is unsurprising then that the two sampled breeding colonies of the Barau's Petrel also contain moderate genetic diversity, including a large proportion of rare (frequency < 0.20) and private alleles.

3.4.3. Conclusive management implications, in relation to genetic diversity

Extinction is a fundamental ecological process and is considered the ultimate fate of all species (Griffen & Drake 2008). As a general rule in conservation biology, a species composed of several genetic populations should be more resilient to global extinction than one consisting of only a single genetic population (reviewed in Griffen & Drake 2008). There are many exceptions to this, one of which is species consisting of fragmented island populations. The disintegration of populations on islands can, for example, lead to reductions in genetic diversity and results in more limited adaptation and recovery potential with a concomitant increase in the risk of extinction (Griffen & Drake 2008). Smaller populations are considered more likely to go extinct because of demographic stochasticity (Belovsky *et al.* 1999; Lande *et al.* 2003), while lower levels of migration among populations are believed to increase extinction risk through a diminished 'rescue effect' (Allen *et al.* 1993). Increased genetic diversity (regardless of population size) will decrease extinction risk, however, by increasing adaption ability (Reed & Frankham 2003). In this sense, the moderate level of genetic diversity in the Barau's Petrel is suggestive of a degree of resilience to environmental variability.

However, the two sampled breeding colonies show limited (and possibly no) admixture and are genetically distinct from one another, both containing a high proportion of rare and private alleles. The loss of even a single one of these breeding colonies will therefore result in the loss of overall genetic diversity, possibly interrupting current evolutionary processes, and will result in decreased adaption potential. Both colonies also support a significant (> 5%) proportion of the species' total breeding population total breeding population, though in this case the long-term implications of losses in genetic diversity will likely outweigh those linked to losses in population size. The two genetic populations of Barau's Petrel should therefore be considered as separate MUs, demanding that conservation measures be implemented on a colony-to-colony basis, rather than working under the current assumption that the species' population is homogeneous.

Molecular techniques, such as microsatellite analysis, are now commonly used to disentangle taxonomic relationships among species. The treatment of the gadfly petrels has provoked considerable debate within the scientific community and is frequently revised (Brown *et al.* 2011). Traditional approaches used to classify species in this group have been dependant on anatomical features, behaviour, call structure, and their ectoparasites (Imber 1985; Brown *et al.* 2011). The present results further emphasize the usefulness of molecular techniques in the clarification of taxonomic relationships among species, and importantly in the identification of diversification processes below the species level. Inter-colony diversification processes in most other gadfly petrel species are currently unrecognized, and (in virtually all cases) are impossible to detect with traditional, non-genetic, approaches. Present results highlight the potential role of philopatric behaviour to diversification in the gadfly petrels, and emphasize the importance of scale-specific management strategies for their breeding colonies in general.

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CHAPTER 4: GENETIC META-POPULATION STRUCTURE OF THE SOOTY TERN (*ONYCHOPRION* FUSCATUS) IN THE TROPICAL WESTERN INDIAN OCEAN: IMPLICATIONS FOR REGIONAL HARVEST MANAGEMENT AND CONSERVATION.

4.1. Introduction

The islands of the western Indian Ocean host globally significant populations of seabirds, though the region's seabird community is dominated by the Sooty Tern (*Onychoprion fuscatus*; Feare *et al.* 2007; Danckwerts *et al.* 2014). Current estimates suggest that around 6 500 000 pairs of the Sooty Tern occupy 26 breeding colonies in the western Indian Ocean, altogether accounting for more than 70% of the region's total seabird biomass (Danckwerts *et al.* 2014). The species' breeding colonies are typically located on offshore coralline and granitic islands, and range in size from about 26 breeding pairs on Tromelin (Le Corre, M. pers. comm.) to about 2 000 000 pairs on Juan de Nova (northern Mozambique Channel; Feare *et al.* 2007; refer to Figure 4.1). Other notable colonies in the region include Cosmoledo (approximatey 1 000 000 pairs; northern Mozambique Channel), Europa (approximatey 750 000 pairs; southern Mozambique Channel), and Bird Island (approximatey 600 000 pairs; Seychelles; Feare *et al.* 2007; Danckwerts *et al.* 2014; refer to Figure 4.1). These four main colonies (i.e. Juan de Nova, Cosmoledo, Europa, and Bird Island) altogether account for more than 25% of the Indian Ocean Sooty Tern population and are perhaps among the best surveyed colonies in the world (Feare *et al.* 2007). Nevertheless, during the past two centuries, the Sooty Tern populations of the western Indian Ocean have exhibited an overall negative trend and many colonies are now greatly reduced in size (reviewed in Feare *et al.* 2007). Fifteen additional colonies, collectively amounting to some 600 000 pairs, are known to have been extirpated altogether including apparently large breeding populations on Ile

Pierrot and Ile Combrani (Rodrigues), Diego Garcia (Chagos), Agalega, and Ile Platte (Seychelles; Feare *et al.* 2007; refer to Figure 4.1). The drivers of these changes are reviewed in Feare *et al.* (2007), but habitat alteration and human exploitation of adults and their eggs are believed responsible.

Sooty Tern eggs have been commercially exploited on islands in the Seychelles and Amirantes since at-least the beginning of the 20th century (Fryer 1910; Ridley & Percy 1958). Eggs are typically collected for local consumption, where they constitute a major delicacy (locally known as '*dizef zwazo'*), though yolks were separated and barrelled for export in addition to normal egg harvesting in the 1930s. Sooty Tern eggs are sold locally at a retail price of between US$0.40 (5 Seychelles Rupees [SCR]) and US$0.70 (9SCR) per piece (Vannier *et al.* 2015), implying that the many hundreds of thousands of eggs taken each year contribute significantly to the Seychelles economy and to the livelihoods of the impoverished local communities. Following claims of over exploitation, numerous authors studied the species' demography and population status to inform best-practice harvest management protocols. Vesey-Fitzgerald (1941) first drew attention to the declining populations of the Sooty Tern in the Seychelles, and considered that it was due largely to the over-exploitation of eggs. Ridley and Percy (1958) then published governmental data on the scale and frequency of egg harvesting, noting that exceptionally high numbers of eggs (> 3 000 000 pieces in total) were taken annually during the 1930s. It was on this basis that both Vesey-Fitzgerald (1941) and Ridley and Percy (1958) stressed the need for stringent conservation measures to stop ongoing population declines in the Sooty Tern. The islands from which eggs were collected are debatable, and many of the claimed harvests were impossibly high given the species' breeding behaviour (i.e. breeding density) and the sizes of the islands in question (Feare 1976b). Feare (1976b) therefore concluded that the evidence for over-exploitation, and resulting declines in Sooty Tern numbers, was unconvincing and noted that, although some colonies had decreased or become extinct, factors other than over-exploitation were more likely responsible. Feare and Lesperance (2002) later studied the inter- and intra-colony movement dynamics of Sooty Terns in the Seychelles and, in amalgamation with an experimental study that assessed the behaviour of Sooty Terns subsequent to egg harvesting (Feare 1976a), noted that considerable exchange of adults occurred among islands. This result contrasted with a study of Sooty Tern site fidelity on the Dry Tortugas (Florida; Robertson & Wilmers 1996), where most individuals remained faithful to a single colony, and suggested that the Seychelles birds are units within a single subpopulation and that considerable genetic exchange occurs among islands.

Nevertheless, the recommendations made by Ridley and Percy (1958, 1966), Feare (1976a, b), Feare *et al.* (1997) and Feare and Lesperance (2002), have been largely accepted and are implemented to the present day. Regulations now in operation specify that the annual harvest should be restricted to no more than 20% of the area of each of the Seychelles and Amirantes colonies, that the vegetation within colonies should be managed to maximise the area available for nesting, that no more than half of any island should be cropped of vegetation to ease harvesting and the non-cropped part should be strictly protected, and finally that 15% of the gross income from the eggs should be returned to the Division of Environment of the Seychelles as a levy to fund monitoring, policing, and research (Feare *et al.* 2007). It was further suggested that harvesting be limited to only two islands within the Seychelles per breeding season, and that all harvesting cease before the end of the main laying period on each island (Feare 1976b). Since 1976, all available data indicate that the breeding populations of the major colonies have remained stable, suggesting that the current scale and frequency of commercial egg harvesting is sustainable (Feare *et al.* 2007). However, several recent observations suggest that the recommendations made by Feare *et al.* are no longer being adhered to and, as such, there is growing concern about over-harvesting (Feare, C. pers. comm.). In addition to the commercial harvests, illegal harvesting also occurs on many islands within and outside (e.g. Madagascar, Mauritius) of the Seychelles (Feare *et al.* 2007; Voogt, N. pers. comm.). Driven by unmet demands, and despite its national park status, annual attempts are made to poach the breeding colony on Aride in the Seychelles (Feare *et al.* 2007). Other small colonies (e.g. Booby Islet and African Banks) suffer such profound poaching that few chicks ever manage to fledge. Some of these smaller breeding colonies have been eliminated altogether (e.g. Ile aux Vaches [Grand Anse], Mammelles, and Ile Seche) as a direct result (Feare *et al.* 2007). Colonies outside of the Seychelles, mainly on Madagascar and on small islets off Mozambique, also suffer unidentified magnitudes of illegal harvesting and its effect on the demographic trajectories of these breeding colonies is not currently known (Feare *et al.* 2007; Le Corre & Bemanaja 2009). In combination with the eggs that are poached, eggs already present on islands have sometimes been destroyed to ensure that all eggs collected subsequently are fresh (Feare *et al.* 1997). Adult Sooty Terns have also been taken for consumption by poachers, sometimes in their thousands, together with the eggs, chicks, and adults of other seabird species (e.g. Masked [*Sula dactylatra*] and Brown [*S. leucogaster*] Boobies; Feare *et al.* 1997). These illegal poaching practices are wasteful, unregulated, and are unlikely to be sustainable.

Several recent shifts have occurred concerning the ways in which seabird populations are being managed. One of the most important is the use of molecular techniques to inform the correct scale at which conservation measures need to be implemented, and in the assessment of the demographic and evolutionary trajectories of populations (reviewed in Chapter 1.3). To date, no such studies have been performed on the Sooty Tern in the western Indian Ocean.

Avise *et al.* (2000) did, however, assess the global matrilineal history of Sooty Terns using mitochondrial deoxyribonucleic acid (mtDNA, DNA) restriction sites and mtDNA control region (mtDNA CR) sequences. Their results suggested that a shallow genealogical partition exists between Atlantic and Indo-Pacific populations of the Sooty Tern, though colonies within each of the three ocean basins are poorly differentiated from one another. Importantly though, only two breeding colonies were considered from the Indian Ocean and the sample size of each of these was negligible (Chagos n = 6; Seychelles [island unspecified] n = 12). Further to this, though Feare (1976a, b) and Feare and Lesperance (2002) noted considerable exchange of individual Sooty Terns among islands of the Seychelles, the number of banded birds and the level of effort invested into finding recaptures is currently inadequate to confirm whether colonies outside of the Seychelles are a part of the same breeding unit as those within the Seychelles. It is therefore necessary to revisit the genetic population structure of the Sooty Tern using a finer technique (i.e. microsatellite analysis) at the scale of the entire western Indian Ocean. This information is urgently required to properly assess the influence of egg harvesting on the demographic and evolutionary trajectory of the species in the region, especially since recent information has highlighted a lack of synchrony in the breeding schedules of birds among different island groups in relation to local oceanographic conditions (Jaquemet *et al.* 2007).

The aim of this chapter is to assess the population structure of the Sooty Tern, using polymorphic nuclear microsatellite analysis. The overall objective of this is to inform scale-dependant conservation of its populations in the western Indian Ocean, and regional harvest management protocols. Four of the main western Indian Ocean breeding colonies (i.e. Europa, Bird Island [Seychelles], Glorieuses, and Juan de Nova) are compared with one another to assess the genetic relationships among them. It is predicted that low natal philopatry in the region, combined with the species' high dispersal capabilities, will have maintained gene flow among the different breeding colonies thereby minimizing genetic structuration. That said, however, low levels of genetic differentiation are expected among seasonally segregated breeding colonies in that these birds should be isolated in their breeding schedules which are related to spatio-temporal variation in marine productivity in the western Indian Ocean (Jaquemet *et al*. 2007). Birds on Europa and Bird Island (Seychelles) typically lay in June/July (winter), and are thus expected to differ genetically from birds breeding at Juan De Nova since the laying period at this latter colony is in November/December (summer; Jaquemet *et al.* 2007; refer to Figure 4.1). The breeding population on Glorieuses is distinctively aseasonal, with birds recorded laying in February, March, April, August, and October (Jaquemet *et al.* 2007; refer to Figure 4.1). This colony should therefore show a degree of genetic relation or ancestry to all other breeding colonies in the western Indian Ocean, and could also act as a stepping stone for indirect geneflow between seasonally segregated populations. In furtherance to the genetic information, banding records (1974 – 2015) for Sooty Terns at breeding colonies throughout the western Indian Ocean were collated to assess philopatry and quantify levels of inter-colony exchange. Finally, a breeding colony (i.e. Clipperton; 10° 18'N, 109° 13'W) from the Eastern Pacific was included into an additional genetic analysis to assess gene flow through the Indo-Pacific basin.

4.2. Materials and methods

4.2.1. Sample collection

One-hundred and sixty-six adult Sooty Terns were captured at their nests, on five breeding colonies in the Indo-Pacific region (Table 4.1), by means of a fishing landing net. All were banded, facilitating individual identification. A small sample of whole blood (approximatey 0.5 millilitres [mL]; maximum of 1.0% of body weight; Fair *et al.* 2010) was then collected through medial metatarsal or basilic venipuncture using sodium-heparin lined BD Micro-Fine^{TM} 1.5mL 27-gauge syringes. Blood samples were placed in 2mL Eppendorf tubes, in an insulated box containing ice packs. In some cases, whole blood was centrifuged within four hours for serum collection and serological analyses (Lebarbenchon *et al*. 2015; Jaeger *et al.* 2016). Whole blood, or blood cells, were then preserved in 70% ethanol and held at -80 degrees Celsius (°C) at the *Centre de Recherche et de Veille sure les Maladies Emergentes dans l'Océan Indien* (CRVOI; Saint Denis, Réunion) upon return to Réunion Island pending further analysis.

Table 4.1: One-hundred and sixty-six adult Sooty Terns were sampled at five breeding colonies in the Indo-Pacific region.

Breeding colony	Ocean basin (Sector in parentheses)	Breeding period	Latitude/Longitude	Approximate colony size (pairs)	Sample size
Juan de Nova	Indian Ocean (West)	Summer	17° 03'S 42° 43'E	2 000 000	39
Bird Island	Indian Ocean (West)	Winter	03° 43'S 55° 12'E	500 000	51
Glorieuses	Indian Ocean (West)	Aseasonal	11° 34'S 47° 17'E	27 000	16
Europa Island	Indian Ocean (West)	Winter	22° 22'S 40° 21'E	760 000	51
Clipperton Island	Pacific Ocean (East)	N/A	10° 17'N 109° 12'E	2 000	9

The techniques employed to capture and sample blood from Sooty Terns were approved by the Ethics Committee at Rhodes University (reference Number ZOOL-01-2013). Bird capture, handling, and collection of biological material (including Clipperton) were performed under research programs approved by the *Centre de Recherches sur la Biologie des Populations d'Oiseaux* (CRBPO; Muséum Paris; permits 616, 044). Sample collection on

Juan de Nova was conducted with additional approval of *Direction de l'Environnement, de l'Aménagement et du Logement de la Réunion* (DEAL Réunion), and *Terres Australes and Antarctiques Françaises* (TAAF). Biological material from Glorieuses was collected with support from *Prefecture de La Réunion*, and *Météo-France*. Sampling at Bird Island (Seychelles), and sample export to Réunion Island, were performed under the approval of the Seychelles Bureau of Standards and Ministry of Environment, Energy and Climate Change.

4.2.2. DNA extraction technique

Total DNA was extracted from the whole blood, or blood cell, subsamples using the QIAmp Blood & Tissue kit (QIAGEN) following the procedure developed for animal tissue with minor adjustments (refer to Chapter 2.2.3). Quantification of DNA in two microlitre (µL) aliquots of each sample was subsequently performed using a Thermo Scientific NanoDrop 2 000c UV-Vis Spectrophotometer. Measured nucleotide concentration varied between 3.1 and 71.6 nanograms per microlitre (ng/µL). Samples with concentrations higher than 40ng/µL were diluted with Buffer AE (QIAGEN). Extracted DNA was stored at -20°C pending further analysis. This, and all subsequent sample manipulation for the Sooty Tern, was performed at the *Université de La Réunion*.

4.2.3. Oligonucleotide primer development

Until the present study, microsatellite loci had not previously been isolated for the Sooty Tern (results from this study published in Danckwerts *et al*. 2017). A genomic library was therefore constructed using DNA from a single individual collected at Juan De Nova. Microsatellite sequences were isolated by Ecogenics GmbH (Zurich, Switzerland) using this genomic library. Size-selected fragments from genomic DNA were enriched for simple sequence repeat (SSR) content using magnetic streptavidin beads and biotin-labelled GATA and GTAT repeat oligonucleotides. The SSR-enriched library was analysed on an Illumina MiSeq platform using the Nano 2x250 version 2 format.

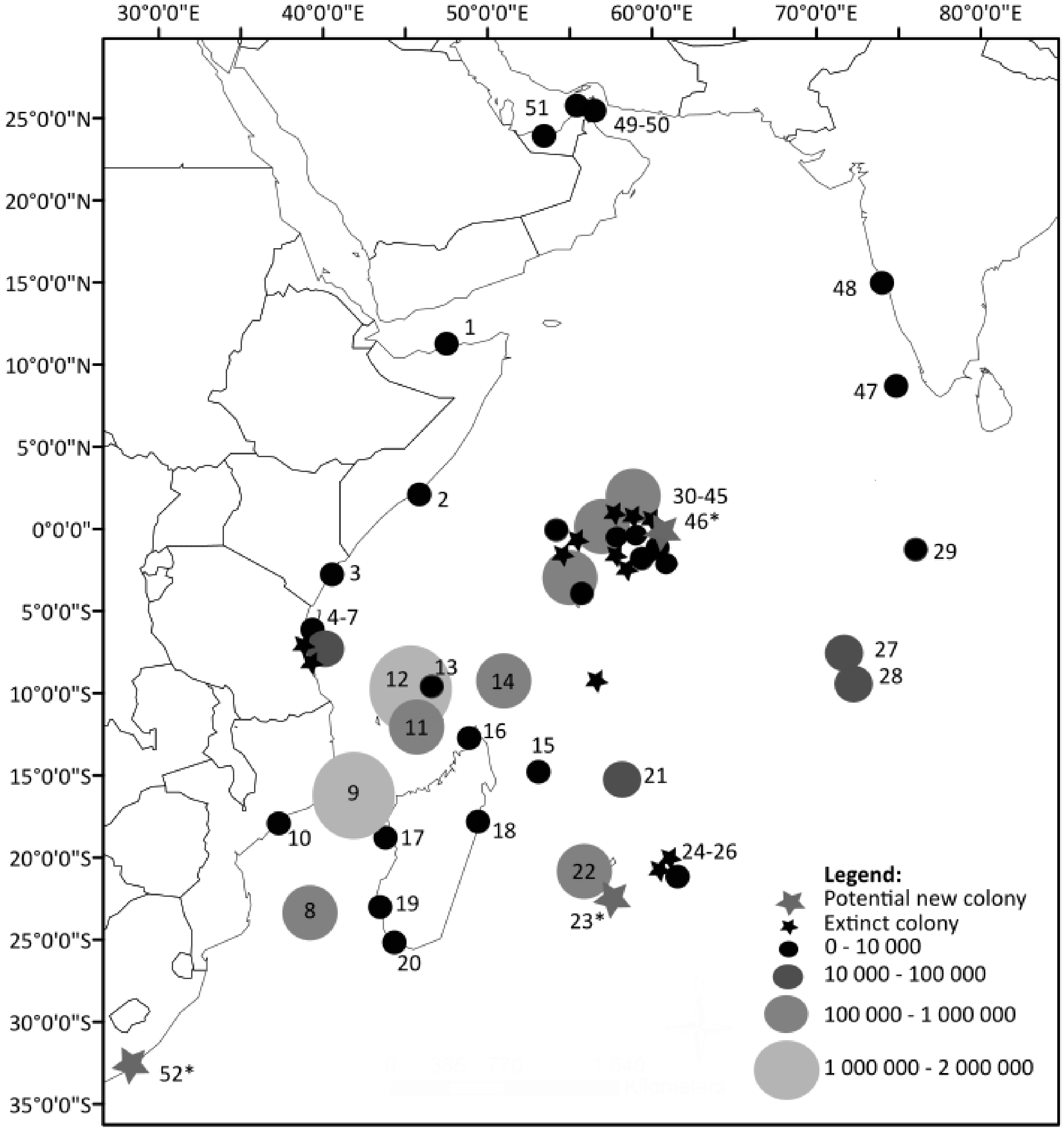

Figure 4.1: Distribution of the active and extinct breeding colonies of the Sooty Tern in the western Indian Ocean (adapted from Feare *et al.* 2007), with spot size indicating the approximate population (in breeding pairs) sizes of all extant colonies. Breeding colonies are as follows: (1) Maydh Island, (2) Islets off Moqdisho, (3) Tenewe Islands, (4) Mafia Island, (5),

Kisite Island, (6) Dar es Salaam, (7) Latham Island, (8) Europa, (9) Juan de Nova, (10) Islands off Mozambique, (11) Glorieuses, (12) Cosmoledo, (13) Astove, (14) Goelette/Farquhar, (15) Tromelin, (16-20) Islets off Madagascar, (21) Saint Brandon, (22) Serpent Island, (23) Iles aux Aigrettes, (24-26) Islets off Rodrigues, (27-28) Chagos Archipelago, (29) Seenu Atoll (Maldives), (30) Desnoeufs, (31) Etiole, (32) Remire, (33) north African Banks, (34) south African Banks, (35) Ile Plate, (36) Ile Seche, (37) Mammelles, (38) Ile aux Vaches, (39) Recif, (40) Fregate, (41) Aride, (42) Booby Islet, (43) Cousin, (44) Cousine, (45) Bird Island (Seychelles), (46) Denis Island, (47) Pitti, (48) Vengurla Rocks, (49) Umm al Fayyarin, (50) Daymaniats, (51) Qarnein Island, and (52) Bird Island (South Africa). Asterisks denote colonies that may soon be naturally colonised or that are part of ongoing restoration efforts.

After assembly, 2 611 contigs or singlets contained a microsatellite insert with a tetra- or a trinucleotide repeat motif of at-least six units, or a dinucleotide repeat motif of at-least 10 units. Suitable primer design was possible in 1 220 microsatellite candidates, of which 36 were tested for functionality and polymorphism. Polymorphism was tested using DNA from an additional four individuals from Juan de Nova, and three from Bird Island (Seychelles). Among the candidate microsatellite loci, 34 contained a high number of stutters comprising between two and 11 alleles, whilst two candidates failed to amplify and were difficult to interpret. Allelic diversity, fragment sizes, and peak patterns in electropherograms were used to select the best markers for primer development (Table 4.2). These microsatellite loci all exhibited tetranucleotide repeat motifs of at-least seven repeat sequences (Table 4.2).

Table 4.2: Characteristics of 16 tetranucleotide repeat microsatellite loci developed for Sooty Tern (adapted from Danckwerts *et al*. 2017). Nucleotides are abbreviated as follows: A = adenine, G = guanine, C = cytosine, T = thymine.

Name	Primer Sequences (5'-3')	Repeat motif	Fragment size (base pairs)	GenBank Accession No.	Mix no.	Dye
Onyfus_282	F: TCAGTCTCGAACAGGCTTCC R: TGCAAGCAACAGGACTTCATC	$(TCTA)_{12}$	223 – 283	KY781353	1	6-FAM
Onyfus_244	F: CTCCACCAGCACAATGACAG R: ACAGAAGCACAGTAGACGGG	$(TTCT)_{12}$	113 – 229	KY781354	1	NED
Onyfus_614	F: ACGGAAAAGATGCTGGCTTTC R: TAGAAACCCCAGGTGATGGC	$(TATC)_{16}$	186 – 250	KY781355	1	PET
Onyfus_5474	F: TGGGTGTTTGCATGAGCTTC R: GTGGTGGCGTTACTGAATGG	$(TATC)_{13}$	255 – 295	KY781356	1	VIC
Onyfus_89	F: GCTCATGCTGATAATGGTTTGC R: ACTCTTCCCTGTTACTGCCC	$(TCTA)_{12}$	166 – 214	KY781357	2	6-FAM
Onyfus_722	F: TGACCAGTTACGTTGTAGGG R: TGATGCCAATTCTGCTGTGC	$(GATA)_{10}$	207 – 263	KY781358	2	NED
Onyfus_1263	F: CCCGTCAGACTTCAAGAAAGG R: AGGAGTTCCTGTGCTCATCC	$(GTTT)_{7}$	239 – 255	KY781359	2	PET
Onyfus_5118	F: TCTCAGCGAAGACAAACACAAG R: GCAGGAAGCATTTCTGTGGG	$(AGAA)_{10}$	238 – 282	KY781360	2	VIC
Onyfus_1916	F: TGCACCAAACCCCTGTACTC R: ACCTATGTCTAGTTTTTGCTTTGC	$(TAGA)_{10}$	223 – 259	KY781361	3	6-FAM
Onyfus_778	F: TTTTGTTTTCCCCTCCAGCC R: TCCATGTTGTAGCTGGGGAC	$(ATAC)_{10}$	206 – 234	KY781362	3	NED
Onyfus_918	F: TTACCTGACGAGGCCATCTG R: GTAGATCATACTGGAAACACCTG	$(GATA)_{11}$	242 – 302	KY781363	3	PET
Onyfus_1205	F: TGGCATTCTTTGGCTTATTTGTC R: CAGTATGGGTCCCTATTCCCC	$(ATCT)_{9}$	193 – 213	KY781364	3	VIC
Onyfus_1123	F: GGAAACCCTGATAAGCTCGC R: GGTGATCCAGGGTCCAGTC	$(CTTT)_{11}$	200 – 300	KY781365	4	6-FAM
Onyfus_5649	F: TGCTCGTCAGTATAGCACAGG R: TCATAACAGGGGCTGGTTGC	$(ATCT)_{12}$	200 – 240	KY781366	4	NED
Onyfus_5155	F: TTATGGGAAAGGGCACTTGG R: CTCAGAAAACATCTGGGAAGCC	$(GATA)_{15}$	220 – 292	KY781367	4	PET
Onyfus_6079	F: ACTTACCTTGGCAGCCTCTC R: GCTTCGACACCCCTTTTGTG	$(TCTA)_{11}$	191 – 267	KY781368	4	VIC

4.2.4. Polymerase chain reaction (PCR) technique

A 3-primer polymerase chain reaction (PCR) approach, using the universal M13 tail (5’-CACGACGTTGTAAAACGA-3’) for the forward primer, was used for microsatellite loci amplification following Schuelke (2000). Four fluorescently-labelled dyes (6-FAM, PET, VIC, and NED) were used alongside the M13 tail, enabling fragment analysis multiplexing. Simplex

PCR amplifications, targeting one locus at a time, were performed using a GeneAmp PCR System 9 700 (Applied Biosystems) in a 10µL reaction volume containing 5µL of GoTaq® G2 Hot Start Colorless Master Mix applied 2x (Promega), 0.25µL of the forward primer with M13 5'-tail [1 micromole {µM}], 0.25µL of the reverse primer [10µM], 0.25µL of the dye (6-FAM, NED, PET or VIC; [10µM]), and 2µL of genomic DNA [approximatey 40ng/µL]. Reaction volumes were completed with sterile deionised water. PCR amplifications were carried out as follows: an initial denaturing step at 95°C for five minutes, followed by 43 primer annealing cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. Final elongation occurred with samples held at 72°C for 20 minutes. PCR products were stored at -20°C prior to product size determination.

Four different simplex PCR plates, each with a different dye (Table 4.2), were mixed and PCR product sizes determined using a 3 730XL DNA analyser (Applied Biosystems), by Gentyane platform (Clermont-Ferrand, France). Product sizes were estimated with the LIZ(500) standard using GeneMapper version 4.0 (Applied Biosystems). Null values of allele sizes, not exceeding 5% of the total dataset, were accepted.

4.2.5. Data analysis

4.2.5.1 Genetic diversity

Mistyped allele sizes, deviations from the tetranucleotide repeat motifs, and evidence of null alleles, large-allele dropout, and stutter peaks were first examined among all loci, in all populations, using MicroChecker version 2.2.3 (van Oosterhout *et al*. 2004). Each sample-locus combination was then tested for linkage disequilibrium (LD) using GenePop version 4.0.10 (Rousset 2008), with the exact probability test (10 000 dememorizations, 100 batches, 1 000 iterations per batch). The resulting p-values were corrected using sequential Bonferroni correction for multiple comparisons (Rice 1989). Presence/absence of LD among loci was confirmed with the method of Index of Association (Agapow & Burt 2001), in the package poppr (Kamvar *et al*. 2014, 2015), in R version 3.4.0 (R Development Core Team 2017). Additionally, to assess the overall power of all subsequent analyses, a multi-locus genotype accumulation curve was constructed using the package poppr, in R version 3.4.0.

Standard estimates of genetic diversity, including the mean number of alleles per locus (N_a), private allele richness (P_A) in each population, the effective number of alleles (A_E), observed heterozygosity over all loci (H_o), and unbiased expected heterozygosity over all loci (uH_e; Nei 1978), were calculated for all breeding colonies and the three seasonally segregated populations (i.e. winter, summer, aseasonal) of the Sooty Tern using GenAlEx version 6.6 (advance notice based off Peakall & Smouse 2012). The statistical significance of differences in medians of N_a and A_E were estimated using Kruskal-Wallis tests for equal medians as implemented in PAST version 3.1.5 (Hammer *et al*. 2001). Where p-values were less than 0.05, pairwise Mann-Whitney tests were used post hoc to explore the origins of significant differences. AR (El Mousadik & Petit 1996) was additionally calculated for each breeding colony and seasonally segregated group using FSTAT version 2.9.3 (Goudet 1995, 2001), and was compared among populations using 10 000 permutations of the alleles at each locus. Clipperton was excluded from these analyses since too few samples were collected. Deviations from the Hardy-Weinberg proportions were assessed at each locus, in all breeding colonies, with the package pegas (Paradis 2010), in R version 3.4.0, using the exact test based on 1 000 Monte Carlo permutations of the alleles. Finally, Wright's inbreeding coefficient, within individuals relative to the subpopulation (F_{IS}; Wright 1922, 1965), was also computed for each breeding colony and seasonally segregated group using GenAlEx version 6.6 (advance notice based off Peakall & Smouse 2012).

4.2.5.2 Genetic differentiation among populations

Wright's overall multi-locus fixation index (F_{ST}; Wright 1922), and its associated p-value, was computed over all loci and populations following Weir and Cockerham (1984) using the package adegenet (Jombart 2008; Jombart & Ahmed 2011), in R version 3.4.0. The statistical significance of these indices, between each pair of populations, was then tested using 10 000 random permutations of the raw genotypes, again with the package adegenet, in R version 3.4.0.

4.2.5.3 Population structure

Genetic diversity within, and genetic differentiation among, colonies of the Sooty Tern were initially examined using Principal Coordinate Analyses (PCoA) based on scaled overall multi-locus genotypes. These analyses were implemented using a Euclidean distance matrix among all individuals, using the package adegenet in R version 3.4.0.

Assignment tests, based on the multi-locus genotypes, were additionally used to assess differentiation and clustering among breeding colonies of the Sooty Tern. These were evaluated using a model-based Markov Chain Monte Carlo (MCMC) clustering procedure, implemented with a Bayesian algorithm, in STRUCTURE version 2.3.3 (Pritchard *et al.* 2000). Multiple independent runs, using a burn-in period of 5 000 simulations followed by 10 000 repetitions, were first performed using different settings (e.g. admixture versus no-admixture model, LOCPRIOR verses no-LOCPRIOR) to assess convergence and determine the importance of model choice. An admixture model (Dirchelet parameter; $\alpha = 1.44 \pm 0.28$), using sampling location as prior information (LOCPRIOR; $r = 6.50 \pm 1.66$; Hubisz *et al*. 2009), was finally used. Correlated allele frequencies were assumed (Pritchard *et al.* 2000; Falush *et al.* 2003). To estimate the number of genetic clusters (K), 10 independent runs of 10 000 iterations each (after a burn-in of 5 000 steps) were performed for K = 1 to K = 7. The optimal value of K was subsequently determined using the mean overall log-likelihood given the number of clusters (lnP(X|K); Pritchard *et al*. 2000), and the second-order rate of change of lnP(X|K) (ΔK; Evanno *et al*. 2005). Ten independent simulations, at the optimal value of K, were then run using 1 000 000 iterations each (after a burn-in of 500 000 steps). The simulation with the highest value of lnP(X|K), at the optimal value of K, was finally used to visually represent the posterior assignment probabilities for all individuals from the five breeding colonies. A second clustering procedure was implemented in STRUCTURE version 2.3.3 to assess clustering irrespective of sampling location. Breeding colonies were categorised according to breeding seasonality (i.e. summer, winter, aseasonal) for the purposes of this second model. An admixture model was once again used (Dirchelet parameter; $\alpha = 1.91 \pm 0.31$), with breeding season employed as the LOCPRIOR factor ($r = 5.37 \pm 0.77$). Membership coefficients (termed *Q*) were assigned to all individuals for each genetic cluster at the optimal value of K.

4.2.5.4 Ringing/banding information

Ringing/banding records for Sooty Terns, at colonies within the western Indian Ocean, were gathered from as many ringing authorities as possible. This included the British Trust of Ornithology (Feare), the South African Bird Ringing Unit (SAFRING), and the CRBPO (Muséum Paris). Subsetting functions in R version 3.4.0 were then used to extract the total number of birds ringed at each colony, the number of birds resighted as breeding individuals at the colonies in which they were first banded, and the number of individuals from each colony that were recorded breeding away from where they were first banded.

4.3. Results

4.3.1. Description of the data, tests of assumptions, and genetic diversity

All sixteen nuclear microsatellite loci successfully amplified in more than 87% of all samples from the five breeding colonies of the Sooty Tern. Only 0.98% of the global dataset consisted of null values, with a maximum of 11.16% missing data at any one locus for any breeding colony. This reasonably high proportion of missing data existed for loci Onyfus_89, Onyfus_918, and Onyfus_1123 in the Clipperton population. Only 9 individuals were sampled at this colony, however, and all samples were dated (January 2005). The proportion of missing data (across all loci) in each breeding colony never exceeded 2.08%. In all of the western Indian Ocean breeding colonies, the proportion of missing data at any one locus never exceeded 6.25% with a maximum of 1.59% missing data in each population across all loci.

No significant LD was observed among any loci (Markov Chain probability test, all p-values > 0.05; Index of Association Method, p-value > 0.05). Null allele frequency was generally low, ranging between -0.19 and 0.39, among all breeding colonies. Presence of null alleles was detected at loci Onyfus_244 and Onyfus_89 for Juan de Nova, Bird Island, Glorieuses, and Europa, for loci Onyfus_282 and Onyfus_5474 in the Europa breeding population only, and at locus Onyfus_1123 in the Bird Island, Glorieuses, and Europa colonies. This pattern was only consistent for loci Onyfus_244 (mean r = 0.1258) and Onyfus_89 (mean r = 0.2718), however. Presence of null alleles was always associated with homozygote excesses. No large-allele dropout or stutter peaks were observed among any loci. N_a was generally very high in the global dataset, ranging between 11.25 ± 1.53 and 12.00 ± 1.04 at Juan de Nova and Bird Island, respectively (grand mean across populations 10.05 ± 0.05; Table 4.3a). Differences in N_a among the three main colonies (Juan de Nova, Europa, and Bird Island) were insignificant (Kruskal-Wallis test: H = 17.74, overall p-value < 0.01; all pairwise p-values

< 0.05), though these colonies all exhibited significantly higher N_a than for both Clipperton and Glorieuses (Kruskal-Wallis test: all pairwise p-values < 0.01; Table 4.3a and b). These significant differences were almost certainly an artefact of the lower sampling effort for the Glorieuses and Clipperton breeding populations. A reduced dataset, omitting loci Onyfus_244 and Onyfus_89 (see below), also exhibited high N_a per locus for all colonies with between 11.21 ± 1.24 and 11.79 ± 1.03 alleles in the Juan de Nova and Bird Island breeding populations, respectively (grand mean across populations 9.99 ± 0.49; Table 4.3b). Patterns of significance matched those in the global dataset (Kruskal-Wallis test: $H = 15.21$, p-value < 0.01), with all main colonies showing significant departure from Glorieuses and Clipperton (Kruskal-Wallis test: all pairwise p-values < 0.02; Table 4.3a and b). Estimates of A_E were also similar among populations in the global and the reduced datasets (grand mean across populations 8.53 ± 0.70 and 8.56 ± 0.73 for the global and the reduced datasets, respectively; Table 4.3a and b), and all breeding colonies were not significantly different from one another (Kruskal-Wallis test: $H = 1.84$, p-value $= 0.76$). Differences between N_a and A_E, along with the allelic frequency distributions (Figure 4.2), indicated a very high proportion of rare (< 0.20 of the total allelic variation) alleles within each of the breeding colonies. AR in the global dataset, based on a minimum sample size of 15 diploid individuals, ranged between 8.03 ± 0.78 and 8.55 ± 0.66 at Glorieuses and Bird Island, respectively (grand mean across populations 8.53 ± 0.70; Table 4.3a). Differences in AR were not statistically significant among the colonies (p-value $= 0.62$). Similar estimates of AR were recorded for the reduced dataset; the range of AR estimates was 8.07 ± 0.74 and 8.61 ± 0.69 at Glorieuses and Bird Island, respectively (grand mean across populations 8.56 ± 0.73; Table 4.3b), with no significant difference among the breeding colonies (p-value $= 0.53$). All breeding populations contained P_A, with Bird Island and Juan de Nova showing the highest and lowest counts (Table 4.3a and b). Clipperton and Glorieuses exhibited a low proportion of P_A, but this was once again reflective of the low sample size (Table 4.3a and b). Only four P_A had a frequency greater than 0.05 in the global dataset, two each for Clipperton and Glorieuses, though all others accounted for more than 1% of the total allelic variation at each locus within each breeding population. Only two P_A had a frequency of greater than 0.05 in the reduced dataset, one each for Clipperton and Glorieuses. Locus specific deviations from Hardy-Weinberg proportions, at the 99% confidence interval, were observed at loci Onyfus_244, Onyfus_89, Onyfus_1123, and Onyfus_778 implying that the global dataset showed significant deviations from Hardy-Weinberg proportions (p-value = 0.01). This highlighted an adverse effect of null alleles on these data. The removal of loci Onyfus_244 and Onyfus_89 (for the reduced dataset) dramatically improved this overall result to within acceptable limits (p-value = 0.02). F_{IS} was generally low, never exceeding 0.09 ± 0.05 in either dataset, suggesting random mating in all breeding populations (Table 4.3a and b). H_o and uH_e were similar among all colonies in the global dataset, ranging from 0.64 ± 0.07 to 0.73 ± 0.05 and between 0.75 ± 0.06 and 0.78 ± 0.04, respectively (grand means across populations for H_o and uH_e were 0.74 ± 0.02 and 0.77 ± 0.02; Table 4.3a). Similar values of H_o and uH_e were estimated from the reduced dataset (grand means across populations for H_o and uH_e were 0.69 ± 0.03 and 0.79 ± 0.02, respectively; Table 4.3b). The number of multi-locus genotypes recorded across all breeding colonies remained stable after six loci, suggesting that the use of 14 polymorphic microsatellite loci was sufficient to describe the genetic diversity and genetic population structure of the Sooty Tern (Figure 4.3).

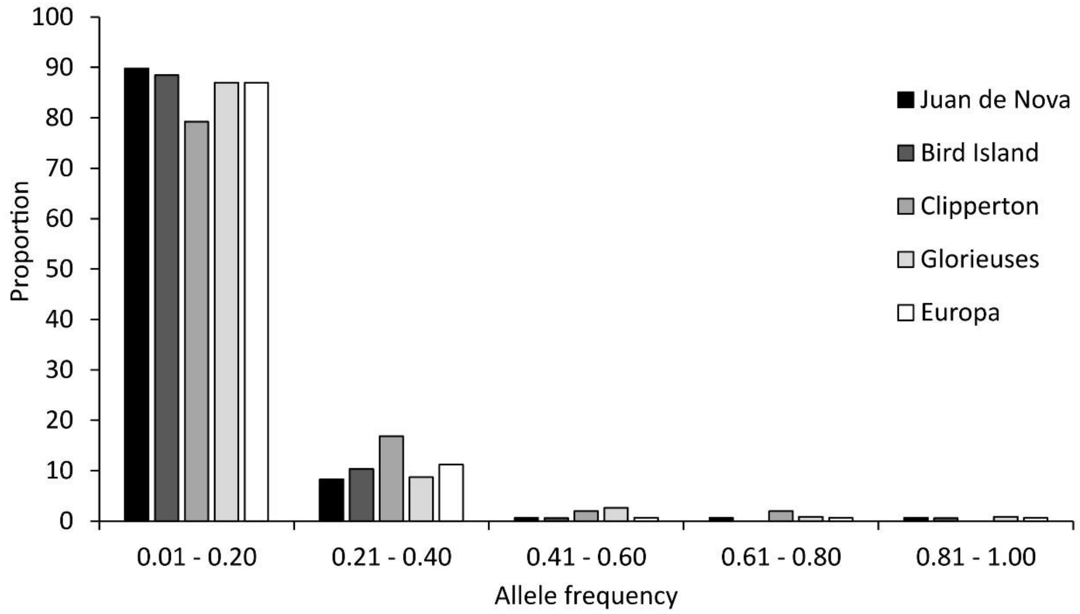

Figure 4.2: Allele frequency distributions, standardised across 14 microsatellite loci, of Sooty Terns, sampled at five breeding colonies in the Indo-Pacific region, based on a reduced dataset.

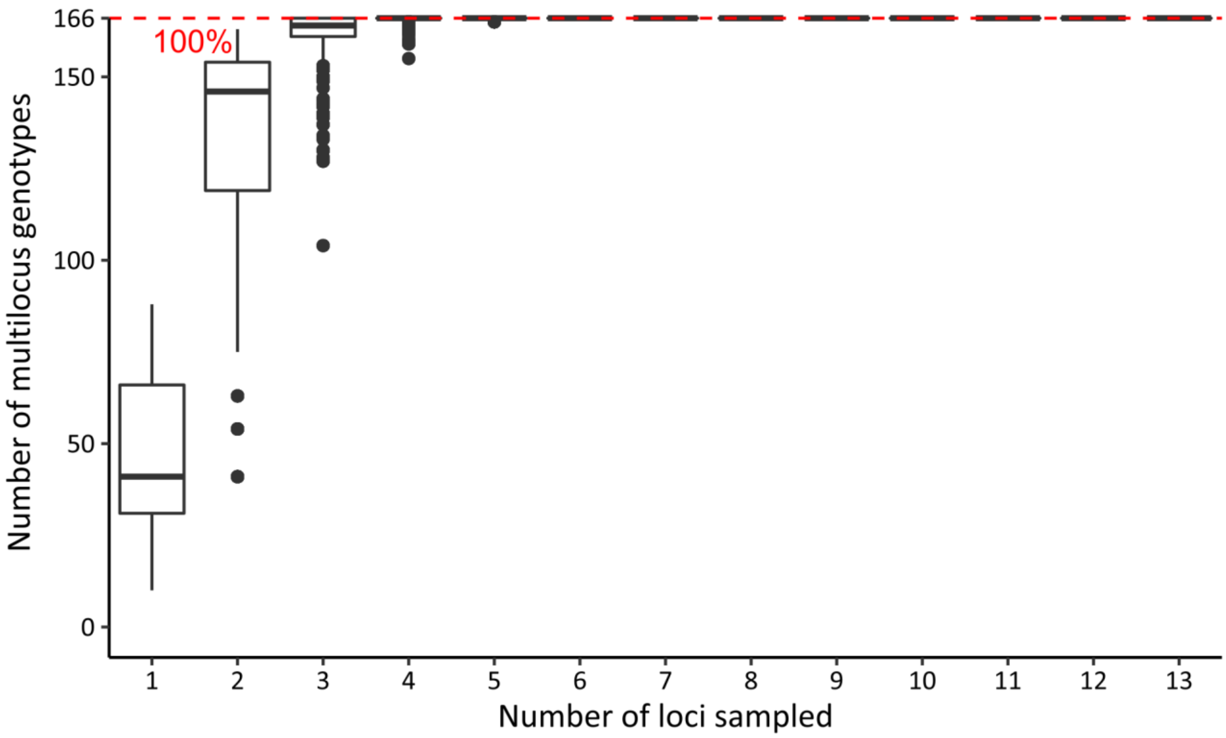

Figure 4.3: Multi-locus genotype accumulation curve for 14 tetranucleotide repeat microsatellite loci, employing 166 individuals sampled from five breeding colonies.

Concerning genetic diversity among the seasonally segregated populations. N_a ranged between 11.21 ± 1.24 in the summer breeding population and 13.21 ± 1.40 in the winter breeding population, while lower N_a (8.29 ± 0.68) was reported from the aseasonal group presumably because of the lower sample size (grand mean across populations 10.90 ± 0.72; Table 4.3c). The difference in N_a between the three populations were mostly insignificant, however (Kruskal-Wallis test: H = 7.12; p-value = 0.2), with only a single significant difference between the winter and aseasonal populations (pairwise Mann-Whitney p-value = 0.03). A_E was also similar, though not significantly different (Kruskal-Wallis test: H = 2.75; p-value = 0.25), among the three seasonally segregated populations; estimates of A_E were 5.95 ± 0.61 and 6.00 ± 0.71 in the summer and winter populations, respectively. The differences between estimates of N_a and A_E, combined with the allele frequency distribution (data not shown), indicated many rare alleles (< 0.20 of the total allelic variation) within all three of the seasonally segregated populations. AR, based on a minimum sample size of 15 diploid individuals, ranged between 8.55 ± 0.76 and 8.59 ± 0.74 in the summer and winter populations, respectively (grand mean 8.55 ± 0.73; Table 4.3c), though these differences were statistically insignificant (p-value > 0.90). P_A were found in all three of the seasonally segregated populations (Table 4.3c), though only one in the aseasonal group had a frequency of greater than 0.05. Allelic frequency of most other P_A exceeded 0.01 of the total variation at each locus within each breeding group, however. F_{IS} was generally low, never exceeding 0.04 ± 0.02, in all three populations of the Sooty Tern (Table 4.3c). H_o and uH_e were similar among the three morphs, ranging from 0.74 ± 0.04 to 0.78 ± 0.04 and 0.78 ± 0.04 to 0.79 ± 0.04, respectively (Table 4.3c)

Table 4.3: Estimates of allelic diversity in five breeding colonies of the Sooty Tern, based on a) 16 and b) 14 tetranucleotide repeat microsatellite loci. Estimates of allelic diversity among the three seasonal populations of the Sooty Tern are presented in c) based on 14 microsatellite loci.

a)

Breeding colony	**n**	**N_a**	**AR**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
Juan de Nova	38.75 ± 0.11	11.25 ± 1.53	8.52 ± 0.72^{a}	13 (7%)	5.57 ± 0.60	0.73 ± 0.05	0.77 ± 0.04	0.04 ± 0.03
Bird Island	50.19 ± 0.23	12.00 ± 1.04	8.55 ± 0.66^{a}	15 (8%)	5.52 ± 0.67	0.69 ± 0.05	0.76 ± 0.04	0.09 ± 0.05
Clipperton	8.81 ± 0.10	6.94 ± 0.59	N/A	2 (2%)	4.78 ± 0.51	0.71 ± 0.05	0.78 ± 0.04	0.05 ± 0.05
Glorieuses	15.94 ± 0.06	8.18 ± 0.81	8.03 ± 0.78^{a}	3 (2%)	4.92 ± 0.51	0.64 ± 0.07	0.75 ± 0.06	0.01 ± 0.07
Europa	50.69 ± 0.20	11.86 ± 1.24	8.33 ± 0.71^{a}	14 (7%)	5.51 ± 0.65	0.69 ± 0.06	0.77 ± 0.04	0.10 ± 0.06
Total	32.88 ± 1.96	10.05 ± 0.05	8.53 ± 0.70	N/A	5.26 ± 0.26	0.69 ± 0.03	0.77 ± 0.02	0.08 ± 0.02

b)

Breeding colony	**n**	**N_a**	**AR**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
Juan de Nova	38.79 ± 0.11	11.21 ± 1.24	8.58 ± 075^{b}	12 (7%)	5.95 ± 0.61	0.78 ± 0.04	0.79 ± 0.04	0.001 ± 0.01
Bird Island	50.14 ± 0.25	11.79 ± 1.03	8.61 ± 0.69^{b}	14 (8%)	5.88 ± 0.70	0.74 ± 0.04	0.78 ± 0.05	0.03 ± 0.03
Clipperton	8.86 ± 0.10	7.21 ± 0.61	N/A	1 (1%)	5.09 ± 0.54	0.74 ± 0.05	0.81 ± 0.04	0.03 ± 0.05
Glorieuses	15.93 ± 0.07	8.21 ± 0.77	8.07 ± 0.74^{b}	2 (2%)	5.05 ± 0.54	0.68 ± 0.06	0.76 ± 0.06	0.06 ± 0.04
Europa	50.71 ± 0.22	11.50 ± 1.22	8.33 ± 0.74^{b}	11 (7%)	5.86 ± 0.69	0.74 ± 0.04	0.79 ± 0.04	0.03 ± 0.03
Total	32.89 ± 2.10	9.99 ± 0.49	8.56 ± 0.73	N/A	5.57 ± 0.27	0.74 ± 0.02	0.79 ± 0.02	0.03 ± 0.02

c)

Breeding population	**N**	**N_a**	**AR**	**P_A**	**A_E**	**H_o**	**uH_e**	**F_{IS}**
Summer	38.79 ± 0.11	11.21 ± 1.24	8.55 ± 0.76^{c}	12 (8%)	5.95 ± 0.61	0.78 ± 0.04	0.79 ± 0.04	0.001 ± 0.01
Winter	100.93 ± 0.25	13.21 ± 1.40	8.59 ± 0.74^{c}	35 (19%)	6.00 ± 0.71	0.74 ± 0.04	0.78 ± 0.05	0.04 ± 0.02
Aseasonal	15.86 ± 0.10	8.29 ± 0.68	8.15 ± 0.66^{c}	4 (3%)	5.11 ± 0.11	0.74 ± 0.05	0.78 ± 0.04	0.02 ± 0.04
Total:	51.86 ± 5.61	10.90 ± 0.72	8.55 ± 0.73	N/A	5.69 ± 0.35	0.75 ± 0.02	0.79 ± 0.02	0.02 ± 0.02

n = mean number of individuals per locus ± s.e.; N_a = mean number of alleles per locus ± s.e.; AR = mean allelic richness per locus ± s.e. based on a minimum sample size of 21 diploid individuals (superscripts indicate statistically homogenous groups); P_A = private allele richness (proportion of private alleles from total allelic richness in parenthesis); A_E = mean number of effective alleles ± s.e.; H_o = observed heterozygosity over all loci ± s.e; uH_e = unbiased expected heterozygosity ± s.e.; F_{IS} = fixation index calculated based on Weir and Cockerham (1984)

4.3.2. Genetic differentiation and overall population structure

A global F_{ST} of 2.0 x10^{-3} (p-value = 0.07) was estimated among the five breeding colonies of the Sooty Tern. Pairwise F_{ST} values ranged between 6.55 x10^{-3} and 0.02, though all but one of these were statistically insignificant (p-value > 0.05; Table 4.4). The significant pairwise F_{ST} value was reported between Clipperton and Glorieuses (p-value = 0.01; Table 4.4). These results altogether indicated a total absence of genetic structure across the sampled breeding colonies of the Sooty Tern.

Table 4.4: Pairwise F_{ST} estimates (below diagonal), and levels of statistical significance (above diagonal), among five Indo-Pacific breeding colonies of the Sooty Tern. Significant differences are signified by asterisks.

	Juan de Nova	**Bird Island**	**Clipperton**	**Glorieuses**	**Europa**
Juan de Nova	N/A	0.32	0.12	0.06	0.89
Bird Island	7.16 x10^{-3}	N/A	0.23	0.41	0.84
Clipperton	9.88 x10^{-3}	8.67 x10^{-3}	N/A	0.01*	0.86
Glorieuses	0.01	8.48 x10^{-3}	0.02	N/A	0.89
Europa	6.55 x10^{-3}	7.07 x10^{-3}	7.13 x10^{-3}	7.46 x10^{-3}	N/A

A similar global F_{ST} of 2.0 x10^{-3} (p-value = 0.02) was estimated among the three seasonally segregated populations of the Sooty Tern. This value of F_{ST} indicates a total absence of genetic structure. Pairwise F_{ST} values ranged between 0.02 and 0.69 (Table 4.5). No differences, except the comparison between Juan de Nova (summer) and Glorieuses (winter; p-value = 0.02), were statistically significant (p-values > 0.05; Table 4.5).

Table 4.5: Pairwise F_{ST} estimates (below diagonal), and levels of statistical significance (above diagonal), among five Indo-Pacific breeding colonies of the Sooty Tern. Significant differences are signified by asterisks.

	Summer	**Winter**	**Aseasonal**
Summer	N/A	0.30	0.02*
Winter	4.00×10^{-3}	N/A	0.69
Aseasonal	0.01	5.00×10^{-3}	N/A

The first two axes of the Euclidean distance-standardised PCoA, representing individuals from all five breeding colonies of the Sooty Tern, accounted for less than 8% of the total genetic variation (Figure 4.4). Similarly, the first two axes of the PCoA, representing individuals from the three seasonally segregated population of the Sooty Tern, accounted for less than 5% of the total genetic variation (figure not presented). This indicates an almost complete lack of genetic differentiation among the sampled breeding colonies and seasonally segregated populations of the Sooty Tern.

The best-supported model for the Bayesian clustering analysis performed in STRUCTURE version 2.3.3, representing breeding colonies and based on Pritchard *et al.* (2000), was that of one genetic cluster (lowest standard error [s.e.] for $\ln P(X|K)$ at $K = 1$; Figure 4.5). The results based on Evanno *et al.* (2005) were less clear (Figure 4.5), presumably as ΔK cannot be used to estimate K where $K = 1$. For illustrative purposes, the posterior probabilities for the simulation exhibiting the highest $\ln P(X|K)$ at $K = 2$ is presented in Figure 4.6. Values of $Q_{cluster1}$ and $Q_{cluster2}$ were similar for all individuals sampled at all five breeding colonies. The second Bayesian clustering analysis (not illustrated), representing colonies by their breeding period, similarly indicated a total absence of genetic structure in the Sooty Tern.

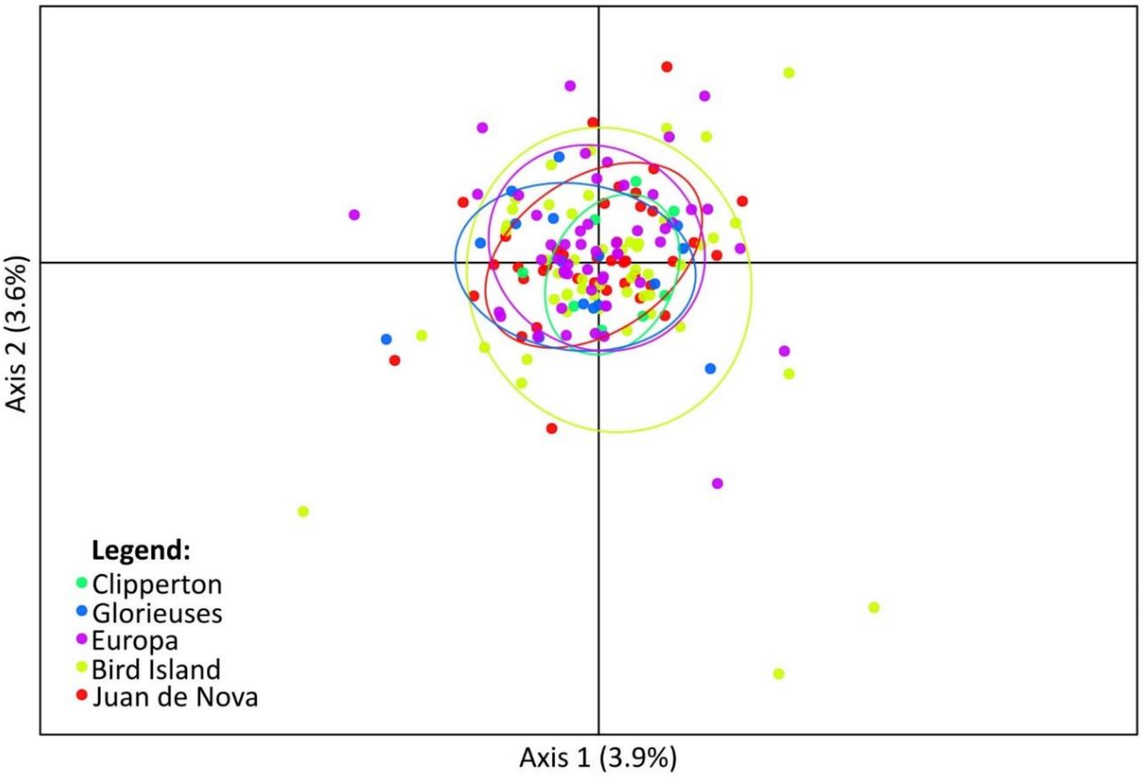

Figure 4.4: First and second axes of the Euclidian-distance standardised PCoA, including standard inertia ellipses, visualising the genetic diversity within, and genetic differentiation between, individual Sooty Terns sampled at five breeding colonies.

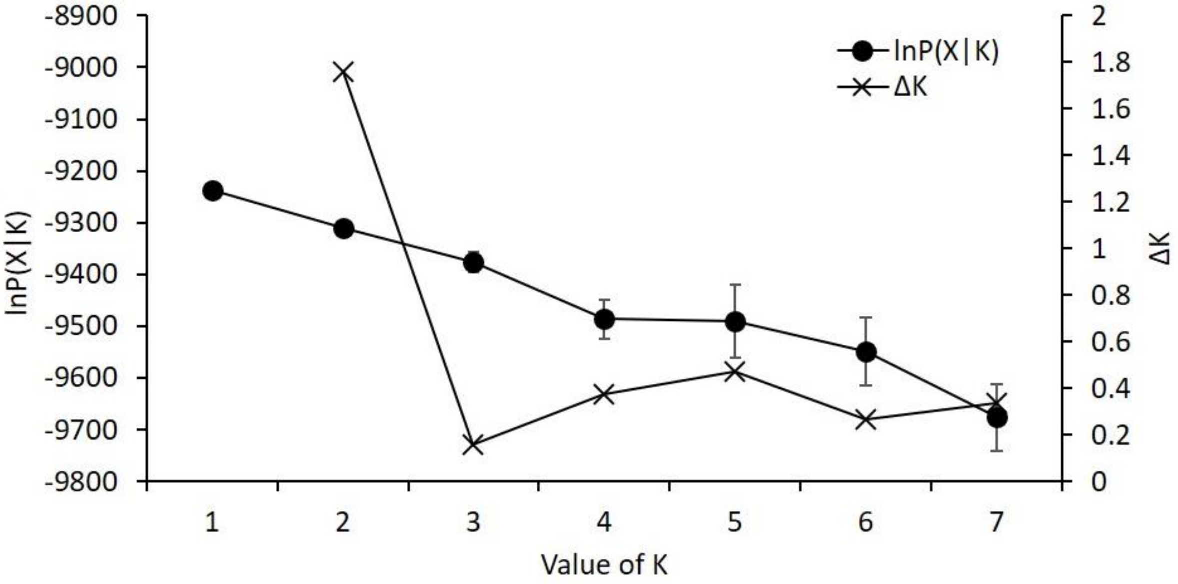

Figure 4.5: lnP(X|K; ± s.e.; Pritchard *et al.* 2000) and ΔK (Evanno *et al*. 2005), as obtained in STRUCTURE version 2.3.3, with Kmax ranging between 1 and 7. Each value was obtained by averaging the posterior probabilities of 10 independent runs.

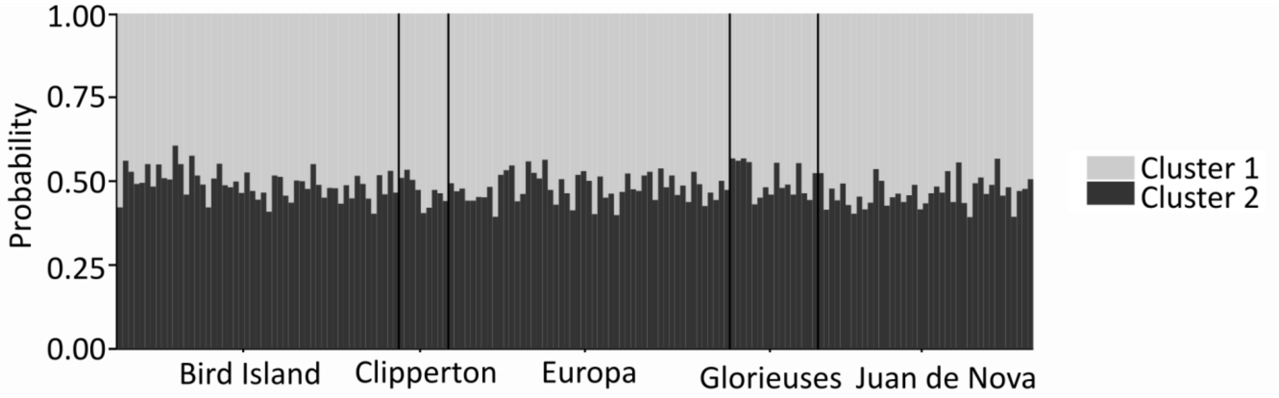

Figure 4.6: Results from the MCMC clustering procedure, performed in STRUCTURE version 2.3.3, visualising the overall membership of individuals sampled at five Sooty Tern breeding colonies, assuming sampling location, to two genetic clusters.

4.3.3. Ringing/banding information

More than 26 000 individual Sooty Terns were ringed/banded at 15 breeding colonies in the western Indian Ocean between 1974 and 2015 (Table 4.6). The proportion of birds that have been resighted varies considerably among colonies and in relation to the distribution of effort (Table 4.6). The number of re-sightings is highest at Bird Island (Seychelles), for example, as this is the colony where the greatest effort has been invested (Table 4.6; refer to Appendix 1). Re-sightings are lowest on îles Eparses, and at Cosmoledo, Cousin, Goelette, and Serpent Island. Effort has been rather minimal in each of these colonies, often limited to just a single study period. Looking at the total dataset, around 11% of all individuals have been recaptured as breeding birds at the colonies in which they were first banded (Table 4.6). A smaller proportion of birds (0.2% of the total banded population), including individuals from most colonies in the Seychelles, have dispersed among islands (Table 4.6). This information is summarised in greater detail in Appendix 1. Dispersal rates were highest from African Banks and Recif, and lowest from Bird Island (Table 4.6).

Table 4.6: Summary of banding information at 10 breeding sites of the Sooty Tern in the western Indian Ocean.

Colony	Region	Authorities	Individuals ringed	Period covered	Recaptured within colony	Recaptured elsewhere
African Banks	Seychelles	Feare	226	1974 - 2011	0 (0%)	10 (4%)
Aride Island	Seychelles	Feare	2617	1976 - 2012	27 (1%)	14 (0.5%)
Bird Island	Seychelles	Feare	12 978	1972 - 2015	2 335 (17%)	2 (0.01%)
Cousine	Seychelles	Feare, SAFRING	533	1994 - 2012	0 (0%)	2 (0.4%)
Cosmoledo	Seychelles	Feare	850	1999	0 (0%)	0 (0%)
Cousin	Seychelles	Feare, SAFRING	23	2005	0 (0%)	0 (0%)
Desnoeufs	Seychelles	Feare	7681	1976 - 2012	519 (7%)	13 (0.2%)
Europa	îles Eparses	CRBPO	740	2003 - 2012	0 (0%)	0 (0%)
Glorieuses	îles Eparses	CRBPO	58	2003 - 2004	0 (0%)	0 (0%)
Goelette	Seychelles	Feare	20	1976	0 (0%)	0 (0%)
Juan de Nova	îles Eparses	CRBPO	660	2003 - 2011	16 (2%)	0 (0%)
Nosy Manampaho	îles Eparses	CRBPO	20	2008	0 (0%)	0 (0%)
Recif	Seychelles	Feare	317	1994	2 (0.6%)	11 (3%)
Serpent Island	Mauritius	SAFRING	21	2013	0 (0%)	0 (0%)
Tromelin	îles Eparses	CRBPO	15	2016 - 2017	0 (0%)	0 (0%)
Total:		Feare, SAFRING, CRBPO	26 759	1974 - 2015	2 899 (11%)	52 (0.2%)

4.4. Discussion

4.4.1. Overall population structure: regional and global perspectives

Avise *et al.* (2000) first highlighted the global genetic relationships among Sooty Tern breeding colonies. Their principal finding was that a shallow phylogenetic partition exists, separating populations in the Atlantic from those in the Indian and Pacific Oceans. Assuming the rate of divergence in the Sooty Tern mtDNA CR is within the same range of the evolutionary rates identified in other avian species (eight to 20% per million years; Quinn 1992; Wenink *et al.* 1996), the Indo-Pacific and Atlantic lineages of the Sooty Tern are theorised to have diverged between about 180 000 and 450 000 years ago (Avise *et al.* 2000). Lineage separations often pre-date population-level splits (Avise 2000), such that Avise *et al.* (2000) stressed the need for a correction of the aforementioned dates to account for within ocean basin nucleotide diversity. In this regard, the net genetic distance among Sooty Tern breeding colonies indicated that historical population sundering probably occurred between about 7 500 and 187 000 years ago (Avise *et al.* 2000). This structuring was theorised to have been driven primarily by major physical barriers and channels to gene flow (Avise *et al.* 2000). The main physical barriers responsible for the divergence of the Atlantic lineage, from the Indo-Pacific population, were the Isthmus of Panama and the Cape of Good Hope. The rise of the Isthmus of Panama, about 3 Million Years Ago (MYA), limited gene flow between Atlantic and Pacific populations of many marine species including several tropical seabirds (e.g. Avise *et al.* 2000; Steeves *et al*. 2003, 2005; Friesen *et al*. 2007; Morris-Pocock *et al*. 2010, 2011). Though population divergence in the Sooty Tern postdates the rise of the Isthmus of Panama, historical connection between the Pacific and Atlantic Ocean basins appears to have occurred despite a complete lack of contemporary gene flow (Avise *et al.* 2000). The southern parts of Africa constitute a similar phylogeographic break to tropical species (Briggs 1974; Zardi *et al.* 2015), though the Sooty Tern occurs as an annual vagrant on the East coast of South Africa and a single pair of an unknown lineage attempted to breed on Bird Island (Algoa Bay, South Africa) in 2015 (pers. obs.; refer to Figure 4.1). Thus, based on vagrancy alone, there is potential for rare gene flow between the Indian and Atlantic breeding colonies of the Sooty Tern. This was not supported genetically, however (Avise *et al.* 2000). Furthermore, Avise *et al.* (2000) noted that the Pacific and Indian Ocean breeding colonies of the Sooty Tern were interspersed throughout the estimated matrilineal tree indicating that these breeding populations are of a common mtDNA lineage. While populations of several other marine taxa are limited by the Indonesian straits (e.g. Williams & Benzie 1998; Barber *et al.* 2002; Otwoma & Kochzius 2016), many seabirds including the Sooty Tern appear to have an ability to cross it thereby maintaining such Indo-Pacific phylogenetic relationships. Concerning within ocean basin genetic diversity, Avise *et al.* (2000) noted that Sooty Tern colonies were, at best, weakly differentiated in their matrilineal ancestry. Almost identical mtDNA CR haplotypes were shared among all breeding colonies within each ocean basin, including some separated by nearly 16 000 kilometres (Km; Avise *et al.* 2000). Altogether, this implies that the evolutionary forces imposed on Sooty Tern populations act at extremely large scales and, in this regard, it was unsurprising that no genetic structure was detected from the present microsatellite genotype data.

Though the five sampled breeding colonies of the Sooty Tern all contained extremely high genetic diversity, including moderate proportions of private alleles, Wright's overall multi-locus fixation index (F_{ST}; Wright 1922, 1965) failed to detect genetic differentiation among the breeding colonies and seasonally segregated groups of birds. The Bayesian assignment tests, performed in STRUCTURE version 2.3.3, similarly indicated a complete lack of genetic clustering among all individuals sampled, as was also supported by the PCoA. In the context of these results, and the species' behavioural traits (e.g. high dispersal capabilities, low assumed natal philopatry), it appears that considerable dispersal occurs among all islands of the western Indian Ocean. This said, accurate estimation of the number of migrants per generation (*Nm*) in each direction among all pairs of breeding populations was not feasible in that F_{ST} values of greater than 0.05 are necessary to obtain reliable estimates of the migration rate (*m*; Faubet *et al.* 2007). To further complicate the estimation of *Nm*, it was not possible to accurately calculate effective population size (N_e) based on these genetic data. Virtually all estimates of N_e for the Sooty Tern were infinite (data not presented), which is assumed equivalent to failing to reject the null hypothesis in the LD model at a significance level proportionate with the confidence interval (CI) chosen (Do *et al.* 2014; Jones *et al.* 2016). The hypothesis in question is that the studied population has the same coefficient of determination (r^2) as an infinitely-sized ideal population, and is therefore indistinguishable from it based on the sample estimate (Jones *et al.* 2016). Considerable inter-colony exchange of adult Sooty Terns is known to occur among islands separated by between 74 and 370Km in the Seychelles, however (Feare & Lesperance 2002). In a five-year period between 1996 and 2001, 19 individuals banded as breeding adults were resighted nesting in colonies other than those in which they were initially marked (Feare & Lesperance 2002). The more comprehensive dataset employed in the present study provides additional evidence for contemporary dispersal among breeding colonies of the Sooty Tern, indicating individual-exchanges among virtually all occupied islands in the Seychelles. This involved the movement of birds banded as both adults and pre-fledglings (refer to Appendix 1). Larger scale dispersal among colonies, outside of the Seychelles, is not currently known from banding recoveries (Le Corre, M. pers. comm). In this context, Avise *et al.* (2000) found that birds from the Seychelles and Chagos Archipelagos (separated by about 1 700Km) were of the same mtDNA stock. These two contained some of the highest within-colony mtDNA haplotype and nucleotide diversity, and yet only 3.1% of the total genetic variation could be attributed to genetic differences among colonies (Avise *et al.* 2000). This implies that contemporary gene flow may occur among geographically distant islands within the Indian Ocean, or alternatively, that sundering of Sooty Tern populations has occurred too recently to be detected using mtDNA CR sequences (Avise *et al.* 2000). Present microsatellite information supports the former hypothesis, expanding off the banding information, and highlights considerable genetic movement among distant islands of the western Indian Ocean. In further support of Avise *et al.* (2000), the four colonies from the western Indian Ocean were genetically inseparable from birds sampled on Clipperton Island in the eastern Pacific. Though a greater sampling effort is required from Pacific colonies, this result indicates that at-least some contemporary gene flow occurs between the two ocean basins. This is supported by the discovery of a single individual (D-8767), banded as a pre-fledging on Raoul Island in the Kermadec group (29° 16’S, 177° 55’E; South-West Pacific Ocean), breeding on Aride Island in the Seychelles (Cossee 1995).

Avise *et al.* (2000) related several aspects of Sooty Tern natural history to that of the Green Turtle (*Chelonia mydas*). To illustrate – both species are long lived, occur circumtropically, and nest in discrete colonies on scattered islands. The two species also often utilize the same islands for nesting. In comparing the genetic histories of the two species, using the information provided by Bowen *et al.* (1992, 1993), Avise *et al.* (2000) noted that both the Sooty Tern and the Green Turtle exhibited a phylogeographic partition between the Atlantic and Indo-Pacific basins. This suggested a major influence of global geology on historical gene flow in the two species. Weaker and more recent genetic differentiation was recorded among populations of the Sooty Tern than the Green Turtle, however, implying that the latter species’ population structure is more deeply rooted (Bowen *et al.* 1992, 1993; Avise *et al.* 2000). Bourjea *et al.* (2007) more recently studied the population genetic structure of Green Turtles, among 10 rookeries in the South-western Indian Ocean, using mtDNA CR sequences. Despite the conservative nature of mtDNA markers (refer to Chapter 1.3), Bourjea *et al.* (2007) noted significant phylogenetic structure and a distinct break between colonies in the northern (i.e. Glorieuses, Comoros, Seychelles, and Nosy Iranja) and the southern and central (i.e. Europa and Juan de Nova) Mozambique Channel. These phylogenetic patterns are attributable to recent colonization of the southern Mozambique Channel from rookeries in the Atlantic Ocean (Roberts *et al.* 2004; Bourjea *et al.* 2007), and are maintained by oceanic conditions that influence early stages in the species’ life cycle (Bourjea *et al.* 2007). Much like the Sooty Tern, the breeding schedule of Green Turtles in the western Indian Ocean also differs in relation to the local oceanographic conditions surrounding rookeries (Lauret-Stepler 2006; Dalleau *et al.* 2012). Europa and Tromelin, hosting two of the most important colonies of the Green Turtle in the western Indian Ocean, both exhibit similar patterns in seasonality with well-defined summer breeding peaks (Lauret-Stepler 2006; Dalleau *et al.* 2012). The peak in breeding activity occurs four to five months later on rookeries in the northern Mozambique Channel, including Aldabra and Glorieuses, yet seasonality there is distinctively less marked (Lauret-Stepler 2006; Dalleau *et al.* 2012). Though the influence of this breeding asynchrony on genetic structuration was not investigated by Bourjea *et al.* (2007), the patterns of peak breeding activity seem to broadly match the species' overall genetic population structure. These findings altogether contrast with the present microsatellite information on the Sooty Tern, in which absolutely no genetic differentiation was detected among the sampled breeding colonies. This distinction, as first theorised by Avise *et al.* (2000), is attributable to behavioural differences between the two species. Sooty terns are generally considered roamers, in that they move constantly across the pelagic realm (Jaeger *et al.* 2017) and breed when conditions are favourable and where there is sufficient conspecific stimulus to initiate laying (refer to Chapter 4.4.2). Green turtles, by contrast, are migrators that move between specific feeding and breeding areas (Bourjea *et al.* 2013; Robinson *et al.* 2017). Individual Green Turtles also often establish specific home ranges, where they remain throughout the year when not breeding (Robinson *et al.* 2017). On this basis alone, Sooty Terns have a greater probability of encountering other non-natal breeding sites where they may potentially remain to breed (Friesen *et al.* 2007). In fact, banded Sooty Terns are regularly seen in colonies other than those in which they were initially marked and, while most are thought to return to their natal colonies to breed, some appear to join breeding populations elsewhere (Robertson & Wilmers 1996; Feare & Lesperance 2002; refer also to Chapter 4.4.2).

4.4.2. Mechanisms underlying gene flow among populations

Despite the general reproductive and behavioural characteristics of seabirds that lend themselves towards population structuring, panmixia has been reported in several species other than the Sooty Tern. Examples include the Grey-headed Albatross (*Thalassarche chrysostoma*; Burg & Croxall 2001), Great Frigatebird (*Fregata minor*; Levin & Parker 2012), Grey-faced Petrel (*Pterodroma gouldi*; Lawrence *et al.* 2014), and Peruvian Booby (*Sula variegata*; Taylor *et al.* 2012). One of the key drivers of population differentiation in seabirds is natal philopatry (Friesen *et al.* 2007; Levin & Parker 2012; Friesen 2015). But, whilst philopatric tendencies of seabirds tend to vary among taxonomic groups (Coulson 2016), individual terns are typically more faithful to colonies where they have previously bred successfully (cf breeding philopatry) than to the colonies in which they were born (cf natal philopatry; Lebreton *et al.* 2003; Møller *et al.* 2006; Draheim *et al.* 2010). In support of this, there are two documented cases (DB11155 and D61550) of individual Sooty Terns that left their natal colony as fledglings only to breed years later in an alternate colony (see Appendix 1). Several adults also dispersed from the islands on which they were first banded in the Seychelles, only to become faithful to another colony for multiple successive seasons (DB11378, DB11389, DB11084, DB08844, and DB23880; see Appendix 1). One could assume that this site fidelity was the result of successful breeding attempts among adults in the new colonies, or that a collective set of features existed on the islands in question that lent themselves to higher breeding success. This then begs the question of, if not natal philopatry, what determines where and when individual Sooty Terns choose to breed?

Virtually all seabirds nest in large and highly synchronous colonies (e.g. Feare *et al.* 2007), though the onset of breeding usually shows a degree of temporal plasticity (Jaquemet *et al.* 2007). This said, the factors underlying the decision of when birds choose to breed are not yet fully understood (Mougin *et al.* 2001; Coulson 2002; Jovani & Grimm 2008). Reproduction in many populations/species often commences in response to or anticipation of favourable extrinsic factors of either the occupied islands or, as is more typical for seabirds, the marine environments that are important for foraging (Le Corre 2001; Hamer *et al.* 2002; Jaquemet *et al.* 2007). This is especially the case in tropical systems where marine productivity is generally low, and where food availability is patchy and ephemeral (Ashmole 1971; Jaquemet *et al.* 2007). Le Corre (2001) and Jaquemet *et al.* (2007) described such a relationship between the oceanographic conditions surrounding islands of the western Indian Ocean and spatial patterns in seabird breeding phenology (including that of the Sooty Tern). The sea-surface temperature (SST) and chlorophyll *a* concentration of the Mozambique Channel and the Seychelles show clear seasonal differences, relating mainly to the monsoon phenomenon and the southern climate (Donguy & Meyers 1996; Schott *et al.* 2009). In relation to this, though the breeding of Sooty Terns and other seabirds is generally highly synchronised within each colony (Feare 1976a; Le Corre 2001), the timing of breeding is often different among the various island groups (Jaquemet *et al.* 2007). Seasonal reproduction in several species occurs in austral winter at Europa and on most islands in the Seychelles (Feare 1976a, 1981; Diamond & Prys-Jones 1986; Le Corre 2001; Ramos *et al*. 2002), in the austral summer at Juan de Nova and on the Mascarene islands (Le Corre 2001), and aseasonally in a few populations such as the Sooty Tern colony on Glorieuses (Diamond 1976; Jaquemet *et al.* 2007). A large monthly shift in SST typically occurs just prior to the onset of laying, around each of the seasonal breeding populations, driving the annual phytoplankton bloom and boosting food availability (Flint 1991; Piontkovki & Williams 1995; Jaquemet *et al.* 2007). This variation is further accompanied by the development of physical oceanographic features (i.e. fronts and eddies; Ridderinkhof & de Ruitjer 2003) that boost productivity and the aggregations of mesopelagic prey (McGillicuddy *et al*. 1998; Lima *et al*. 2002). These features also often determine the presence of migratory schooling tuna with which most tropical seabird species associate while foraging (Jaquemet *et al.* 2004, 2005). Extrinsic factors of the colonies/marine environment may therefore enable opportunistic breeding and enhance gene flow in seabird species, such as the Sooty Tern, that exhibit lower than average natal philopatry and wide dispersal patterns.

Another feature of critical importance as a determinant of seabird breeding activity, particularly for colonial and roaming/migratory species, is the arrival date of individual birds at breeding colonies. This variable has cascading effects on reproductive timing (e.g. length of post-fledging dependency) and breeding success, with resultant significant impacts on several evolutionary and demographic parameters (e.g. Becker *et al.* 2008). Peak laying, a function of the mean arrival date at colonies, is additionally dependant on the stimuli provided by conspecifics (Waas *et al.* 2000; Coulson 2002; Mougin *et al.* 2001). These may include both auditory (e.g. sounds produced by the colony) and visual cues (e.g. nest construction, and displaying) provided by other birds within or near a colony (Coulson 2002). The rationale behind social stimulation of breeding, or the conspecific attraction hypothesis, is that the mere presence of other birds at a breeding site indicates safer and more productive conditions than at other possibly unused localities (Darling 1938). To expand on this, larger breeding colonies are indicative of more stable and favourable conditions where opportunities for conspecific facilitation and location of prey capture are also increased (Darling 1938). Breeding in many seabird species, even those that are not truly colonial (e.g. the Yellow-eyed Penguin [*Megadyptes antipodes*]), often depends on social cues (Coulson 2002; Setiawan *et al.* 2007). Over 75% of the estimated 395 000 Sooty Tern eggs produced on Bird Island (Seychelles) in 1973 were laid within a brief 9-day period, emphasizing the importance of social stimulation to this species' breeding regime (Feare 1976a; Feare *et al.* 2003). A recent attempt to attract Sooty Terns to Denis Island (Seychelles), where the species has never bred previously (Feare *et al.* 2007), also employed artificial stimuli including two (2D) and three-dimensional (3D) decoys and playback of recorded calls (Feare *et al.* 2003, 2015). Prospectors were attracted by the broadcast calls, with some circling over the artificial colony and several individuals landing among the decoys. Large 3D plastic models, considerably larger in size than adult Sooty Terns, proved superior to the life-size 2D painted cutouts and were described as 'superstimuli' (Feare *et al.* 2015). In the first year of the project (2008), most of the birds that landed remained on the ground for only a short time (two seconds to 20 minutes) before flying off (Feare *et al.* 2015). Nest territory acquisition and courtship behaviours (including copulation) were observed in the second and third years of the restoration effort, and several eggs were laid although none hatched due to predation by indigenous Common Moorhens (*Gallinula chloropus*; Feare *et al.* 2015). Numbers of up to 250 individual Sooty Terns were then reported in both 2011 and 2012, though the establishment of a self-sustaining breeding colony is expected to take much longer (Feare *et al.* 2015). This colony restoration effort further emphasizes the importance of social cues in determining when and where seabirds breed. On this basis, provided the environmental conditions are favourable to do so, social stimuli may attract and prompt individual Sooty Terns into breeding in colonies other than those in which they were born and may thus encourage gene flow (Feare *et al.* 2003).

Several features of the nesting islands also seem to promote dispersal within and among colonies of the Sooty Tern. One is the frequency, scale, and timing of egg harvesting. Many Sooty Terns will re-lay once their eggs have been harvested, though the ability of birds to produce replacement eggs shows a marked seasonal decline (Feare 1976a). Feare (1976a) noted that 94% of birds from which eggs were taken just prior to the peak laying period re-laid within 12 or 13 days. Only 38% of birds re-laid if their first eggs were harvested 10 days subsequent to peak laying, and fewer than 10% re-laid if their eggs were taken 25 days after the peak egg laying period (Feare 1976a). By repeated removal of eggs in an experimental study, some individuals were stimulated into laying as many as three times in the same season (Feare 1976a). Though birds often stay in the same colony to do so, there is a definite tendency for intra-colony movement of individuals where replacement eggs are typically laid in areas that are less disturbed or where laying is locally synchronised among multiple individuals (Feare 1976a; Feare & Lesperacne 2002). Several individual Sooty Terns have also been stimulated into switching colonies in response to intensive and repeated egg harvesting activities. Feare and Lesperance (2002) noted that at-least five individuals dispersed from each of the Desnoeufs and African Banks colonies in a four-year period between 1993 and 1997. This is believed to have occurred in response to anthropogenic disturbance associated with egg harvesting (Feare & Lesperance 2002). Additional evidence for dispersal among colonies, as a result of egg harvesting activities, exists from the more comprehensive banding database employed in the present study (see Appendix 1). Ten individual Sooty Terns, from which eggs were taken on Desnoeufs, were found breeding two or more years later on Bird Island (Seychelles; see Appendix 1). Two of these individuals became faithful to the latter colony for multiple successive seasons (DB08844 and DB23880; refer to Appendix 1). An additional 10 individuals dispersed from the African Banks colony, where extensive poaching of adults and their eggs occurs annually, to either Bird Island (Seychelles) or Desnoeufs (refer to Appendix 1). Thus, given the scale and frequency to which egg harvesting occurs on some islands of the western Indian Ocean (particularly in the Seychelles), human disturbance appears to act as an influencing factor behind Sooty Tern dispersal. Other variables of known importance include the level of habitat alteration in and around breeding sites, and the presence of ectoparasites and other irritants (Feare 1999).

4.4.3. Conclusive management implications

A lack of genetic population structure has several important consequences on the demographic status, trajectory, management, and recovery of seabird populations (reviewed in Chapter 1). To illustrate – populations of a given species may persist or grow, despite exceptionally low breeding success, when frequent immigration occurs from other areas (e.g. Brommer *et al.* 2017; Payo-Payo *et al.* in prep). Dispersal also facilitates gene flow among breeding colonies, and enables populations to respond to climatic changes or variances in other ecological conditions (Frankham *et al.* 2002; Wan *et al.* 2004). Limited or a complete lack of dispersal among breeding populations drives genetic differentiation and limits adaptation potential, resilience, and population recovery (Frankham *et al.* 2002; Wan *et al.* 2004). In this sense, our capacity to delineate management units (MUs) for seabird populations remains of pivotal importance for defining conservation status, guiding restoration efforts, and in the management of anthropogenic impacts on seabird populations (Frankham *et al.* 2002; Wan *et al.* 2004; Feare *et al.* 2007).

Results from the present study have highlighted a total absence of genetic structure among colonies of the Sooty Tern in the western Indian Ocean, and between breeding sites in the western Indian and Pacific Ocean basins. This implies considerable individual exchanges among breeding colonies, probably due to the roaming behaviour and low natal philopatry of the species, and suggests that at-least some (and possibly all) Indo-Pacific breeding populations are connected as part of the same MU. The consequences of panmixia on the species' management in the western Indian Ocean, particularly in relation to the exploitation of eggs, are therefore few in that the demographic impacts of harvesting (and other threats) within colonies are likely buffered by active movement of birds among breeding sites. However, this should not encourage additional harvests or resumed export of Sooty Tern eggs from breeding colonies in the western Indian Ocean. Several recent population changes, including the collapse of the breeding colony on Aride Island from > 200 000 pairs to only around 25 000 pairs, highlights a sensitivity of Sooty Tern colonies to anthropogenic change (Feare, C. pers. comm.). Colony specific harvest management protocols should therefore be structured based on informed demographic models that include dispersal rates from other breeding sites. Terns are also major hosts of influenza A viruses including seabird-associated virus subtypes (e.g. H16), those more commonly associated with wild ducks (e.g. H3, H6, and H12 subtypes), and those that represent a significant threat to veterinary and human health (H1, H5, H7, and H9 subtypes; Lebarbenchon *et al.* 2015). This should also be considered in population management plans for the Sooty Tern, since there is a relationship between seabird dispersal and the spread of influenza A viruses among oceanic islands. Continued banding efforts may therefore assist in quantifying levels of dispersal among colonies, since it was not possible to do so using current genetic information.

Including the pioneering work at Denis Island, in the Seychelles, there are also several initiatives currently in progress that are attempting to restore or propagate colonies of the Sooty Tern on islands where the species bred previously or has never bred before (Feare *et al.* 2003, 2015; Cole, N. pers. comm.). These efforts have typically employed the use of social stimuli to attract prospecting adults to a predefined area, and the translocation of pre-fledging chicks to the restoration site from colonies elsewhere (Feare *et al.* 2003, 2015; Cole, N. pers. comm.). The complete lack of genetic structure in the Indo-Pacific Sooty Tern population implies that the proximity of the restoration site to the source colony need not be as important a consideration, in translocation efforts, as perhaps the size of the founder population (Lawrence *et al.* 2014). In the sense that anthropogenic disturbance may drive dispersal in the Sooty Tern, an important consideration should always be the use of chicks from larger founder colonies where the impacts of chick-removal may be less (Lawrence *et al.* 2014). The same could also be said for egg harvesting activities. Finally, the high apparent gene flow among the separate breeding colonies, as indicated by genetic panmixia, emphasizes the importance of social stimulation in restoration efforts but also highlights a potential for natural recolonisation of remote islands where active restoration efforts may not actually be feasible. This is exemplified by the rapid passive recolonization of Tromelin Island, where the Sooty Tern last bred in about 1856, following the successful eradication of Norway Rats (*Rattus norvegicus*; Le Corre *et al.* 2015; Le Corre, M. unpub. data). Several other tern species (e.g. White Tern [*Gygis alba*] and Brown Noddy [*Anous stolidus*]) also returned to Tromelin, implying that the lessons learned from the Sooty Tern may apply to other species too.

4.5. References

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CHAPTER 5: GENERAL SYNTHESIS

5.1. Status and management of the world's seabird populations

In a recent review of the demographic trends of the world's monitored seabirds, collectively amounting to around 19% of the global seabird population, Paleczny *et al.* (2015) noted an overall decline in numbers of about 69.7% between 1950 and 2010. Of the 3 213 breeding populations included in their analysis, 61% of the monitored and 27% of the unmonitored colonies were observed to increase (Paleczny *et al.* 2015). These increases were largely attributable to the Hydrobatidae (i.e. northern storm-petrels; +45.4%), Alcidae (i.e. alcids; +9.1%), and Sulidae (i.e. boobies; +1%; Paleczny *et al.* 2015). The discrepancy among these statistics exists in the fact that all populations showing increases were relatively small, and that a substantial proportion of the overall losses were attributable to large declines in five major southern hemisphere populations (Paleczny *et al.* 2015). This included substantial losses in the Sternidae (i.e. terns; -85.8%), Frigatidae (i.e. frigatebirds; -81.7%), Procellariidae (i.e. petrels and allies; -79.6%), Phalacrocoracidae (i.e. cormorants; -73.6%), Diomedeidae (i.e. albatrosses; -69.0%), Stercorariidae (i.e. skuas; -65.4%), Pelecanidae (i.e. pelicans; -35.3%), Spheniscidae (i.e. penguins; -32.3%), Phaethontidae (i.e. tropicbirds; -25.8%), and Laridae (i.e. gulls; -17.4%; Paleczny *et al.* 2015). In another recent review of the conservation status of all 346 seabird species, Croxall *et al.* (2012) identified an overall International Union for Conservation of Nature (IUCN) threat status worse than for any other comparable group of birds. They additionally noted that seabird populations have deteriorated faster, in recent decades, than any other bird groups. The principal current threats to seabirds at sea are related to commercial fisheries, including competition and mortality associated with these practices, and to pollution (Croxall *et al.* 2012). On land – invasive predators constitute the major threat to virtually all breeding colonies, though habitat degradation and human disturbance (including direct exploitation) are also central to the declines in several species (Croxall *et al.* 2012). In light of this, the priority actions and knowledge gaps for seabird populations were reviewed by Lewison *et al*. (2012). These topics broadly included aspects of seabird at-sea spatial ecology, trophic dynamics, and community roles. Emphasis was further placed on the direct and indirect impacts of fisheries on seabird populations, population responses to environmental variability, the management of anthropogenic impacts, and the importance of integrating subpopulation dynamics into conservation action plans. Concerning this last topic, genetic approaches (e.g. mitochondrial deoxyribonucleic acid [mtDNA, DNA] and variable number of tandem repeats [VNTR] analyses) were valued by Lewison *et al*. (2012) for their ability to identify the demographic trends of seabird populations and in the definition of genetic units for management/conservation.

Genetic approaches, such as mtDNA and mini- and microsatellite markers, have increasingly been employed in seabird studies to identify individuals in non-natal breeding colonies (e.g. Morris-Pocock *et al.* 2016), and to test for dramatic changes in effective population size, genetic connectedness, and genetic divergence over time (e.g. Avise *et al.* 2000; Morris-Pocock *et al.* 2010, 2012). In some cases, the information provided by genetic studies has critically affected management decisions for threatened species or populations (Friesen 2007; Taylor *et al.* 2012; Abdul-Muneer 2014). Indeed, the study of intraspecific population genetic differentiation has proven an effective method with which to study the early phases of speciation/divergence (Wan *et al.* 2004). The importance of these studies lies in the fact that the conservation of species usually demands the conservation of diversification processes among seabird populations (Friesen 2007; Taylor *et al.* 2012). It is on this basis that genetic approaches have become valued among conservation biologists for their ability to define units for conservation, below the taxonomic species level (Moritz 1994; Wan *et al.* 2004; Palsbøll *et al.* 2006; Robertson *et al.* 2014). Evolutionary significant (ESUs) and management units (MUs) are recognised as a means through which to partition genetic diversity and assess conservation value, and it is only on this basis that best practice management strategies can be implemented at the appropriate scales to slow or halt the decline of threatened species (Moritz 1994; Wan *et al.* 2004; Palsbøll *et al.* 2006).

5.2. Diversification processes underlying the population structure of seabirds in the tropical western Indian Ocean

Population genetic structure in seabirds is usually explained by the interaction of physical and cryptic barriers to dispersal (e.g. Avise *et al.* 2000; Morris-Pocock *et al.* 2016), natal and breeding philopatry (e.g. Faria *et al.* 2010; Levin & Parker 2012; Rawlence *et al.* 2014), mate fidelity (Friesen *et al.* 2006), mate choice (e.g. Taylor *et al.* 2012), differences in breeding and non-breeding distributions (e.g. Burg & Croxall 2001; Wiley *et al.* 2011), and allochrony (e.g. Monteiro 1998; Smith & Friesen 2007). But, whilst our understanding of the mechanisms underlying seabird population genetics has greatly increased within the last decade or so (reviewed in Friesen *et al.* 2007 and Friesen 2015), most population genetic studies on seabirds have focussed on temperate and polar species while tropical groups have been largely neglected (Friesen *et al.* 2007; Paleczny *et al*. 2015). It is widely believed that the mechanisms underlying population differentiation in seabirds differ by latitude (Friesen *et al.* 2007). In addressing these processes in three tropical species from the western Indian Ocean, the results presented in this thesis offer new insight into the dynamics of and processes affecting seabird populations at various scales in low latitudes.

Overall, the population structure of the three studied species seemed unrelated to scale. However weak, though significant, genetic structure was detected among the sampled breeding colonies of both the Red-footed Booby (*Sula sula*) and the Barau's Petrel (*Pterodroma baraui*) and populations of both of these species were composed of multiple genetic MUs. That said, the mechanisms underlying divergence in the two species were quite different. Genetic population structure exists at exceptionally fine scales in the Barau's Petrel and was largely the result of extreme natal philopatry and mate fidelity. Little or no gene flow appeared to exist between the *Vallee des Deux Miches* (Grand Benare) and *Bras-des-Etangs* (Piton-des-Neiges) breeding colonies and both genetic populations contained distinct allele frequencies and a moderate (5 – 10%) proportion of unique genetic diversity. More than a decade worth of banding records supported this genetic interpretation and highlighted a total absence of individual-exchanges among the two breeding colonies. Though the scale to which this species' populations are genetically structured was surprising, natal philopatry and mate fidelity are known to have driven divergence among populations of several other related tropical gadfly petrels (e.g. Friesen *et al.* 2006; Rayner *et al.* 2010; Wiley *et al.* 2011; Coulson 2016; Iglesias-Vasquez *et al.* 2017). Friesen (2015) additionally noted that philopatry is the second most obvious potential barrier to gene flow in seabirds after the physical isolation of breeding colonies. Divergence among the genetic populations of the Red-footed Booby appeared to be slightly more complex, and the exact underlying forces remain somewhat uncertain and warrant further investigation. Little is known about the foraging and dispersal patterns of Red-footed Boobies (Weimerskirch *et al.* 2005; Mendez *et al.* 2016), though the species was long thought to be sedentary on the occupied islands (Diamond 1974). In contrast to this assumption, present genetic information supported Morris-Pocock *et al.* (2016) and proposed that dispersal does in fact occur among geographically separate colonies. The breeding populations on Aldabra and Europa appear to be largely isolated from one another on either side of the Mozambique Channel, however. Both contained distinct allele frequencies, as well as moderate proportions (7 – 18%) of private alleles. One could assume that differing intrinsic (e.g. foraging ability, kleptoparasitism risk, discreet breeding phenologies, differing temperature regulation abilities, and social cues among the birds themselves) and extrinsic (e.g. ambient temperature, and sea-surface temperature [SST] on and surrounding the breeding colonies) barriers are driving this divergence (Le Corre 1999). This result also confirmed previous speculation and suggested that the warm current flowing through the Mozambique Channel may constitute a cryptic physical barrier to gene flow (Le Corre 1999; Bourjea *et al.* 2007). The colonies on Aldabra and Europa both exhibited a degree of genetic relation to the breeding population on Tromelin, suggesting that more contemporary gene flow occurs around either side of Madagascar. The scale and frequency of dispersal among the three sampled breeding colonies of the Red-footed Booby requires further study, however. Interestingly, in contrast to previous studies on the species, significant assortative mating by colour morph was also detected among birds breeding on Tromelin (Le Corre 1999; Baião & Parker 2008). This could imply that selection against immigrants (or atypical morph birds) may also occur in the Aldabra and Europa breeding colonies. In this context, assortative mating also appears to have maintained genetic differences among white-tailed white (potentially of Aldabra origin) and white-tailed brown (potentially of Europa origin) birds on Tromelin, thus sustaining this breeding populations' unusual but very stable morph ratio.

Microsatellite results from the Sooty Tern (*Onychoprion fuscatus*) surprisingly contrasted from those of both the Red-footed Booby and Barau's Petrel, in that absolutely no genetic population structure was detected among colonies in the western Indian Ocean.

Furthermore, the sampled breeding populations in the western Indian Ocean were genetically indistinguishable from Clipperton some 20 000 kilometres (Km) away in the Eastern Pacific Ocean. This reinforced two banding studies in the western Indian Ocean (Feare 1976a; Feare & Lesperance 2002), and supported the species' global matrilineal history (Avise *et al.* 2000). Considerable gene flow appears to occur among geographically separate breeding colonies of the Sooty Tern in the Indo-Pacific basin, despite differences in breeding phenology among some of the sampled islands. Sooty Terns have extremely low natal philopatry, unlike the Barau's Petrel, and appear to breed wherever conditions are favourable to do so (Le Corre 2001; Hamer *et al.* 2002; Jaquemet *et al.* 2007) and where there is sufficient conspecific social stimulus to initiate laying (Waas *et al.* 2000; Mougin *et al.* 2001; Coulson 2002). Adults and immature birds scatter widely at sea, sometimes over tens of thousands of kilometres (Jaeger *et al.* 2017), implying that individual Sooty Terns may regularly encounter (and possibly visit) non-natal breeding colonies. In one of the most intensive banding studies ever conducted on the species, Robertson and Wilmers (1996) noted that marked birds were regularly sighted on islands other than those in which they were initially marked. Though most birds returned to the colonies where they first bred, empirical studies support the hypothesis that this roaming behaviour lends itself towards panmixia (Friesen *et al.* 2007). More recent banding information supported Robertson and Wilmers (1996) and identified that Sooty Terns may be more faithful to colonies where they have previously bred, rather than to the colonies in which they were born. Again, in this manner, the Sooty Tern contrasted with both other studied species and especially the Barau's Petrel. Robertson and Wimers (1996) doubted whether Sooty Terns ever attempted to breed in colonies other than those in which they first became established. However, aside from environmental and social cues, several extrinsic factors appear to influence the decision of where individual Sooty Terns choose to breed

(Feare 1976a, b; Feare 1999; Feare & Lesperance 2002). Human disturbance within breeding colonies, particularly related to egg harvesting activities in the Seychelles and Amirantes, is a major factor underlying the decisions of adult Sooty Terns to move among breeding sites (Feare & Lesperance 2002). Other features of importance are the level of habitat alteration (Feare & Lesperance 2002), and the presence of ectoparasites and other irritants (Feare 1999).

5.3. Concluding remarks including prospects for future research

As previously theorised (Friesen *et al*. 2007), the mechanisms of population differentiation in seabirds seem to differ by latitude with what appears to be a greater influence of non-physical barriers to dispersal in the tropics. Genetic structure (or lack thereof, in the case of the Sooty Tern) among breeding populations of the three studied species was related to natal or breeding philopatric tendencies and, to a lesser extent, the intrinsic behavioural patterns (i.e. dispersal versus routine migration) of the study species and the conditions on and surrounding the breeding islands. However, this was completely unrelated to scale implying that, before any concrete prediction can be made about the scale-dependant mechanisms underlying seabird population structure, the results presented in this thesis will need to be extended in several directions. Most importantly, studies on other seabird species are necessary to strengthen general interpretations about the processes influencing seabird populations at low latitudes, and how these may compare to the mechanisms driving divergence in temperate and polar systems. Indeed, the conclusions drawn from this thesis were based on three very different species. Already this work has been extended to include the Red-tailed (*Phaethon rubricauda*; Humeau *et al*. in prep) and White-tailed Tropicbirds (*P. lepturus*; Jaeger *et al*. in prep), but other species should also be considered. Resolution of the mechanisms underlying genetic population structure of the Red-footed Booby is also necessary, and could be achieved by studying variables of presumed adaptive significance to plumage polymorphism (outlined in more detail in Chapter 2.4.4). Concerning all three of the study species, continued banding efforts would greatly assist in quantifying the scale and frequency of dispersal among breeding colonies. Global positioning system (GPS) or global location sensor (GLS) tracking at multiple breeding colonies of the Sooty Tern and Red-footed Booby could also be used to determine if intraspecific variation for habitat preference exists, and how this may relate to dispersal among breeding sites in both species. Tracking of non-breeding and fledging seabirds has only recently become possible (Weimerskirch et al. 2015), and therefore remains an exciting avenue for further research.

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APPENDICES:

Appendix 1: Band recoveries for Sooty Terns, in two age groups, at breeding colonies other than those in which the band was fitted. Data sourced from the British Trust of Ornithology (Feare), the South African Bird Ringing Unit (SAFRING), and the *Centre de Recherches sur la Biologie des Populations d'Oiseaux* (Muséum Paris).

Individual	Date banded	Age when first banded	Colony in which banded	Date of first recapture	Time elapsed (months)	Colony in which recovered	Distance travelled (Km)	Notes
DB11308	9 June 1997	Adult	African Banks	1 June 2005	95.54	Bird Island	244	Eggs possibly harvested.
DB11356	9 June 1997	Adult	African Banks	1 June 2005	95.54	Bird Island	244	Eggs possibly harvested.
DB11371	9 June 1997	Adult	African Banks	16 June 1999	24.16	Bird Island	244	Eggs possibly harvested.
DB11376	9 June 1997	Adult	African Banks	21 June 1998	12.36	Bird Island	244	Eggs possibly harvested.
DB11378	9 June 1997	Adult	African Banks	16 June 1999	24.16	Bird Island	244	Found subsequently on Bird Island on 18 June 2003. Eggs possibly harvested.
DB11380	9 June 1997	Adult	African Banks	16 June 1999	24.16	Bird Island	244	Eggs possibly harvested.
DB11389	9 June 1997	Adult	African Banks	1 July 2011	168.36	Desnoeufs	151	Found subsequently on Desnoeufs on 26 June 2012. Eggs possibly harvested.
DB11399	9 June 1997	Adult	African Banks	2 July 2011	168.39	Desnoeufs	151	Eggs possibly harvested.
DB11408	9 June 1997	Adult	African Banks	Not recorded	N/A	Bird Island	244	Eggs possibly harvested.
DB11443	9 June 1997	Adult	African Banks	16 June 1999	24.16	Bird Island	244	Eggs possibly harvested.
DB11034	19 July 1995	Adult	Aride	25 June 2006	130.95	Recif	42	N/A
DB11084	23 July 1995	Adult	Aride	14 June 2006	130.46	Bird Island	74	Found subsequently on Bird Island on 11 June 2007
DB11155	21 August 1995	Pullus	Aride	25 June 2006	129.87	Recif	42	Found subsequently on Recif on 1 June 2011
DB22399	23 August 1995	Pullus	Aride	10 July 2001	58.43	Recif	42	N/A

Appendix 1 continued.

Individual	Date banded	Age when first banded	Colony in which banded	Date of first recapture	Time elapsed (months)	Colony in which recovered	Distance travelled (Km)	Notes
DB22671	26 August 1995	Pullus	Aride	11 June 2006	117.25	Bird Island	74	N/A
DB22692	26 August 1995	Pullus	Aride	14 July 2012	190.20	Bird Island	74	N/A
DB22941	27 August 1999	Pullus	Aride	25 June 2004	93.74	Bird Island	74	N/A
DK89743	13 July 1994	Adult	Aride	17 June 2000	71.02	Bird Island	74	N/A
DK89837	10 July 1994	Adult	Aride	16 June 2000	71.08	Bird Island	74	N/A
DK89920	10 July 1994	Adult	Aride	16 June 2000	71.08	Bird Island	74	N/A
DK89937	10 July 1994	Adult	Aride	22 June 2002	95.21	Bird Island	74	N/A
DK89967	10 July 1994	Adult	Aride	27 June 2002	95.38	Recif	42	N/A
DK89971	10 July 1994	Adult	Aride	17 June 2000	71.11	Bird Island	74	N/A
DK89992	10 July 1994	Adult	Aride	17 June 2000	71.11	Bird Island	74	N/A
DK87328	21 June 1994	Adult	Bird Island	20 June 1996	23.93	Desnoeufs	370	Found subsequently on Bird Island on 17 June 2011
DB77931	26 June 1995	Pullus	Bird Island	26 June 2002	83.84	Recif	113	N/A
D60507	04 July 2004	Adult	Cousine	11 June 2006	23.18	Bird Island	85	N/A
D61550	10 September 2004	Pullus	Cousine	1 January 2011	75.54	Recif	28	Found subsequently on Recif on 1 January 2012
DB07068	30 June 1995	Adult	Desnoeufs	14 June 2008	155.18	Bird Island	370	N/A
DB08444	2 July 1995	Adult	Desnoeufs	21 June 2002	83.48	Bird Island	370	N/A
DB08844	2 July 1995	Adult	Desnoeufs	21 June 1998	35.57	Bird Island	370	Eggs exploited a priori; Found subsequently on Bird Island on 23 June 2002, 18 June 2003, 16 June 2004, 12 June 2006, 10 July 2012
DB09235	3 July 1995	Adult	Desnoeufs	17 June 1999	47.38	Bird Island	370	Eggs exploited a priori
DB09236	3 July 1995	Adult	Desnoeufs	18 June 2010	179.15	Bird Island	370	Eggs exploited a priori
DB09238	3 July 1995	Adult	Desnoeufs	12 July 2010	179.97	Bird Island	370	Eggs exploited a priori
DB09248	3 July 1995	Adult	Desnoeufs	29 June 2010	179.51	Bird Island	370	Eggs exploited a priori

Appendix 1 continued:

Individual	Date banded	Age when first banded	Colony in which banded	Date of first recapture	Time elapsed (months)	Colony in which recovered	Distance travelled (Km)	Notes
DB23436	17 June 1996	Adult	Desnoeufs	16 June 2006	119.70	Bird Island	370	Eggs exploited a priori
DK89635	06 July 1994	Adult	Recif	9 June 2001	82.95	Bird Island	110	N/A
DB23880	21 June 1996	Adult	Desnoeufs	24 June 2002	71.93	Bird Island	370	Eggs exploited a priori; Found subsequently on Bird Island on 20 June 2004, 12 June 2006 and 14 June 2010
DB23882	21 June 1996	Adult	Desnoeufs	16 July 2010	168.46	Bird Island	370	Eggs exploited a priori
DB23885	21 June 1996	Adult	Desnoeufs	23 July 2010	168.69	Bird Island	370	Eggs exploited a priori
DB23887	21 June 1996	Adult	Desnoeufs	26 June 2010	167.80	Bird Island	370	Eggs exploited a priori
DK87212	26 June 1995	Adult	Desnoeufs	16 June 2000	59.57	Bird Island	370	N/A
DK89556	6 July 1994	Adult	Recif	26 June 2004	119.44	Bird Island	110	N/A
DK89603	6 July 1994	Adult	Recif	17 June 2000	71.25	Bird Island	110	N/A
DK89609	6 July 1994	Adult	Recif	15 June 2000	71.18	Bird Island	110	N/A
DK89467	6 July 1994	Adult	Recif	28 May 2005	130.46	Bird Island	110	N/A
DK89504	6 July 1994	Adult	Recif	14 May 2001	82.10	Aride	42	Found dead in colony
DB77956	27 June 2002	Adult	Recif	23 June 2011	107.64	Desnoeufs	353	N/A
DB77963	27 June 2002	Adult	Recif	26 June 2011	107.74	Desnoeufs	353	N/A
DB77968	27 June 2002	Adult	Recif	10 July 2011	108.20	Desnoeufs	353	N/A
DB77971	27 June 2002	Adult	Recif	23 June 2011	107.64	Desnoeufs	353	N/A
DB77992	27 June 2002	Adult	Recif	23 June 2004	23.84	Bird Island	110	N/A
D-8767	19 December 1961	Pre-fledgling	Raoul Island	30 June 1995	401.51	Aride Island	13 200	Indo-Pacific dispersal